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Differential Response of *Azolla microphylla* Kaulf. and *Azolla filiculoides* Lam. to Sodium Fluoride

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We assessed the differential response of *Azolla microphylla* and *Azolla filiculoides* to fluoride stress by growing them in culture media containing 1 to 50 ppm sodium fluoride (NaF). *A. microphylla* had a higher total chlorophyll content than *A. filiculoides*. Both species showed gradual decreases in protein content as the concentration of NaF increased. *A. microphylla* accumulated less proline than *A. filiculoides* when more NaF was added to the culture medium. For all concentrations tested, the amount of residual fluoride in the nutrient media that supported *A. microphylla* was higher than in the nutrient media used for growing *A. filiculoides*.

Keywords: *Azolla filiculoides*, *Azolla microphylla*, sodium fluoride (NaF) stress

Fluoride is ubiquitous in the environment and is considered to be the most phytotoxic of the more common pollutants (Takmaz-Nisancioglu and Davison, 1988). Fluoride affects cellular metabolism in plants and can lead to reductions in yield (Khoshoo, 1988).

The agronomic potential of *Azolla* is quite significant, particularly for rice, where it may be applied as a biofertilizer for increasing yields (Kannaiyan et al., 1982). The nitrogen fixed by the *Azolla-Anabaena* symbiosis becomes a source of nitrogen in rice crops, and can contribute approximately 30 to 40 kg nitrogen/ha (Kannaiyan, 1985). Groundwater in several parts of Tamil Nadu in India contain toxic levels of fluoride, which makes the supply unfit for human consumption and irrigation. Because aquatic plants can accumulate salts and heavy metals, the physiology of these aquatic nitrogen-fixing systems in irrigation water can be altered (Abbas and Nipaney, 1985; Song et al., 1994).

We assessed the effects of NaF on chlorophyll, protein, and proline contents in *Azolla microphylla* and *Azolla filiculoides*. We also studied how manipulating the concentration of residual fluoride in the nutrient media could affect the growth of these two species.

MATERIALS AND METHODS

Sterilized, nitrogen-free growth media (IRR (–) NO_3) were prepared (Watanabe et al., 1977) in distilled water, and NaF was added to achieve concentrations of 1 ppm, 5 ppm, 10 ppm, and 50 ppm. *Azolla* inoculum was taken from the stock cultures maintained at the Botanical Garden of Thiagarajar College. Cultures of *A. microphylla* Kaulf. and *A. filiculoides* Lam. were cleaned thoroughly in distilled water, and the moisture was removed with filter paper. A 500-mg sample of a fresh *Azolla* culture was inoculated into a 250-mL conical flask containing 100 mL of IRRI (–) NO_3 medium, with or without (control) NaF. All flasks were closed with aluminum foil that was pricked with pinholes to facilitate aeration. The flasks were kept at 28°C ± 2°C, in partial sunlight.

Because the doubling time of control *Azolla* is approximately 15 days, under laboratory conditions, the experiments were terminated on the 15th day. Chlorophyll, protein, proline, and residual-fluoride contents were determined on that day. The experimental design was a randomized complete block with three replications.

Chlorophyll content was determined following the method of Wintermans and Demots (1965). Protein was extracted and estimated according to Lowry et al. (1951), and proline content was estimated by using the method of Bates et al. (1973).

The residual fluoride in the nutrient medium was estimated by following the method of Saxena (1987). A 50-mL sample of each nutrient medium, containing a different concentration of NaF, was taken from individual flasks that had been used for growing *Azolla*. Alizarin red solution (2.5 mL) and Zi conyl acid solution (2.5 mL) were added to each sample, and the

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solutions were allowed to stand for 1 h. The absorbance of each solution was read in a Spectronic-20 (Bausch & Lomb, USA) and the residual fluoride was determined.

RESULT AND DISCUSSION

Chlorophyll

Both *A. microphylla* and *A. filiculoides* showed proportional decreases in total chlorophyll content as the concentration of NaF was increased. *A. microphylla* retained 38% of its chlorophyll at 50 ppm NaF; *A. filiculoides* retained only 34% of its initial chlorophyll at the same concentration (Fig. 1). This result agrees with that of Ivinskis and Murray (1984), who reported that Chl-a and Chl-b concentrations and photosynthetic capacity were decreased in the presence of higher concentrations of fluoride in *Eucalyptus*. Because chlorophyll is considered one of the bioindicators of biochemical stress (Godbold et al., 1993), we infer that *A. microphylla* showed a more positive response or tolerance to fluoride stress because it had retained more of its initial chlorophyll content.

Protein

When the concentration of NaF was increased in the nutrient media, a gradual decrease in protein content was found in both species of *Azolla*. At a level of 50 ppm NaF, *A. filiculoides* retained only 69% of its initial protein content (Fig. 2). The decrease in protein content at higher concentrations of NaF may have been caused by the decline in the overall rates of protein synthesis during fluoride stress.

