PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF PLANTS UNDER FLUORIDE STRESS: AN OVERVIEW

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SUMMARY: Fluoride (F) present naturally in soil, water, and atmosphere shows significant interaction with plants and adversely affects their various physiobiochemical parameters, sometimes even without showing any visible symptoms of injury. F affects plant growth and development by interfering with several metabolic pathways associated with photosynthesis, respiration, protein synthesis, carbohydrate metabolism, and nucleotide synthesis. The present review critically discusses the diverse toxic effects on agricultural crops and trees of exposure to F as it is well known that excess F intake through drinking water over a period causes serious irreversible ill effects in the form of osteo-dental fluorosis.

Keywords: Antioxidants; Fluoride accumulation; Fluoride toxicity; Photosynthesis; Respiration.

INTRODUCTION

Fluoride (F) exposure for a prolonged period generates diverse toxic effects not only in livestock[1,2] and in human beings[3,4] but also in plants.[5] These effects in plants and vegetation are mainly through the F contaminated water, soil, gases, and dust altering leaf physiology. The signs of F injury to plants may be acute or chronic and their severity is dependent on the F concentration as well as the duration and frequency of the F exposure.[2] F toxicity in plants results from the gradual accumulation of F into their sub cellular components which then changes the F sensitive biochemical processes. Visible foliar damage is associated with prolonged F exposure. F can also influence the various enzymatic activities and affect, directly or indirectly, the physiological process such as respiration and photosynthesis without showing any visible symptoms of injury. This review provides an overview of information on the cellular and molecular aspects of the interactions between F and plant cells resulting in alteration of various morpho-physiological and biochemical processes.

TOXICOLOGICAL EFFECTS OF F

Entry of F into plants is mainly through two pathways. First, aerial deposition of gaseous F takes place through stomatal diffusion. Through the leaf stomata, F permeates the cell walls and migrates towards the margins and tips that are the sites of greatest evaporation.[6] The second route is from the soil and water into the plant roots through a passive diffusion process. Subsequently F is transported via xylem through the apoplastic and symplastic pathways in a unidirectional distal movement into the shoots.[7] F is also transported through biological membranes via non-ionic diffusion of hydrogen F (HF). The small neutral molecule of HF penetrates cell membranes much faster than the dissociated F ion (F⁻), resulting in

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a more pronounced intracellular intake. Membrane permeability to HF is five to seven orders of magnitude above that of F^-.

The bioavailability of F to plants is influenced mainly by the pH of the solution, the presence of other metal ions such as calcium (Ca), aluminum (Al), and phosphorus (P), and the soil type.[8] Normally, F accumulation follows the order of soil>root>shoot>grain. A pot culture study conducted to evaluate the bio-accumulation of F in terms of bio-concentration factor (BCF) in Lady's finger (*Abelmoschus esculentus*) grown in sodium F (NaF) contaminated alkaline soil showed that the roots accumulated most of the F supplied through the irrigation water, while the fruit accumulated least.[9] A study conducted on the organ-wise distribution of F in three semi arid plant species viz. *Acacia tortilis* (Umbrella thorn)*, Prosopis juliflora* (Honey mesquite)*,* and *Cassia fistula* (Golden shower) indicated significant translocation of F from root into aerial parts.[10] F absorbed by roots was transported to shoots through the transpiration stream and accumulated mostly in the leaf tissues. The stem was the least preferred organ of F accumulation. Similarly, another study on *P. juliflora* showed that the roots accumulated the larger portion of the F (1024.63 µg/g) followed by the shoots (492.30 µg/g).[11]

The initial and visible symptoms of F injury to plants are the genesis of necrosis at the tips and margins of the leaves. On continuous and prolonged F exposure, the tip falls off leaving the leaf notched. In a recent study, symptoms of leaf chlorosis and necrosis in poplar seedlings were induced after treatment with 500 ppm F in irrigation water.[12] In *Eucalyptus, Acacia*, and numerous rain forest species, chlorosis was believed to be sensitive indicator of F toxicity. The rate at which symptoms appeared was influenced by many environmental factors, such as concentration of F, distances from source, length of exposure, and meteorological conditions.[13]

