FLUORIDE-INDUCED CHANGES IN THE ANTIOXIDANT DEFENCE SYSTEM IN TWO CONTRASTING CULTIVARS OF *TRITICUM AESTIVUM* L.

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ABSTRACT: The effects of sodium fluoride (NaF) at 0, 50, 100, 200, 300, and 400 ppm on germination behaviour, membrane stability, and some other biochemical parameters were studied in two wheat (*Triticum aestivum* L.) cultivars, HD 3086 and WH 1105, at the seedling stage on the 7th day of germination. With increasing concentrations of NaF (50–400 ppm), the germination percentage decreased from 100% in the control group with 0 ppm of NaF to 96–81% in HD 3086 and from 99% in the control group to 93–77% in WH 1105. Physiological parameters, viz., root and shoot length, dry weight, and seed vigour, also decreased monotonically with increasing NaF concentrations. Fluoride ion (F) application resulted in a disruption of cellular membrane by increasing lipid peroxidation which was reflected in a rise in the content of the lipid peroxidation marker malondialdehyde. Nonetheless, compared to the WH 1105 cultivar, the HD 3086 cultivar had lower malondialdehyde and higher proline levels in the treated seedlingas owing to the better expression of antioxidants. Activities of antioxidative enzymes, viz., superoxide dismutase and peroxidase, increased with increasing NaF concentrations in both the cultivars while catalase activity was reduced in correspondence with the decrease in the contents of ascorbate and tocopherol. Apparently, the wheat seedlings responded to NaF-induced stress with an increase in superoxide dismutase activity and in proline content. The higher up-regulation of the antioxidant defense system with NaF-induced stress in the HD 3086 cultivar, as compared to the WH 1105 cultivar, possibly contributed to its better tolerance to F toxicity.

Keywords: Antioxidant enzymes; Ascorbate; Fluoride; Growth parameters; Proline; Tocopherol; *Triticum aestivum* L.

INTRODUCTION

Prolonged exposure to the fluoride ion (F) causes diverse mild to severe toxic effects not only in humans[1-5] and domestic animals[6-10] but also in plants.[11,12] Indeed, F induces an array of physiological and biochemical changes in plants which affect plant growth and development and these may lead to a drastic reduction in economic yield. A high F level above >2.6 mM in certain soils causes decreased biomass production and crop productivity.[13] Absorbed F from the soil is transported via xylematic flow to the transpiratory organs like leaves, where it can accumulate and cause adverse effects in plants.[14] F stress induces numerous biochemical responses including increased production of reactive oxygen species (ROS) that disrupt normal metabolism of plants causing lipid peroxidation, protein denaturation, and DNA damage.[15] Content of ROS is controlled by an antioxidant system which include antioxidative enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC1.11.1.7), and catalase (CAT; EC 1.11.1.6) and antioxidants (ascorbate, tocopherol, etc.).[16,17] In a number of studies, it has been observed that ROS scavenging mechanisms play an important vital role in

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protecting plants against oxidative stress[18,19]and different responses have been reported in tolerant and sensitive cultivars.[20] Proline, an amino acid and scavenger of ROS increased in response to diverse types of abiotic stresses. A rise in proline content showed a strong correlation with its biosynthetic enzymes and played a diverse role as a transient source of carbon and nitrogen in the F-stressed plants.[21,22] Evidently, proline is important for the protection and survival of plants by acting as a regulator of cellular osmotic adjustment under stress conditions.

Wheat is one of the major cereals in the world and is one of the main sources of calories and protein. However, crop production is severely affected by adverse environmental stresses. Sodium fluoride (NaF) at 100–200 ppm doses were found to be toxic to all morphological and yield characteristics of the wheat plant.[23] However, the physiological and biochemical basis of this toxicity remains poorly understood. Therefore, the present study was undertaken to ascertain the effect of NaF treatment on the antioxidant defense mechanism in two wheat cultivars at the seedling stage.

