BIOLOGIA PLANTARUM 41 (3): 469-473, 1998

BRIEF COMMUNICATION

Amelioration of lead and mercury effects on germination and rice seedling growth by antioxidants

A. MISHRA and M.A. CHOUDHURI*

Plant Physiology and Biochemistry Laboratory, Department of Botany, University of Burdwan, Burdwan 713 104, W. Bengal, India

Abstract

Germination of two rice (*Oryza sativa* L.) cultivars (Ratna and IR 36) in the presence of 10 μM $PbCl_2$ and 10 μM $HgCl_2$ decreased germination percentage, germination index, shoot/root length, tolerance index and dry mass of shoots and roots. Mercury was more toxic than lead. Reduced glutathione (GSH), cysteine (Cys), ascorbic acid, and α-tocopherol alleviated the adverse effects of these metals on plants in the order GSH > Cys > ascorbic acid > α-tocopherol. The effects were more pronounced in tolerant cultivar IR 36 than in the relatively susceptible cultivar Ratna.

Additional key words: ascorbic acid, cysteine, germination index, *Oryza sativa*, reduced-glutathione, α-tocopherol, tolerance index.

Heavy metals, such as lead and mercury, have been widely reported to inhibit germination percentage and shoot/root elongation thereby decreasing fresh and dry mass of shoots and roots in various plant species including rice (Mukherjee and Maitra 1977, Kačabová and Nátr 1986, Mesmar and Jaber 1991, Hsu and Chang 1992, Mishra and Choudhuri 1997). Cysteine (Cys) is one of the three amino acids constituting phytochelatins and reduced-glutathione (GSH) is the precursor of phytochelatin (Steffens 1990). Ascorbic acid and α-tocopherol have antioxidative role in plant senescence (Grossman and Leshem 1978, Thompson 1988). The ameliorating effects of these chemicals on phytotoxic effects of lead and mercury have not been reported yet.

Received 24 October 1997, *accepted* 21 July 1998.

Abbreviations: AA - ascorbic acid; Cys - cysteine; GI - germination index; GSH - reduced-glutathione; TI - tolerance index; TP - α-tocopherol.

Acknowledgements: One of us (AM) thankfully acknowledges the Council of Scientific and Industrial Research, New Delhi for awarding her SRF-NET.

* To whom correspondence should be sent, fax: (+91) 0342 61260

The present study was therefore undertaken to analyse the possible ameliorating effect of the above-mentioned chemicals on the toxic effects of lead and mercury on germination and seedling growth of two rice cultivars.

Seeds of rice (*Oryza sativa* L. cv. Ratna and IR 36) were surface sterilized in 4 % (m/v) sodium hypochlorite solution and 20 seeds were placed in 9 cm Petri plate on filter paper discs moistened with either 10 cm^3 of sterilized water (control) or 10 cm^3 of $PbCl_2$ (10 μM) or $HgCl_2$ (10 μM) alone or in combination with reduced glutathione (GSH; 100 μM), cysteine (Cys; 100 μM), ascorbic acid (AA; 100 μM), and α-tocopherol (TP; 100 μM). The Petri plates were then covered with the lid and kept in a growth room at temperature of 28 ± 1 °C and irradiance of 29.70 $\mu mol\ m^{-2}\ s^{-1}$ (400 - 700 nm) for 16-h photoperiod. The lids were taken off from the Petri plates and the germination percentage in each set was recorded at intervals of 24 h. The test solutions (10 cm^3) was replenished every alternate day up to 7 d. The control set contained sterilized water only.

Germination index (GI) was recorded after 5 d as sum of total germinated seeds out of 1 800 seeds (Mhatre and Chaphekar 1982). Means of shoot/root length of 7-d-old seedlings were recorded. Tolerance index (TI) of 7-d-old seedlings were calculated according to the following formula (Turner and Marshal 1972):

$$TI = \frac{\text{mean length of longest root in test solution}}{\text{mean length of longest root in control solution}} \times 100$$

The dry mass of shoot/root was taken from 7-d-old seedlings after keeping them in an oven at 80 °C for 72 h. Each experiment was repeated three times with six replications per treatment on each occasion. The data (3 × 6) were statistically analysed for calculation of LSD values at 95 % confidence limits (Panse and Sukhatme 1967).

