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Phytotoxic effects of fluoride on crop plants: a review

Sakuntala Chakrabarti*[1], Pulak Kumar Patra[2], Bhabatosh Mandal[3]

1 Department of Environmental Science, Vivekananda College, Kolkata-700063, West Bengal, India.
2 Department of Environmental Studies, Visva Bharati, Santiniketan 731235, West Bengal, India.
3 Department of Chemistry, Visva Bharati, Santiniketan 731235, West Bengal, India.

E-mail : sakuntala.vb@gmail.com Contact No. : +91 - 9531533310

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Abstract

Fluoride is a common element on the earth's crust and exists naturally in soil, water and air. The elevated concentrations of fluoride in environment have been found to affect adversely the health of humans, animals and plants in many parts of the world. Fluoride is not an essential element for plants. However, plants can uptake fluoride from the fluoride contaminated environment, either through the roots from the soil zone or by the stomata from the atmospheric deposits on leaves. The use of fluoride-contaminated groundwater for irrigation is a major concern in many fluoride endemic areas. It results fluoride accumulation in the plant parts which ultimately enter the food chain and increases the risk of fluorosis in the area which is already affected by F- rich drinking water. Besides F-uptake, plants also undergo major physiological, metabolically and biochemical alterations by prolonged exposure to fluoride. The present review compiles the available information of the effect of fluoro-toxicity on seed germination, growth and productivity, pigments, metabolites and biomolecules in crop plants. The paper highlights the adaptive response of different crop plants to F- stress, the knowledge of which will be useful for future research in developing F-resistant crop varieties or mitigating the F-stress in crop plants.

Key words : Fluoride uptake, Antioxidants; Fluoride toxicity; Growth responses; Oxidative stress

INTRODUCTION

Fluoride (F) contamination in ground water and soils is a great concern in several countries when it is present at levels above the permissible limit[1]. In India 19 states are facing acute fluorosis problems [2]. The most seriously affected areas are Andhra Pradesh, Punjab, Haryana, Rajasthan, Gujarat, Tamil Nadu, and Uttar Pradesh[3]. In many parts of India fluoride-contaminated ground water is resultant of the irrigation of high-fluoride soils[2,4]. The toxic effects of fluoride on plants have been known since 1940s[5,6]. F is not an essential trace element for plants, animals, or humans although some consider it to have a beneficial effect in reducing dental caries when applied topically to the teeth. Chronic exposure to F induces deleterious effects in animals, humans and in several plants[7-14]. Some plants accumulate F and are able to grow even at high F concentrations (4000 μg/g) without showing any signs of injury while several other plants sustain damage if exposed to even low F concentrations (<20 μg/g)[15].

In this review, we emphasized on the findings on the various aspects of plant metabolism in relation to F-toxicity and uptake of F by plant parts which may provide some information regarding the risk of F toxicity to the in plants, especially the crop plants.

