Methods for Analyzing Silica Induced Effects:

Trichome Structure and Leaf Desiccation Rate

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Abstract

Two techniques were tested to determine if they would be suitable for detecting differences between plants grown with or without silica. Soybean (*Glycine max*) and wheat (*Triticum aestivum*) leaves were inspected using microscopes to look for structural differences on trichomes and leaf surfaces. Wheat leaves were weighed over time to determine the leaf desiccation rate, which was then compared across silica and salt treatments. The microscope observations showed that silica enhanced soybean plants have much more pronounced trichomes than control plants. Wheat leaves also showed slight differences in visible leaf structure. The observed leaf desiccation rates suggest that wheat grown with silica has enhanced salt tolerance.

Introduction

In the 1887 J. Sachs, one of the pioneers of mineral nutrition claimed that while silica made up a significant portion of the ash of a plant, it did not appear to be necessary for the complete development of plants (Epstein, 2005). This is the view that has prevailed in plant science since that day, and silica is still excluded from most essential elements lists.

Epstein discusses many ways that silica can be beneficial by providing protection from both biotic and abiotic stresses. He also notes that it is much easier to see a difference between plants grown with silica and those that aren’t when there is a stressor involved (Epstein, 2009). One of the issues facing scientists is that many studies are performed in greenhouses, which reduce or even eliminate entirely the stresses that silica may protect against. However, despite the difficulty of measuring differences, there has been an explosion in the research done on silica over the past fifteen years.

Several studies suggest that silica can help the overall health of stressed plants. In 1998, Agarie et al. found that polysaccharides in cell walls were 1.6 times higher in plants grown with silica. They also found that electrolyte leakage was lower in plants grown with silica, which they attributed to the increase in the strength of the cell wall. (Agarie, Hanaoka, Miyazaki, Kubota, Agata, & Kaufman, 1998). Gong et al. showed that wheat grown with silica had much higher amounts of antioxidant enzymes than control plants did, and that added silica increased the CO_2 assimilation rates of wheat (Gong, Zhu, Chen, Wang, & Zhang, 2005).

One of the most critical subjects studied in silica research is increased drought tolerance. Hattori et al. found that silica increased the ability of sorghum to extract water from dry soil, while maintaining stomatal conductance. They also found that while silica led to an increase of dry mass in drought conditions, it did not increase yield in wet conditions (Hattori, et al., 2005), and there are several other studies that suggest the same results.

Finally, there is increasing evidence that plants actively uptake silica, which further bolsters the argument that plants need this mineral. Mitani and Ma suggest that silica actively taken up by rice, cucumber and tomato, though at significantly different rates. They also showed that the uptake process is energy-dependent (Mitani & Ma, 2005), which suggests that plants are willing to spend a good deal of their energy to get the silica.

To help researchers quickly see differences between control and treatment plants, it is imperative to introduce novel techniques are cheap and easy to run. This study will examine two techniques for suitability in silica research.

Objective

The purpose of this study is to do develop and test new techniques for analyzing the effect of silica on plant growth and health.

Hypotheses

Trichome Structure

There will be visible differences on the leaves of plants that received silica treatments and those that did not.

Leaf Desiccation Rate:

That leaves from plants that received silica treatment will dry out slower than those that did not.

Materials and Methods

The plant tissue tested for both experiments came from two longer-term studies: a soybean study that was grown in peat, and a wheat study that was grown in a hydroponic solution.

Soybeans:

Sixteen five-gallon buckets were filled 21.2 L of peat on 7/31/12. Half of the buckets had 127.2 g Plantuff (Levy Corp.) mixed with the peat, and the other half had 63.6 g dolomitic limestone mixed into the peat. All the buckets had 82 g of Polyon 16-6-13 with a 1-2 month release profile mixed with the peat as well. The buckets were labeled 1-16, with the silica treated buckets being assigned to the even numbers, and the dolomite treated buckets being assigned to the odd numbers. On 8/18/12 about 3-4 cm of perlite was added to the top of each bucket to reduce evaporative loss.

