Alleviation of Copper Toxicity in *Arabidopsis thaliana* by Silicon Addition to Hydroponic Solutions

Copper (Cu) is an essential micronutrient for plants and is the active ingredient in pesticides for some pathogens and algae. Though it is an essential element, elevated doses of Cu can cause toxicity in plants. Toxicity effects of some heavy metals have been shown to be alleviated by the presence of elevated silicon (Si), but the role of Si in reducing symptoms induced by excess Cu is unclear. The objective of this study was to evaluate the effects of Si on Cu detoxification using the model plant, arabidopis by a variety of parameters ranging from morphologic responses to changes in gene expression.

Arabidopsis were grown in four treatments: control, elevated Si, elevated Cu, and elevated Cu and Si. Plants grown under elevated Si conditions appeared healthy and had tissue nutrient levels comparable (except Si) to control plants (Figure 1). Plants grown in elevated Cu showed reduced growth and chlorosis starting at the tips of the older leaves. Elevated Si treatment alleviated the reduction of shoot and root fresh weight and reduced leaf chlorosis caused by Cu toxicity. In control plants, roots appeared whiteish-yellow, while roots of plants grown under elevated Cu were dark brown, indicating the presence of phenolics or metal oxides on the root surface. Roots grown in elevated Cu with Si appeared lighter brown and more vigorous, indicating Si alleviated Cu stress in roots.

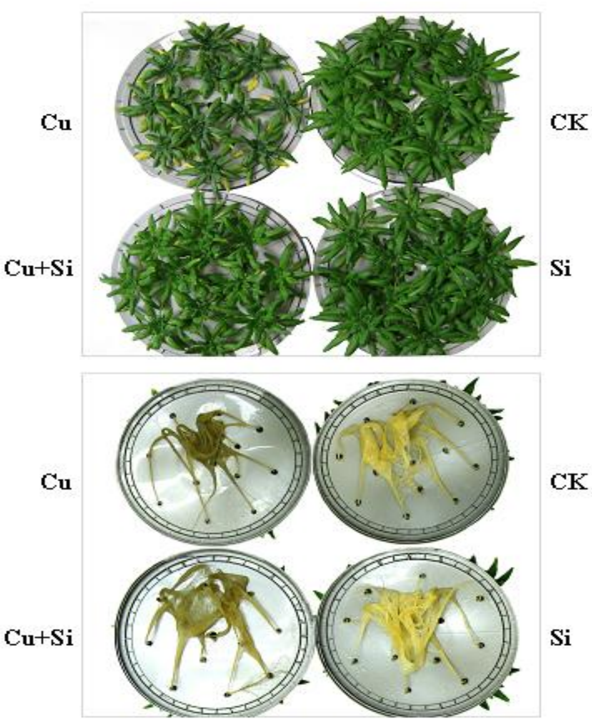

Figure 1. Responses of arabidopsis shoots and roots to elevated Si and Cu. Arabidopsis plants were treated with Si and Cu and analyzed after 3 d. The treatments were: control [CK (0.12 µM Cu + 0.10mM Si)], elevated Si [Si (0.12 µM Cu + 1.5 mM Si)], elevated Cu [Cu (30 µM Cu + 0.10 mM Si)], and elevated Cu and Si [Cu + Si (30 µM Cu + 1.5 mM Si)].

A reduction of phenylalanine ammonia lyase (PAL, a stress-induced enzyme) activity in the shoot occurred in the treatment of elevated Cu and supplemental Si. Real-time reverse transcriptase-polymerase chain reaction analyses indicated that RNA levels of two arabidopsis copper transporter genes, *copper transporter 1 (COPT1)* and *heavy metal ATPase subunit 5 (HMA5)* were induced by high levels of Cu, but were significantly decreased when Si levels were elevated (Figure 2).

Our findings indicate that Si addition can improve the resistance of arabidopsis to Cu stress on multiple levels, from physiological changes to alterations of gene expression.

THE UNIVERSITY OF TOLEDO 1872

For more information, contact: Scott Leisner, sleisne@utnet.utoledo.edu, University of Toledo, Department of Biological Sciences, 2801 W. Bancroft St., Toledo, OH 43606

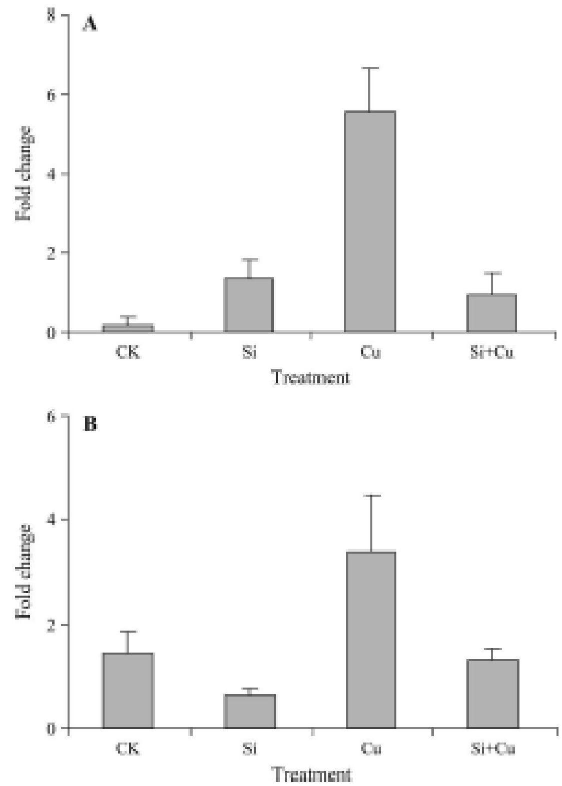

Figure 2. Expression of copper transporter 1 (COPT1; A) and heavy metal ATPase5 (HMA5; B) genes in arabidopsis roots after treatment for 3 d with various combinations of Si and Cu under hydroponic conditions. The treatments were: control [CK (0.12 µM Cu + 0.10mM Si)], elevated Si [Si (0.12 µM Cu + 1.5mM Si)], elevated Cu [Cu (30 µM Cu + 0.10 mM Si)], and elevated Si and Cu [Si +Cu (1.5 mM Si + 30 µM Cu)]. Actin was used to correct for variations in RNA levels. Averages are shown with error bars indicating SD (n = 12).

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