F is reported to cause adverse effects on plant growth and yield. Experiments were conducted on *Raphanus sativus* (radish) grown in soil supplemented with various levels of NaF. The F treated plants exhibited a marked reduction in growth parameters i.e. seedling germination percentage, length of root, length of shoot, plant height, number of leaves, size of leaf, number of flowers per plant, fruit-set percentage, and seed-set percentage as compared to control plants.[14] F treatment showed similar results on various other plants, e.g., *Amygdalus communis* (almond),[15] *Cyamopsis tetragonoloba* (cluster bean),[16] *Medicago sativa* (alfalfa), [17] *Populus deltoides* (poplar),[12] *Cicer arietinum* (Bengal gram),[18] *Triticum aestivum* (wheat),[19] *Vigna radiata* (mung bean),[20] and *Oryza sativa* (paddy).[21]

MOLECULAR MECHANISM OF F TOXICITY

The high internal F concentration disturbs almost all the physiological and biochemical process in plants. A number of cellular processes identified to cause deleterious effects on plants include disruption of enzyme activity involved in metabolic processes, inhibition of protein secretion and synthesis, generation of reactive oxygen species (ROS), and alteration of gene expression.[22]

At micromolar concentrations, F acts as an anabolic agent and promotes cell proliferation, whereas at millimolar concentrations it acts as an enzyme inhibitor.[23] F disrupts enzyme activity by binding to functional amino acid groups that surrounds the enzyme's active centre. Inhibition of protein synthesis and secretion interrupts the signaling pathways involved in cell proliferation and apoptosis. F can increase oxidative stress leading to the degradation of cellular membranes and reduced mitochondrial activity.[22]

The mechanism by which F affects photosynthesis is mainly by reducing the synthesis of chlorophyll, degradation of chloroplasts, and inhibition of Hills reaction.[24] The effects of F on the photosynthetic electron transport chain have been studied in spinach thylakoid membranes. An inhibition by F in the photosystem-II (PS-II) electron transport rate followed by a subsequent increase in the photosystem-I (PS-I) electron transport rate indicated the possibility of state transitions being a mechanism for F toxicity. The photosynthetic capacity of azalea cultivars (*Rhododendron* sp.) was significantly reduced, *in vitro*, at F concentrations of 190 ppm.[25] F treatment on *Salicornia brachiata* (Umari keerai) grown in solution cultures under controlled conditions resulted in a decrease of photosynthetic pigments (chlorophylls and carotenoids), while the anthocyanin content increased significantly.[26] Mulberry plant variety (S54), believed to be sensitive to F, showed a reduction in photosynthetic capacity, chlorophyll-a (Chl-a) and chlorophyll-b (Chl-b) concentrations, and leaf area when grown in soil with a high F content. [27] Similarly in one of the recent studies conducted on growth and development of seedlings of watermelon (*Citrullus lanatus*), it was found that increasing NaF concentration decreased the Chl-a and Chl-b pigments, total chlorophyll, and carotenoids content as compared to control seedlings.[28] The decrease in photosynthetic pigments may be due to inhibition of chlorophyll biosynthesis.[21] Increasing the activity of the chlorophyll degrading enzyme chlorophyllase and the reduction of Fe^{+2} ions are essential for the synthesis of chlorophyll pigments.[15,28] The addition of F during early growth of three semi-arid plant species, viz. *A. tortilis*, *C. fistula*, and *P. juliflora* revealed no major changes in photosynthetic performance, as revealed by electron transport rate (ETR), ETR max, photosynthetic photon flux density (PPFD-sat), and ΔF/Fm'-sat (effective quantum yield at saturating light intensity) values which were higher in these plants, but there were significant decreases in the Chl-a, Chl-b, and total chlorophyll concentrations. For most of the parameters, *C. fistula* was found to be more sensitive to F stress whereas *P. juliflora* showed the least damage from F exposure.[29]

F accumulation in leaves also affects stomatal conductance and gas exchange. In one of the experiments,[30] CO_2 assimilation (*A*), transpiration (*E*), stomatal conductance (*gs*), and chlorophyll fluorescence rates were measured after 27 days of treatment with F in coffee and sweet orange plants. The results were compared with sensitive (gladiolus) and tolerant (ryegrass) reference species. It was found that in gladiolus, there was a significant reduction in chlorophyll fluorescence and consequent damage to the potential quantum efficiency of PS II (Fv/Fm). The

increase of CO_2 assimilation by the coffee plants was possibly due to the onset of stress experienced by this species. Thus, the damage to the photosynthetic system was generally reflected in the species susceptibility to F stress. In the chloroplasts, F is known to affect enzymes, such as ATP synthase, ribulose bisphosphate carboxylase-oxygenase, and sucrose synthase.

