EFFECT OF FLUORIDE ON SUPEROXIDE DISMUTASE ACTIVITY IN FOUR COMMON CROP PLANTS

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SUMMARY: Radish (*Raphunus sativa*), coriander (*Coriandrum sativum*), mustard (*Brassica juncea*), and spinach (*Spinacea oleracea*) plants were grown in earthen pots watered with aqueous solutions containing 0, 5, and 10 mg F/L. Fluoride (F) uptake and superoxide dismutase (SOD) activity (unit/mg protein/min) were estimated from the edible plant parts following harvest after 60 days. The values of both entities rose with increasing F exposure in the order radish > coriander > spinach > mustard. The results indicate that plant species tolerant to F toxicity induce higher antioxidant SOD activity, which may be an adaptive reaction in plant cells to attenuate the damaging effect of reactive oxygen species (ROS) generated during F stress.

Keywords: Coriander; Mustard; Plant fluoride uptake; Radish; Spinach; Superoxide dismutase activities.

INTRODUCTION

Fluoride (F) toxicity is a cause of great concern in many countries, especially where it is found in excessive quantities in the groundwater.[1] In addition, significant accumulation of F in crops irrigated with F-contaminated water is also a matter of growing concern,[2,3] particularly since F causes inhibition of growth and development and reduction in photosynthesis in many plant species.[4,5] In this connection, F-induced oxidative stress is believed to be a key mechanism underlying various toxic effects associated with F exposure,[6] causing increased production of reactive oxygen species (ROS**)** such as superoxide radicals that may damage membrane lipids, cause enzyme inactivation, and increase breakage of DNA strands.[7,8] It is also known that living systems have evolved an intracellular enzymatic defense system for protection against ROS. Among these, superoxide dismutase (SOD: EC 1.15.1.1) is a key enzyme that eliminates superoxide radicals from the cell environment and prevents the formation of toxic ROS and their derivatives.[9-11]

Although there have been many investigations on the effects of F stress on SOD activity in various organs of animals, e.g., on testes of rats,[6] such studies are less frequent in plants, e.g., in tea leaves[10] and mung bean seedlings.[11] The present study investigated the impact of F on SOD activities in the edible tissues of four common winter crops grown in many F-endemic areas of India: radish (*Raphunus sativa*), coriander (*Coriandrum sativum*), mustard (*Brassica juncea*), and spinach (*Spinacea oleracea*).

MATERIALS AND METHODS

Experimental design: Healthy seeds of radish, spinach, coriander, and mustard were collected locally and were germinated under normal temperature in 36 earthen pots filled with 4 kg of loamy soil. Three replicate pots were used with

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each of the four kinds of seeds and were irrigated with 0, 5, and 10 mg F (from NaF)/L, making a total of 36 pots. After germination, three seedlings 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 two months the plants had matured and were harvested. Representative samples (leaves of all plants, along with radish roots and mustard seeds) were collected for estimation of F and SOD specific activity.

Estimation of F: The plant samples were air dried and blended separately. Extracts were prepared in 0.1 M perchloric acid. Water extractable fluoride was measured by the F ion selective electrode (Orion Thermo scientific, Model 1119000, USA) method.[12] Mean values and standard deviations (SD) were calculated from the result of three replicates.

Estimation of SOD activity: The activity of superoxide dismutase was estimated in units/mg protein/min following the method of Paoletti et al. (1986).[13] Plant materials were extracted in 100 mM Tea-Dea buffer, and the solution absorbance at 340 nm was recorded for 10 min. The difference in absorbance value was used to calculate the percentage of inhibition and the activity in enzyme units/mg protein/min, which was determined following the method of Lowry et al.[14]

RESULTS AND DISCUSSION

F uptake: As seen in Table 1, the F uptake by all the studied plant samples increased many fold with increasing F treatment in the order radish root > coriander leaf > radish leaf > spinach leaf > mustard leaf > mustard seed.

Table 1. F uptake (in mg/kg)[a] by selected plant parts at different concentrations of F in the irrigation water

Plant parts	F concentration		
	Control (0 mg F/L)	5 mg F/L	10 mg F/L
Mustard leaf	0.00	4.15±0.01	8.21±0.03
Mustard seed	0.00	2.69±0.03	5.12±0.01
Radish root	0.021 ±0.003	26.20±0.06	59.78±0.05
Radish leaf	0.016±0.001	21.09±0.09	47.13±0.04
Coriander leaf	0.00	22.30±0.08	50.17±0.05
Spinach leaf	0.00	8.61±0.03	18.20±0.04

[a]Values are mean ± SD of 3 replicates

The F uptake at 10 mg F/L exposure, compared to 5 mg F/L, increased by 90.3% in mustard seed, 98% in mustard leaf, 111.4% in spinach leaf, 123.5% in radish leaf, 125% in coriander leaf, and 128.2% in radish root. A trace amount of F was also found in the radish root and leaf in the control. In radish and mustard, the F

content decreased from roots to leaves and from leaves to seeds, respectively, which is in agreement with the general trend of translocation of F in plants.

SOD activity: Superoxide dismutases (SODs) are ubiquitous metalloenzymes that play important role in immune response of organisms, especially in defence against toxic superoxide radicals.[15,16] As noted in the introduction, inhibitory effects of F on SOD activity in tea leaves and mung bean seedlings are on record.[10,11] Moreover, variations in SOD activity in other plant species from exposure to F at different stages of growth have also been reported.[17,18] Here, as seen in Table 2, an increase in SOD activity occurred in all parts of the four plant species studied with increasing concentration of F in the irrigation water.

Table 2. SOD activity (in units/mg protein/min) in selected plant parts at different concentrations of F in the irrigation water[a]

Plant parts	F concentration		
	Control (0 mg F/L)	5 mg F/L	10 mg F/L
Mustard leaf	1.42±0.02	1.52±0.03	1.96±0.02
Mustard seed	1.51±0.02	1.60±0.02	2.02±0.03
Radish root	1.47 ±0.04	1.66±0.04	2.38±0.02
Radish leaf	1.33±0.03	1.48±0.03	2.11±0.04
Coriander leaf	1.37±0.02	1.51±0.02	2.08±0.02
Spinach leaf	1.85±0.03	2.00±0.01	2.69±0.02

[a]Values are mean ± SD of 3 replicates

The spinach leaf and mustard leaf recorded the highest and lowest SOD activity, respectively. However, the increase in activity was nearly parallel to the F uptake by the plant parts. Relative to the control, the SOD activity increased with 5 and then 10 mg F/L exposure in the order: radish root (13% – 62%) > radish leaf (12% – 59%) > coriander leaf (10.2% – 52%) > spinach leaf (8% – 45%) > mustard leaf (7.0% – 38%) > mustard seed (6.0% – 34%).

Increased SOD activity has also been reported in tolerant paddy cultivars owing to salinity stress.[18] This tolerance may be an adaptive reaction to changes in oxidative stress. F exposure possibly increases SOD activity through enhanced metabolism or SOD biosynthesis in the crop plants, which can be considered as a positive feedback mechanism. [17]

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