

## **FLUORIDE TOXICITY STRESS: PHYSIOLOGICAL AND BIOCHEMICAL CONSEQUENCES ON PLANTS**

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### **Review article**

### **ABSTRACT**

Fluoride is one of the most toxic atmospheric pollutants. Accumulation of fluoride in the soil, surrounding plant roots and mesophyll cell disturbs several morphological physiological and biochemical parameters of plants. Fluoride toxicity adversely affects germination, growth, mineral nutrition, photosynthesis, respiration, activity of cellular enzymes, reproduction and yield of crops. Fluoride is also known to inhibit the activity of antioxidative enzyme systems like super oxide dismutase and interfere with cell signalling. Fluoride is known to interfere with calcium which plays essential role in fertilization. Accumulation of fluoride on the stigmatic surface disrupts the calcium gradient in the stigma and style. The symptoms of fluoride toxicity in plants include depressed growth and development, chlorosis, necrosis, abscission of leaves, flowers, fruits and decreased seed production. In this paper, consequences of fluoride toxicity on different morpho-physiological and biochemical characters have been discussed. This paper reviews the literature supporting evidence for the morphological, physiological, and biochemical effect of fluoride toxicity on crop plants.

**Key words:** HF, Fluoride, biochemical, physiological, toxicity

### **INTRODUCTION**

Air pollution is a serious problem to human life and agriculture. Fluoride is the most phytotoxic of the common air pollutants (Haidoutiet al., 1993). High levels of fluorine in acid soils reduce crop yield due to increasing aluminum and decreasing phosphate uptake (Elrashidiet al., 1997). The Al-F complex formed in acid soil is thought to be an effective vehicle for the intracellular uptake of Al and F. Inside the cell, these elements dissociate from each other to exert toxic effects (Kinraide, 1997). The toxicity of aluminum is enhanced in acidic environment contaminated with fluoride (Rai et al., 1996). Fluoroaluminate could play a role in the mechanism of aluminum toxicity and inhibition of plant growth observed in acid soils (Facanha and Meis, 1995).

Fluoride has long been known as a potent metabolic inhibitor. Fluoride is the most phytotoxic of known air pollutants on the basis of atmospheric concentrations required to injure plants. Only peroxyacetyl nitrate, a constituent of photochemical smog, can rival this extreme phytotoxicity (Weinstein, 1983). Depending on plant species and on concentration, hydrogen fluoride is 10 to 1000 times more harmful than sulfur dioxide (Guderian, 1977). The threshold concentration of fluoride causing metabolic or physiologic change and produce lesions on leaves of the most sensitive species at 0.001 ppm (1 ppb v/v, or  $0.8 \mu\text{gHFm}^{-3}$ ) or less, whereas threshold concentrations for ozone or sulfur dioxide that will produce an irreversible effect were found to be generally above 0.05 ppm and more than double that concentration and time for nitrogen dioxide for same exposure periods. This is largely due to the tendency of fluoride to accumulate in plant foliage. Leaves are extremely efficient absorbers of gaseous fluorides entering through the stomata. When once it is inside the leaf, little fluoride is translocated out of the organ by the way of the conducting tissues (Heggstad and Bennett

,1984). Gaseous fluorides, such as hydrogen fluoride (HF) or silicon tetrafluoride (SiF<sub>4</sub>), are among the most toxic of all pollutants important to agriculture (McCune and Weinstein, 1971).

The incidence of fluoride above permissible levels of 1.5ppm occur in 14 Indian states, namely, Andhra Pradesh, Bihar, Gujarat, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal affecting a total of 69 districts, according to some estimates. Some other estimates find that 65 per cent of India's villages are exposed to fluoride risk (Kumar and Saha, 2011). Gaseous fluorides, such as hydrogen fluoride (HF) or silicon tetrafluoride (SiF<sub>4</sub>), are among the most toxic of all pollutants important to agriculture (McCune and Weinstein,1971).Fluoride toxicity affects the most of morphological, physiological and biochemical parameters in the plant starting from the germination and early seedling growth. Fluoride affects a wide range of physiological processes including germination, growth, mineral nutrition, photosynthesis, respiration, carbohydrate metabolism, protein synthesis and lipid metabolism. Fluoride also interferes with the metabolism of proteins, lipids, and carbohydrates. Fluoride often inhibits enzymes that require such cofactors as Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Mn<sup>2+</sup> ions. The inhibition is attributed, in part, to removal of the cofactor Ca<sup>2+</sup>. Changes in enzyme activity and intermediary metabolism caused by chronic fluoride exposure may lead to altered growth, development, and reproduction of the organism.

