

Original Research Article

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Biochemical Responses of Elevated Level of Fluoride in Nutrient Medium on Wheat and Barley

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ABSTRACT

The present study response of wheat and barley to fluoride toxicity. 40 days old hydroponically grown plants were exposed to different levels of fluoride stress by supplementing normal Hoagland's solution with 100 ppm (T₁) and 200 ppm fluoride (T₂) using NaF. Observations were recorded after 25 days of imposing fluoride treatments. In wheat and barley starch content in leaves increased with increased fluoride concentration in root zone. Soluble sugar content followed a decline trend in wheat but in barley it creased up to T₁ level and then declined. In both crops soluble protein content declined but proline and free amino acid contents increased with increased fluoride concentration in root. In wheat malondialdehyde (MDA) content decreased significant in plants under T₁ treatment, but further increased markedly at 200 ppm of NaF. In barley MDA content increased progressively and significantly as the level of NaF increased. As compared to barley, 100 ppm fluoride was lesser toxic to wheat for measured parameters while at 200 ppm both crops were equally sensitive to fluoride toxicity. It is opined that MDA content may be taken as a parameter to identify fluoride toxicity tolerant and sensitive crops.

Keywords

Fluoride toxicity, Morphological parameters, Sugar content, Starch content, Amino acids content, Protein content, MDA content

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Introduction

Fluorine is one of the most common elements in the natural environment. It is the 13th most abundant element in the earth crust (Loganathan *et al.*, 2011). The uptake of fluoride by plants from the substrate is typically low because soil-borne fluoride most

frequently occurs in a form unavailable to plants; hence plants absorb considerable amounts of this element under natural conditions (Snioszek *et al.*, 2009). Fluorides are common phytotoxic air and soil pollutant (Guderian, 2012). However, in soils polluted with fluoride; plants may take up its excessive quantities. Soils exposed to large emission of

fluoride tend to accumulate it, which eventually has an unfavorable impact on agricultural production. The negative effect of fluoride on plants is manifested, for example, by chlorosis (yellowing) and necrosis of leaves as well as a decrease in content of chlorophyll in leaves. The end result of which is the inhibited growth of plant and less biomass production (Ram *et al.*, 2014). The influence of fluoride on plants ought to be viewed from two angles. On one hand, the effect of this element contained in industrial gases and dusts and on the other hand, the effect of fluoride absorbed by the root system of plant (Sabal *et al.*, 2006). Fluoride toxicity affects physiological and biochemical parameter in plant viz. seed germination (Farooq and Bara, 2010) and growth, nutrient uptake, photosynthesis, respiration, yield and yield attributes (Sodani *et al.*, 2018). Fluoride in higher concentration is also injurious to animals and human beings (Choubisa and Choubisa, 2016). Earlier it was thought that the chief source to fluoride toxicity in human beings is through drinking water, but now it is well documented that plants contribute significantly to fluoride toxicity in organisms (Choubisa and Choubisa, 2010). Crop and crop varieties are reported to respond differently to increased fluoride concentration in soil and accumulate differential amounts of fluoride in their vegetative and reproductive parts (Mondal and Gupta, 2015). Barley is reported to be more sensitive to fluoride toxicity than wheat (Singh *et al.*, 2017; Arya and Rao, 1978). Toxicity of fluoride is expected to intensify with increased atmospheric pollution and depleting soil water table. It is, therefore, expected that in days to come hazard of fluoride will increase. Therefore, it is essential to identify differential responses of crops and their genotypes to fluoride toxicity and the effect of elevated levels of fluoride on biochemical processes of plants so that parameters associated with fluoride toxicity may be

visualized and crops genotypes resistant to fluoride toxicity may be identified/developed.