Soybean seeds (*Glycine max* 'Hoyt') were planted into the peat, and were grown under greenhouse conditions with 25°C days and 20°C nights. The buckets were watered automatically using a drip system throughout the study, with the different rows getting water at different times to simulate drought stress.


Figure 1. Soybeans growing in individual buckets.

Wheat:

Six 40cm wide by 56cm long by 24cm deep plastic tubs were divided into two groups of three each. To cover the tops of the tubs, a foam board was cut to the size of each tub, and fifteen 1.5" holes were drilled into each board to hold the plants. The tubs were placed into a greenhouse where they received 25̊ C day and 20̊ C nights.

A standard monocot hydroponic stock solution was created, using 1mM of $Ca(NO_3)_2$, 4mM $K(NO_3)$, 0.05 mM KH_2PO_4, 0.5mM $MgSO_4$, 20µM $FeCl_3$, 10µM Fe-HEEDTA, 3µM $MnCl_2$, 6µM $ZnCl_2$ 2µM H_3BO_3, 3µM $CuCl_2$, and 0.1µM Na_2MoO_4.

The six tubs were divided into two groups. One group received a salt treatment, and the other received no salt. Each tub in the both groups had a different silica treatment level. The first tub had no silica added; the second tub was maintained at a level of 0.4 mM silica; the third tub was maintained at a level of 0.8 mM silica.

Initially, all six tubs were filled with 45 L of the stock solution. Every 2-3 days throughout the study this solution was used to refill any transpired or evaporated water for the first half of the study. Midway through the study, a separate stock solution was created for each silica concentration. These solutions had 0.0, 0.4, and 0.4 mM Si added to them, but were otherwise identical to the original stock solution.

Finally, an aeration system was installed into each tub to allow for constant oxygen supply to the roots. This system was comprised of PVC pipes that had small holes drilled to allow the air to escape. The pipes were attached to the greenhouse air supply, and air was pumped into the pipe and allowed to bubble into the hydroponic solution.

Several hundred wheat seeds (*Triticum aestivum*, 'USU-Apogee') were germinated in wet paper towels on 9/10/12. Once the cotyledons emerged, and sufficient roots had developed to reach the hydroponic solution, the most robust of the seedlings were transplanted into the hydroponics solution on 9/18/12. One plant was transplanted into each hole, and was secured using a foam plug. A few days later, the transplants were evaluated, and those that did not survive were replaced by other seedlings that had been saved for that purpose. These seedlings were then randomized between the different tubs before the treatments began.

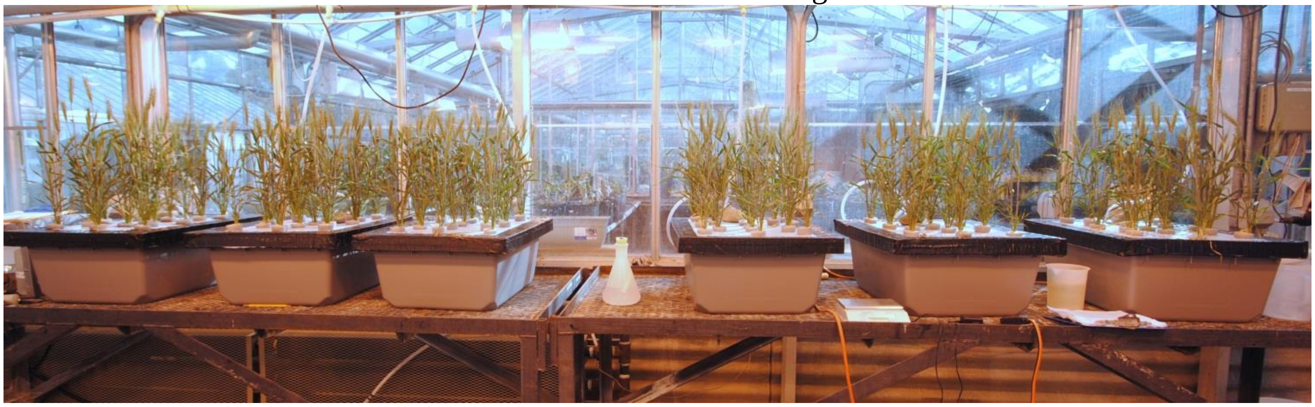

Figure 2. Wheat grown in the hydroponics study.