### **Effect of Fluoride on Seed Germination and Seedling Growth**

During germination, phytin is broken down by the activity of the enzyme phytase to supply the young seedling with inorganic phosphate. Fluoride is known to prevent the dephosphorylation of phytin compound in the tissues by inhibiting phytase enzyme and retards the rate of seedling root growth during germination. Phytin originated inorganic phosphates which are sources of orthophosphates, are essential for RNA metabolism. Limited supply of phytin–originated orthophosphates may possibly be one of the factors which inhibit the growth rate of fluoride-treated seedlings(Chang,1967).

Shadad et al.(1989) studied the effect of NaF in various concentrations on seed germination, seedling growth, transpiration rate and growth criteria of *Zea mays*, *Helinathusannuus* and *Vicia faba*. The germination of the treated seeds significantly dropped as the concentration of the applied inhibitors increased, however, low doses of the applied inhibitors stimulated the germination of maize grains. The radicle and plumule lengths were considerably reduced at all levels of sodium fluoride. Low concentrations of the inhibitor used had nearly small effect, if any, on transpiration rate, while the high levels strongly inhibited transpiration rate. Growth criteria (leaf area, and dry matter gain) of the different organs of bean and sunflower plants were sharply reduced; more prominently at moderate and high doses of fluoride. The failure of the treated seeds to germinate at the high concentrations of fluoride may be consequence of retarded water uptake, inhibited cell divisions and enlargements in the embryo and or an overall decrease in metabolic activity relevant to these steps. The blockage of any one of the phases leading to germination may, and very likely will, completely inhibit the process of germination. Higher concentration of sodium fluoride (NaF) is known to affect germination, seedling growth and total biomass of cluster bean (*Cyamopsis tetragonoloba*) under controlled condition (Sabal et al.,2006).Fluoridetoxicity reduces the percent seed germination, root and shoot length, and total biomass significantly a compared to control. Phytotoxicity of fluoride also affects the germination and decreases different physiological

parameters like root length, shoot length, and dry weight of paddy (*Oryza sativa*) (Gupta et al., 2009).

Impaired root growth in germinating seeds has been known as a manifestation of phytotoxicity caused by many environmental chemicals including fluoride. The viability and soluble proteins of germinating wheat seeds are reported to be reduced by exposure to NaF. The germination of mung bean (*Vignaradiata*) is found to be inhibited when the seeds were treated with 1.0 mM NaF and that the inhibition might be related to lipase and amylase activity, and reducing sugar contents. It has been reported that fluoride (NaF) has inhibitory effect on DNA synthesis in germinating mung bean seeds (Narita et al., 1996). NaF suppresses DNA synthesis in mung bean seedlings.

According to Nitsan and Lang (1965), a decrease in DNA synthesis in higher plants leads to decreased RNA and protein synthesis, and to reduced cell division and cell elongation. Inhibition of germination, lowered biomass or impaired root elongation of mung bean in the presence of NaF, may be attributed to NaF dependent depression of DNA synthesis.

### **Effect of Fluoride Toxicity on Mineral Nutrition**

Mineral nutrition not only affects the toxicity of fluoride but also fluoride toxicity affects the mineral nutrition of crops. The main elements with which interactions might be expected are those with which it forms complexes (Ca, Mg, Fe, Mn, Cu and Zn). There is evidence that chronic fluoride injury symptoms are associated with a lower foliar content of Mn and Mg in citrus (Brewer et al., 1967) and symptoms of chronic fluoride injury in many broad-leaved species resemble deficiency of Mn, Zn or Mg; for this reason, Brewer et al. (1960) suggested that chronic fluoride injury was synonymous with a deficiency of certain nutrient elements. Interactions with other elements (*e.g.* N, P, K, Mo) are likely to be indirect and due to changes in the uptake, accumulation or movement of the fluoride. Fluoride sensitivity was found to be increased by low calcium (Brennan et al., 1950; Daines et al., 1952). It was found that low or deficient supplies of N, Ca and P reduced the absorption of toxic amounts of fluoride in tomato, while very high concentrations of N and Ca prevented fluoride injury. Conversely, symptoms of fluoride toxicity are found in bean plants exposed to HF grown under N-deficient medium (Adams and Sulzbach, 1961). Guderian (1977) reported that moderate levels of N fertilizer resulted in reduced fluoride injury but not reduced uptake in potted spruce trees. Excess fertilizer decreased fluoride uptake and enhanced foliar injury.