MATERIAL AND METHODS

PLANT MATERIAL: Seeds of two wheat cultivars, HD 3086 and WH 1105, were obtained from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India. The seeds were surface sterilized with 0.1% solution of mercuric chloride for 1 min followed by thorough rinsing with distilled water. Seeds were then germinated in distilled water (control) and in NaF solutions, with 50, 100, 200, 300, and 400 ppm of NaF, in petri plates (10 seeds for each cultivar) containing germination paper moistened with 4 mL of distilled water. These petri plates were kept at 25±1ºC in BOD incubator. Uniform size wheat seedlings were sampled after 6th day of germination for analysis of physiological and biochemical parameters. In the control, as well as in the NaF-stressed seedlings, all measurements were performed on root and shoot in triplicate.

PHYSIOLOGICAL PARAMETERS: The root and shoot lengths of seedlings raised under six different conditions were measured on 7th day of germination. The time of 7 days for these parameters was taken to allow clear differences to occur in the cultivars between root and shoot under a stress condition. Dry weight of root and shoot was estimated after drying the tissue at 60ºC for four days. Germination percentage was determined according to following formula:

$$\text{Germination percentage} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

$$\text{Vigour index} = (\text{root length} + \text{shoot length}) \times \text{germination percentage}$$

BIOCHEMICAL PARAMETERS:

Enzyme extraction and assays: Root and shoot samples (0.5 g fresh weight) were homogenized in 3 mL of ice-cold 50 mM sodium phosphate buffer pH 6.5 for

POD and SOD activities, and at pH 7.5 for CAT activity. The homogenate was centrifuged at 10,000 × g for 20 min at 4ºC and clear supernatant was used for assaying activities of POD, SOD, and CAT as previously described.[24]

Antioxidant, proline, and malondialdehyde contents: The contents of ascorbate, tocopherol, proline, and the lipid peroxidation marker malondialdehyde were determined as previously described.[25]

RESULTS

The seedling growth data representing germination percentage of root and shoot lengths and dry weight in two wheat cultivars HD 3086 and WH 1105 were recorded in Tables 1 and 2.

Table 1. Effect of NaF on the growth parameters of the shoots of wheat seedlings on the 7th day of treatment. (Values are mean±SE of three replicates, FW=fresh weight, DW=dry weight)

Cultivar	Treatment with NaF (ppm)	Shoot				
		Germination (%)	Length (cm)	FW (mg)	DW (mg)	Vigour index
HD 3086	Control	100	16.3±1.37	105.6±2.5	20.13±1.82	3956±249
	50	96±1.0	12.6±1.20	92.01±2.0	16.56±1.55	3092±152
	100	93±1.0	9.21±1.05	83.18±1.5	13.70±1.36	2290±199
	200	90±2.0	7.84±1.30	78.40±2.8	12.43±1.25	1840±230
	300	86±2.0	6.76±1.15	69.03±3.6	11.20±1.15	1249±169
	400	81±1.0	3.72±0.56	42.34±2.5	8.26±1.04	704.1±60
WH 1105	Control	99±0.6	11.4±1.40	89.06±2.9	14.40±1.41	3113±232
	50	93±3.0	9.96±1.38	71.64±2.1	13.54±1.60	2569±122
	100	90±1.5	7.76±1.02	63.04±2.6	12.60±1.40	1885±210
	200	85±2.6	6.41±1.05	53.05±2.0	11.66±1.33	1422±110
	300	83±2.0	5.48±0.70	38.71±1.5	8.26±1.10	979.4±77
	400	77±4.5	3.34±0.50	27.66±2.5	7.20±1.00	564.93±30
CD value (5%)		A (1.45), B (2.52), AB (NS)	A (0.75), B (1.31), AB (1.85)	A (1.69), B (2.93), AB (4.14)	A (0.93), B (1.61), AB (2.28)	A (116.26), B (201.37), AB (284.79)

Table 2. Effect of NaF on the growth parameters of the roots of wheat seedlings on the 7th day of treatment. (Values are mean±SE of three replicates, FW=fresh weight, DW=dry weight)