Beginning on 9/22/12, each tub was treated with the prescribed amount of silica. The silica levels were measured every week using the Lamotte Low Range Silica Colorimetric test, and silica was added to replace what the plants took up.

Starting 10/10/12, the first three tubs had a NaCl solution added gradually over a period of eight days until the tub solution reached a EC reading of 15 mS/Cm^2 , while the other three tubs were given no salt.

Trichome Structure

For the soybeans, leaves were taken from the 2nd and 3rd buckets. The leaves were then visually examined using a Lomo МБС-10 dissection microscope at a magnification of 56x. Pictures were then taken using a LG VM670 camera phone handheld to the microscope eyepiece.

For the wheat, leaves were taken from the 0.0 and 0.8 mM Si salt treatment. These leaves were visually inspected using the Lomo МБС-10 dissection microscope at a magnification of 56x. Pictures were taken using a Nikon E4500 camera handheld to the microscope eyepiece. Leaves were taken from the 0.0, 0.4, and 0.8 mM Si without salt treatment. These leaves were visually inspected using a Leitz Laborlux 12 POL microscope using 40x magnification. Pictures were taken using a Nikon E4500 camera through the camera phototube.

Leaf Desiccation Rate

For the leaf desiccation rate experiment, an Ohous Explorer scale with a precision of .01g was used to weigh the leaves over time. A few trial runs were conducted using soybean leaves, but the plants went into decline before the main experiment could be conducted, so wheat was used instead. A few trial runs of wheat leaves were also run to determine the minimum amount of leaf material that would provide reliable results.

One salt treatment was chosen for each cycle of the experiment. Four wheat leaves were taken at random from each tub, for a total of twelve leaves. Because of the physical distance between the greenhouse and the scale, the initial weight was taken at sixty seconds. Leaf weights were then taken every minute up to the five minute mark, and following readings were taken at the ten, twenty, and thirty minute marks. Finally, the leaves were dried to determine the initial water fraction.

The experiment was run six times between the dates of 11/12/12 and 11/29/12; three times each for the salt and no salt treatments, with a total of 144 total individual measurements. The data was analyzed using a Repeated Measures design in SAS 9.3.

Results

Trichome Structure

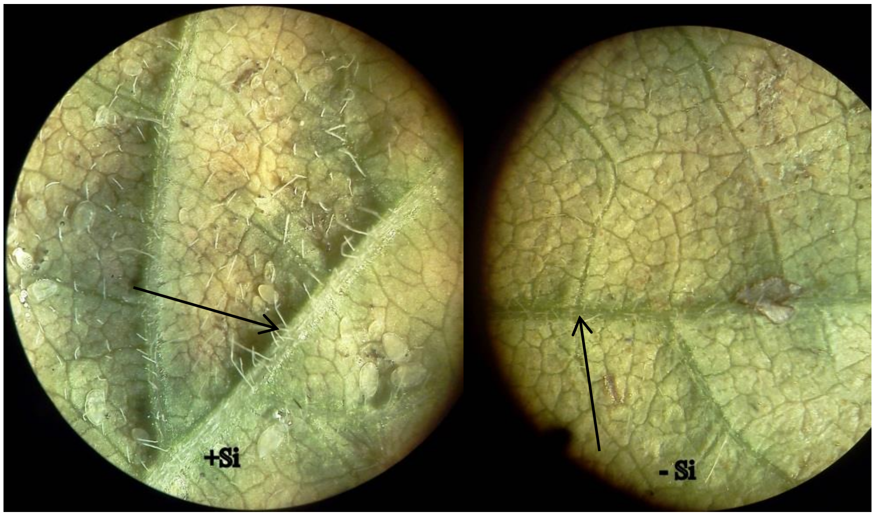

Figure 3. Underside of soybean leaves under microscope. With Silica and without Silica

The soybean leaves showed significant differences in the trichome development between the silica treatment and the control leaves. The most visible differences were noted on the underside veins of the leaves, as shown in Figure 1. The veins of the silica treated leaf have much more pronounced hairs than the veins of the control leaf.

