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Effects of salicylic acid on heavy metal-induced membrane deterioration mediated by lipoxygenase in rice

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Abstract

Deterioration of membranes caused by lipoxygenase (LOX) activity under 10 μM $PbCl_2$ or 10 μM $HgCl_2$ was partially alleviated by the exogenous application of 100 μM salicylic acid (SA). In two cultivars of rice (*Oryza sativa* L. cvs. Ratna and IR 36), the presence of SA ameliorated the increased leakage of electrolytes, injury index, and the content of malondialdehyde caused by these heavy metals. Lead decreased H_2O_2 content whereas Hg increased it in both cultivars. Application of SA increased H_2O_2 in presence of Pb, while decreased it in presence of Hg. Both Pb and Hg decreased superoxide dismutase activity, while increased peroxidase activity. The activity of catalase was decreased by Hg but increased by Pb and SA reversed their effects. Thus, SA ameliorated the damaging effects of Pb and Hg on membranes.

Additional key words: catalase, electrical conductivity, injury index, lead, lipid peroxidation, malondialdehyde, membrane permeability, mercury, *Oryza sativa*, peroxidase, superoxide dismutase.

Introduction

Salicylic acid (SA), a phenolic compound, has recently qualified as a plant hormone due to its various physiological and biochemical roles in plants (Raskin 1992). For example, SA inhibited ethylene biosynthesis in pear cell culture (Leslie and Romani 1988), induced pathogenesis-related (PR) proteins (Raskin 1992) and alleviated the inhibitory effects of heavy metals on germination of seeds of two rice cultivars (Mishra and Choudhuri 1997). Heavy metals induce proteins having structural similarities to PR proteins (Ortega and Ownby 1993).

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Abbreviations: EC - electrical conductivity; EDTA - ethylenediamine tetracetate; LOX - lipoxygenase; MDA - malondialdehyde; PR - pathogenesis-related; SA - salicylic acid; SOD - superoxide dismutase.

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Lipoxygenase (LOX) is a universally occurring enzyme that catalyzes the hydroperoxidation of unsaturated fatty acids of biomembranes (Axelrod *et al.* 1981). It is involved in membrane lipid peroxidation during plant senescence as well (Kar and Feierabend 1984, Lynch *et al.* 1985). The degradative products include free radicals, peroxides, malondialdehyde and jasmonic acid (Vick and Zimmerman 1984). All these substances cause further deterioration of membrane lipids (Thompson 1988) leading to increased leakage of solutes (Pauls and Thompson 1984). The membrane damage caused by senescence and different abiotic stresses including heavy metals is largely mediated through membrane lipid peroxidation (Lynch *et al.* 1985, Roy Chowdhury and Choudhuri 1985, De Vos *et al.* 1981, Chaudhuri and Choudhuri 1993, Bhattacharjee 1997/98). Reports concerning participation of free radicals in membrane deterioration caused by heavy metals are relatively few (Somashekaraiah *et al.* 1992, Bhattacharyya and Choudhuri 1995, Bhattacharrjee *et al.* 1996), though the importance of the maintenance of membrane integrity for better stress tolerance cannot be denied. We have shown that heavy metals such as lead and mercury caused loss of membrane integrity in rice (Mishra and Choudhuri 1996).

The aim of this paper was to report the effects of SA on lead- and mercury-induced membrane deterioration caused by lipoxygenase and also its effect on free radical scavenging enzymes such as superoxide dismutase, peroxidase and catalase under lead and mercury stress.

Materials and methods

Healthy seeds of rice (*Oryza sativa* L. cv. Ratna and cv. IR-36) procured from the Crop Research Farm of Burdwan University, were surface sterilized in 4 % (m/v) sodium hypochlorite solution. Seeds were placed in Petri plates containing filter paper discs moistened with either 10 cm^3 of sterile water (control) or 10 cm^3 of one of the following test solutions: 100 μM SA, 10 μM $PbCl_2$, 10 μM $HgCl_2$, 10 μM $PbCl_2$ + 100 μM SA, 10 μM $HgCl_2$ + 100 μM SA. These concentrations of lead, mercury and SA were selected from a previous germination tests (Mishra and Choudhuri 1997). The Petri plates were then kept in a growth room where the temperature was 28 ± 1 °C, irradiance 29.71 μmol(photon) m^{-2} s^{-1} (400 - 700 nm), and 16 h photoperiod. After 5 d, the seedlings were transferred to beakers containing sterile distilled water for control or one of the test solutions and kept for 10 more days. Then, the seedlings were taken out, washed thoroughly and the biochemical parameters were analysed from randomized shoot and root samples of 15-d-old rice seedlings.