UPTAKE OF FLUORIDE

Significant uptake of F has been observed under F exposure in various seedlings and mature plant parts, including cereals like paddy and leafy vegetables like mustard *(Brassica juncea*), spinach (*Spinacia oleracea*), coriander (*Coriandrum sativum*), potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), brinjal (*Solanum melongena*), onion (*Allium cepa*) and beans (*Phaseolus vulgaris*), where accumulation increases with increasing F concentrations.[16-23]. F enters into plants mainly through two pathways. Initially, F deposited over leaves enters *via* stomata, and secondly, through the soil and water into the roots by a passive diffusion process.[24] Subsequently, F is transported via xylem tissues through the apoplastic and symplastic pathways into the shoots.[25] Leafy and roots vegetables like spinach, radish (*Raphunus sativa*) are good accumulator of fluoride, greater accumulation in root may be due to low permeability through endodermis or dilution of fluoride due to increased shoot biomass or restricted translocation from root to shoot[16]. The mean fluoride accumulation decreased in the order: root > leaf > stem > seeds[18]. Maximum fluoride accumulated in tips and margins of leaves[19]. The variable fluoride accumulation in was reported[10] where accumulation in leafy vegetables were reported to be higher than the fruiting and tuber vegetables as well as seed crops. Under controlled condition in onion, more accumulation in roots and shoots than in bulbs was inferred[10]. In healthy grape leaves (*Vitis vinifera L*) exposed to F, phosphate (PO_4^{3-}), calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentration increases to trap and detoxify F in form of CaF_2 and MgF_2, where as in damaged leaves Ca^{2+} and Mg^{2+} concentration declines [26]. Tea plants (*Camellia sinensis*) are inherently able to accumulate large quantities of fluoride, this was affected both by pH and by Ca^{2+} levels in the medium (uptake highest at pH 5.5) and this reduction due to Ca^{2+} application may be due to the effect of calcium on the properties of cell wall or membrane permeability in the solution experiments and also due to alteration of fluoride speciations and their quantities in soil solutions and precipitation of CaF_2 in solution and soil or to the complexing of calcium and fluoride in roots [27]. All the vegetables as well as all the vegetative parts do not accumulate fluoride to same extent. The increased concentration of fluoride in the plant shoot beyond a certain added level of fluoride may be attributed to the fact that when a high concentration of the fluoride is added to the soil or soil solution, pH becomes more alkaline, fluoride could increase in the soil solution and more fluoride would be potentially available for uptake by the plant root [10]. Fluoride uptake by roots affects growth and metabolism at root level [28]. Moreover, aquaporins may be involved in the transmembrane transport of F [29].

EFFECT ON PLANT GERMINATION AND SEEDLING GROWTH

A number of researchers have experimentally shown that elevated levels of Fluoride exposure reduces the germination % , shoot length, root length, dry weight in many crop plants such as paddy (*Oryza sativa)*[17, 22] bengal gram (*Cicer arietinum*) [23] *and* wheat (*Triticum aestivum)*[30]. Significant reductions in leaf area, NAR (Net assimilation rate) as well as relative growth rate have been observed in mung bean (*Vigna radiata*) [31], pea seedling [32], barley (*Hordeum vulgare)*, maize (*Zea mays)*[33] and oats (*Avena sativa*)[34]. It suppresses root growth of germinating corn seedlings by decreasing cell number or cell extension[35]. Variation in NAR, pulpy, pithy, succulent and even rotten roots may be due to the accumulation of fluoride in the root zone which cuts off the supply of mineral nutrients and water[19]. In germinating corn seedlings fluoride induces changes of RNA structures and significantly depressed adenine only in the root tissue treated by the fluoride concentration[35]. The failure of germination have been accounted as a consequence of reduced water uptake, inhibited cell division and enlargement in the embryo, and/or an overall fall in the metabolic activity associated with these processes. F-imposed prevention of the dephosphorylation of phytin compounds through inhibited phytase enzyme, mineral nutrition, and amylase activity, have been held responsible for the reduced rates of seed germination.[36, 37]

EFFECT ON PLANT GROWTH AND PRODUCTIVITY

The growth and productivity of many mature crops such as *Vicia faba* (fava bean/ broad bean)[38, 39], *Allium cepa* (onion)[38] and grain crops like wheat[40, 41] have been found to be adversely affected by fluoride. At low level fluoride exposure a number of physiological and biochemical changes may be initiated in plant without the appearance of visible injury symptoms and some of these changes may have important consequences such as reduction in growth or yield[42]. Common visible symptoms include depressed growth and development, chlorosis, decreased photosynthetic activity, necrosis, abscission of leaves, flowers or fruits, impaired cone and seed production[43]. The effects of fluoride on growth and yields, tissue destruction, photosynthesis rates, inhibition of enzymes, respiration in citrus plants[44], bean seedlings[45] have been observed. Growth, biomass, productivity, dry matter and leaf area were also significantly reduced in pea (*Pisum sativum*), tomato (*Solanum lycopersicum*), pinto bean (*Phasoelus vulgaris*) and alfalfa (*Medicago sativa*)[46,47, 48]. Tip burning and even death of the onion plant was noticed in highly contaminated soils (>400 mg NaF kg^{-1} soil) and the phyto-toxic threshold limit (LC_{50}) in onion shoot was found to be 55 mg F kg^{-1}, beyond which the biomass yield decreased by 50%.[10] Fluoride toxicity also influences production and quality of flower [49]. High levels of fluoride in acid soils reduce crop yield in barley (*Hordeum vulgare*) due to increasing aluminium and decreasing phosphorus uptake[50]. F affects the rate of photosynthesis chiefly by reducing chlorophyll synthesis or by degradation of the ultra-structure of the chloroplasts and an inhibition of the Hill reaction.[24]. In wheat leaves C_6/C_1 ratio (direct oxidation pathway of plant respiration i.e, glucose/carbon dioxide) decreased and this accompanied by a decreased sensitivity of O_2 uptake or $14CO_2$ production due to fluoride exposure [51]. Accumulation of fluoride in the soil, surrounding plant roots and mesophyll cells disturbs the mineral metabolism and other morphological and physiological characters [38, 52, 53]