The percentage of seed germination as well as germination index of two rice cultivars decreased significantly under lead and mercury stress. Application of GSH, Cys, AA and TP (100 mM) ameliorated the negative effects of lead and mercury (Table 1). Both lead and mercury decreased shoot/root length. Treatment with above-mentioned compounds mitigated this decrease in both cultivars thereby increasing their tolerance index (Table 1).

Both lead and mercury had an inhibitory effect on dry mass of shoot and root. The inhibition of root growth was greater compared to shoot growth as evidenced from the higher shoot/root ratio. GSH, Cys, AA and TP alleviated this effect in both cultivars (Table 2).

In all the above cases the degree of alleviation was GSH > Cys > AA > TP and lead was less inhibitory than mercury. The cultivar IR 36 was more tolerant than the cultivar Ratna against these metals.

All the ameliorants used here have antioxidative properties and thus seem to influence the intracellular antioxidative mechanism of cells (Tappel 1980, Alscher 1989). GSH is a well known antioxidant playing a prominent role in defence against free radicals in plants (Alscher 1989). Moreover, GSH is a precursor of phytochelatin which is thought to detoxify metals (Steffens 1990). Cys also can alleviate the

Table 1. Effects of reduced glutathione (GSH, 100 μM), cysteine (Cys, 100 μM), ascorbic acid (AA, 100 μM), and α-tocopherol (TP, 100 μM) on germination and seedling growth of two cultivars of rice (*Oryza sativa* L. cvs. Ratna and IR 36) under lead ($PbCl_2$, 10 μM) and mercury ($HgCl_2$, 10 μM) stress.

Treatment	Germination [%]		Germination index		Shoot length [cm]		Root length [cm]		Tolerance index	
	Ratna	IR 36	Ratna	IR 36	Ratna	IR 36	Ratna	IR 36	Ratna	IR 36
Control (H_2O)	45.66	47.8	1760	1800	14.00	10.50	8.70	11.00	-	-
$PbCl_2$	39.65	44.25	1520	1680	13.20	10.00	5.70	8.00	46.77	67.10
$PbCl_2$ + GSH	42.71	49.30	1640	1800	16.00	12.75	10.00	14.20	108.87	109.86
$PbCl_2$ + Cys	41.52	47.80	1600	1800	15.10	12.10	9.89	13.90	105.64	106.57
$PbCl_2$ + AA	41.80	47.80	1560	1800	15.40	12.50	9.40	13.55	82.25	103.28
$PbCl_2$ + TP	41.20	47.67	1560	1800	15.20	11.98	9.20	13.46	81.45	100.00
$HgCl_2$	36.65	42.65	1400	1600	13.00	9.80	3.50	6.20	39.51	59.86
$HgCl_2$ + GSH	40.16	47.75	1620	1800	14.25	12.14	7.10	12.80	100.00	102.63
$HgCl_2$ + Cys	39.16	47.30	1580	1780	13.90	12.11	6.80	12.10	97.58	100.00
$HgCl_2$ + AA	38.12	45.77	1520	1760	13.99	11.99	6.80	12.10	68.54	89.47
$HgCl_2$ + TP	38.00	44.80	1480	1720	13.86	11.62	6.40	11.71	65.32	82.89
LSD at $P = 0.05$	4.10	4.30	159	162	1.14	1.21	0.91	0.67	7.10	8.90

adverse effects of mercury on some enzymes as reported by Vyas and Puranik (1993). AA is a scavenger of oxygen free radicals (Buckland *et al.* 1991). TP is an effective protector against peroxidation (Tappel 1980). AA and TP have been identified as non-enzymatic protectors against lipid peroxidation (Tappel 1980, Kunert and Böger 1984) and hence may counteract membrane lipid peroxidation.

Table 2. Effects of reduced glutathione (GSH, 100 μM), cysteine (Cys, 100 μM), ascorbic acid (AA, 100 μM) and α-tocopherol (TP, 100 μM) on dry mass [g] of shoot and root of two cultivars of rice (*Oryza sativa* L. cvs. Ratna and IR 36) under lead ($PbCl_2$, 10 μM) and mercury ($HgCl_2$, 10 μM) stress.