F inhibition of the mechanism of respiration may be linked to inhibition of respiratory enzymes and stimulation may be linked to an uncoupling of phosphorylation. Respiratory enzymes such as succinate, malate, and NADH dehydrogenases are all sensitive to F, with succinate dehydrogenase, in the presence of phosphate, being the most sensitive**.** Fumigation of young soybean plants (*Glycine max* Merr. cv. Hawkeye) with 9–12 µg/m^3 HF caused a stimulation of respiration at about 2 days of treatment followed by inhibition 2 days later. Higher respiration rates, greater ATPase activity, and lower P/O ratio (Phosphate/ Oxygen Ratio) was observed in mitochondria isolated from the stimulated tissue, while in mitochondria from inhibited tissue, all three were reduced.[31] Enolase was found to be the most important enzyme of carbohydrate metabolism inhibited by F. F competition with Mg^{+2} resulted in a slow decrease of enzyme activity and subsequently, in a complete loss of enzyme activity.[32] In one of the recent findings on six planktonic algae, enolase was inhibited by F, with K_i values ranging from 27 to 319 µM.[33] Similarly, inhibition of phosphoglucomutase, another enzyme participating in sucrose biosynthesis, in higher plants could account for the inhibition of sucrose synthesis in F-fumigated plants.[34]

F also affects energy metabolism in higher plants by inhibiting the ATP synthase enzymes in chloroplasts, mitochondria, and plasma membrane that are responsible for ATP formation. The plasma membrane associated ATPase (P-ATPase) and tonoplast associated ATPase (V-ATPase) enzymes are known to be the sites of functional alterations during environmental stress. Studies have indicated that P-ATPase and V-ATPase may be among the initial sites of F injury to plants as well as the initial sites of the defense reaction.[35]

The reactions of plants to F stress are complex and involve changes in many biochemical processes associated with amino acids, antioxidant enzyme activity, and proteins. Proteins, being one of the important organic nitrogenous constituents of plants, play a great role in the compensatory metabolism of a plant species during F stress conditions. Recently a study was conducted to ascertain the rate of protein content in senescing leaves of mulberry under F treatment.[36] The decreased protein content can be explained by a decrease in protein synthesis, enhanced protein degradation, or the use of protein for the purpose of providing energy. It was postulated that the decrease in protein synthesis could be attributed to the ability of F to modify the ratio of free nucleotides and RNA, to decrease of the rate of RNA synthesis, and/or to enhance ribonuclease activity. The addition of F also increases the proline content, which is an important stress indicator of protein synthesis and this effect has been shown in *Vigna* seedlings.[20] Researchers have demonstrated that eukaryotic resistance to F toxicity is mediated by a wide spread family of F export proteins. A protein called FEX (fluoride exporter) in

many fungi is essential for cell survival in the presence of high F concentrations. The protein is required for the rapid expulsion of cytoplasmic F, indicating that many eukaryotic species that carry FEX genes are likely to avoid F toxicity by purging cellular F.[37]

Plants have antioxidant defense mechanisms comprising the enzymes catalase, peroxidases, superoxide dismutase, and non enzymatic constituents. Antioxidant treatment consistently protects cells from the lipid peroxidation caused by F exposure, suggesting that oxidative damage is the major mode of action of F. Many researchers have studied the adverse effect of F on the metabolic processes of plants due to effects on these antioxidant enzymatic systems. A study conducted on tea leaves, one of the F hyperaccumulator plants, showed a decrease in the activity of superoxidase dismutase whereas catalase and guaiacol peroxidase activities at first increased at low F concentrations, reaching their peak values at 0.21 and 0.32 mM F respectively, and then decreased at the higher F concentration of 0.53 mM, thus lowering the antioxidative effect.[38] In mulberry leaves, concomitant with an increase in F exposure levels, there was a decrease in catalase activity and increases in guaiacol peroxidase and ascorbate oxidase activity.[36,39] Glutathione levels are also altered by F, often resulting in the excessive production of reactive oxygen species (ROS) at the mitochondrial level, leading to the damage of cellular components. It is known that excessive ROS production leads to macromolecule oxidation, resulting in free radical attack of membrane phospholipids with resulting membrane damage via the induction of lipid peroxidation, mitochondrial membrane depolarization, and apoptosis.[22]

Although experimental evidence provides a good understanding of F stress on plant physiology and biochemistry, it does not suffice for predictive purposes. In contrast to the abundant research that has been done to determine the effects and mechanisms of F toxicity in animal systems, for plants, more in-depth investigations are required to understand the uptake, accumulation and mechanisms of F toxicity.

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