Levels of nitrogen, calcium, and phosphorus nutrition are known to affect the susceptibility of crop plants to injury from NaF applications to the roots and HF fumigation of the tops of the plants. Medium levels of nitrogen, calcium, and phosphorus favored absorption and translocation of fluorine in sufficient quantities to cause visible leaf injury by root treatments and also absorption of toxic quantities of fluorine in plants fumigated at the higher fluorine level. A low or deficient supply of nitrogen, calcium and phosphorus to the plant aided in preventing the absorption of toxic amounts of fluorine through the roots or through fumigation. Low phosphorus seemed to have the least inhibitory effect. Excessive amounts of nitrogen and calcium prevented fluorine injury, as did deficiency of these elements. Calcium has tendency to precipitate fluorine in the form of insoluble compounds within or around the roots reducing the injury to the foliage (Brennan et al., 1950; Daines et al., 1952). Symptoms of severe fluorine toxicity on the leaves were invariably associated with the highest fluorine foliage content. Root injury occurred in the same plants which showed foliage injury in the root-treated series. There was no indication of fluorine injury to roots as a result of

fumigation. Root injury is not associated with a high F content, many of the highest fluorine values occurring within the healthiest root systems. When NaF was added to the substrate, although fluorine was translocated to the leaves, the roots almost invariably showed a greater fluorine accumulation than did the leaves. HF fumigated plants show a higher accumulation of fluorine in the leaves and a normal amount in the roots, suggesting the absence of downward translocation of fluorine from the leaves. The lower fumigation, below critical level produced no injury on crop plants. The higher fumigation rate produced definite injury. Plants grown under similar environmental conditions absorb fluorine in proportion to the amount present in the atmosphere. Where foliage accumulation of fluorine was brought about by fumigation, there appeared to be a loss in fluorine from the leaf tissue within a seven-day period. No such loss was obvious where fluorine accumulation resulted from fluorine added through the roots. The inhibitory activity of fluoride varies, depending on the  $Mg^{2+}$  concentration in the medium. Murphy and Coll (1992) and Murphy and Hoover (1992) proposed that this inhibition is promoted by the binding of free fluoride to the magnesium bound to the enzyme. The fluoroaluminate complex seems to act as a Pi analog that strongly binds to the E<sub>i</sub> conformation of the two transport ATPases in a quasi-irreversible process that

### **Fluoride and Cell Signalling**

Fluoride is a potent, rapidly reversible activator of the trimeric G proteins. As a result, numerous G protein mediated signal transduction pathways are stimulated in membrane preparations or crude cell lysates by fluoride ions. Activation of purified G proteins by non-hydrolysing GTP analogues like  $AlF_4^-$  results in a change in the conformation of the G subunit (Higashijima et al., 1987) which promotes its dissociation from the trimeric complex and its association with the effector(s) (Northup et al., 1983a, b). Compelling evidence indicates that fluoride acts as an aluminium complex,  $AlF_4^-$  (Higashijima et al., 1987; Sternweis and Gilman, 1982) that mimics the  $\gamma$ -phosphate of GTP when bound to the GDP-bound form of G (Bigayet et al., 1985, 1987). Fluorides like  $AlF_4^-$  ions can mimic GTP analogues to promote G protein-induced processes in plants (Higashijima et al., 1987). The incubation of tomato plasma membranes with  $AlF_4^-$  resulted in the dephosphorylation of the plasma membrane  $H^+$ -ATPase in the absence of active elicitors or in the presence of a dilution of the elicitor that does not induce dephosphorylation (Xing et al., 1997).