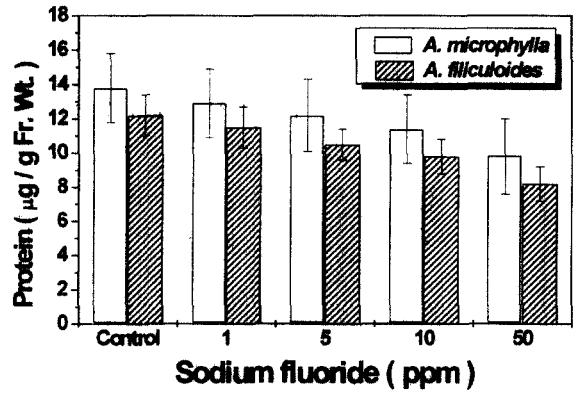

Figure 2. Effect of sodium fluoride on protein content of *A. microphylla* and *A. filiculoides*.

Chang (1970) stated that fluoride decreased the number of ribosomes and destroyed the structure of ribosomal proteins, which negatively affected the entire protein synthesis. We can reach the same inference with our results.

Proline

Proline accumulated gradually in *A. microphylla* in response to the increase in NaF concentration in the nutrient medium. In *A. filiculoides*, the proline accumulation was much more pronounced, but the rate of accumulation was significantly more at a concentration of 50 ppm than at 5- or 10-ppm concentrations (Fig. 3). *A. filiculoides* had 33.3% more proline than did *A. microphylla*, and was probably more sensitive to fluoride stress.

The higher proline accumulation in *A. filiculoides*, in response to NaF stress, may be reflected in the poor growth of this species (results not shown).

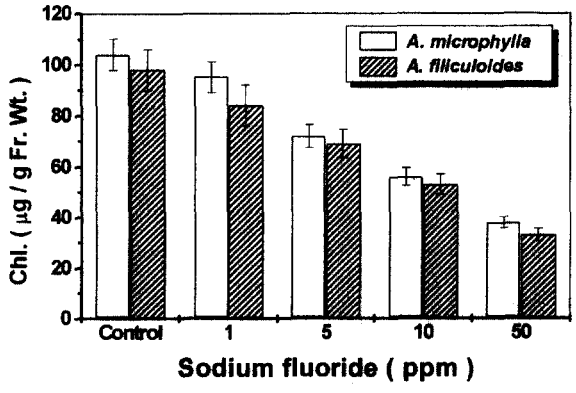

Figure 1. Effect of sodium fluoride on chlorophyll content of *A. microphylla* and *A. filiculoides*.

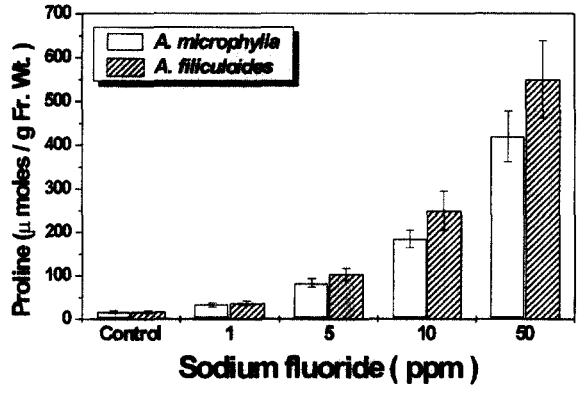

Figure 3. Effect of sodium fluoride on proline content in *A. microphylla* and *A. filiculoides*.

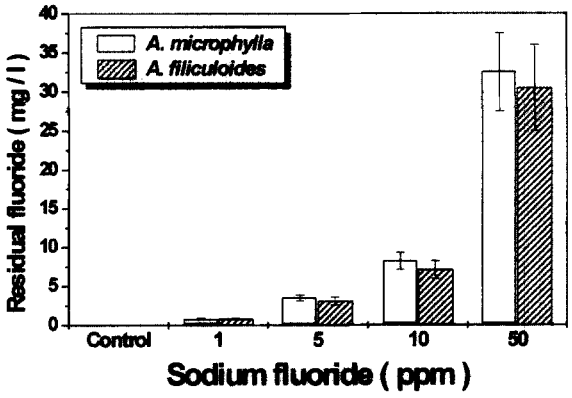

Figure 4. Effect of sodium fluoride on the uptake of residual fluoride by *A. microphylla* and *A. filiculoides*.

Greenway and Munns (1980) supported this observation by suggesting that most plants accumulate proline in substantial concentrations only when growth is severely reduced. The role of proline appears to be related to survival rather than to maintenance of growth. Similar results were reported by Hanson et al. (1977).

Residual Fluoride

In the nutrient media supporting *A. microphylla*, the level of residual fluoride was higher in all the concentrations, which indicates that fluoride uptake by *A. microphylla* was comparatively less than by *A. filiculoides*. At 50 ppm NaF, the solution supporting *A. microphylla* had 61% more residual fluoride than did the solution that supported *A. filiculoides*. Therefore, *A. microphylla* and *A. filiculoides* differed in their ability to absorb fluoride from the culture medium (Fig. 4).

Studies on the effect of absorption and accumulation of fluoride in plants exposed to HF emission (Satoh et al., 1970) demonstrate that accumulated fluorine can negatively influence photosynthesis and growth. Thus, the better performance of *A. microphylla*, under the same amount of fluoride stress as experienced by *A. filiculoides*, may be correlated with its lower rate of absorption of fluoride and a consequently lower level of toxicity. Our results strengthen the view that a species sensitivity to stress is related to the rate of absorption of the particular stress factor.

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