Materials and Methods

A hydroponic experiment was carried out in the Net House of the Agricultural Farm of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, situated at 25°18' N latitude, 83°3' E longitude and at an altitude of 75.7 meter above the mean sea level during *rabi* (winter season) 2016-17 taking wheat (HUW-234) and barley (EMBSN-34) genotypes. Seeds were germinated on *Petri* dishes in an incubator for 3 days at 28±1°C. Plastic containers (18×18×9cm) were taken and filled with 21 L distilled water. Containers were covered with steriofoam sheet in such a way that it was 1 cm above the upper level of water in the containers. Steriofoam sheets were having holes (2 cm diameter) at a distance of 5 cm. Three days old seedlings of uniform growth were selected carefully from *Petri* dishes and planted in each hole on steriofoam sheet with the support of sterilized cotton plug. For three days seedlings were grown on distilled water and then supplied with N/2 Hoagland's (Epstein, 1972) nutrient solution for the next 3 days. Thereafter, it was replaced by N-Hoagland's solution. Plants were allowed to grow in N-Hoagland's solution up to the age of 40 days. During this period Hoagland's solution was replaced at an interval of every three days. Trays were grouped into 3 sets and after 40 days supplemented with N-Hoagland (T₀), N-Hoagland+100ppm fluoride (T₁) and N-Hoagland + 200ppm fluoride (T₂) using NaF. Observations pertaining to soluble sugar (Dubois *et al.*, 1956), starch (Dubois *et al.*, 1956), protein (Kruger, 2002), amino acids (Dubois *et al.*, 1956), proline (Bates *et al.*, 1973) and malondialdehyde (Heath and Packer, 1968) contents were recorded after 25 days of imposing NaF treatment on first fully expanded leaf from top. At the time of

observation, total plant age was 65 days. All observations were recorded in three replications and mean values were calculated. Data were analyzed following completely randomized design. Critical difference (CD) values were calculated at 1% level.

Results and Discussion

In wheat soluble sugar content though decreased with increased leave of fluoride in the root zone (Table 1) but differences between treatments were not significant. Starch content increased significantly, i.e., from 29.36 mgg⁻¹ fresh weight in control (T₀) to 43.822 mgg⁻¹ fresh weight in T₁ and 45.760 mgg⁻¹ fresh weight in plant under T₂ treatment. The maximum protein content (0.136 mgg⁻¹ fresh weight) was observed in T₀ plants and the minimum (0.103 mgg⁻¹ fresh weight) in plant under T₂ treatment (Table 1). Nevertheless, differences were significant between T₀ and T₂. In this crop, as compared to T₀, proline content though increased in plants under T₁ treatment, but difference was not significant. However, in plant under T₂ treatment proline content of 0.346 mgg⁻¹ fresh

weight was significant high than those in T₀ plants (Table 1). Free amino acids content in leaves increased under NaF treatments (Table1). Nevertheless, difference between treatments did not differ significantly. The MDA content, as compared to control (T₀), decreased markedly in plants under T₁ treatment in wheat, but further increased significantly in plant treated with 200 ppm fluoride (Table 1).

In barley, as compared to T₀ plants, soluble sugars increased significantly in plant under fluoride treatment and the increment was of higher magnitude in plants under T₁ treatment than in plants under T₂ (Table 2). Starch content also increased significantly with added doses of NaF in the Hoagland's solution (Table 2). Protein content, as compare to T₀, decreased in plant as the level of fluoride increased in the nutrient medium (Table 2). Proline content, as compared to T₀, increased in plants under T₁ treatment which further declined in plant under T₂ treatment (Table 2). In this crop, MDA content increased significantly as the level of NaF increased in the Hoagland's solution.

Table.1 Effect of different levels of sodium fluoride treatment on soluble sugar, starch, proline, protein, free amino acids (mg g⁻¹ fresh weight) and MDA (nM g⁻¹ fresh weight) contents in wheat (HUW-234)

S.No.	Treatments*	Soluble sugar	Starch	Protein	Proline	MDA	Free amino acids
1.	T ₀	13.668	29.360	0.136	0.242	14.849	0.153
2.	T ₁	12.105	43.822	0.123	0.336	9.548	0.167
3.	T ₂	11.472	45.780	0.103	0.346	27.591	0.192
	SEm±	1.374	3.549	0.010	0.035	0.585	0.027
	C.D. at 1%	4.758	12.282	0.036	0.119	2.025	0.092

*T₀ = Normal Hoagland (Control), T₁ = Normal Hoagland + 100 ppm fluoride, T₂ = Normal Hoagland + 200ppm fluoride

Plant was imposed with above treatments after 40 days of growth. Observation was taken after 25 days of imposing fluoride treatments (at observation total plant age 65 days).

