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BRIEF COMMUNICATION

Fluoride-induced alteration of carbon and nitrogen metabolism in developing wheat grains

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Abstract

Effect of fluoride (10 and 50 mM) on the activities of sucrose metabolizing enzymes, alkaline inorganic pyrophosphatase, and transaminases in relation to the accumulation of free sugars, starch, and soluble protein was studied in detached ears of wheat cultured in a liquid medium. Culturing for 5 d in the presence of fluoride reduced the amount of grain starch whereas contents of total free sugars, particularly sucrose, and soluble protein increased. Fluoride inhibited the activities of soluble acid and neutral invertases, as well as sucrose synthase acting in the cleavage direction. Uptake of uniformly labelled ^{14}C-sucrose or fructose was also drastically reduced by fluoride. Glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) activities also increased with fluoride addition in correspondence with an increase in soluble protein. Apparently, the wheat grain responds to fluoride-mediated disruption of carbon metabolism by a compensatory effect on nitrogen metabolism.

Additional key words: enzyme activities, liquid culturing, protein, starch, sugars, *Triticum aestivum*.

High fluoride concentrations in certain soils (Weinstein 1977) are proved to be toxic for plants (Leone *et al.* 1948) and thereby for animals and human beings (Chinoy 1991). Fluoride concentration above 2.5 mM decreased dry matter production and grain yield in wheat (Singh *et al.* 1979). However, the physiological and biochemical basis of fluoride toxicity remains poorly understood.

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Abbreviations: BSA - bovine serum albumin; DPA - days post anthesis; DTT - dithiothreitol; EDTA - ethylene diamine tetraacetic acid; GOT - glutamate oxaloacetate transaminase; GPT - glutamate pyruvate transaminase; Hepes - hydroxyethylpiperazinylethanesulfonic acid; TCA - trichloroacetic acid.

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Fluoride inhibits starch biosynthesis in potato tubers (Viola and Davies 1991) and sorghum grains (Asthir and Singh 1995), possibly via elevated levels of P_i in the amyloplasts. Whether the uptake of sugars is also reduced by fluoride ions has not been investigated. On the other hand, both the inhibitory (Quick *et al.* 1989) and promotory (Viola and Davies 1991) effects of fluoride on sucrose biosynthesis have been reported. Information regarding fluoride effects on other metabolic pathways is scarcely available, especially in relation to C/N balance. The biochemical consequences of a fluoride-mediated disruption in carbon metabolism especially in terms of an altered flow towards nitrogen metabolism are worthy of investigation.

The objectives of the present study are, therefore, to investigate the fluoride-induced quantitative changes in free sugars, starch and soluble protein in conjunction with the activities of sucrose metabolizing enzymes and the transaminases using detached ears of wheat cultured in a liquid medium.

Ears from field-grown wheat (*Triticum aestivum* L. cv. WL 1562) were collected at mid-milky stage, *i.e.* 12 d post anthesis (DPA), cut under water and cultivated according to the method of Donovan and Lee (1977) as modified by Singh and Jenner (1983), keeping six replications for each treatment. The concentrations of sucrose and L-glutamine in the culture medium were 117 mM and 17 mM, respectively. The effect of sodium fluoride (NaF) on grain metabolism was studied at 10 and 50 mM concentration. Culture solution devoid of fluoride was taken as control.

The pH of the culture medium was adjusted to 5.0 followed by filtration through a *Millipore* membrane (0.22 μm). Before culturing the ear-heads, the flag leaf and its sheath were removed and the stems were surface-sterilized with 40 % ethanol followed by quick washing with distilled water. Ear-heads carrying 20 cm peduncle length from the cut end were placed in culture tubes (one ear-head/tube) containing 35 cm^3 cold sterilized liquid medium under aseptic conditions. The cultured ear-heads were then kept in an water bath maintained at temperature of 4 °C (Asthir and Singh 1995). In the growth chamber, an irradiance of 4000 μmol(photon) m^{-2} s^{-1} (measured with a quantum meter *Licor 188B*, Lincoln, USA) was provided with cool white fluorescent tubes (*TL 40 W/54 Philips*, Calcutta, India) and incandescent lamps (60 W, *Sylvania*, New Delhi, India) for a 14-h photoperiod.

After 5-d cultivation, grains were separated from the bracts-pedicel and plunged immediately into hot 80 % EtOH and stored below 0 °C till used for biochemical analysis.

