EFFECT OF FLUORIDE ON ALMOND SEEDLINGS IN CULTURE SOLUTION

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SUMMARY: Effects of different NaF concentrations on the growth and certain metabolic parameters of almond seedlings (*Amygdalis communis*) were studied under strictly controlled growth conditions in nutrient solutions containing increasing NaF concentrations ranging from 0 to 10 mM NaF. After 14 days, production of material measured as dry matter was significantly reduced in the root system, which accumulated large amounts of F. The chlorophyll, calcium, and magnesium content of the leaves showed a significant decrease, and the leaf content of starch and sugar was also reduced, especially at the higher F concentrations. Mineral concentration changes in the roots were minor except for manganese, which showed a major decrease at 2.5 mM NaF. Overall, the nutritional status of the leaves appeared to be affected more than that of roots.

Keywords: Almond seedlings; Chlorophyll content; Fluoride; Growth; Nutrient distribution; Reducing sugars; Starch.

INTRODUCTION

To most plants, fluoride (F) is phytotoxic through altering a series of metabolic pathways.[1-3] Fluoride can be deposited into soil from several anthropogenic sources, both directly through phosphate fertilizers or indirectly through atmospheric pollution from industrial activities and burning of fossil fuels.[4,5] From the soil, F is absorbed by plant roots and then transported via xylematic flow to the transpiratory organs, mainly the leaves, where it can accumulate with adverse effects that have been described in the literature.[6,7] The rate at which symptoms appear depends on many environmental factors, such as the type and concentration of pollutants, distance from the emission source, length of exposure, and meteorological conditions.

Owing to its high adaptability to semi-arid area climatic conditions and because of the high nutritional value and widening market of its product, the almond tree is receiving increased agricultural attention in Tunisia. In this report we record changes in some ecophysiological parameters of the F-sensitive almond plant, *Amygdalis communis*,[8] following treatment of almond seedlings with F under controlled conditions. To our knowledge, no such previous study has been made. This work, therefore, provides a laboratory study on the effects of F on certain metabolic activities of almond seedlings.

MATERIALS AND METHODS

Nutrient solution: Almond seeds, *Amygdalis communis*, were germinated on wet filter paper, in the darkness at 4ºC for 15–20 days. Following their germination, seedlings of approximately the same size were transferred to a Lang-Ashton nutritive medium[9] that was continuously aerated and renewed

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weekly. The seedlings were cultivated in a growth chamber under the following conditions: 26/22ºC day/night temperature, 16/8-hr photoperiod, and 300 μmol photons/m^2/s light intensity. After a 2-week acclimatization phase, NaF was added to the nutritive medium at concentrations of 1, 2.5, 5, and 10 mM. On the 14th day after the start of F exposure, eight plants from each treatment were harvested. Plant organs were separated into leaves, stems, and roots. Dry matter of the different tissues was determined after drying in an oven at 80ºC for 48 hr. Dried samples were then ground to a fine powder.

Fluoride analysis: Powdered plant samples (500 mg) were ashed at 550ºC for 1 hr with 4 g of a sodium-potassium carbonate mixture, and the temperature was raised to 950ºC for an additional 30 min. The cooled ashed material was then dissolved in 20 mL of 1 M HCl, filtered into a volumetric flask, and the volume was diluted to 60 mL with demineralized water.[10] For potentiometric measurement of total F, the diluted solution supernatant was mixed with TISAB-buffer solution (1:10) to dissociate F complexes, stabilize the pH, and maintain a constant ionic strength.[11]

Chlorophyll content: The chlorophyll concentration in the ground leaves was determined according to the method of Moran and Porath.[12]

Carbohydrates: Reducing sugars in the ground leaves were analysed according to the procedure of Ashwell,[13] and leaf starch was determined according to the method of McCready et al.[14]

Macro- and micronutrients: The dried leaves and roots were wet-ashed in a 2:1 v/v nitric and perchloric acid mixture, and Ca, Mg, K, and Fe were determined by atomic absorption spectrophotometry.

