INFLUENCE OF FLUORIDE-CONTAMINATED IRRIGATION WATER ON PHYSIOLOGICAL RESPONSES OF POPLAR SEEDLINGS (*POPULUS DELTOIDES* L. CLONE-S_7C_{15})

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SUMMARY: When exposed to 100–500 ppm fluoride-contaminated irrigation water for six weeks, six-week old poplar (*Populus deltoides* L. clone-S_7C_{15}) seedlings showed decreases in the following physiological characteristics: growth, leaf expansion, photosynthetic CO_2 assimilation, stomatal conductance, chlorophyll fluorescence yield, plant biomass, and harvest index. An enhanced electrolyte leakage triggered susceptibility of the seedlings to loss in the harvest index. The 500-ppm F level induced marked inter-vein chlorosis, leaf-margin necrosis, and leaf-curl on younger leaves with a xerophytic condition for rhizosphere that altered the tree habit of poplars in scrub vegetation. With increasing F concentrations, the harvest index and other growth parameters were successively reduced.

Keywords: Chlorophyll fluorescence yield; Fluoride irrigation water; Photosynthetic CO_2 assimilation; Plant growth; Poplar; *Populus deltoides*; Stomatal conductance

INTRODUCTION

Fluoride (F) in the atmosphere enters plants through stomatal diffusion.[1] F is also taken up by plant roots and is then transported via xylem to different parts of the plant where it accumulates.[2-6] Moreover, aquaporins may be involved in the transmembrane transport of F.[7] The effect of F on germination and physiological and biochemical characteristics in different plant species is negatively correlated.[7,8] Excess F absorption induces phytotoxicity symptoms with ultrastructural changes in leaves[9] and may enhance enzymes that mitigate reactive oxygen species.[10]

F is therefore a common pollutant in air and soil that may cause reduction in growth,[11] photosynthesis, root hydraulic conductivity[12] involving water flow affecting gas exchange with leaf area expansion.[4] Significant amounts of air-borne CO_2 probably cannot be utilized owing to a decline in leaf area and stomatal conductance accompanied by an inability to acquire expected biomass-productivity by impaired CO_2 diffusion and carboxylation. With graded solutions of F, decreased relative growth rate occurs[13] affecting physio-agronomic attributes, i.e., canopy photosynthesis with inhibited light use efficiency associated with plant performance.[14]

In the present study, an expanding phytoremediation technology that employs higher plants for cleanup of F-contaminated sites is explored with poplar (*Populus deltoides*) seedlings.

MATERIALS AND METHODS

Plant material: In view of its high agro-economic value, poplar (*Populus deltoides* L. clone S_7 C_{15}) plants are largely accepted by agro-forestry programs in northern India. Here poplar seedlings were raised from stem cuttings uniform in

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diameter and ca. 18–20 cm in length, purchased in February 2010 from WIMCO Seedlings (Pvt.) Ltd, Bagwala (Kashipur Road), Rudrapur, Udham Singh Nagar (Uttarakhand, India). The cuttings were kept vertically for 3 weeks in a plastic tray filled with water to promote rooting-sprouting under weak light exposure (200 µmol/m^2-s) for 12 hr at 25ºC with daily change of the tray water.[15,16] The sprouted-seedlings were then shifted on March 10, 2010 into 30-cm diameter earthen pots filled with fertile soil for their proper root establishment under field conditions for a period of 3 weeks (photoperiod 12 hr, PPFDs 500–1500 µmol/m^2-s, day–night temperature 30–20ºC) under an average relative humidity of 65%.

NaF treatment: The six-week-old poplar seedlings were subjected to 100, 200 and 500 ppm F-contaminated irrigation water for a period of 6 weeks by the applying F solutions (prepared from NaF) up to field capacity once each week. The control seedlings were raised identically except without F in the irrigation water. Ten independent replications were made for each treatment.