Table.2 Effect of different levels of sodium fluoride treatment on soluble sugar, starch, proline, protein, free amino acids (mg g⁻¹ fresh weight) and MDA (nM g⁻¹ fresh weight) in barley (EMBSM)

S.No.	Treatments*	Soluble sugar	Starch	Protein	Proline	MDA	Free amino acids
1.	T ₀	14.843	29.360	0.118	0.325	21.505	0.853
2.	T ₁	24.358	43.823	0.085	0.594	32.903	0.409
3.	T ₂	18.022	45.780	0.078	0.340	42.043	0.056
	SEm±	1.158	3.549	0.015	0.045	2.258	0.130
	C.D at 1%	4.009	12.282	0.054	0.155	7.811	0.450

*T₀ = Normal Hoagland (Control), T₁ = Normal Hoagland + 100 ppm fluoride, T₂ = Normal Hoagland + 200ppm fluoride Plant was imposed with above treatments after 40 days of growth. Observation were taken after 25 days of imposing fluoride treatments (at observation total plant age 65 days)

Table.3 Per cent increase (+) or decrease (-) in soluble sugar, starch, proline, protein, free amino acids and MDA contents in plants under T₁ and T₂ treatments over T₀ in wheat and barley

Crop	Treatments*	Soluble sugars	Starch	Protein	Proline	MDA	Free amino acids
Wheat	T ₁	11.42	49.25	9.55	38.84	37.71	9.15
	T ₂	16.03	55.92	24.26	42.97	85.86	25.49
Barley	T ₁	64.08	46.34	27.96	82.76	53.03	52.05
	T ₂	21.42	54.78	33.89	4.61	95.53	93.43

*T₀ = Normal Hoagland (Control), T₁ = Normal Hoagland + 100 ppm fluoride, T₂ = Normal Hoagland + 200ppm fluoride

The maximum amount of MDA was found in leaves of those plants which were under T₂ treatment (Table 2). Free amino acids content in barley reduced significantly as the amount of NaF in Hoagland's solution increased progressively (Table 2).

Phytotoxicity due to NaF is one of the severe ecological problems in the world. Presence of fluoride in soil or irrigation water causes an array of physiological and biochemical changes in plants, affecting plant growth and development and may lead to a drastic reduction in economic yield (Szostek and Ciecko, 2017). Present investigation indicated that increased fluoride level in root zone caused derangement in the levels of soluble sugars, starch, protein, proline and free amino acids contents in leaves of wheat at barley.

Such observations have been reported by others (Kumar, 2000; Murray and Wilson, 2014; Panda, 2015). A central role of sugars depends not only on direct involvement in the synthesis of other compound and production of energy but also on stabilization of membrane action such as regulators of gene expression and as signalling molecules (Mohammadkhani and Heidari, 2008). Phosphoglucosyltransferase, a key enzyme in the sucrose biosynthesis, is reported to be very sensitive to fluoride toxicity, which results in inhibition of sucrose biosynthesis in plants (Yang and Miller, 1963). Soluble sugar content proved to be a better marker for selecting improvement of F toxicity tolerance in wheat and barley (Gadi *et al.*, 2012). In present investigation, in wheat crop, soluble sugar content as compare to control plant (T₀)

decreased under both T₁ and T₂ treatments which suggested the inhibition of sucrose synthesis by NaF.

In barley at T₁ level of F, soluble sugar content increases while at T₂ level of F it decreased (Table 3) which indicated that as compared to wheat soluble sugar biosynthesis in barley is lesser sensitive to fluoride toxicity. Literature is available to indicate variable trend in soluble sugar level under fluoride toxicity in plants (Greenway and Munns, 1980). Increased starch content in wheat and barley under fluoride toxicity indicated that starch breakdown is suppressed by fluoride. It is reported that fluoride inhibits activity of α -amylase (Sethy and Ghosh, 2013). In the presence of fluoride activity of α -amylase declines due to binding of calcium with fluoride making calcium non-available. Probably this is the major cause for high starch content in plants supplemented with F.