Lipoxygenase (LOX, EC 1.13.11.12) was extracted and assayed according to Peterman and Siedow (1985). Malondialdehyde (MDA), a peroxidation product of fatty acids from membrane lipid, was determined following Heath and Packer (1985).

The membrane damage was assessed by measuring the leakage of electrolytes from shoot and root tissues of equal fresh mass immersed in equal volume of deionized water by the method of Biswas and Choudhuri (1976) as changes in

(electrical conductivity, EC) in a *Direct Reading Conductivity Meter 304* (*Systronics*, Ahmedabad, India). The injury index data relating to membrane damage were recorded according to the formula of Sullivan (1972):

$$\text{Injury (\%)} = [1-(1-T_1/T_2) / (1-C_1/C_2)] \times 100$$

where C_1 and C_2 are EC of control sample before and after autoclaving and T_1 and T_2 are treated samples before and after autoclaving.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined as described by Roy Chowdhury and Choudhuri (1985), peroxidase (EC 1.11.1.7) activity was assayed by the method of Kar and Mishra (1976) and catalase (EC 1.11.1.6) activity was determined according to Snell and Snell (1971). Hydrogenperoxide was extracted and estimated by the method described by Mondal and Choudhuri (1981).

All spectrophotometric readings were taken in a UV-VIS spectrophotometer (*Beckman DU-64*, Geneva, Switzerland). In all the cases, the percentage change over control was determined. Each experiment was repeated at least three times with six replicates per treatment on each occasion. The data were statistically analysed for determination of least significant difference (LSD) at 95 % confidence limits (Panse and Sukhatme 1967).

Results and discussion

The activity of LOX, MDA content, electrolyte leakage, and injury index increased in the shoots and roots of two cultivars of rice treated with Pb or Hg (Tables 1 and 2).

Table 1. Effect of 100 μM SA on membrane lipoxygenase activity [μmol(linolenic acid) g^{-1}(d.m.) s^{-1}] and malondialdehyde content [nmol g^{-1}(d.m.)] of shoots and roots of 15-d-old *Oryza sativa* cvs. Ratna and IR 36 under 10 μM Pb^{2+} or 10 μM Hg^{2+} treatment.

Treatments	Lipoxygenase activity				Malondialdehyde content			
	Ratna		IR 36		Ratna		IR 36	
	shoots	roots	shoots	roots	shoots	roots	shoots	roots
Control	5.13	0.77	1.00	0.70	34	89	54	79
SA	5.00	0.74	0.95	0.66	32	82	50	72
Pb	5.88	1.10	1.12	0.95	54	169	75	128
Pb + SA	5.52	0.97	1.02	0.80	43	123	62	99
Hg	5.91	1.14	1.23	1.03	64	214	78	141
Hg + SA	5.63	1.04	1.14	0.94	51	171	63	103
$LSD_{0.05}$	0.28	0.04	0.02	0.05	3	6	4	6

The effects of these metals were more pronounced in the cv. Ratna. SA alone significantly reduced LOX activity and MDA content and ameliorated the effect of Pb or Hg. The ameliorating effect of SA was more pronounced in root than in shoot of Ratna and IR 36. Further, Hg was more effective in increasing LOX activity than Pb and SA markedly reduced the metal-induced rise in LOX activity. The

counteraction of SA on increased LOX activity in presence of a heavy metal and the consequent effect on membrane lipid peroxidation is reported for the first time. One of the mechanisms of SA action may be a suppression of ethylene formation due to correlation between metal-induced LOX activity and ethylene formation (Kacperska and Kubacka-Zebalska 1985, Bhattacharjee 1997/98), and SA has been reported to inhibit ethylene evolution (Raskin 1992). Another explanation may be linked with the

Table 2. Effect of 100 μM SA on electrical conductivity [mS cm^{-1}] and injury index of shoots and roots of 15-d-old *Oryza sativa* cvs. Ratna and IR 36 under 10 μM Pb^{2+} or 10 μM Hg^{2+} treatment.