Statistical analysis: Each determination was repeated at least twice. The mean SE values are shown in the figures. Significant differences between the controls and treatments were determined using the Student's t test.

RESULTS

1. Effects on growth: As seen in Figure 1, after 14 days of exposure to F in nutrient solution, the dry weight of the leaves and roots of the almond seedlings decreased with increasing F concentration which was significant only for the roots at 5 and 10 mM NaF.

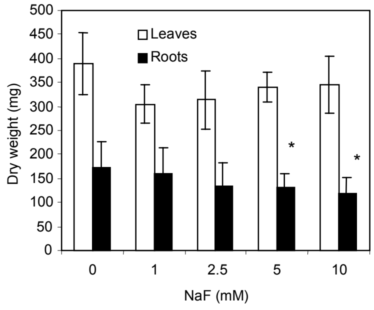

Figure 1. Effect of different fluoride concentrations on the dry weight of roots and leaves of almond seedlings after 14 days of exposure. Values are means SE (n = 8). *p=0.05.

2. Fluoride concentration in plant tissues: As seen in Figure 2, the F concentration increased significantly in both the leaves and roots of the almonds seedlings. The F concentration in roots, however, increased considerably more than in the leaves, ranging from 645 to 1767 µg/g dry matter (DM) in the roots and from 27 to 189 µg/g DM in the leaves.

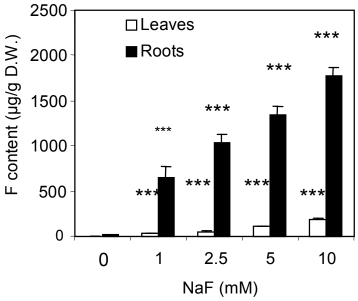

Figure 2. Fluoride accumulation in roots and leaves of almond seedlings after 14 days of exposure to various fluoride concentrations. ***p=0.001.

3. Chlorophyll content: As seen in Figure 3, the chlorophyll content of the leaves was significantly lower at 1 mM F but then showed a small nonsignificant increase with increasing nutrient F concentration.

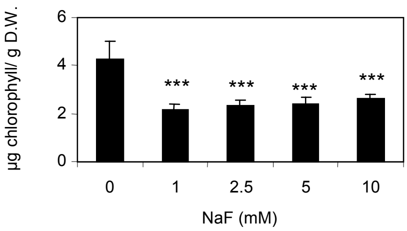

Figure 3. Effect of different F concentrations on chlorophyll content of almond seedling leaves after 14 days of exposure. Values are means SE (n = 6). ***p=0.001.

4. Leaf sugar content: As seen in Figure 4, carbohydrate metabolism showed a decrease in reducing sugars and starch in the leaves with increasing F concentration in the nutrient solution.

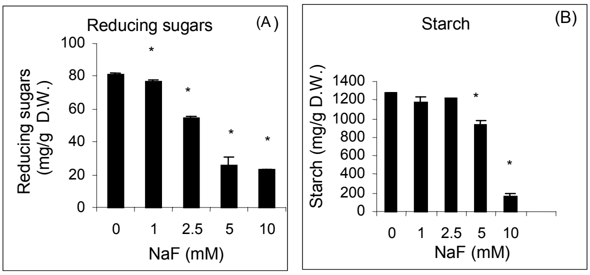

Figure 4. Effect of different fluoride concentrations on leaf reducing sugars contents (A) and leaf starch contents (B). Values are means SE (n = 6). *p=0.05.

5. Distribution of nutrients: As seen in Figure 5A, B, and C, root magnesium, calcium, and iron contents appeared to be hardly affected by increasing F concentration. However, a highly significant decrease in the leaf content of Ca and Mg was observed (Figure 5A, B). Whereas the Fe content did not change significantly, decreasing leaf Fe content was detected with 5 and 10 mM NaF. By contrast, Mn content did not show any significant changes in the leaves, but it decreased significantly in the roots with 2.5 mM F (Figure 5C).