Free sugars were extracted and estimated according to Singh and Asthir (1988), and the reducing sugars and sucrose by the method of Nelson (1944) and Roe (1934), respectively. From the sugar-free grain samples, starch was determined according to Asthir and Singh (1995). Soluble proteins extracted in 0.1 M NaOH and precipitated with TCA were estimated by the method of Lowry *et al.* (1951).

For determination of ^{14}C-sugars incorporation ears of wheat at 12 DPA were cut under water, keeping a stalk length of 2.5 cm in each spikelet. The spikelets were then aseptically incubated in triplicate with 14.6 mM ^{14}C-sucrose or 27.7 mM ^{14}C-fructose solution (radioactivity 222 kBq), containing 10 or 50 mM NaF (two spikelets per 3 cm^3) at 28 °C for 6 h. Sugar solution without NaF was kept as the control. After incubation, grains were separated and plunged immediately into hot

80 % EtOH and free sugars and starch were quantitatively extracted from the radiolabelled samples as described by Singh and Asthir (1988) and their ^{14}C activity was measured in a *Packard* liquid scintillation spectrometer using Bray's scintillant (Bray 1960).

For extracting soluble invertases (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) and sucrose synthase (UDP-glucose : D-fructose 2 glucosyl-transferase, EC 2.4.1.13), and alkaline inorganic pyrophosphatase (pyrophosphate phosphohydrolase, EC 3.6.1.1) the harvested wheat grains were homogenized at 0 - 4°C in 50 mM Hepes-NaOH buffer (pH 7.5) containing 5 mM $MgCl_2$, 1 mM disodium EDTA, 2.5 mM DTT, 0.5 mg cm^{-3} BSA and 0.05 % (v/v) Triton X-100. Homogenates were centrifuged at 10 000 *g* for 15 min and the extraction procedure was repeated with the pellet obtained. The pooled supernatants were passed through *Sephadex G-25* column equilibrated with the above buffer without EDTA and Triton X-100. For the extraction of glutamate-oxaloacetate/glutamate-pyruvate transaminase (GOT/GPT, EC 2.6.1.1/2.6.1.2, L-aspartate/L-alanine : 2-oxoglutarate aminotransferase), 50 mM Tris-HCl buffer (pH 7.5) containing 100 mM β-mercaptoethanol, 2 mM $MgCl_2$, 2 mM EDTA and 10 mM cysteine was used.

Alkaline inorganic pyrophosphatase was assayed by the method of Heppel (1955). The P_i released was measured by the method of Taussky and Shorr (1953). The activity of sucrose synthase was assayed according to Morrell and Copeland (1985). Activities of soluble acid invertase (pH 4.8) and neutral invertase (pH 7.5) were assayed as described previously (Asthir and Singh 1995). The activities of GOT and GPT were determined according to Tonhazy *et al.* (1960a,b). In all enzyme assays, the conditions for linear rates with respect to substrate concentration, time, optimum temperature and pH were determined in preliminary assays.

Wheat grains harvested from ears cultured for 5 d in liquid medium with NaF showed a progressive increase in the contents of total free sugars, sucrose, and soluble proteins, concomitant with an increase in fluoride concentration from 10 to 50 mM. The converse was true in case of starch and reducing sugars (Table 1).

Table 1. Effect of NaF on the contents [mg g^{-1}(d.m.)] of free sugars, starch and soluble proteins in the wheat grain. The detached ears at mid-milky stage (12 DPA) were cultured for 5 d in complete liquid medium in the presence or absence of NaF. Means ± SE, $n = 5$.

NaF [mM]	Total free sugars	Reducing sugars	Sucrose	Starch	Soluble proteins
0	24.6 ± 3.1	6.4 ± 1.0	15.1 ± 1.4	232.4 ± 6.3	8.3 ± 0.9
10	32.0 ± 3.4	3.8 ± 0.9	23.0 ± 2.0	201.1 ± 5.4	18.3 ± 2.3
50	38.4 ± 2.4	1.6 ± 0.2	29.2 ± 2.4	174.8 ± 6.0	21.1 ± 1.8

During the short-term (6 h) incubation of detached ears with ^{14}C-sucrose or ^{14}C-fructose, the inhibitory effect of fluoride on grain starch biosynthesis was much more apparent (Table 2). Fluoride also inhibited ^{14}C incorporation into total free sugars (Table 2). Therefore, the decreased incorporation of ^{14}C into starch could

partly be a consequence of an inhibition of ^{14}C-sucrose or ^{14}C-fructose uptake rather than a consequence of a specific inhibition of starch synthesis alone. A higher build-up of free sugars during 5-d cultivation is suggestive of a sequential response to fluoride. The response in first hours involving a drastic reduction in starch

Table 2. Effect of NaF on ^{14}C-incorporation [Bq g^{-1}(d.m.)] from uniformly labelled ^{14}C-sucrose or ^{14}C-fructose into free sugars and starch in the wheat grain. The detached ears at mid-milky stage (12 DPA) were incubated in radiolabelled sugars for 6 h. Means ± SE, $n = 5$.