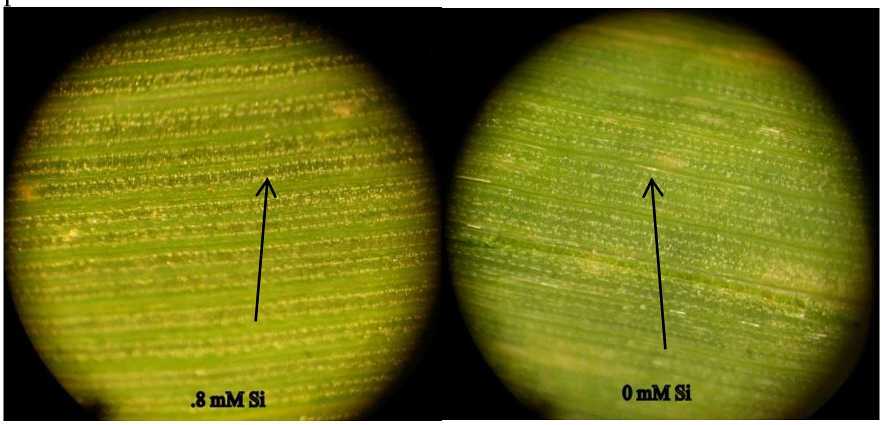

Figure 4. Wheat leaves under microscope. 0.8 mM Si and 0.0 mM Si. with Salt.

Likewise, the wheat leaves also showed a significant treatment difference. While not as pronounced as the effect on the soybean leaves, the silica treated leaves do have larger structures on the leaf veins than the control leaves do.

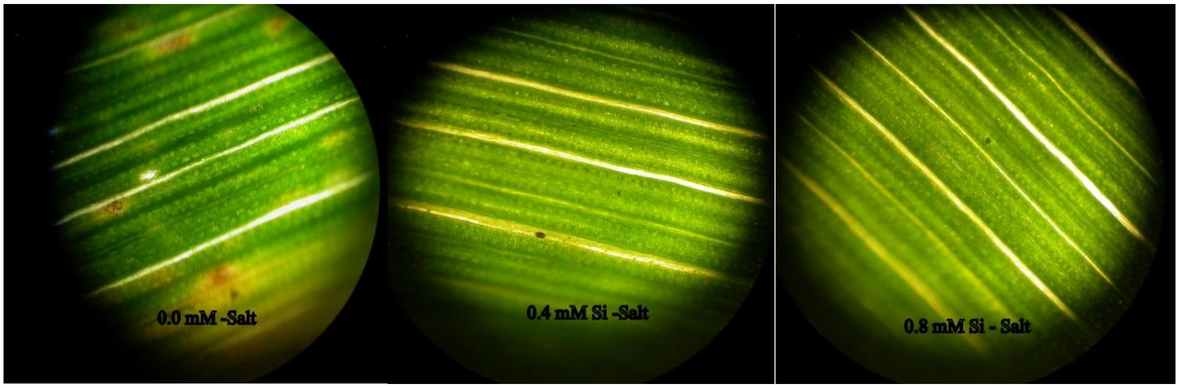

Figure 5 Wheat leaves under microscope. 0.0 mM Si, 0.4 mM Si, and 0.8 mM Si without Salt.

Because it is a slide microscope, and the depth of field is so narrow, the Leitz microscope proved to be unsuitable for the task of showing silica differences. There were no major differences noted between treatments using this microscope.