Cultivar	Treatment with NaF (ppm)	Root		
		Length (cm)	FW (mg)	DW (mg)
HD 3086	Control	23.2±1.46	149.6±5.03	25.34±2.20
	50	19.6±1.15	115.3±1.52	20.33±1.85
	100	15.4±1.20	85.66±4.04	13.35±1.55
	200	12.63±1.02	72.33±2.51	12.40±1.65
	300	7.84±1.15	48.67±3.21	18.63±1.40
	400	4.95±1.10	34.33±4.04	7.84±1.22
WH 1105	Control	20.03±1.30	158.3±2.88	26.50±1.31
	50	17.56±1.00	129.0±3.60	20.85±1.30
	100	13.23±1.02	116.6±2.08	19.46±1.35
	200	10.32±0.75	102.3±2.51	18.30±1.31
	300	6.46±1.22	45.00±2.64	9.35±1.25
	400	4.06±1.05	25.33±1.52	6.25±1.05
CD Value (5%)		A (0.78), B (1.35), AB (NS)	A (2.16), B(3.74), AB (5.29)	A (0.02), B (1.77), AB (2.50)

In comparison to control, 50–400 ppm of NaF led to a decrease in germination percentage, root/shoot lengths, and fresh and dry weights accompanied by a decrease in vigour index. At the maximum NaF concentration (400 ppm), the germination percentage was reduced to 77–81% followed by 300 ppm NaF (83–86%), 200 ppm NaF (85–90%), 100 ppm NaF (90–93%), and 50 ppm NaF (93–96%) in both the cultivars as compared to seedlings maintained under controlled conditions, i.e., without NaF (100%). It was found that the highest NaF concentration (400 ppm) also decreased shoot length by 70–77% and root length by 76–80% in WH 1105 and HD 3086, respectively. Similarly, fresh and dry weights of the seedlings also decreased with increasing F concentrations. Fresh

weight of shoot was decreased by 60–68% over control at 400 ppm NaF concentration while in root it decreased by 77–83%. Table 1 shows that vigour index was decreased to 18% with the higher concentration 400 ppm NaF treatment compared to the control group.

Fluoride stress caused a significant elevation in the content of the lipid peroxidation marker malondialdehyde in root and shoot of both the cultivars (Figure 1). In WH 1105, the increase in MDA content was more in shoot than in r root whereas in HD 3086 it increased more in root than in shoot.

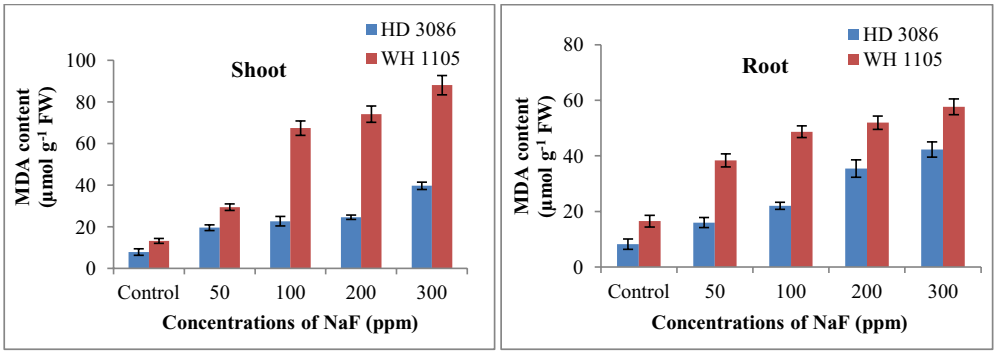

Figure 1. Effect of NaF on malondialdehyde (MDA) content in shoot and root of wheat on 7th day of germination. Shoot (G=1.23, T=1.95, G×T=2.76) and root (G=1.76, T=2.78, G×T=3.94) where G is genotype, T is treatment and G×T is genotype and treatment interaction. The values of G, T, and G×T are the critical differences at the 5% level of significance while the vertical bar on the graph represents the standard error.

The activities of antioxidant enzymes (POD, SOD, and CAT) in root and shoot of the two wheat cultivars under the influence of F stress were assayed (Figure 2). In comparison to control seedlings, SOD and POD activities increased with increasing NaF concentration in both the cultivars. In contrast, specific activity of CAT decreased with increase in NaF concentration.

The contents of ascorbate, tocopherol, and proline in root and shoot of the two wheat cultivars under the influence of F stress were assayed (Figure3). Seedlings treated with 50–300 ppm of NaF showed a decrease in the ascorbic acid and tocopherol content. However, the proline content increased from 64–89% with increasing NaF concentration from 50–300 ppm.

