BIOCHEMICAL AND ANTIOXIDANT RESPONSES OF PADDY (*ORYZA SATIVA* L.) TO FLUORIDE STRESS

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SUMMARY: Fluorotoxicosis was investigated in saplings of two varieties of paddy crops, *Oryza sativa* L. var. Swarno and *O. sativa* L. var. IR-36, grown in earthen pots watered with aqueous solution containing 0, 10, 20, and 30 mg NaF/L. Eight common biochemical parameters, viz., chlorophyll, carotenoid, catalase activity, peroxidase activity, ascorbic acid, free sugar, superoxide dismutase (SOD) activity, and free amino acids were estimated from the paddy plants following harvest after 90 days. The first four parameters were estimated from the leaves only, whereas the remaining four parameters were estimated from the roots, leaves, and grains. The chlorophyll, carotenoid, free sugar, and catalase activities decreased with increasing fluoride (F) treatment. The peroxidase and SOD activities as well as the free amino acids content showed an increasing trend. Ascorbic acid initially decreased but showed a higher value for 30 mg F/L treatment. Among these paddy varieties, the IR-36 was found to be slightly more tolerant to F stress as compared to the Swarno variety. The antioxidative defense mechanisms operating in the plant cells may be responsible for the observed biochemical changes in the paddy plant parts under the F stress. The study shows that the grains produce a larger antioxidant response than the leaves and roots for the same F uptake.

Keywords: Antioxidants; Biochemicals; Fluoride stress; Paddy (*Oryza sativa* L.); Reactive oxygen species (ROS).

INTRODUCTION

The toxic effects of chronic fluoride (F) exposure in humans,[1-3] domestic animals,[4-6] and plants[7] are well documented. Physiobiochemical changes due to F toxicity have been studied and reported in many plant species.[8,9] However; the exact mechanisms for these changes are not well understood. Various environmental stresses lead to excessive production of reactive oxygen species (ROS) in plants due to disruption of cellular homeostasis.[10] In order to avoid the oxidative damage, higher plants possess a complex antioxidative defence system comprising of non-enzymatic and enzymatic components.[11] The study of the biochemical changes in the plant cells in stressed conditions can improve our understanding of this defense mechanism. In an earlier study with four plant crops, we observed that F toxicity induced higher antioxidant superoxide dismutase (SOD) activity to attenuate the damaging effect of ROS.[12] In the present study we investigated the response of eight biochemical parameters to different degrees of F stress in two varieties of paddy crop (*Oryza sativa* L.).

MATERIALS AND METHODS

Experimental design: One-month-old paddy saplings of two high yielding varieties (i.e., *Oryza sativa* L. var. IR-36 and *O. sativa* L. var. Swarno) were transplanted in 24 earthen pots (35×40 cm) filled with loamy soil. Three replicated pots were used with each of the two varieties and were irrigated with 0, 10, 20, and

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30 mg F (NaF)/L, making a total of 24 pots. After transplantation, the saplings were allowed to grow in each pot. The pots were kept in open field conditions and were irrigated regularly with the above F solutions. After three months the plants had matured and were harvested. Representative samples of roots, leaves, and whole grains were collected for the estimation of the biochemical parameters. Chlorophyll, carotenoids, catalase activity, and peroxidase activity were estimated from leaf samples only. Ascorbic acid, free sugar, free amino acids, and superoxide dismutase **(**SOD) activity were measured from the roots, leaves, and grains separately.

Biochemical analysis: Chlorophyll pigments (chl-a and b and total chl) and ascorbic acid were estimated by following the methods as described earlier.[13] Carotenoid in the leaf extracts (in 80% acetone) was estimated spectrohotometrically[14] whereas catalase and peroxidase enzyme activities in the plant tissues were measured by colorimetric methods.[15,16] Simultaneously, superoxide dismutase activity was also estimated[12] in the extracts from root, leaf, and grain samples. Total water soluble sugar (monosaccharides and disaccharides) and free amino acids were determined by following the methods as described earlier.[17,18] Mean values and standard deviations (SD) were calculated from the result of three replicates for each of the parameters. The presence of significant variations among the means for each of the 32 groups was tested for with one-way analysis of variance (ANOVA) using GraphPad Instat™, version 3.10.

RESULTS AND DISCUSSION

Chlorophyll (Chl): Chl-a, chl-b, and total chl pigments were found to be decreased with increasing F concentration in paddy crop varieties, *O. sativa* L. var. Swarno, and *O. sativa* L. var. IR-36 (Table 1.)

