Stress-Induced Silicon Accumulation: Are "Low"-Accumulators Really "Inducible"-Accumulators?

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Abstract

Silicon (Si) is an abundant element in the lithosphere and is implicated in the tolerance to abiotic as well as biotic stresses across multiple angiosperm taxa. Plants have classically been characterized based on Si acquisition under favorable environmental conditions into three categories: high-, intermediate-, or low- accumulators. However, our recent data show induced accumulation of the element in the "low accumulators" *Nicotiana tabacum* (tobacco) and *Antirrhinum majus* (snapdragon) in response to stress. Tobacco plants inoculated with *Tobacco ringspot virus* (TRSV) and supplemented with Si exhibited significantly higher foliar Si content when compared to control Si-treated/mock-inoculated plants. The Si-supplemented plants also showed lower TRSV systemic symptom formation. In contrast to TRSV, *Tobacco mosaic virus* infection was also tested in *N. tabacum*, and resulted in no significant changes in foliar Si content or symptoms, indicating that the inducibility is stress-specific. Si-induced accumulation is not restricted to biotic stress. Snapdragon plants treated with high levels of copper (Cu) showed significantly higher foliar Si concentrations compared to control plants. In contrast, the "high" Si accumulator, *Zinnia elegans*, treated under similar conditions did not show a significant increase in Si content of leaves, suggesting the Cu-induced Si accumulation may be limited to "low" or what we describe as "inducible" accumulators. We are currently investigating other types of abiotic stress-induced Si accumulation.