EFFECT ON PLANT PIGMENTS

Chlorophyll and carotenoids are the most important photosynthetic plant pigments. The high electronegativity of fluoride destroys the chlorophyll molecule and accelerates the disintegration of chloroplasts[54]. Along with accumulating in chloroplasts and reducing the chlorophyll concentration, ultra structural have shown that fluoride disrupts chloroplasts membrane [55]. The fluoride induced inhibition of chlorophyll synthesis was found to be directly related to the degradation of magnesium atom attached to the chlorophyll ring structure [29] as Mg is the central component of chlorophyll and important compound of cellwall pectin [26]. The effects of fluoride on the activities of chloroplast ATP-ase[56] and chlorophyll destruction have been reported[57, 45, 58,59]. The chlorophyll content of leaves in several crop plants reduces with increasing concentration of NaF exposure[30, 60] including leaf necrosis with chlorophyll destruction[52, 53, 61, 38]**.** The reduction in leaf chlorophyll and significant correlations were found in between fluoride flux in the soil, foliar fluoride concentrations and in photosynthetic rate of mullberry leaves[62]. Significant reduction in chl-a, b and total chlorophyll content in bengal gram seedlings was recorded[23]. 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 fluoride induced reduction in Fe^{+2} which is essential for biosynthesis of chlorophyll [22]. The reduction in carotenoids may be due to fluoride induced stress which inhibits carotenoid formation in the plant cells [63]. F accumulation leads to inhibition in the electron transport rate, particularly at the photo system- II, is also one of the mechanisms of F-injury.[24] It has also been demonstrated that oxygen is continuously produced during the photolysis of water. Substitution of Cl ions of the photo system-II, by the F, inhibits the photo-oxidation of water and causes the generation of new free radicals in the proteins of this system, which is incapable of compensating by the process of photolysis.[61] It has been proposed that the inhibited photosynthesis may possibly be the result of the loss of sub cellular organization, granulation of the chloroplasts, and low stomatal conductance linked a limited CO_2 uptake.[62] In addition, in the chloroplasts, F is also shown to affect the activities of RUBISCO (ribulose 1,5-biphosphate carboxylase), sucrose synthase, sucrose synthetase, and the enzymes associated with CO_2 fixation.[62]

