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BRIEF COMMUNICATION

Salt tolerance of two aquatic macrophytes, *Pistia stratiotes* and *Salvinia molesta*

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Abstract

The physiological responses to NaCl salinity were investigated in two floating aquatic macrophytes, *Pistia stratiotes* L. and *Salvinia molesta* L. With the increasing NaCl concentration a decrease in chlorophyll and carotenoid contents was recorded in *Salvinia* as compared to *Pistia*. Also a greater increase in H_2O_2 accumulation and lipid peroxidation was observed in the shoot and root tissues of *Salvinia* as compared to *Pistia*. The superoxide dismutase, glutathione reductase, catalase and guaiacol peroxidase activities, and ascorbate and glutathione contents increased in *Salvinia* and *Pistia* shoot and root tissues in response to NaCl.

Additional key words: catalase, glutathione reductase, NaCl-salinity, peroxidase, superoxide dismutase, thiobarbituric acid reactive substance.

An inevitable product of aerobic cellular metabolism is generation of reactive oxygen species (ROS) and abiotic stresses are known to act as catalyst in producing free radical reactions causing oxidative stress in plants where reactive oxygen species (ROS), *i.e.*, superoxide radicals, hydroxyl radicals, alkoxyl radicals, and hydrogen peroxide are produced (for details see Scandalios 2002). These ROS cause lipid peroxidation and consequent membrane damage, protein and nucleic acid degradation and pigment bleaching (for details see Hendry and Crawford 1994). Plants are well equipped with both enzymatic [superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), dehydro-ascorbate reductase (DHAR), glutathione reductase (GR)] and non-enzymatic (carotenoid, ascorbate, glutathione, α-tocopherol) antioxidants to overcome the oxidative stress.

Pistia stratiotes L., an angiosperm and *Salvinia molesta* L., a pteridophyte, are two fast growing aquatic floating macrophytes and are important components of the natural ecosystems which are badly affected now-a-days with the ever-changing anthropogenic activities (Arber 1963). To test the hypothesis that NaCl-salinity induces oxidative stress in aquatic macrophytes and the possible involvement of antioxidant regulation in the differential salt tolerance of both the aquatic floating macrophytes, the present investigation was undertaken.

Floating macrophytes of two types (*Pistia stratiotes* L. and *Salvinia molesta* L.) were collected from the uncontaminated pond nearby university (90° 40' E longitude and 20° 04' N latitude) and grown under laboratory condition. The plants were washed with double distilled water several times and soaked dry without damaging the tissues. Plants were then transferred to Petri plates with different concentrations (0, 50, 100 and 200 mM) of NaCl solution with three replicates each. The Petri plates were incubated under white fluorescent tubes (*Philips* 36 Watt TLD, Bombay, India) giving a photon flux density of 52 μmol m^{-2} s^{-1}, temperature of 29 °C and relative humidity of 67 % for 3 d. After the treatments, the shoot and root were separated out, soaked dry and sampled for various biochemical and enzymic estimations.

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Abbreviations: APX - ascorbate peroxidase; CAT - catalase; DHAR - dehydroascorbate reductase; GPX - guaiacol peroxidase; GR - glutathione reductase; SOD - superoxide dismutase; TBARS - thiobarbituric acid reactive substance.
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Chlorophylls (Chl) and carotenoids (Car) were extracted using 80 % cold alkaline acetone and estimated spectrophotometrically by *Systronics* (Gujarat, India) *UV-106* spectrophotometer as per the method of Arnon (1949). Shoots and roots (0.2 g) were homogenized with 5 % trichloroacetic acid and the homogenate was used for the extraction and estimation of total peroxide (Sagisaka 1976) and malonyldialdehyde (MDA) determined by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Extraction and assay of CAT, GPX and SOD were done as per the methods of Chance and Maehly (1955) and Giannopolitis and Ries (1977). Enzyme activity are expressed as μmol(H_2O_2 destroyed) g^{-1}(f.m.) min^{-1}. GR was assayed by the method of Smith *et al*. (1980). The activity is expressed as ΔA_{412} g^{-1}(f.m.) s^{-1}. For the extraction and estimation of glutathione (Glu) and ascorbate (Asc) methods of Griffith (1980) and Oser (1979), respectively, were used. All observations were done in triplicates and repeated thrice and data represented mean ± SE.

Table 1. Changes in contents of chlorophyll *a*+*b* and carotenoids [mg g^{-1}(f.m.)] in two aquatic macrophytes subjected to different concentrations of NaCl for 3 d. Means ± SE.

Species	NaCl [mM]	Chl	Car
Pistia	0	1021.25 ± 52.41	15.33 ± 1.48
	50	684.11 ± 44.22	16.81 ± 1.14
	100	655.18 ± 42.48	15.22 ± 1.22
	200	622.48 ± 32.18	15.19 ± 1.44
Salvinia	0	985.51 ± 63.49	16.33 ± 1.48
	50	841.22 ± 56.46	16.81 ± 1.14
	100	79.44 ± 44.22	15.22 ± 1.22
	200	44.78 ± 39.91	15.90 ± 1.44

In both the macrophytes, Chl and Car contents decreased with the increase in NaCl concentration and treatment duration. Chl content dropped more in *Pistia* than in *Salvinia* at 200 mM NaCl. On the other hand, the

Table 2. Changes in contents [μmol g^{-1}(f.m.)] of H_2O_2, TBARS, Asc and Glu in two aquatic macrophytes subjected to different concentration of NaCl for 3 d. Means ± SE.