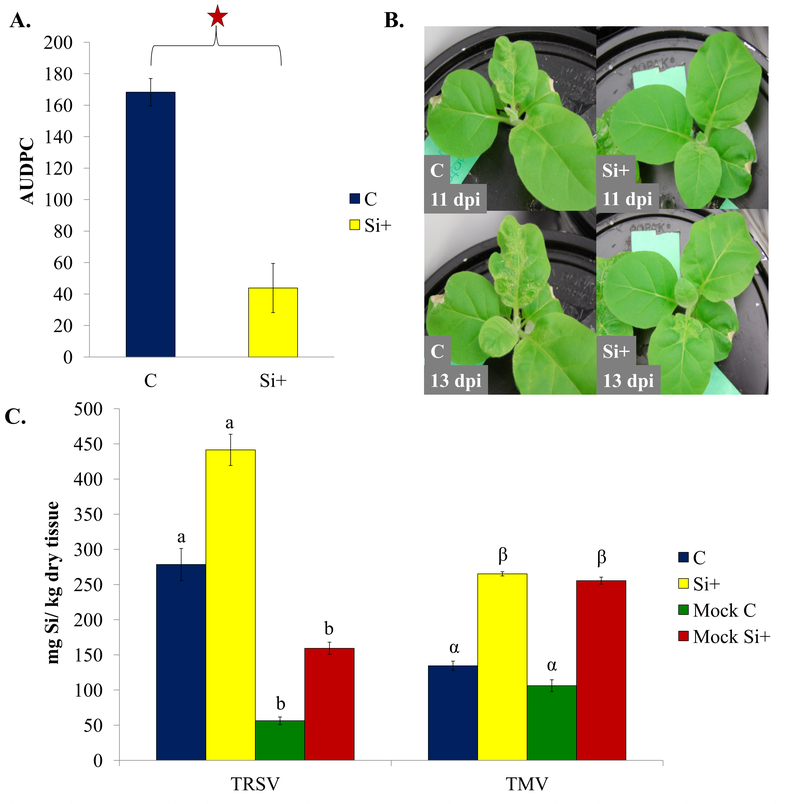

Figure 1: Si reduces TRSV infection in *N. tabacum* and TRSV infection induces foliar Si accumulation. (A) TRSV symptoms were reduced over a 15 day period up to 25 days post inoculation. Graph shows area under the disease progression curve (AUDPC) for control (blue; 0.1 mM Si) and Si supplemented (yellow; Si+; 1.0 mM Si) tobaccos. Star in B indicates a pValue =0.013. (B) TRSV symptoms at 11 and 13 days post inoculation (dpi) on tobaccos grown with control (C; 0.1 mM) or elevated (Si+; 1.0 mM) Si. (C) Foliar Si levels, as determined by ICP-OES, were increased in TRSV infected plants supplemented with Si (Si+; yellow bar) compared to non-infected tobaccos supplemented with the element (Mock Si; red bar), but not for plants infected with TMV. Plants inoculated with TRSV grown under control (0.1 mM) Si levels (C; dark blue bar) show a significant difference from non-inoculated control plants (Mock C; green bar). Foliar Si levels are the same for plants inoculated with TMV and grown under control Si levels (C; dark blue bar) or non-inoculated plants (Mock C; green bar). Different letters represent significant differences based on Tukey's HSD with pValue < 0.05.

Introduction

The literature contains numerous articles highlighting the beneficial effects of Si treatment on various abiotic and biotic stressors in plants (1). For silicon to be beneficial, it must be acquired by the plant. Silicon acquisition in higher plants varies greatly (1). Since the early 1900s, plants have been defined as high-, intermediate-, or low-accumulators based on Si content in leaves that were > 1%; 1-0.1% or <0.1% Si/dry weight, respectively (2,3). However, the determination was based on plants grown under optimal environmental conditions, even though many effects of Si are not observed until the plants encounter some type of stress (4). Under these classifications, tobacco is currently considered a low-accumulator.

In general, Si supplementation benefits plants by enhancing resistance to pathogens (5). However, the literature describing the effects of the element on virus infection indicates that Si can also cause detrimental effects. *Belladonna mottle virus* (BeMoV) infection in *Nicotiana tabacum* is enhanced by Si supplementation (6). Other than this one report, data reporting the effects of Si on viral infections is lacking.

The nepovirus *Tobacco Ringspot Virus* (TRSV) and the tobamovirus *Tobacco mosaic virus* (TMV) are single-stranded RNA viruses that infect a wide variety of food and ornamental crops (7). As such, they are good models for understanding virus-plant interactions. However these two viruses are only distantly related to each other. During pathogen challenge, plants produce a variety of signaling molecules modulating plant defenses (8). Hormones, such as abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA), have been implicated in a number of stress responses in plants.

In addition to aiding plants against biotic stress, Si can also help plants to better deal with abiotic stress. Cu is an essential microelement that has the ability to accumulate in many plants, leading to toxicity (1). Si supplementation reduces the toxicity of elevated copper in *Arabidopsis thaliana* (9).

While most of the work done on Si in plants has focused on the beneficial effects of the element on stress, little mention is made of the effects of stress on Si accumulation. Here we examined Si accumulation in *N. tabacum* challenged with abiotic and biotic stresses. We also tested the effects of hormone treatment on Si accumulation in plants.

Material and Methods

N. tabacum plants were grown hydroponically as previously described with *A. thaliana* (9). Once plants reached the 4 leaf stage, three plants were transplanted to 4 L buckets and nutrient solution was changed every 5-7 days. At least three buckets were used per treatment per experiment. Si concentrations for the viral studies were 0.1 mM for control (C) and 1.0 mM for excess Si (Si+). Viral symptoms were documented with a digital SLR camera and the symptomatic leaf area was calculated using Assess software (American Phytopathological Society; APS). The Area Under the Disease Progression Curve (AUDPC) was then calculated using the R program (10). Leaf tissue was harvested after 25 dpi, dried and analyzed for Si content via Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES).