Measurement of physiological responses: After 6 weeks of growth in the pots, an infrared gas analyser (IRGA-6400, portable photosynthesis system, USA) was used for monitoring CO_2 assimilation and stomatal conductance, while the chlorophyll fluorescence yield was monitored by a plant efficiency analyzer (Handy PEA, Hansatech, UK). Photosynthetic CO_2 assimilation was measured with fully extended leaves (6th position from apex) on clear days in the forenoon at 9:00–10:00 am under saturating PPFDs ~1500 µmol/m-s, air flow 500 µmol/s, and CO_2~360 ppm. Fv/Fm measured by handy PEA to assess photosynthetic efficiency of PS II. Chlorophyll was estimated spectrophotometrically in 80% acetone.[17]

Electrolyte leakage): Measurement of membrane leakage in root, stem and leaf tissues was made by the method of Valentoric et al.[18] The central portions of the root, stem, and leaf were cut into 1-cm pieces weighing about 1 g each, which were placed in test tubes containing 15 mL of deionized water. The tubes were then tightly capped and shaken for 24 hr at 25–30ºC. The electrical conductivity (EC_1) of the solution was measured with an electrical conductivity meter (HI 8733, Hanna Instruments Inc., Woonsocket, USA). Afterward, the samples were autoclaved at 120ºC for 20 min and allowed to cool (25ºC) to record the electrical conductivity (EC_2) of the solution. The electrolyte leakage is expressed as:

$$\text{EL (\%)} = \frac{\text{EC1}}{\text{EC2}} \times 100$$

Shoot-root length, leaf area and harvest index: The leaf area expansion (cm^2), number and shoot-root length (cm) were measured after terminating the F treatment. The leaf area was calculated by the paper-weight method with the help of a calibration curve. The harvest index was calculated by harvesting at least 10 seedlings of each treatment.[19]

RESULTS AND DISCUSSION

As seen in Figure 1, F phytotoxicity was expressed through inter-vein chlorosis and leaf-margin necrosis followed by leaf-curl[20] in the younger leaves after exposure of the seedlings to the 500-ppm F irrigation water for six weeks. The peripheral leaf-margins exhibited F induced necrosis similar to *Callistemon* and *Eucalyptus*.[21,22]

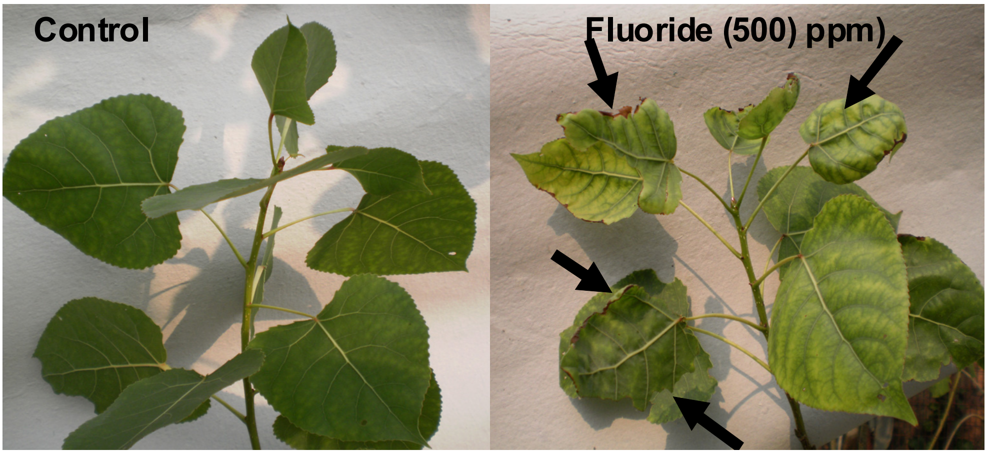

Figure 1. Symptoms of leaf chlorosis and necrosis in poplar seedlings induced after 500-ppm F irrigation water treatment (up to field capacity for six weeks, F irrigation frequency, n = 6, once each week). The photographs were obtained 20 days after terminating F treatment. Symptoms appeared through leaf lamina inter-veinal chlorosis, necrosis at leaf margins, and leaf curl, as indicated by arrows.