The existence and the possible function for G proteins in plants have been increasingly documented. G proteins have been shown to be involved in the control of  $K^+$  channel opening in guard cells and mesophyll cells (Fairley-Grenot and Assmann, 1991; Wu and Assmann, 1994), the transmission of red and blue light-induced signals (Warpeha et al., 1991; Neuhaus et al., 1993) and the pathogen-induced resistance reactions (Legendre et al., 1992).

The  $AlF_4^-$  complex circumvents the need for GDP dissociation by binding alongside GDP and mimicking the terminal phosphate group of GTP, thereby activating the G protein. Typical for the effect of fluoride upon G proteins is also the lack of an effect at high fluoride concentrations (Chabre and Vuong, 1987). These authors noted that the active species in G-protein activation is  $AlF_4^-$  and  $AlF_4^-$  is the predominant form of complexed aluminum at fluoride concentrations of 1-10 mM. At high  $F^-$  concentration (100 mM) the form is  $AlF_5^{2-}$  and at low fluoride concentrations the form is  $AlF_3$ . Both  $AlF_5^{2-}$  and  $AlF_3$  are unable to mimic the terminal phosphate of GTP and do not activate G proteins, even when GDP is in the nucleotide-binding pocket (De Boer et al., 1994).

## Effects of Fluoride on Enzyme Activities

Fluoride has an effect on enzymes associated with glycolysis, respiration, photosynthesis and other reaction systems. Enolase is particularly sensitive to fluoride and is inhibited *in vitro* at concentrations as low as  $10^{-4}$  M. Membrane ATPases are also quite sensitive to low fluoride concentrations. Some enzymes such as glucose-6-phosphate dehydrogenase, catalase and peroxidase are enhanced *in vitro* by fluoride. Fluoride has dual effects on the enzyme system of higher plants *i.e.* inhibitory or stimulatory. If fluoride reaches toxic concentrations in a plant tissue or organelle, it may be expected that enzymes that are activated by divalent cations would be inhibited, so there have been many studies of enzymes such as enolase and phosphoglucomutase. Fluoride inhibition of enolase (2-phosphoglyceric acid  $\rightleftharpoons$  Phosphoenolpyruvic acid) is perhaps the best known of the effects of fluoride *in vitro* and was first studied in yeast (Warburg and Christian, 1942). The fluoride concentration needed for 50% reduction in enzyme activity was found to increase with increasing  $Mg^{2+}$  concentration.  $Mg^{2+}$  was required to activate the enzyme and, in the presence of fluoride and phosphate, an inactive dissociable magnesium fluorophosphate complex was postulated to be formed and to be inhibitory to the enzyme, although fluorophosphates itself was shown not to be inhibitory (Peters et al., 1964), and it may be necessary for the complex to be formed on the enzyme to have any effect. In plants, the sensitivity of enolase to fluoride was described by Miller (1958); the pea-seed enzyme also had an  $Mg^{2+}$  requirement, while  $Mn^{2+}$ ,  $Co^{2+}$ , and  $Zn^{2+}$  were less effective. Fumigation of intact plants with HF was found to have stimulatory effect on enzyme activity (McCune et al., 1964; Lee et al., 1966). This apparent contradiction may be explained in a number of ways. Apparent stimulation may be an artefact caused by the extraction or assay procedures, but it may simply be that the fluoride did not reach toxic concentrations in the mitochondria or that there was increased respiration due to an extra demand for maintenance. There may also be several isozymes that differ in sensitivity. An important observation made by McCune et al. (1964), after exposure of bean plants to relatively low concentrations of HF (1.7 and 7.6 mg/m<sup>3</sup>), was that, although fluoride increased the activities of enolase and catalase, they tended to approach the control levels after a recovery period. Unfortunately, this is one of the few studies that examined recovery. The mechanism of fluoride inhibition of phosphoglucomutase (glucose-1-phosphate  $\rightleftharpoons$  glucose-6-phosphate) appears to be similar to that for enolase (Chung and Nickerson, 1954). A magnesium-fluoride complex is formed with glucose-1-phosphate and the enzyme. Like enolase, inhibition depends upon the  $Mg^{2+}$  concentration, but, in the one study in which it was measured in plants that had been exposed to HF, phosphoglucomutase was inhibited. The HF exposure, however, was extremely high, at 25 mg/m<sup>3</sup>, for 3–5 days. Fluoride also combines readily with many haem enzymes and there has been some research on catalase (McCune et al., 1964; Lee et al., 1966). In the case of cytochrome oxidase, catalase and peroxidase, the reaction is with the enzyme in the ferric iron state, which is freely reversible and involves one atom of iron and one molecule of fluoride (Hewitt and Nicholas, 1963). Fluoride reacts weakly with many other metalloenzymes. Thus, the zinc-containing enzyme, carbonic anhydrase, and the molybdenum-containing enzymes, nitrate reductase and other molybdo-flavoproteins (which also contain iron), are not very sensitive to fluoride. From *in vitro* studies, it is apparent that multiple sites of fluoride action exist, so the distribution and concentrations of fluoride within the cells determine the degree to which metabolism is affected.