NaF [mM]	^{14}C-sucrose free sugars	starch	^{14}C-fructose free sugars	starch
0	5114.6 ± 112.3	953.4 ± 18.6	3268.3 ± 95.3	1027.8 ± 47.3
10	312.7 ± 11.4	60.9 ± 6.3	751.4 ± 18.7	83.8 ± 9.2
50	279.3 ± 7.3	58.0 ± 4.4	396.6 ±10.1	71.1 ± 8.1

biosynthesis is followed by a accumulation of soluble sugars and proteins, as a consequence of starch inhibition. Inhibition of alkaline inorganic pyrophosphatase (a marker enzyme of amyloplasts) by fluoride (Table 3) possibly results in an accumulation of P_i (Asthir and Singh 1995), causing a decrease in the rate of conversion of glucose-1-P to ADP-glucose and hence starch biosynthesis (Viola and Davies 1991).

Table 3. Effect of NaF on the activities of acid and neutral invertase [μg(sucrose hydrolyzed) g^{-1}(f.m.) s^{-1}], sucrose synthase [μg(sucrose cleaved) g^{-1}(f.m.) s^{-1}], alkaline inorganic pyrophosphatase [μg(Pi formed) g^{-1}(f.m.) s^{-1}], glutamate oxaloacetate transaminase [μg(OAA formed) g^{-1}(f.m.) s^{-1}], and glutamate pyruvate transaminase[μg(Pyr formed) g^{-1}(f.m.) s^{-1}] in the wheat grain. The detached ears at mid-milky stage (12 DPA) were cultured for 5 d in complete liquid medium in the presence or absence of NaF. Means ± SE, $n = 5$.

NaF [mM]	Acid invertase	Neutral invertase	Sucrose synthase	Phosphatase	GOT	GPT
0	2.46 ± 0.12	0.34 ± 0.02	1.11 ± 0.05	6.84 ± 0.24	1.78 ± 0.12	4.84 ± 0.17
10	0.72 ± 0.05	0.10 ± 0.01	0.87 ± 0.03	5.61 ± 0.20	2.21 ± 0.11	7.52 ± 0.25
50	0.82 ± 0.05	0.07 ± 0.00	0.78 ± 0.05	4.99 ± 0.16	2.30 ± 0.07	9.94 ± 0.23

Seemingly, the pathways of sucrose and starch biosynthesis are interrelated in wheat grains. It is worth noting that the activities of grain soluble acid invertase and neutral invertase decline markedly with fluoride additions (Table 3) along with a decrease in the amount of reducing sugars (Table 1). The cleavage activity of sucrose synthase also declined (Table 3). Fluoride-induced decreases in both degradation of sucrose and transformation to starch might result in its increased levels in wheat grains (Table 1). A similar increase in sucrose content of potato tubers was observed by Viola and Davies (1981) when the tuber discs (preincubated in 10 mM NaF) were incubated for 2 h in radiolabelled sucrose or glucose or glycerol.

Variable effects of fluoride on biosynthesis of sucrose have been reported, *e.g.*, sucrose biosynthesis is inhibited in spinach leaves (Quick *et al.* 1989), whereas the sucrose content of potato tubers increased in proportion to the inhibition of starch biosynthesis (Viola and Davies 1991). The pathways of sucrose metabolism and starch biosynthesis in developing potato tubers and in wheat grains are apparently similar (Viola and Davies 1991, Keeling *et al.* 1988). Evidently, sucrose synthase might be responsible for sucrose biosynthesis in the wheat grain.

As in case of sucrose, cytosolic amino acid synthesis is also affected by fluoride as reflected in increased activities of transaminases. For example, GOT and GPT activities were enhanced by fluoride (Table 3), correlating with an increase in soluble protein content (Table 1). Enzymes of nitrogen metabolism are often up-regulated by elevated sugar contents in plant tissues (Lam *et al.* 1996). The resulting increase in amino acids would in turn affect the protein biosynthesis.