Table 1. Chlorophyll, carotenoids, catalase activity, and peroxidase activity in paddy leaves at different concentrations of F treatment. Values are mean±SD of 3 replicates

Treatment (mg F/L)	Chl-a (mg/g)	Chl-b (mg/g)	Total chl (mg/g)	Carotenoids (mg/g)	Catalase activity (unit/min/g)	Peroxidase activity (unit/min/g)
			Var. Swarno			
Control	3.69±0.15†	2.510±0.10†	6.49±0.20†	0.779±0.04†	2.91±0.03†	54.02±1.40†
10	2.57±0.03†	1.340±0.02†	4.48±0.10†	0.661±0.02†	2.03±0.02†	105.51±1.00†
20	1.83±0.05†	0.627±0.02†	3.58±0.05†	0.518±0.03†	1.05±0.01†	146.21±2.00†
30	0.89±0.01†	0.189±0.01†	2.16±0.04†	0.371±0.02†	0.55±0.01†	205.50±1.30†
			Var. IR-36			
Control	3.71±0.1†	2.560±0.10†	6.65±0.21†	0.788±0.06†	2.98±0.031†	53.25±1.00†
10	2.71±0.1†	1.572±0.05†	4.60±0.12†	0.665±0.01†	2.11±0.014†	99.46±1.80†
20	1.94±0.1†	0.709±0.02†	3.64±0.14†	0.526±0.01†	1.28±0.011†	141.70±1.90†
30	0.98±0.1†	0.256±0.05†	2.46±0.07†	0.394±0.02†	0.61±0.005†	195.81±2.10†

Comparing the column means for the control, 10, 20, and 30 mg F/L treatment groups: *p<0.001, †p<0.0001. Chl-a = chlorophyll a, Chl-b = chlorophyll b, Total chl = total chlorophyll.

However, Chl-b pigment recorded the maximum reduction (38 to 92%) as compared to the control value. The possible causes for the decreasing of the pigment content are break down of chlorophyll, inhibition of chlorophyll biosynthesis, a stress-induced increase in the activity of the chlorophyll degrading enzyme chlorophyllase, and a F-induced reduction in Fe^{+2} which is essential for biosynthesis of chlorophyll.[19]

Carotenoids: The carotenoid pigments decreased up to 50% of the control value (Table 1). Carotenoid is an important antioxidant which plays a protective role during photosynthesis. It avoids the production of singlet oxygen by quenching the excited states of chlorophyll.[20] The reduction in carotenoids may be due to F-induced stress which inhibits carotenoid formation in the plant cells.

Catalase activity: The activity of catalase was found to be reduced up to 80% of the control value with increasing F concentration (Table 1). Catalase scavenges reactive oxygen species (ROS) such as H_2O_2 by breaking them down directly into water and oxygen. The observation indicates that F inhibits catalase activity in paddy plants. Possibly, the hydroxyl ions (OH^-) attached to iron atoms in catalase compounds are replaced by low molecular weight anions in sufficient concentration leading to this inhibition.[21]

Peroxidase activity: The peroxidase activity increased significantly (86% to 280% of the control value) with increasing F exposure (Table 1). Peroxidase decomposes H_2O_2 by oxidation of phenolic compounds. Peroxidases are ubiquitous antioxidant enzymes that participate in cellular redox homeostasis and have also been shown to increase under several abiotic stresses.[22]

Superoxide dismutase activity (SOD): The activity of SOD (unit/mg protein/ min) was increased by 75% of the control value in the paddy grains. The increasing value in roots and leaves was found about 60% (Table 2).

Table 2. SOD activity and free amino acids in roots, leaves and grains of paddy at different concentrations of F treatment.
Values are mean±SD of 3 replicates

Treatment (mg F/L)	SOD activities (unit/mg protein/min)			Free amino acids (mg/g)		
	Root	Leaf	Grain	Root	Leaf	Grain
			Var. Swarno			
Control	1.112±0.001†	1.354±0.002†	1.099±0.001†	4.51±0.03†	2.94±0.04†	2.48±0.01†
10	1.362±0.002†	1.633±0.003†	1.42±0.002†	4.77±0.01†	3.12±0.01†	2.62±0.03†
20	1.551±0.001†	1.876±0.004†	1.67±0.002†	5.19±0.02†	3.39±0.01†	2.85±0.02†
30	1.815±0.002†	2.168±0.001†	1.93±0.002†	5.73±0.01†	3.74±0.01†	3.14±0.01†
			Var. IR-36			
Control	1.137±0.002†	1.361±0.003†	1.109±0.002†	4.6±0.015†	2.98±0.02†	2.52±0.02†
10	1.375±0.003†	1.636±0.002†	1.43±0. 003†	4.87±0.02†	3.16±0.03†	2.66±0.02†
20	1.569±0.004†	1.846±0.002†	1.674±0.001†	5.30±0.06†	3.43±0.01†	2.89±0.01†
30	1.137±0.002†	1.361±0.003†	1.109±0.002†	4.6±0.015†	2.98±0.02†	2.52±0.02†

Comparing the column means for the control, 10, 20, and 30 mg F/L treatment groups: *p<0.001, †p<0.0001.