Treatments	Electrical conductivity				Injury index			
	Ratna		IR 36		Ratna		IR 36	
	shoots	roots	shoots	roots	shoots	roots	shoots	roots
Control	1.20	0.73	1.15	1.42	-	-	-	-
SA	1.10	0.66	1.04	1.25	8.5	10.2	13.5	16.9
Pb	1.90	1.48	1.30	2.01	52.1	63.8	7.1	34.1
Pb + SA	1.60	1.10	1.20	1.70	31.1	37.1	4.2	22.8
Hg	2.14	1.53	1.33	2.12	9.6	47.7	7.0	22.5
Hg + SA	1.78	1.20	1.24	1.95	4.1	30.5	3.7	12.5
$LSD_{0.05}$	0.09	0.06	0.06	0.05	2.5	3.1	1.0	2.1

chelating action of SA on metals (Oota 1975). This might be a reason also for decreased MDA content in presence of SA under heavy metal stress. The lower availability of the metals at the target site due to reported inhibition of uptake of metals by SA (Glass 1973, Harper and Balke 1981) cannot be ruled out either. The presence of SA protects membrane integrity under Pb or Hg stress and was also involved in alleviation of increased EC of the bathing medium and injury index (Table 2).

Table 3. Effect of 100 μM SA on superoxide dismutase [U g^{-1}(d.m.) s^{-1}] and peroxidase [μmol(H_2O_2 reduced) g^{-1}(d.m.) s^{-1}] activities of shoots and roots of 15-d-old *Oryza sativa* cvs. Ratna and IR 36 under 10 μM Pb^{2+} or 10 μM Hg^{2+} treatment.

Treatments	SOD activity				Peroxidase activity			
	Ratna		IR 36		Ratna		IR 36	
	shoots	roots	shoots	roots	shoots	roots	shoots	roots
Control	0.209	0.144	0.106	0.128	103.7	35.5	75.5	88.8
SA	0.220	0.156	0.117	0.143	93.2	31.6	66.2	76.9
Pb	0.148	0.117	0.102	0.120	159.4	101.2	99.37	103.9
Pb + SA	0.176	0.127	0.104	0.122	136.7	82.1	83.24	97.1
Hg	0.115	0.116	0.101	0.116	192.5	111.8	118.5	122.5
Hg + SA	0.163	0.132	0.101	0.121	164.5	87.5	92.19	101.2
$LSD_{0.05}$	0.020	0.030	0.010	0.020	5.2	3.5	4.0	4.2

Treatment of Pb and Hg decreased SOD activity in shoots and roots of both the cultivars of rice. SA could partially erase their inhibitory effect. Peroxidase activity increased in presence of Pb or Hg and SA treatment reduced the metal-induced rise in peroxidase activity (Table 3).

Table 4. Effect of 100 μM SA on catalase activity [μmol(H_2O_2 decomposed) g^{-1}(d.m.) s^{-1}] and H_2O_2 content [nmol g^{-1}(d.m.)] of shoots and roots of 15-d-old *Oryza sativa* cvs. Ratna and IR 36 under 10 μM Pb^{2+} or 10 μM Hg^{2+} treatment.

Treatments	Catalase activity				H_2O_2 content			
	Ratna		IR 36		Ratna		IR 36	
	shoots	roots	shoots	roots	shoots	roots	shoots	roots
Control	2.72	5.27	3.03	6.55	58	75	200	150
SA	2.59	5.00	2.78	6.12	89	95	204	182
Pb	4.71	6.74	4.49	7.67	46	62	145	135
Pb + SA	3.82	6.02	3.84	6.99	76	89	171	168
Hg	2.36	3.27	2.49	4.00	105	112	205	167
Hg + SA	4.27	5.11	2.98	5.90	81	87	176	132
$LSD_{0.05}$	0.20	0.25	0.23	0.04	3	3	4	4

Pb treatment increased while Hg treatment decreased catalase activity in shoots and roots in both cultivars but the effect was more pronounced in roots and in cv. Ratna (Table 4). SA reversed the above effect, *i.e.*, SA + Pb decreased catalase activity whereas SA + Hg increased its activity over their individual treatment. Pb treatment also decreased H_2O_2 content while Hg increased it over control in shoots and roots of rice cultivars. Here also SA in presence of Pb or Hg produced opposite effects on the endogenous content of H_2O_2 thereby showing a distinct correlation between catalase activity as influnced by the heavy metals and the corresponding H_2O_2 content (Table 4). The rise in catalase activity in presence of Pb was reported by Mukherjee and Maitra (1977). Hoxha *et al.* (1986) have shown that application of EDTA, a metal chelating agent, decreased catalase activity by chelating Pb.

In all cases, roots suffered more injury than the shoots in presence of heavy-metals and showed higher amelioration of deleterious effects by SA, and the cv. IR 36 showed greater tolerance than the cv. Ratna to the metals under study. The results reported here thus led to the general conclusion that SA could be used to alleviate the toxic effects of Pb and Hg on membrane integrity in rice plants.

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