EFFECT ON ESSENTIAL METABOLITES

To maintain homeostasis, cells are armed with an integrated ROS scavenging system network that comprises enzymic (SOD, CAT, POX, APX) and non-enzymic (AA, α-tocopherol, flavonoids, Pro, and glutathione) candidates.[63,64] Among the enzymatic components, SOD, a family of metalloenzymes, catalyzes the disproportionation of O_2 into H_2O_2 and O_2, and is present in almost all aerobic organisms.[65] Three isoforms of it namely; Mn-SOD (mitochondria), Fe- SOD (chloroplasts), and Cu/Zn-SOD (cytosol, chloroplasts, peroxisomes, and mitochondria) are shown to exist.[65, 66] CAT, a heme-containing enzyme, catalyzes the dismutation of H_2O_2 into H_2O, and O_2.[65, 66] POX, a heme containing protein, has four conserved disulfide bridges and takes part in lignin biosynthesis and organogenesis via the decomposition of auxin or the biosynthesis of ethylene.[65, 66] Additionally, APX, a central component of the ascorbate-glutathione cycle, uses two moles of ascorbate to reduce H_2O_2 into water, and generates two moles of monodehydroascorbate[65, 66]. An excessive availability of F has been demonstrated to inhibit the ATP synthase enzyme, thus affecting energy metabolism in the higher plants [56] The plasma membrane is quite sensitive to F and hence is considered to be a critical site for attack.[14] Moreover, the membranes of the tonoplasts were shown to be most susceptible to F.[14] The rate of symptoms appears to depend on the weather and the time of exposure to the fluoride. Fluoride induced inhibition of essential plant enzymes like acid phophatase[67, 68,69], enolase, phosphoglucomutase, hexokinase, PEPcarboxylase, pyruvate kinase, succinic dehydrogenase, malic dehydrogenase, pyrophosphates, phytase, nitrate reductase, mitochondrial ATPase and urease leads to the inhibition of photosynthesis, protein synthesis, lipid metabolism.[69] which can be attributed to binding of fluoride to Mg^{2+} and Ca^{2+} thus, decreasing the availability to plant for enzymes activity[70, 71] where as marked increase was observed in several organic acids, including malic acid, malonic, succinic and citric acids[71].

The reactive oxygen species triggered by fluorine compounds include super oxide radical (Co G), hydroxyl radical (OHG) and hydrogen peroxide (H_2O_2), in turn cause damage to the biomolecules such as membrane lipids proteins, lipids, plant pigments, enzymes, nucleic acids[72]. A significant alleviation in activities of peroxidase, catalase and ascorbic acid oxidase was recorded in Mullberry (*Morus alba*) on exposure to fluoride with simultaneous increase in tissue levels of peroxidases[62]. Exposure to F was shown to reduce SOD activity in *H. annuus*.[61] In contrast, an increase in it was recorded in F-treated seedlings of *O. sativa*.[63] This fluctuating behavior of SOD may probably be related to altered metabolic status or its biosynthesis, in F-affected tissues.[63] However, the enhanced activity of antioxidants may be an adaptive strategy which can be considered as a positive feedback mechanism against oxidative stress.[63]. The reduction in the catalase activity of the fluoride treated leaves is a result of inhibition of the enzymatic system or a change in the actual catalase concentration in the fluoride-treated tissues as compared to the non-treated tissues[73, 74, 75]. CAT was also reduced with F-stress in *O. sativa*.[63] The findings suggested that with F-toxicity, the OH attached to the Fe^{2+} atoms of CAT are replaced by low molecular weight anions thereby inhibiting enzyme activity.[63] In contrast, enhanced POD and APX levels were measured in *O. sativa*.[63] Like the enzymatic candidates, the content of ascorbic acid fell initially in response to F, but then rose drastically with increased F concentration, in *O. sativa* and *C. arietinum*[23, 63] The binding of F with ascorbic acid oxidase allowed an increased accumulation of ascorbic acid with low F. At higher F doses, the bond(s) between F and ascorbic acid oxidase may broken down allowing low levels of AA.