Overall, it appears that fluoride-mediated inhibition of starch biosynthesis in wheat grain is accompanied by an alteration of carbon and nitrogen metabolism leading to accumulation of sugars and soluble protein.

References

Asthir, B., Singh, R.: Fluoride-induced changes in the activities of sucrose metabolizing enzymes in relation to starch accumulation in sorghum caryopsis, raised through liquid culture. - Plant Physiol. Biochem. **33**: 219-223, 1995.

Asthir, B., Singh, R.: Invertase-mediated interconversion of sucrose and hexoses during their translocation in growing pearl millet plant. - J. Plant Biochem. Biotechnol. **4**: 23-28, 1995.

Bray, G.A.: A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. - Anal. Biochem. **1**: 279-285, 1960.

Chinoy, N.J.: Effects of fluoride on physiology of animals and human beings. - Indian J. Environ. Toxicol. **1**: 17-32, 1991.

Chinoy, N.J., Barot, V.V., Michael, M., Barot, J.M., Purohit, R.M., Ghodasara, N.G., Parikh, D.J.: Fluoride toxicity studies in Mehsana district, North Gujarat. - J. environ. Biol. **3**: 168-170, 1994.

Donovan, G.R., Lee, J.W.: The growth of detached wheat heads in liquid culture. - Plant Sci. Lett. **9**: 107-113, 1977.

Heppel, L.A.: Inorganic pyrophosphate from yeast. - Methods Enzymol. **2**: 570-576, 1955.

Keeling, P.L., Wood, J.R., Tyson, R.S., Bridges, I.G.: Starch biosynthesis in developing wheat grains. Evidence against the direct involvement of triose phosphates in the metabolic pathway. - Plant Physiol. **87**: 311-319, 1988.

Lam, H.M., Coschigano, K.T., Schultz, C., Melo-Oliveira, R., Coruzzi, G.M.: The molecular genetics of nitrogen assimilation into amino acids in higher plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **47**: 569-593, 1996.

Leone, I.A., Brennan, E.G., Danies, R.H., Robbins, W.R.: Some effects of fluorine on peach, tomato and buckwheat when absorbed through the roots. - Soil Sci. **55**: 259-266, 1948.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with Folin-phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Morrell, M., Copeland, L.: Sucrose synthase of soybean nodules. - Plant Physiol. **78**: 149-154, 1985.

Nelson, N.: A photometric adaptation of the Somogyi method for the determination of glucose. - J. biol. Chem. **153**: 375-380, 1944.

Quick, P., Neuhaus, E., Feil, R., Stitt, M.: Fluoride leads to an increase of inorganic pyrophosphate and an inhibition of photosynthetic sucrose synthesis in spinach leaves. - Biochim. biophys. Acta **973**: 263-271, 1989.

Roe, J.H.: A colorimetric method for the determination of fructose in blood and urine. - J. biol. Chem. **107**: 15-22, 1934.

Singh, A., Chhabra, R., Abrol, I.P.: Effect of fluorine and phosphorus applied to a sodic soil on their availability and on yield and chemical composition of wheat. - Soil Sci. **128**: 90-97, 1979.

Singh, B.K., Jenner, C.F.: Culture of detached ears in liquid culture: Modification and extension of the method. - Aust. J. Plant Physiol. **10**: 227-236, 1983.

Singh, R., Asthir, B.: Import of sucrose and its transformation to starch in the developing sorghum caryopsis. - Physiol. Plant. **74**: 58-65, 1988.

Taussky, H.H., Shorr, E.: A microcolorimetric method for the determination of inorganic phosphorus. - J. biol. Chem. **202**: 675-685, 1953.

Tonhazy, N.E.: Glutamate-oxaloacetate-transaminase. - In: Bergmeyer, H.U. (ed.): Methods of Enzyme Analysis. Pp. 695-698. Akademie-Verlag, Berlin 1960a.

Tonhazy, N.E.: Glutamate-pyruvate-transaminase. - In: Bergmeyer, H.U. (ed.): Methods of Enzyme Analysis. Pp. 727-731. Akademie-Verlag, Berlin 1960b.

Viola, R., Davies, H.J.: Fluoride-induced inhibition of starch biosynthesis in developing potato, *Solanum tuberosum* L., tubers is associated with pyrophosphate accumulation. - Plant Physiol. **97**: 638-643, 1991.

Weinstein, L.H.: Fluoride and plant life. - J. Occup. Med. **19**: 49-77, 1977.