Species	NaCl [mM]	H_2O_2 root	shoot	TBARS root	shoot	Asc root	shoot	Glu root	shoot
Pistia	0	13.8 ± 1.21	14.3 ± 1.92	1.21 ± 0.14	0.26 ± 0.20	0.48 ± 0.01	0.51 ± 0.02	28.42 ± 1.42	32.89 ± 2.21
	50	14.9 ± 1.31	16.6 ± 2.42	1.30 ± 0.28	1.31 ± 0.32	0.66 ± 0.03	0.62 ± 0.04	30.54 ± 1.70	39.81 ± 2.14
	100	15.8 ± 1.42	17.6 ± 2.81	1.35 ± 0.44	1.34 ± 0.48	0.71 ± 0.05	0.75 ± 0.04	36.91 ± 2.24	38.33 ± 3.91
	200	18.4 ± 1.35	19.6 ± 3.84	1.43 ± 1.81	1.49 ± 0.62	0.79 ± 0.08	0.98 ± 0.09	48.98 ± 3.21	68.99 ± 4.81
Salvinia	0	15.4 ± 1.64	15.4 ± 1.92	1.28 ± 0.41	1.30 ± 0.22	0.66 ± 0.04	0.66 ± 0.-08	2.41 ± 1.08	3.22 ± 1.10
	50	16.5 ± 1.92	17.9 ± 2.01	1.32 ± 0.14	1.36 ± 0.31	0.72 ± 0.03	0.86 ± 0.02	4.83 ± 1.11	11.02 ± 1.10
	100	16.8 ± 8.80	18.3 ± 2.06	1.57 ± 0.28	1.46 ± 0.34	0.77 ± 0.06	0.88 ± 0.07	7.79 ± 1.07	11.83 ± 1.09
	200	17.2 ± 2.11	19.8 ± 2.09	1.65 ± 0.64	1.61 ± 0.55	0.85 ± 0.05	0.92 ± 0.08	8.06 ± 1.08	17.75 ± 1.03

Table 3. Changes in activities [U g^{-1}(f.m.)] of superoxide dismutase, glutathione reductase, catalase, guaiacol peroxidase in two aquatic macrophytes subjected to different concentration of NaCl for 3 d. Means ± SE.

Species	NaCl [mM]	SOD root	shoot	GR root	shoot	CAT root	shoot	GPX root	shoot
Pistia	0	0.24 ± 0.08	0.21 ± 0.06	0.03 ± 0.06	0.04 ± 0.07	253.4 ± 22.2	263.5 ± 22.3	62.51 ± 1.81	65.11 ± 1.21
	50	0.85 ± 0.12	0.30 ± 0.07	0.47 ± 0.10	0.46 ± 0.09	358.4 ± 31.2	358.4 ± 32.2	92.60 ± 1.42	99.42 ± 1.22
	100	1.60 ± 0.28	0.41 ± 0.11	0.48 ± 0.13	0.49 ± 0.12	439.1 ± 34.2	438.4 ± 34.0	95.28 ± 2.48	115.48 ± 2.48
	200	2.01 ± 0.14	1.50 ± 0.18	0.58 ± 0.20	0.54 ± 0.15	512.3 ± 40.2	482.3 ± 39.8	139.98 ± 2.35	137.98 ± 2.82
Salvinia	0	0.90 ± 0.09	0.08 ± 0.10	0.11 ± 0.10	0.10 ± 0.03	270.7 ± 21.0	93.1 ± 20.2	5.61 ± 0.14	25.30 ± 0.44
	50	0.85 ± 0.14	0.81 ± 0.41	0.21 ± 0.18	0.23 ± 0.05	255.3 ± 28.4	299.3 ± 31.4	10.20 ± 0.18	28.31 ± 0.33
	100	1.60 ± 0.27	0.44 ± 0.11	0.22 ± 0.14	0.28 ± 0.12	277.2 ± 38.4	377.5 ± 30.4	11.54 ± 0.61	30.88 ± 0.64
	200	1.88 ± 0.16	0.50 ± 0.15	0.41 ± 0.13	0.32 ± 0.15	300.2 ± 41.2	480.2 ± 34.2	13.49 ± 0.66	37.74 ± 0.77

Car content decreased sharply in *Pistia* and slightly in *Salvinia* (Table 1). The decrease in Chl and Car content may be attributed to a salt stress mediated degradation associated with a slower pigment synthesis (Bassi *et al*. 1990, Khan 2003/4). An increased H_2O_2 accumulation (Table 2) initiated localized oxidative damage. Increase in TBARS content (Table 2) indicated increased plasma membrane lipid peroxidation resulting in loss of membrane integrity, elasticity and fluidity (Paul and Thompson 1984, Panda and Upadhyay 2004). Simultaneously H_2O_2 acted as an oxidative stress signal that led to appropriate response of cellular protection system (Foyer 1997). The increase in SOD, GR, CAT and GPX activities at 200mM NaCl was recorded, with higher increase in *Pistia* than in *Salvinia* (Table 3). Ascorbate and glutathione contents also increased more in *Pistia* than in *Salvinia* at higher NaCl concentrations (Table 2). Increase in enzymatic as well as non-enzymatic antioxidants indicated good cellular capacity in preventing oxidative stress in these plants (Alscher *et al*. 1997, Hernandez *et al*. 2001, Scandalios 2002, Arbona *et al*. 2003).

In conclusion, our results showed the development of the antioxidative defense system in studied macrophytes against salt stress depended on plant species, NaCl concentration and day of treatment. *Salvinia* was found to be more sensitive to NaCl salinity possibly due to less effective antioxidant regulation mechanism.

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