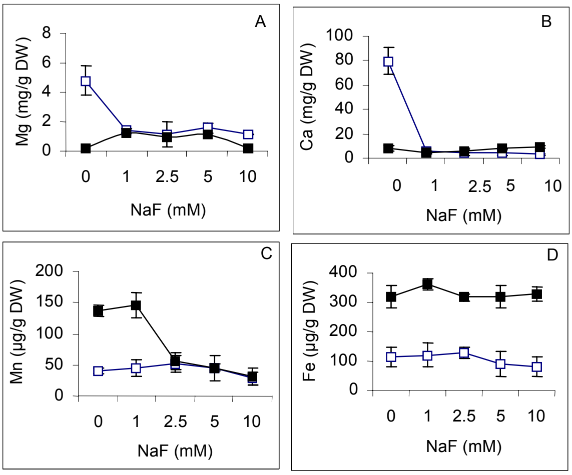

Figure 5. Effect of F treatment on macronutrients and micronutrients in leaves (open squares) and roots (closed squares) of almond seedlings.

DISCUSSION

This investigation demonstrated that F is taken up through the roots of almond seedlings. However, only 5 to 10% of the absorbed F seems to be transported to the leaves. Thus, F retention by the roots might be a mechanism for F tolerance operating in almond root cells. High F accumulation in roots compared to that found in leaves has been reported in previous studies.[15,16] Since formation of reducing sugars such as glucose, fructose, and mannose in leaves is thought to be inhibited by F, the tendency of plants exposed to F to decrease the concentrations of such sugars in their leaves indicates the possible conversion of these sugars to non-reducing sugars, such as sucrose and raffinose or sugar alcohols. Under these conditions, increased levels of non-reducing sugars in tissues might be a mechanism adopted by plants to reduce F toxicity.[17]

On the other hand, addition of F to the nutrient media also caused various changes in Mg^{2+}, Ca^{2+}, and Fe^{2+} levels in almond seedling leaves. Such changes in mineral content might be expected to induce secondary effects on plant metabolism.

However, in F-exposed almond seedlings, root Mg, Ca, and Fe contents seem to be unaffected. These results suggest that F may not interfere with the translocation of these nutrients to the leaves, although Mn uptake was depressed in the roots at 2.5 mM F.

Low Ca in the leaves of almond seedling grown in elevated F nutrient solution may be due either to the complexation of Ca^{2+} in the form of CaF_2 within the roots or to the lower Ca^{2+} activities in the nutrient solution arising from its precipitation with F. The mechanisms by which F is toxic are thought to involve inhibition of enzymes or interference with membrane permeability through F precipitation with Ca.[18] Changes in membrane permeability could overcome the barrier to F uptake in the root cortex, thereby increasing F concentration in plant to phytotoxic levels.

Leaf Mg content was also decreased significantly, suggesting that the uptake and upward translocation of Mg^{2+} ions could be disturbed by high levels of F. The toxic action of F toward Mg is also thought to involve the inactivation of Mg^{2+} at its sites of physiological activity.[19] Mg^{2+} is an important cofactor in almost all enzymes related to or involved in phosphorylating processes, and it is also required for ribulose-1,5-biphosphate carboxylase activation,[20] CO_2 assimilation, and production of carbohydrates. As expected, the marked decrease in leaf Mg concentration of almond seedlings treated with F also led to decreased photosynthesis as evidenced by the relatively low levels of sugars found in F-treated seedlings compared to the controls.

On the other hand, the decrease in Fe^{2+} content in F-treated almond seedlings might have other physiological implications, such as a decrease in ferridoxin necessary in the light-induced oxido-reduction process in photosynthesis leading to a reduction in chlorophyll content. Inhibition of photosynthesis is also expected to occur with lower Mg^{2+} and Fe^{2+} concentrations, and this could explain the relatively low levels of reducing sugars and starch in the leaves.

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