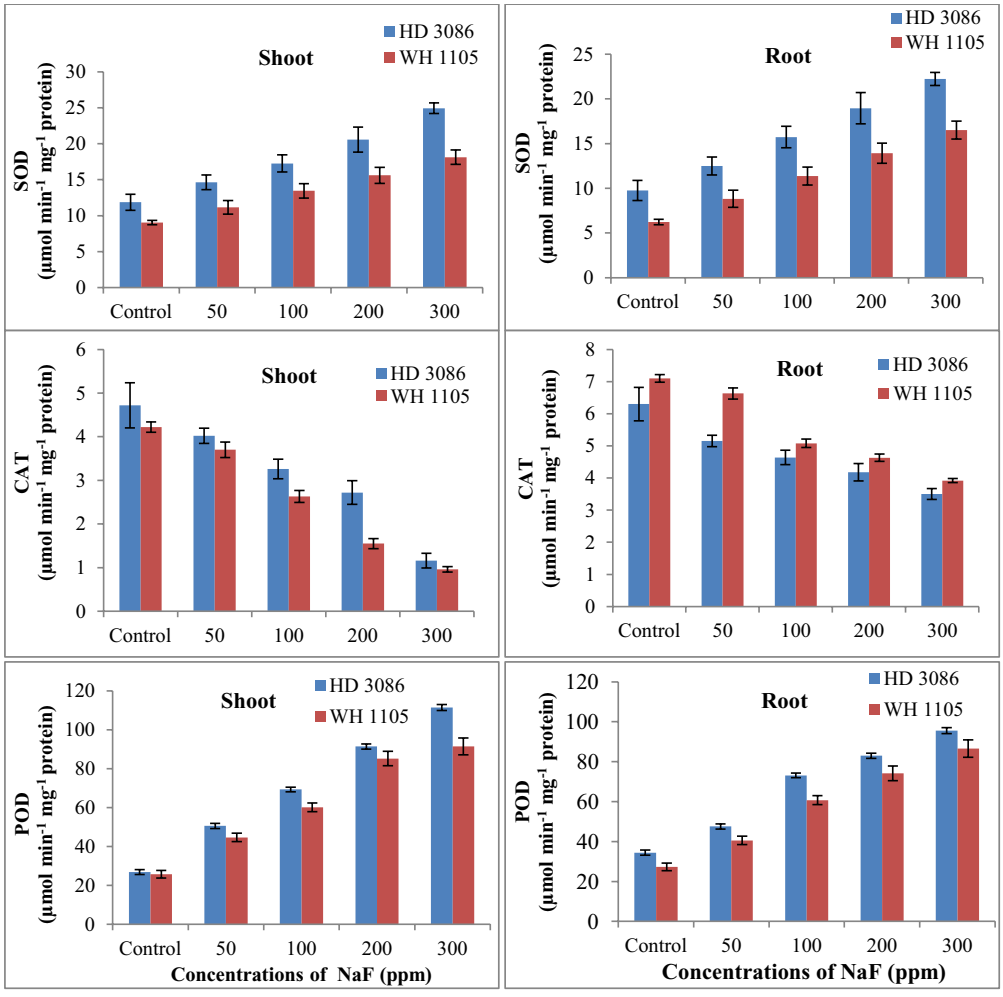

Figure 2. Effect of NaF on specific activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in shoot and root of wheat on 7th day of germination. (SOD) Shoot (G=0.81, T=1.29, G×T=1.83) Root (G=0.80, T=1.27, G×T=NS), (CAT) Shoot (G=0.17, T=0.27, G×T=0.39) Root (G=0.24, T=0.38, G×T=0.55), (POD) Shoot (G=1.78, T=2.82, G×T=3.99) Root (G=1.71, T=2.71, G×T=NS) where G is genotype, T is treatment and G×T is genotype and treatment interaction. The values of G, T and G×T are the critical differences at the 5% level of significance while the vertical bar on the graph represents the standard error.