## Effect of Fluoride Toxicity on the Anti-oxidative Enzyme System

Among the many biochemical effects of fluoride, one that has attracted much recent attention is the generation of superoxide free radicals ( $O_2^{\cdot-}$ ). Superoxide free radical ( $O_2^{\cdot-}$ ) is produced from  $O_2$  by plants under oxidative stress induced by different biotic and abiotic stress. Superoxide free radical is both an oxidant and a reductant and has the potential to cause adverse effects on biomolecules. For example, it can damage membrane lipids through lipid peroxidation and cause enzyme inactivation and DNA strand breakage. Living systems have evolved an intracellular enzymatic defense system to protect themselves against  $O_2^{\cdot-}$ . Superoxide dismutase (SOD) is an enzyme responsible for the breakdown of  $O_2^{\cdot-}$ . It is a metalloprotein and catalyzes the dismutation of  $O_2^{\cdot-}$  to  $O_2$  and  $H_2O_2$ . By altering the concentration of  $O_2^{\cdot-}$ , SOD helps prevent both direct toxicity from  $O_2^{\cdot-}$  and secondary toxicity from  $\cdot OH$  and  $H_2O_2$ . Fluoride has been shown to inhibit the activity of SOD. Wilde (1998) reported decrease in SOD activity in germinating mung bean seedlings by higher concentration of fluoride (NaF). The activity of antioxidant enzymes like catalase and peroxidase was found to be increased with increase in F concentration. Saini et al. (2013) reported increase in catalase (3.2 folds) and peroxidase (2.7 folds) enzymes activity in *Prosopis juliflora* plant with increase in F concentration.

## Effect on Photosynthesis

Photosynthesis is found to be affected by fluoride toxicity above a threshold level. The threshold concentration of fluoride above which photosynthesis is inhibited varies from plant to plant. At very high concentration of fluoride in plant tissue net photosynthesis is affected but at very low concentration it has negligible effect on photosynthesis. This may be just due to chance but it may also suggest that photosynthesis is not particularly sensitive to HF. This could be due to the sharp gradients in fluoride concentrations within leaves or possibly chloroplasts are not such a strong sink for fluoride as models suggest. A few indicate the relationship between effects on photosynthesis and visible symptoms.

There is some evidence that effects on photosynthesis are causally linked to visible symptoms (Thomas and Hendricks, 1956; Hill and Pack, 1983; Doley, 1988). Thomas and Hendricks (1956) found that the reduction in photosynthesis in *Gladiolus* was proportional to the injury and Doley (1988) found that differences in photosynthetic rates of two pine species (*Pinus elliottii*, *Pinus caribaea*) were correlated with the concentrations of chlorophylls a, b and a+b. In Hill and Pack's (1983) experiments, HF treatments either had no significant effect on the rate of photosynthesis or the effect was proportional to the amount of leaf necrosis or chlorosis. Continuous fumigation of strawberry plants for 18 days at average daily fluoride concentrations ranging from 1 to 9 mg m<sup>-3</sup> had no effect on photosynthesis, although a small amount of necrosis was induced. But, when the HF concentration was increased to 36 mg/m<sup>3</sup> for 24 h, there was an abrupt drop in the photosynthetic rate of about 50%. As the HF concentration was decreased, the rate returned to about 75% of the previous rate within 24 h and then recovered more slowly for about 3 weeks until the rate was about 95% of the control. Considering that 4 to 5% of the leaf area was necrotic, this was considered to be complete recovery. In maize, the photosynthetic rate was reduced by 12 to 14% after exposure to 5.1 mg/m<sup>3</sup> for 15 days. This treatment induced chlorotic mottling typical for the species and was considered to be the reason for the photosynthetic decline. Several other authors have reported that the effects of fluoride were reversed after exposure (Thomas and Hendricks, 1956; Bennett and Hill, 1973; McCune et al., 1976; Horvath et al., 1978), but this is difficult to understand if the inhibition was caused by accumulation of fluoride in the

chloroplasts. Perhaps the inhibition was caused by greatly reduced stomatal conductance, which limited CO<sub>2</sub> uptake, or, more indirectly, through some feedback system (Weinstein and Davison, 2004).