As with *Eucalyptus, Acacia*, and numerous rain forest species, chlorosis may be a much more sensitive indicator of F toxicity in poplar than necrosis.[21] In many species of *Eucalyptus*, F injury symptoms are expressed in the form of reduced leaf-area expansion, with depressed chlorophyll biosynthesis and its organization in the younger leaves.[21] Irregular effects similar to chewing-injury and sap-sucking insect attacks have also been found among leaves of similar age.[20] The chlorosis induced by F may also resemble symptoms of iron, magnesium, and boron deficiency along with loss of apical dominance. The phyto-hormone auxin that plays a crucial role to sustain apical dominance seemed to be considerably perturbed, which might have resulted in scrub-vegetation becoming a serious demerit for agro-forestry.

As seen in Table 1, exposure of the poplar seedlings to 100, 200, and 500 ppm F in the irrigation water for six weeks monotonically increased impairment of leaf area expansion and leaf number. Thus, loss in leaf-area,[4] chlorosis, and necrosis reduced canopy photosynthesis.[14] A proportional reduction in root and shoot lengths also correlated with these increasing concentrations of F in the irrigation water. The stimulation of root growth in wheat, shoot growth in mustard, and both root and shoot growth in Bengal gram have been observed in studies with exposure of seedlings of these plants to NaF.[5] The reduction in root growth at higher dosages of F may be associated with direct injury to the roots.[8,23,24]

Consequently, as portrayed in Figure 2, extended F irrigation of poplar seedlings triggered a substantial decrease in photosynthetic CO_2 assimilation, stomatal conductance, and chlorophyll fluorescence yield.[4]

Table 1. Effect of F-contaminated irrigation water on growth-development, leaf area expansion-number, shoot-root length, and photosynthetic pigments in poplar seedlings with a total of six weeks of weekly irrigation. Observations were made after terminating the F treatment.

Fluoride concentration (ppm)	Physiological responses (% decrease)						
	Leaf number	Leaf area (cm^2)	Shoot length (cm)	Root length (cm)	Chl a	Chl b	Chl (a+b)
0 (control)	-	-	-	-	-	-	-
100	20	9	4	6	3	6	3
200	55	46	32	26	26	18	24
500	61	60	42	62	47	34	43

Physiological responses

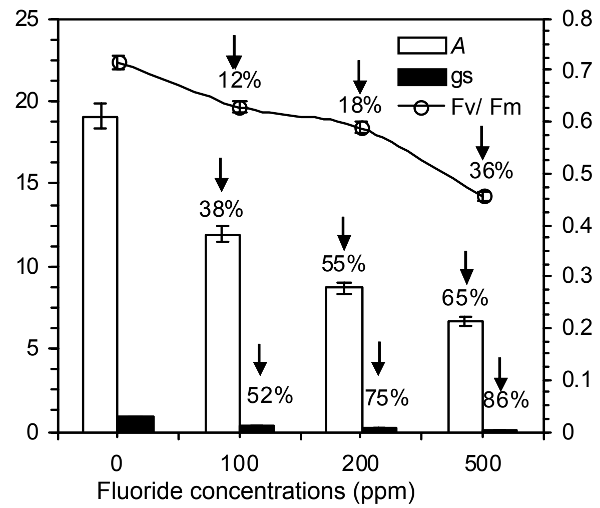

Figure 2. Effect of F-contaminated irrigation water on physiological responses in poplar seedlings: A–photosynthetic CO_2 assimilation (μmol/m^2-s), gs–stomatal conductance (mmol/m^2-s), and Fv/Fm–chlorophyll fluorescence variable per maximum yield. Observations were made after terminating the F treatment. Data represent the means of 5–7 independent observations with ± SEM.