For the Cu and hormone studies, plants were germinated in the absence of Si and prior to treatment, Si concentrations were increased to 2 mM. In the Cu studies for *Antirrhinum majus*, plants were treated with 0.12 µM (for control) or 150 µM (Cu+) Cu, while Si concentrations were 3.4 µM. *N. tabacum* were treated with 0.12 µM (control) or 75 µM (Cu+) Cu in addition to 2 mM Si. Cu-treated plants were exposed to the element for three weeks. Following treatment, tissue was collected, dried and analyzed for total Si by ICP-OES. For the hormone studies, *N. tabacum* were sprayed every two days with either 25 µM ABA, 50 µM SA, or H_2O to the point of run-off. For the volatile substances, enclosed buckets were treated with 50 µM MeJA or 95% EtOH (control) placed in culture tube lids and replenished every two days. Treatment occurred for 21 days, after which leaves were harvested, dried and analyzed through ICP-OES for total foliar Si.

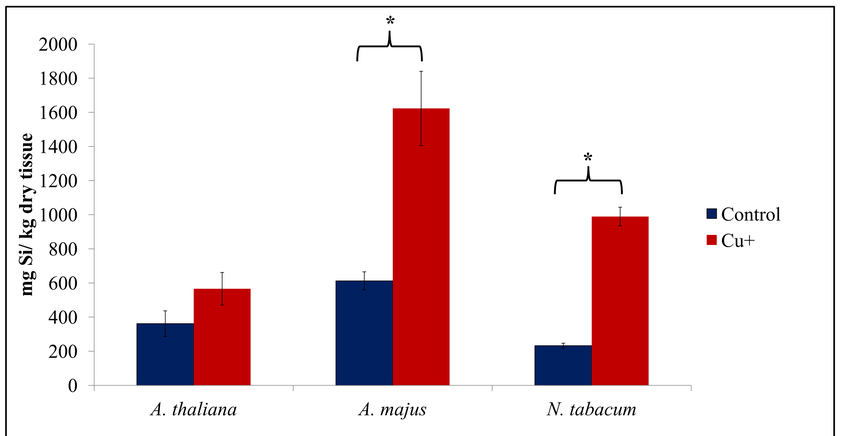

Figure 2: Cu toxicity-induced Si foliar accumulation in *A. thaliana*, *A. majus* and *N. tabacum*. Plants were treated with control (blue bar) or excess Cu (Cu+, red bar) leaves were harvested, and analyzed for total Si. Shown are the average Si concentrations ± SEM; a * indicates a significant difference with a pValue < 0.01.

Results:

To determine if Si could influence virus infection, *N. tabacum* were inoculated with TRSV, TMV, or mock-inoculated. Hydroponic solution Si concentrations were either maintained at 0.1 mM (C; control) or increased to 1.0 mM Si (Si+). The AUDPC was significantly reduced in TRSV/Si+ plants compared to TRSV/C treated plants **(Fig. 1A)**, indicating that the element delayed viral systemic symptom formation **(Fig. 1B).** More importantly, TRSV-infected plants showed significantly higher foliar Si levels than mock-inoculated plants both in cases where the plants were provided with supplemental Si and under control conditions (**Fig. 1C**). In contrast, supplemental Si did not protect plants against TMV infection nor did the virus affect Si content of tobacco leaves (**Fig. 1A**). This suggests that in tobacco, a specific pathogen induced Si accumulation in leaves. We termed this pathogen-induced Si uptake-SISA for Stress-Induced Si Accumulation.

SISA was also activated by an abiotic stress. *N. tabacum*, plants treated with 75 µM Cu and supplemented with Si contained significantly increased Si concentrations in leaves compared to control plants **(Fig. 2)**. A re-examination of some of our previous work showed that *A. majus* treated with elevated levels of Cu and Si showed significantly higher foliar Si concentrations than plants treated with elevated Si alone (11). In the intermediate Si accumulator *A. thaliana,* plants treated with high Cu and Si showed a slight but statistically insignificant increase in above ground Si tissue levels compared to control Si treated plants (9). In contrast, our prior study showed that the high-accumulator, *Z. elegans*, exhibited nosignificant change in foliar Si levels in response to Cu toxicity (11; data not shown). This suggests that SISA is involved in both biotic and abiotic stress response in at least two "low" or what we now refer to as "inducible"-accumulators.