### **Effect on Respiration**

Mitochondrial respiration provides the energy for synthesis of new biomass, translocation of photosynthates, ion uptake, assimilation of elements, such as nitrogen, protein turnover and maintenance of ion gradients (Weinstein and Davison, 2004). Low concentrations or short durations of fluoride exposure is known to stimulate O<sub>2</sub> uptake, while exposure to higher concentration or for longer duration inhibits respiration (McLaughlin and Barnes, 1975). In contrast, two studies showed that respiratory activity was relatively insensitive to fluoride (Hill et al., 1959; Givan and Torrey, 1968) and in others it was inhibited. The effect of fluoride is found to depend upon the age of the plant or tissue (Béjaoui and Pilet, 1975), the duration of exposure (Applegate and Adams, 1960a), nutrient status (Applegate and Adams, 1960a) and concentration of fluoride in the tissue (Applegate et al., 1960). The relationship between inhibition and the age of the tissue can perhaps be explained by the shift from glycolysis to the pentose phosphate pathway, which occurs with ageing of tissues (Gibbs and Beevers, 1955), and by the greater sensitivity of some glycolytic enzymes to fluoride. Fluoride inhibited tissue respiration may be due to large part to inhibition of respiratory enzymes like Ascorbic acid oxidase, polyphenol oxidase (Lee et al., 1965) and succinate dehydrogenase, malate dehydrogenase, peroxidase (Lovelace and Miller, 1967), have been shown to be inhibited by physiological concentrations of F. The reasons for F-stimulated respiration are less obvious. Special species of plants have shown an increased use of the pentose phosphate pathway when exposed to F. The activities of glucose-6-phosphate dehydrogenase, cytochrome oxidase, catalase and peroxidase were enhanced in F-injured tissues (Baunthiyalet al., 2014).

### **Effects on Other Metabolic Functions**

Fluoride is known to affect the functioning of plasma membranes. However there have been very few studies of this important aspect of fluoride toxicity. In white pine (*Pinus strobus*) plants treated with HF for 2 days, ratios of plasma membrane free sterols: phospholipids, sterols: proteins and plasma membrane adenosine triphosphatase (ATPase) activity were higher than in control plants (Rakowski and Zwiazek, 1992). Exposure to HF at 1.6 mg/m<sup>3</sup> for 28 days produced a drastically reduced activity of plasma membrane ATPase activity. Changes at 1.6 mg/m<sup>3</sup> in sterol and phospholipid levels after only 2 days of fumigation suggested that plasma membrane composition was affected even during dormancy and that these membranes may be among the early sites of HF injury (Rakowski et al., 1995; Rakowski, 1997). Effects of HF on partitioning of <sup>14</sup>C between transport and non-transport photoassimilates from source (treated) leaves to sink regions were studied in soybean plants (*Glycine max* cv. 'Hodgson 78') fumigated with HF at 0, 1 and 5 mg/m<sup>3</sup> for 8–10 days at three stages of development (vegetative, flowering and early fruit set, and pod filling) (Madkour and Weinstein, 1987). In plants exposed to HF, there was a greater retention of <sup>14</sup>C-labelled assimilates in the treated leaf and reduced export of these compounds to other plant parts (sink tissues). There was also a greater incorporation of <sup>14</sup>C into ethanol-insoluble assimilates at the expense of ethanol-soluble assimilates in the treated leaf. Compared with control plants, HF also increased the proportion of <sup>14</sup>C incorporated into sugars in treated (source) tissues and a decrease in <sup>14</sup>C sugars in sink tissues. Results suggest that fluoride

inhibits the processes involved in assimilation and transport of sugars, i.e. phloem loading (Madkour and Weinstein, 1987).