Leaf Desiccation Rate

No Salt											
Rep 1				Rep 2				Rep 3			
Time	0.8	0.4	0	Time	0.8	0.4	0	Time	0.8	0.4	0
60	1.00	1.00	1.00	60	1.00	1.00	1.00	60	1.00	1.00	1.00
120	0.98	0.99	0.98	120	0.96	0.95	0.98	120	1.00	0.98	0.96
180	0.95	0.96	0.93	180	0.92	0.93	0.95	180	0.97	0.96	0.96
240	0.93	0.96	0.93	240	0.94	0.95	0.98	240	0.94	0.96	0.96
300	0.91	0.94	0.93	300	0.94	0.95	0.98	300	0.94	0.94	0.92
600	0.84	0.90	0.85	600	0.85	0.90	0.90	600	0.91	0.92	0.79
1200	0.77	0.86	0.80	1200	0.77	0.83	0.83	1200	0.82	0.86	0.75
1800	0.72	0.81	0.76	1800	0.69	0.80	0.71	1800	0.76	0.80	0.67
Salt											
Rep 1				Rep 2				Rep 3			
Time	0.8	0.4	0	Time	0.8	0.4	0	Time	0.8	0.4	0
60	1.00	1.00	1.00	60	1.00	1.00	1.00	60	1.00	1.00	1.00
120	1.00	1.00	1.00	120	0.97	0.98	1.00	120	0.98	0.98	1.00
180	0.98	0.98	0.98	180	0.94	0.98	0.98	180	0.96	0.98	1.00
240	0.98	0.98	0.98	240	0.94	0.95	0.97	240	0.94	0.98	1.00
300	0.98	0.98	0.98	300	0.94	0.93	0.95	300	0.94	0.98	0.98
600	0.95	0.98	0.98	600	0.81	0.91	0.93	600	0.92	0.93	0.96
1200	0.90	0.93	0.95	1200	0.83	0.86	0.90	1200	0.85	0.85	0.95
1800	0.85	0.93	0.93	1800	0.81	0.81	0.88	1800	0.79	0.83	0.91

Table 1. Leaf water fraction over time.

The data from Table 1 shows that the leaf desiccation rate for the six replicates**.**