Since a signal component is likely involved in SISA, we tested a number of hormones involved in stress responses for their ability to induce Si accumulation in *N. tabacum* leaves. Plants were grown hydroponically in growth chambers under no Si. Prior to treatment, Si concentrations were increased to 2 mM. Plants were sprayed every two days with either ABA, SA or water (Control). A second set of plants was exposed to either methyl jasmonate (MeJA) or EtOH (MeJA carrier). Following 21 days of treatment, leaves were collected, harvested and analyzed for total Si via ICP-OES. ABA significantly reduced Si content in tobacco leaves compared to water-sprayed controls **(Fig. 3A)**. SA showed a slight, but statistically insignificant inhibition in Si accumulation. MeJA had no significant effect on Si accumulation in the plants **(Fig. 3B)**.

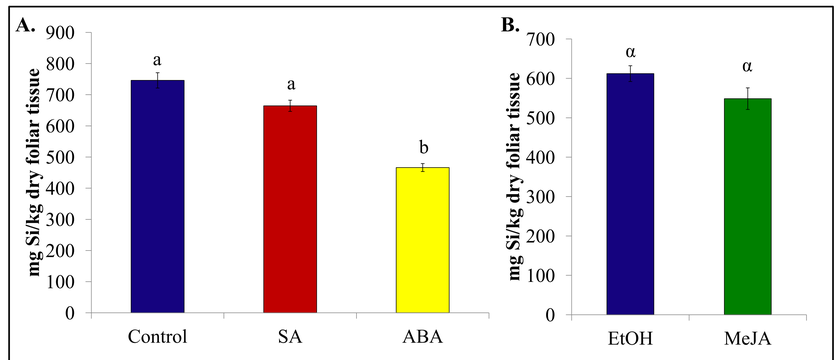

Figure 3: ABA reduces Si levels in *N. tabacum*. Plants were sprayed with ABA, SA or H_2O (Control) (A) or exposed to MeJA or EtOH (B) every two days for three weeks. Leaves were then harvested and total Si determined using ICP-OES. Shown are the average Si concentrations ± SEM. Different letters indicate a significant difference based on Tukey's HSD with a pValue < 0.01.

Conclusions

Si has the ability to protect a number of plants against biotic as well as abiotic stresses and the protective property is related to the ability of the plant to take up the element. Earlier classifications of plants as high- intermediate- or low-accumulators can be misleading. The studies presented here support the need to include a fourth category, "inducible"-accumulators to the above list of Si nomenclature. This is especially true because the data presented supports the idea that stress can change a plant from one classification to another. In our studies, SISA permitted previously defined "low"-accumulator species to acquire foliar Si to levels where the plants would be classified as intermediate-accumulators. Cu toxicity treatment of *N. tabacum* results in a consistent increase in foliar Si levels and is a versatile system to study the effects Si might have at a molecular level in response to various stressors. The viral studies suggest that SISA is only induced under certain stresses, since TMV infection did not alter Si content in the leaves. However, whether abiotic and biotic stresses induce foliar Si accumulation by the same pathway is currently unknown.

Since Si was applied to the roots of tobaccos, but acquired by leaves, the plants likely utilize a signaling pathway to regulate the process. We hypothesized that the pathway likely utilized plant growth regulators, so we tested the three most likely candidates based on their role in virus resistance. ABA significantly inhibited Si accumulation in leaves. These data are consistent with earlier work showing a similar reduction of foliar Si levels in rice plants treated with ABA (12). Taken together, these results suggest that the accumulation of Si in some species is low until plants are challenged with an appropriate stress, that then induces accumulation of the element.

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Acknowledgments

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