The F-dependent concentration decline in photosynthetic CO_2 assimilation correlated with loss of stomatal conductance. Thus, impaired stomatal-dynamics reduced atmospheric CO_2 diffusion into the cell-chloroplast for carboxylation integrated with the loss in chlorophyll and Fv/Fm values.[4] Hence, F affects chloroplast membrane and stromal enzymes associated with CO_2 fixation,[25] and also blocks the dephosphorylation of chlorophyll a/b protein complex phosphorylated by the light-activated kinase enzyme for distribution of absorbed excitation energy from photosystem (PS) II to PS I due to loss in Fv/Fm, suggested lowered quantum efficiency of the reaction centre II. The decrease in PS II activity occurred due to distinct decrease in the peak intensities at 685 nm and 695 nm associated with CP 43 and CP 47 (chlorophyll binding proteins of core antenna subunits of PS II), as affected by F treatment with increase in PS I peak (735 nm) intensities. An inhibition of oxygen evolution is observed in case both chloride and iodide get substituted by F.[26-28] Thus, F irrigation impaired chlorophyll pigments and biomass characteristics. The loss in leaf area affected by F treatment resulted in chlorosis, also found to be proportional to the live leaf area in a plant canopy. The mechanism by which F inhibits apparent photosynthetic CO_2 assimilation and stomatal conductance is yet to be revealed.[14,29]

Electrolyte leakage from 500 ppm F irrigation greatly enhanced membrane kinetics in the roots, stems, and leaf tissues (Table 2).

Table 2. Effect of F-contaminated irrigation water on electrolyte leakage in poplar seedlings after six weeks. Observations were made after terminating the F treatment.

Fluoride concentration (ppm)	Electrolyte leakage (% increase)		
	Leaf	Stem	Root
0 (control)	-	-	-
100	67	39	25
200	182	97	60
500	224	134	106

At lower F concentration, less electrolyte leakage was observed. Hence, an increased electrolyte leakage with enhanced exposure to F, thereby apparently impairing structural integrity of the bio-membrane with loss in photosynthetic CO_2 assimilation.[30] Thus, as seen in Figure 3, after F irrigation, loss in leaf area-expansion, leaf number, shoot length, chlorophyll, PS II efficiency, stomatal conductance, and CO_2 assimilation occurred with an increase in efficiency of water use and enhanced electrolyte leakage in the root, stem and leaf cells, leading to a reduction in harvest index (HI). Therefore, an attractive possibility is the exploration of poplar cultivation in F-affected (~50 ppm F) agro-climatic zones to extend phytoremediation by using this tree species to sustain the agro-socio economy.

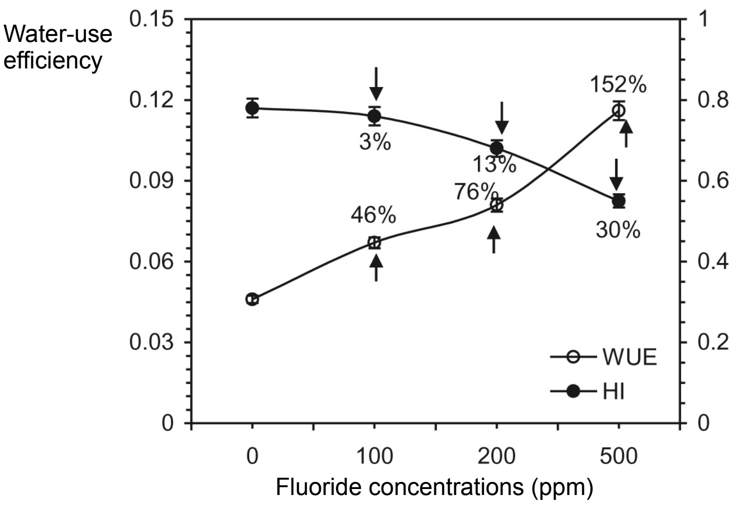

Figure 3. Effect of F-contaminated irrigation water on water-use efficiency (WUE) and biomass in poplar seedlings (harvest index, HI) at the termination of F treatment. Data represent the means of 5–7 independent observations with ± SEM.

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