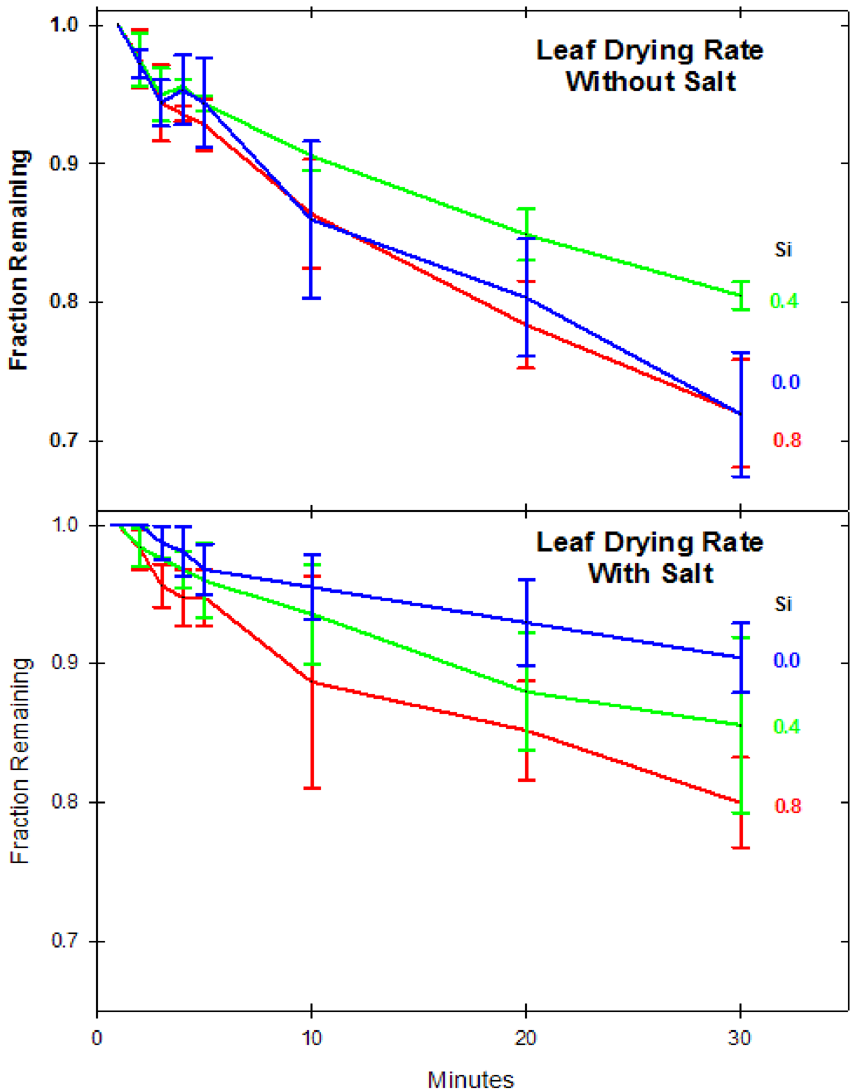

Figure 6. Leaf Drying Weight over Time.

Figure 6 is a graph of the average water fraction left for the different treatments. It shows some of the more subtle trends that showed up during the study. The most notable deviation is the fact that the 0.4 mM Silica without salt transpired much slower than the other treatments. Another observation worth making is that the trend in transpiration rates of the plants that got the salt treatment was opposite of what was predicted in the hypothesis. The more silica a plant got, the faster it lost water.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Salt	1	12	21.12	0.0006
Silica	2	12	2.38	0.1348
Salt*Silica	2	12	2.89	0.0946

Table 2. Statistical Analysis of Results

Table 2 shows that the difference between the silica treatments was not significant, nor was the overall interaction between the salt and the silica, so a more careful look at the least square means table is warranted.

Differences of Least Squares Means											
Effect	**Salt**	**Silica**	**Salt**	**Silica**	**Estimate**	**Std Err**	**DF**	**t Value**	**Pr > |t|**	**Adjustment**	**Adj P**
Salt*Silica	No	0	Yes	0	0.075	0.016	12	-4.60	0.0006	Tukey	0.006
Salt*Silica	No	0.4	Yes	0.4	0.024	0.016	12	-1.46	0.1702	Tukey	0.694
Salt*Silica	No	0.8	Yes	0.8	0.031	0.016	12	-1.90	0.0814	Tukey	0.445

Table 3. Least Square Means Table

The least square means table (Table 3), illuminates some of the subtle interactions between the salt and the silica treatments. The wheat plants that had no silica added transpired much more rapidly when they were grown without salt. This was not the case with the silica treated plants. While they did transpire more quickly when grown without salt, the difference was much smaller, and was not statistically significant.

Discussion

Trichome Structure

The microscope study provided fairly straightforward results. The differences between the two silica treatments for the soybean leaves was easy to see under the right conditions, and quite dramatic. The wheat leaves proved harder to analyze because the trichome structures are smaller and closer to the leaf, but they also showed a significant effect across the different silica treatments.

Leaf Desiccation Rate

The desiccation rate study showed some surprising trends. In the salt treatment, the more silica a leaf had, the faster it lost water. In the no salt treatment, there was not a significant difference between treatments. Another interesting observation is that the salt treated plants that got silica did not lose water at a significantly different rate. This suggests that the silica protected the plants from the salt stress.

Future Research

Trichome Structure

The main problem with the microscope study was the inability to quantify the differences that were so easily seen under the microscope. In future studies, it would be beneficial to develop techniques to determine the size and density of the trichomes, so that a quantitative analysis could be conducted.

Leaf Desiccation Rate

The desiccation rate experiment showed some intriguing possibilities. It was very easy to run, and didn't take much time. Because of this, multiple replications are possible in a very short period of time. Now that the technique has been proven and refined, fewer measurements will be needed to determine differences. The only change that would need to happen is that the first weighing needs to take place immediately after harvesting the leaves. Sixty seconds is long enough for a significant amount of water to escape from the leaves, skewing the results slightly.

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