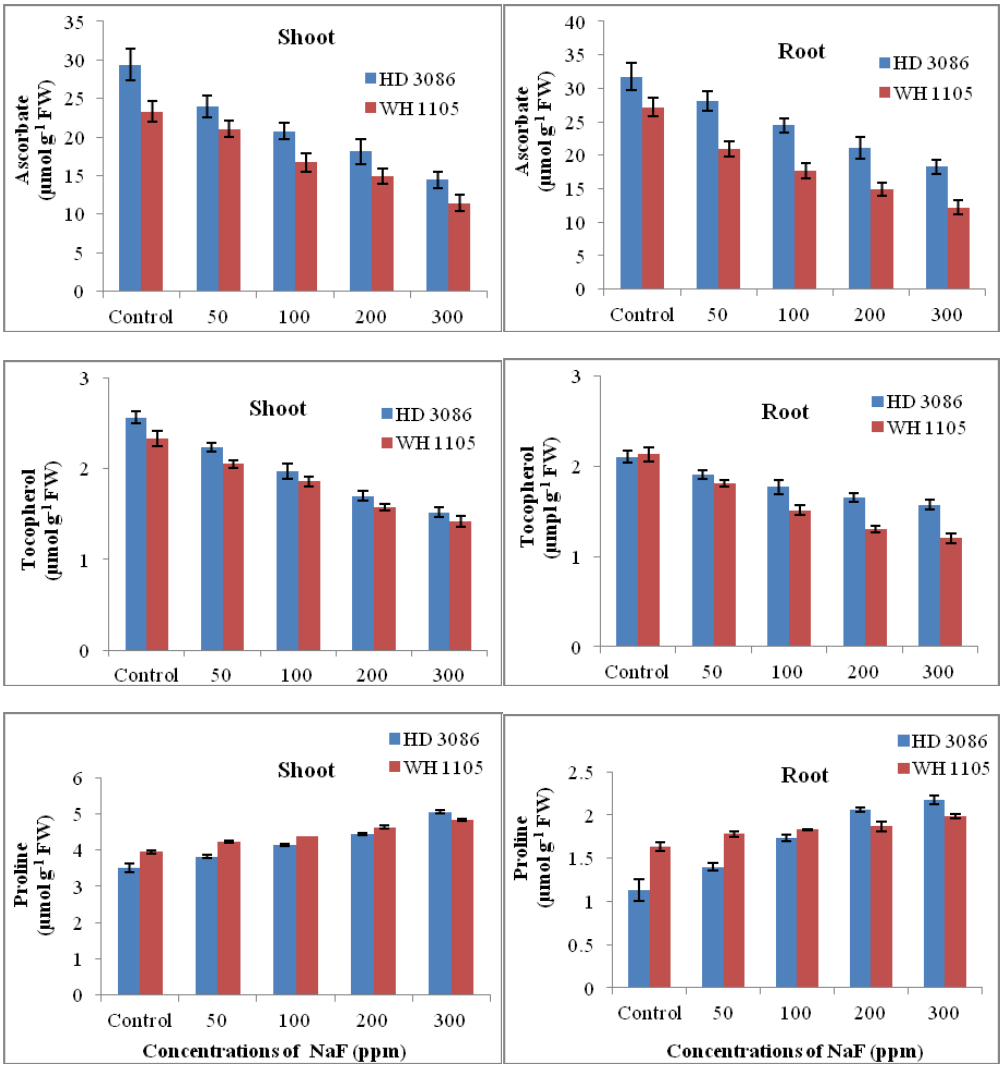

Figure 3. Effect of NaF on contents of ascorbate, tocopherol, and proline in shoot and root of wheat on 7th day of germination. (Ascorbate) Shoot (G=1.00, T=1.58, G¥T=NS) Root (G=1.10, T=1.75, G¥T=NS), (a-tocopherol) Shoot (G=0.44, T=0.70, G¥T=NS) Root (G=0.47, T=0.75, G¥T=0.10), (Proline) Shoot (G=0.41, T=0.65, G¥T=0.91) Root (G=0.62, T=0.69, G¥T=0.140) where G is genotype, T is treatment and G¥T is genotype and treatment interaction. The values of G, T and G¥T are the critical differences at the 5% level of significance while the vertical bar on the graph represents the standard error.