Treatment	Shoot dry mass		Root dry mass	
	Ratna	IR 36	Ratna	IR 36
Control (H_2O)	0.051	0.056	0.018	0.021
$PbCl_2$	0.049	0.056	0.016	0.021
$PbCl_2$ + GSH	0.060	0.068	0.022	0.026
$PbCl_2$ + Cys	0.056	0.063	0.021	0.025
$PbCl_2$ + AA	0.056	0.064	0.020	0.025
$PbCl_2$ + TP	0.053	0.061	0.019	0.023
$HgCl_2$	0.047	0.054	0.015	0.020
$HgCl_2$ + GSH	0.058	0.063	0.022	0.026
$HgCl_2$ + Cys	0.054	0.060	0.021	0.025
$HgCl_2$ + AA	0.054	0.063	0.019	0.024
$HgCl_2$ + TP	0.051	0.062	0.018	0.023
LSD at $P = 0.05$	0.005	0.005	0.002	0.003

All these chemicals might have some protective action on enzymes required during germination under lead and mercury stress. Thus, it may be concluded that these chemicals can act as good ameliorants to detoxify the harmful effects of lead and mercury. Therefore, GI, TI, and ratio of dry mass of shoot and root served as good indicators to observe the alleviating effects of different chemicals on heavy metal stress condition of rice cultivars in the order TI followed by the ratio of dry mass of shoot and root and others.

References

Alscher, R.G.: Biosynthesis and antioxidant function of glutathione in plants - Physiol. Plant. **77**: 457-464, 1989.

Buckland, S.M., Adam, H.P., George, A.F.H.: The role of ascorbate in drought-treated *Cochlearia atlantica* Poped. and *Armeria maritima* (Mill.) Willd. - New Phytol. **119**: 155-160, 1991.

Grossman, S., Leshem, Y.Y.: Lowering of endogenous lipoxygenase activity in *Pisum sativum* foliage by cytokinin as related to senescence. - Physiol. Plant. **43**: 359-362, 1978.

Hsu, F.H., Chang, H.C.: Inhibitory effects of heavy metals on seed germination and seedling growth of *Miscanthus* species. - Bot. Bull. Acad. sin. **33**: 335-342.

Kačabová, P., Nátr, L.: Effect of lead on growth characteristics and chlorophyll content in barley seedling. - Photosynthetica **20**: 411-417, 1986.

Kunert, K.J., Böger, P.: The diphenyl ether herbicide oxyfluorfen: action of antioxidants. - J. agr. Food Chem. **32**: 725-728, 1984.

Mesmar, M.N., Jaber, K.: The toxic effect of lead on seed germination, growth, chlorophyll and protein contents of wheat and lens. - Acta biol. hung. **42**: 331-344, 1991.

Mhatre, G.N., Chaphekar, S.B.: Effect of heavy metals on seed germination and early growth. - J. environ. Biol. **33**: 53-63, 1982.

Mishra, A., Choudhuri, M.A.: Ameliorating effects of salicylic acid on lead and mercury-induced inhibition of germination and early seedling growth of two rice cultivars. - Seed Sci. Technol. **25**: 263-270, 1997.

Mukherjee, S., Maitra, P.: Growth and metabolism of germinating rice (*Oryza sativa* L.) seeds as influenced by toxic concentration of lead. - Z. Pflanzenphysiol. **81**: 26-33, 1977.

Panse, V.G., Sukhatme, P.T.: Statistical Methods for Agricultural Works. 2nd Edition. - ICAR, New Delhi 1967.

Steffens, J.C.: The heavy metal binding peptides of plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **41**: 533-575, 1990.

Tappel, A.L.: Measurement of and protection from *in vivo* lipid peroxidation. - In: Pryor, W.A. (ed.): Free Radicals in Biology. Vol. IV. Pp. 1-47. Academic Press, New York 1980.

Thompson, J.E.: The molecular basis of membrane deterioration during senescence. - In: Noodén, L.D., Leopold, A.C. (ed.): Senescence and Aging in Plants. Pp. 52-77. Academic Press, San Diego 1988.

Turner, R.C., Marshal, C.: Accumulation of zinc by sub-cellular fraction of root of *Agrostis tenuis* Sibth. in relation to zinc tolerance. - New Phytol. **71**: 671-676, 1972.

Vyas, J., Puranik, R.M.: Inhibition of nitrate reductase activity by mercury in bean leaf segments. - Indian J. Plant Physiol. **35**: 57-60, 1993.