### **Cellular and Structural Damage**

Histochemical studies of fluoride-injured plants have indicated that the damage to leaves first occurs in the spongy mesophyll and lower epidermis followed by distortion and disruption of chloroplast in the palisade cells. The upper epidermis is last to exhibit any distortion or collapse. Wei (1972) described a slight disorganization of mesophyll cells in HF-fumigated soybean (*Glycine max* Merr.) after 3 days of fumigation at 40 ppb. Chlorosis developed later and after five days, a few spongy mesophyll cells and lower epidermal cells collapsed. After six days, necrosis was evident, cellular organization was disrupted and no chloroplasts were recognizable. The first noticeable cellular change consisted of increased and aggregated endoplasmic reticulum. Subsequently (second day of fumigation), small vacuoles were formed in the cytoplasm, and phytoferretin accumulated in chloroplasts. With further fumigation, lipid droplets accumulated in the cytoplasm, mitochondrial membranes showed a slight swelling and a few mitochondria had lost the electron density of the matrix. At this time the tonoplast appeared to break up into vesicles and many multi vesicular bodies appeared in the cytoplasm. Mitochondria later began to degenerate but the chloroplasts remained unaffected until after 5 days of fumigation. Tonoplast breakdown releases phenols and organic substances, which induce osmotic changes in the cytoplasm as well as exerting toxic effects on it, thereby hastening degeneration of other cell organelles.

Fink (1988) reviewed the sequence of cellular injury from HF and listed the responses of the chloroplasts to fluoride toxicity and other stresses. These responses include granulation of the stroma and crystalline inclusions, curling of internal membranes, resulting in an undulated appearance of thylakoids, stretching of the chloroplast envelope, as a consequence of shrinkage of the chloroplast, has been reported for HF, swelling of thylakoids by dilatation of their membranes, dilatation of the parallel alignment of the thylakoid systems and formation of electron-transparent areas of the swollen stroma, incomplete differentiation of the plastids, with reduced membrane systems, accumulation of electron-dense plastoglobuli induced by HF.

### **Effect on Growth of Vascular Plants**

Accumulation of fluorides has clearly been demonstrated to reduce plant growth and general vigor (Brewer et al., 1966). Leaves and portions of green stems can accumulate higher concentrations of fluoride from the atmosphere than other exposed parts due to greater surface area, presence of stomata, and minimal retranslocation from the exposed tissue (Ledbetter et al., 1960). Fluoride becomes concentrated in the tips of monocotyledonous and in the margins of dicotyledonous leaves which may result in extensive chlorosis or necrosis. Under such conditions the yield, growth rates and general plant vigor are adversely affected (Pushnik and Miller, 1990). Reported reductions in shoot and root growth are well correlated with exposure to airborne fluorides; these effects on growth were shown to precede visible damage expression in the foliage (Stocks, 1960). HF fumigation at booting and anthesis significantly reduces the yield of crops (MacLean and Schneider, 1981). The fumigation at anthesis reduces yield because of smaller, fewer spikes. The grain or fruit fluoride concentration does not increase by the fumigation indicating that the fluoride does not translocate from the leaves to the developing seeds (Murray, 1983). It is observed that HF affects leaf injury and fruiting independently. Exposure of crops to fluoride (HF) during

anthesis severely reduces yield, indicating an adverse effect of fluoride on the fertilization process.