Proline levels in plants may cause augmentation of antioxidant response, as proline has been shown to maintain protein integrity and enhance the activities of different enzymes (Ahmad *et al.*, 2010). ROS scavenging activity of proline has been suggested as it acts as a singlet oxygen quencher (Gadi, 2016). It is well known that proline plays a key role in conferring tolerance to the plants against various abiotic stresses (Weinstein and Davison, 2004; Wang *et al.*, 2004). Proline content increased under T₁ and T₂ in wheat in increasing order but in barley significant increment (82.76%) was observed in plants under T₁ treatment and it declined (4.16%) in plants under T₂ treatment, which is in agreement with other work (Datta *et al.*, 2012).

Proteins, being one of the important organic nitrogenous constituents of plants, play a important role in the compensatory metabolism of a plant species during F stress conditions. In wheat and barley content of soluble protein was higher in the control

plants which decreased to 9.55 and 24.26 % in wheat and 27.96 and 33.89% in barley with increased NaF concentrations from 100 to 200 ppm, respectively. Though at these F treatments free amino acid content increased concomitantly (9.15 and 25.49%, respectively) in wheat but declined significantly in barley (Table 3). Increased free amino acid content in fluoride supplemented plants is, therefore, concluded due to increased hydrolysis of proteins. It is also possible that incorporation of free amino acids into protein is decreased. It is documented that fluoride has toxic effects on protein synthesis; as a result, as compared to normal plant fluoride stressed plants have lower protein content (Gadi, 2016). Fluoride is reported to inhibit chain elongation of polypeptides (Rao *et al.*, 2013). Significant increase in free amino acid content and reduction in protein content in barley than in wheat indicated that protein synthesis, is more sensitive in barley than in wheat to fluoride toxicity. Reference is also available to indicate that fluoride toxicity leads to increased tissue protein content (Asthir and Tak, 2017).

The concentration of lipid peroxidation products was determined in roots and shoots in term of content of thiobarbituric acid reactive substances (TBARS) and this is widely used as marker for evaluating oxidative stress which varies in responses to abiotic stresses (Bansal and Srivastava, 2012; Mirzaee *et al.*, 2017). Active oxygen metabolism plays a very important role in plant defence and tolerance to abiotic stress (Mittler, 2002). In abiotic stresses conditions such as drought, high salinity, extreme temperatures, high irradiance, UV light, nutrient deficiency, and air pollution, the production of reactive oxygen species (ROS) viz. singlet oxygen (O₂), superoxide (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (OH[•]) takes place in plant cells.

Within a certain range ROS levels do not damage the plants but excessive ROS accumulation results in oxidative damage. Oxidative damage to membranes is quantified in terms of malondialdehyde (MDA) content. The MDA content is considered as an index of extent of membrane injury due to stress (Yamazoe, 1962). Under fluoride stress, it is reported that the level of ROS is increased (Saini *et al.*, 2013; Wang *et al.*, 1991). Derangement in proper functioning of plasma membrane by elevated level of fluoride has been reported in plants (Rakowski and Zwiazek, 1992). Mulberry genotypes with low MDA content under fluoride stress have been reported to tolerate higher levels of fluoride (Kumar *et al.*, 2013). Increased MDA content under fluoride toxicity has been observed in present investigation. At T₁ treatment MDA content decreased in wheat which indicated that this treatment of sodium fluoride was not lethal to bio-membranes in wheat but at T₂ the MDA increased to 85.86%. It indicated that 200 ppm fluoride is lethal to wheat (Table 3). In barley at both sodium fluoride treatments MDA content increased, indicated that in this crop even T₁ treatment is lethal to bio-membranes.

It is concluded that in wheat and barley fluoride toxicity resulted in derangement in soluble sugars, starch, protein, free amino acid, proline and MDA contents. As compared to barley, 100 ppm fluoride was lesser toxic to these parameters while at 200 ppm both crops were equally sensitive to fluoride toxicity. In wheat as well as in barley MDA content increased under fluoride toxicity therefore it may be taken as a parameter to identify fluoride tolerant crop.

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