DISCUSSION

Environment influences germination and seedling growth which encompasses several physiological and biochemical processes.[12] The present study revealed that the NaF application led to significant inhibition of germination which may be due to reduction of amylase activity.[12] Similar reduction in root/shoot lengths under F stress was associated with the suppression of cell expansion and cell growth in

response to low turgor pressure.[26] Likewise, reduction of root and shoot length due to F toxicity has been reported in *Triticum aestivum*[27] and *Oryza sativa*.[28] Results of the present study are also supported by the view that F induces alterations in metabolism resulting in diminished plant growth.[29,30] Seed vigour is an indicator of plant health and can be computed for all the germination and growth parameters. In this study, germination percentage and root and shoot lengths showed a decreasing trend with addition of NaF, and so the seed vigour, measured as vigour index, also had a decreasing trend with increasing F concentrations (50–400 ppm NaF). Similarly, reduction in seed vigour has also been reported in *Cicerarietinum* under F stress.[12]

Plant cell membranes are disrupted from normal functions if exposed to certain stresses. Hence, the maintenance of their integrity and stability under stress conditions is a major component of stress tolerance mechanisms in plants.[31] Lipid peroxidation is commonly taken as an indicator of oxidative stress and is quantified by the malondialdehyde (MDA) content.[32] F treatment causes the production of superoxide free radicals and ROS like the peroxides of polyunsaturated fatty acids which, on decomposition generate MDA, the most abundant aldehydic lipid breakdown product.[32] MDA content was higher in shoot than root of F-stressed seedlings which led us to suggest that membrane of root is better protected from oxidative stress than shoot. Invariably, the level of POD and SOD were higher in shoot while CAT predominated in root of both cultivars under stress conditions. It may thus be inferred, that a major quantity of ROS are scavenged by POD route in shoot, whereas in root the CAT pathway operates. Peroxidases (PODs) are ubiquitous antioxidant enzymes that participate in cellular redox homeostasis and have also been shown to increase under several abiotic stresses.[33]

A higher POD and SOD activity in HD 3086 over WH 1105 under normal and the NaF stress condition demonstrates its superior tolerance mechanism in terms of H_2O_2 production and utilization. CAT is important in the removal of H_2O_2 generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration, and purine catabolism.[12] Interestingly, CAT activity decreased tremendously with NaF application in both root and shoot of F tolerant cultivar HD 3086. Superoxide dismutase is an enzyme responsible for the dismutating of $O_2^{-\bullet}$, thus maintaining the cell membrane integrity. Our results are in agreement with the reports in paddy where significant up-regulation of the specific activity of SOD by exogenous F (10–30 mg/L) was observed.[21] Ascorbate helps in regeneration of reduced glutathione via ascorbate glutathione cycle and thus helps in elimination of ROS. It helps in maintaining a balance between the generation and quenching of ROS in plant systems.[34] A decrease in α-tocopherol content following F stress in both cultivars was evident. Ascorbate maintains the regeneration of α-tocopherol, providing synergetic protection of the membrane under stress conditions.[35] α-tocopherol levels change differentially in response to environmental stress. Changes in α-tocopherol levels result from altered gene expression of pathway-related degradation and recycling that contribute to plant

stress tolerance, while decreased levels favour oxidative damage.[36] Higher content of proline in shoot reflects their better protective mechanism under oxidative stress over root. Proline plays an important vital role as an osmolyte by activating water uptake to maintain cellular turgor.[37]Wheat cultivar HD 3086 had a higher level of proline in root and shoot as compared to WH 1105 which supports our earlier observations of higher membrane integrity in the former cultivar.

CONCLUSION

Overall, it appears that physiological parameters, viz., root/shoot lengths and their weights, get significantly disrupted with increasing NaF concentrations. The up-regulation of the antioxidant system by NaF in wheat cultivars contributes to better protection of membrane integrity in root and shoot against F through reduced lipid peroxide content. NaF also decreases ascorbate and tocopherol contents in wheat seedlings. Furthermore, shoots are better protected against the destructive effects of ROS than roots. This differential response of two cultivars under different F concentrations gives a clear indication that metabolic pathways differ in these cultivars to counteract ROS generated during stress condition and WH 1105 was more affected than HD 3086 with increasing F concentration. The present findings add significantly to our existing knowledge of F-induced toxicity in crops.

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