### **Effects on Fertilization, Seed Set and Fruit Yield**

Fluoride is known to interfere with calcium which plays essential role in fertilization (Pack, 1966). The fluoride injury on the leaves of low-calcium plants is more. An interactive effect of fluoride and calcium on tomato pollen germination and growth were confirmed by Sulzbach and Pack (1972). Although most fruits are relatively tolerant to HF concentrations up to 2-3mg/m<sup>3</sup>, but some fruits like peach, are exceptionally sensitive. A condition known as *black tip* has been described in peaches in an area in which fluoride was present (McCornack et al., 1952), but more commonly HF induces a condition known as *suture red spot or soft suture*. It is characterized by premature ripening of the flesh on one or both sides of the suture towards the styler (blossom) end of the fruit. The ripening of this tissue precedes that of the normal fruit and is characterized by external and internal reddening of the suture area. The affected area may enlarge faster for a short time before ripening. The suture symptoms are often accompanied by splitting of the flesh along the suture line. At harvest, the affected areas are soft and often decomposing (Griffin and Bayles, 1952; Benson, 1959; Drowley et al., 1963; Bolay et al., 1971; Mezzetti and Sansavini, 1977; MacLean et al., 1984). Continuous exposure to concentrations above about 0.3 mg F/m<sup>3</sup> for longer period results in a high proportion of abnormal fruit in peach (MacLean et al., 1984). Continuous exposure to HF also causes fruit deformation in strawberry (Pack, 1972). HF is known to affect fruiting in tomato and bean by interfering with fertilization and seed development (Pack, 1966, 1971). The flowering stage is known to be most sensitive to HF toxicity. Carpels are more sensitive to HF than pollen-producing anthers and result in more fruit deformity. The stigmatic surface is known to be altered by exposure to HF, affecting pollen-tube growth and subsequent fertilization (Sulzbach and Pack, 1972). Accumulation of fluoride on and just inside the stigmatic surface disrupts the calcium gradient in the stigma and style (Bonte and Garrec, 1980).

### **Aluminum Fluoride Toxicity in Plants**

The main form of fluoride in soils with pH below 6 is different AlF<sub>x</sub> complexes (Arnesen, 1997). AlF mimics phosphate, reduces root uptake of phosphate, and causes a reduction in the plant's energy and yield similar to the effects seen in cases of *phosphate starvation*. Al-F complexes have been shown to be toxic to plants. The F<sup>-</sup> toxicity is attributed to a phosphate-mimicking property of Al-F complexes under this condition. Phosphorus is acquired by plant roots primarily via high-affinity Pi transporters. Several pieces of evidence support a model where AlF<sub>x</sub> complexes can mimic the tetrahedral phosphate group competing with it for the same binding sites on the Pi carriers and possibly stabilizing an inactive conformation. AlF<sub>x</sub> complexes inhibit Pi uptake which can be antagonized by raising the Pi concentration in the reaction medium. The Pi uptake is stimulated in corn roots after AlF<sub>x</sub> pretreatment which is similar to that observed after Pi starvation. It is proved that fluoroaluminates act as physiological Pi analogs by competing directly for the same binding sites of Pi transport rather than any indirect effect on the proton motive force of the process. These evidences support the proposal that the property of AlF<sub>x</sub> to mimic Pi may describe the most important mechanism of AlF<sub>x</sub> toxicity whenever AlF<sub>3</sub> and AlF<sub>4</sub> are the dominant species (Facanha and Okorokova-Facanha, 2002).

## Roles of Calcium, Magnesium, Phosphate and Nitrate in the Phytotoxicity of Fluoride

Under fluoride toxicity condition, Ca form complexes and precipitates with F<sup>-</sup> resulting in lower activity or deficiency of calcium in the plant body. The mechanisms by which F<sup>-</sup> is toxic are thought to involve inhibition of enzymes and interference with membrane permeability through precipitation with Ca (Suttie, 1977). If Ca concentrations in plants are already low, the plants would be more sensitive to F<sup>-</sup> exposure. Changes in membrane permeability could overcome the barrier to F<sup>-</sup> uptake in the cortex of the root and increase F<sup>-</sup> concentrations in plant to phytotoxic levels. The toxic action of F<sup>-</sup> is also thought to involve the inactivation of Mg at its sites of physiological activity (Weinstein and Alscher-Herman, 1982). However, concentrations of Mg in plant shoots showed no changes with F<sup>-</sup> treatment suggesting that F<sup>-</sup> concentrations in solutions had no effect on Mg nutrition in the plant (Stevens et al., 1998).

## CONCLUSION

Fluoride toxicity is a worldwide problem, which adversely affects both plants and animals. While studies on the effects of fluoride toxicity on animal and human health has received greater attention in recent years, the studies on the consequences of fluoride toxicity stress on crop plants needs more attention to understand the physiological, biochemical and molecular basis of fluoride tolerance of crops. Fluoride in air and soil adversely affects the crop growth and yield and has serious repercussion on world agriculture. Therefore, further research at molecular level can only help to understanding fluoride toxicity stress in plants and development tolerant genotypes for sustainable crop production under abiotic stresses like fluoride toxicity.

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