The increased activity of SOD may be partly due to an increased metabolic activity or an increased rate of SOD biosynthesis under the influence of F exposure.[23] This enhanced activity may be an adaptive reaction to changes in oxidative stress which can be considered as a positive feedback mechanism.[12]

Free amino acids: The free amino acids also showed an increasing trend with increasing F treatment (Table 2). An increase of approximately 27% increase from the control values occurred in all the three plant parts *i.e.* roots, grains, and leaves. The free amino acids pool in the plant tissues depends on the relative contributions of degradation of storage protein, amino acid synthesis, and amino acid utilization for protein synthesis and respiration. F stress is known to enhance the respiratory rate in plants, which might have contributed towards the increased production of free amino acids.[24]

Ascorbic acid: The ascorbic acid content initially decreased with increasing F treatment (Table 3).

Table 3. Ascorbic acid and free sugar content in roots, leaves, and grains of paddy at different concentrations of F treatment.
Values are mean±SD of 3 replicates

Treatment (mg F/L)	Ascorbic acid (mg/g)			Free sugar (mg/g)		
	Root	Leaf	Grain	Root	Leaf	Grain
			Var. Swarno			
Control	0.347±0.03†	0.567±0.02†	0.612±0.02†	4.16±0.05†	8.94±0.2†	1.58±0.03†
10	0.201±0.01†	0.403±0.01†	0.431±0.01†	3.78±0.08†	8.42±0.15†	1.47±0.02†
20	0.126±0.04†	0.323±0.05†	0.334±0.01†	3.18±0.02†	7.48±0.05†	1.26±0.02†
30	0.189±0.01†	0.501±0.01†	0.535±0.02†	2.38±0.04†	6.22±0.04†	0.99±0.01†
			Var. IR-36			
Control	0.348±0.07*	0.56±0.031†	0.611±0.03†	4.21±0.20†	8.91±0.12†	1.56±0.02†
10	0.198±0.01*	0.39±0.012†	0.427±0.02†	3.85±0.12†	8.51±0.12†	1.46±0.01†
20	0.106±0.01*	0.312±0.04†	0.325±0.01†	3.25±0.11†	7.56±0.10†	1.25±0.03†
30	0.173±0.03*	0.485±0.04†	0.515±0.03†	2.39±0.12†	6.34±0.11†	1.01±0.01†

Comparing the column means for the control, 10, 20, and 30 mg F/L treatment groups: *$p<0.001$, †$p<0.0001$.

At 20 mg F/L treatment, the ascorbic acid content in the roots was reduced by 70% of its value compared to the control whereas in the leaves and grains the reduction was about 45%. However, in the leaves and grains at 30 mg F/L treatment the ascorbic acid showed a higher value, even exceeding its value at 10 mg F/L treatment. A similar changing pattern in ascorbic acid with increasing F stress has also been observed and reported from other plant species.[13] This is perhaps due to binding of F with ascorbic acid oxidase enzyme which leads to inhibition of the degradation of ascorbic acid.

Free sugar: The free sugar content gradually decreased with increasing F exposure. The reduction was found to be highest (45%) in the roots followed by

the grains (35%) and leaves (30%) compared to the control value (Table 3). Soluble sugars have an important role in ROS scavenging mechanisms as increased glucose levels can increase the production of nicotinamide adenine dinucleotide phosphate (NADPH). The reduction in free sugar may be due to inhibition of photosynthesis or due to F-induced oxidative stress.[10]

In summary, the F stress activates the defence mechanism in paddy crops by apparently changing of peroxidase and SOD enzyme activities as well as the content of ascorbic acid and amino acids. At high F level exposure, production of free sugar, chlorophyll, and carotenoid pigments, and the activities of catalase enzyme were found to be restricted. These changes are relatively less in the *O. sativa* L. var. IR-36 and therefore this variety can be considered to be a tolerant variety or to be less sensitive to F exposure. However, the maximum biochemical changes occurred in the roots as compared to the other parts of paddy plants. Grains of paddy crops are comparatively less susceptible to F accumulation as they can produce the maximum antioxidant response.[25] The present findings have significance for technology development in selection of F tolerant paddy cultivars.

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