IMPACT ON BIOMOLECULES

***Carbohydrates**:* Low F was found to raise the sugar content in *Citrullus lanatus* (watermelon) while at high dose it resulted in reduced sugar accumulation in *Oryza sativa* (rice) seedlings.[36, 63] Soyabean leaves exposed to HF exhibited lower sucrose content, while the levels of both glucose and fructose were elevated[76]. Starch and sugar content of the almond (*Amygdalis communis)* leaves showed a significant decrease at the higher fluoride concentrations and the nutritional status of the leaves appeared to be affected more than that of roots[53]. *Helianthus* seedlings showed significant decreased carbohydrate content, as well as amylase activity.[61] The actions of F in inhibiting photosynthesis, reducing the activities of invertase and amylase, and increasing oxidative stress may possibly be responsible for this reduced sugar accumulation.[61] The activities of more than 300 enzymes are shown to be inhibited severely by F *via* removal of a cofactor Mg^{2+}.[61] Moreover, the partitioning of the photo assimilates for sucrose and starch synthesis, is directed by F-mediated pyrophosphate (PPi) accumulation.[77] Accumulation of sugars under stress condition may act as osmotic and protect specific macromolecules and contribute to the stabilization of membrane structure [78]. Total soluble sugars may increase under fluoride stress in plants and its level is directly related to stress factors [79]. Fluoride stress also increased soluble sugars content in *Oryza sativa* [17] and *Cicer arietinum* [80]**.** The increased sugars may act as osmolytes which increase the water potential of seedlings for survival under F stress since fluoride inhibits root water transport [81, 36]

Lipids: The most damaging and key process known to occur prominently in the plasma membranes is the oxidation of the poly unsaturated fatty acid (PUFA) fractions of lipids.[82] The peroxidation reaction of this macromolecule includes three important steps viz., initiation, progression, and termination.[65] Peroxidation of membrane lipids is initiated when a OH radical abstracts one hydrogen atom from a PUFA.[82]. The lipid peroxidation reaction also exacerbates the oxidative damage through the production of lipid-derived secondary free radicals that themselves can react with and damage both proteins and DNA.[83] Additionally, the PUFAs get oxidized from ROS, and generate a number of cytotoxic by-products namely malondialdehyde (MDA), 4-hydroxy-2-nonenal (4- HNE), and hydroxyl and keto fatty acids.[84] A significant change in the pace of the lipid peroxidation reaction with increasing F and time of exposure has been observed in *Helianthus annuus* (sunflower).[61]

Proteins : F reduces the protein content in both a dose and time dependent manner as observed in a variety of seedlings.[80, 85] Fluoride interferes with phosphorylation of phosphoproteins in cellular membranes[86, 87]. The toxic fluoride effect include an induction of inflammatory reactions, cell contractile responses, inhibition of protein synthesis and cell cycle progression, oxidative stress, and DNA damage[88]. It has been observed that with increasing fluoride exposure during germination and seedling growth stages in *Helianthus*, total protein content decreases whereas the proline cotent increase [61]. Reduced synthesis, enhanced degradation, and/or usage for energy production are held to be responsible for this lowering of protein in stressed seedlings.[24]. Like lipids, proteins are also prone to ROS attack, which may cause deleterious modifications via nitrosylation, carbonylation, formation of disulphide bonds, and glutathionylation.[83]. F has also been shown to be involved in the synthesis of misfolded proteins in the endoplasmic reticulum and consequent ROS generation.[88] The expressions of genes associated with stress response factors (e.g., heat shock proteins), signal transduction components, and apoptosis related proteins have been demonstrated to be up-regulated by exogenous F application.[88]. Fluoride triggers the disruption of mitochondria outer membrane and release of cytochrome c into cytosol, what activates caspases-9 and -3 (intrinsic) apoptotic pathways and changes the expression profile of apoptosis-related genes and causes endoplasmic reticulum stress leading to inhibition of protein synthesis[89].

CONCLUSION

The review of published literature on the effects of flurotoxicity on crop plants reveals that almost all crops are affected significantly by exposure to fluoride. The high electronegativity of fluoride destroys the chlorophyll molecule and inhibits plant growth and productivity. It alters the plant defence enzymes and antioxidant activities and concentration. It damages cell ultrastructures, pigments and metabolites. It exerts its negative effect from germination to crop yield and even crop quality; however the degree of impact varies from plant to plant. Even the different cultivars of a single crop are affected to different degrees. The deleterious effects of F on a variety of plants have been experimentally studied in the last few decades by a number of researchers. Still our understanding of the mechanisms involved in the uptake, translocation and accumulation of F in the plants is not clear. Further research at molecular levels is needed to identify the genes involved in the adaptive repair systems. The findings will assist in developing efficient management of F-toxicity using potential molecules and also in developing F-tolerant genotypes.

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