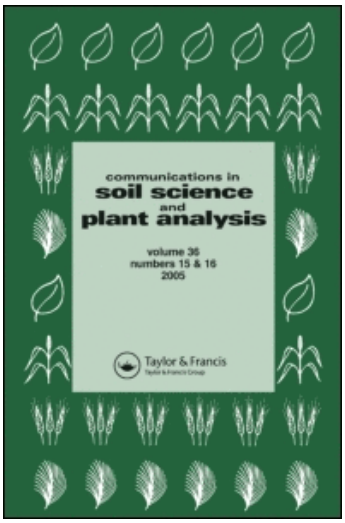

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Detection, Distribution, and Quantification of Silicon in Floricultural Crops utilizing Three Distinct Analytical Methods

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Abstract: Silicon (Si) detection, distribution, and quantification in plants was compared using electron beam analysis (EBA; scanning electron microscopy coupled with energy dispersive X-ray analysis), colorimetric analysis, and inductively coupled plasma–optical emission spectroscopy (ICP-OES) in 14 economically important floriculture species. Using EBA, Si was identified most commonly around the base of trichomes and along the leaf margins. The ICP-OES processing and analysis for Si using sodium hydroxide (NaOH) resulted in damaged torches and microwavable Teflon® vessels that required expensive replacement at the end of each run, but this was not the case in the colorimetric method or with a potassium hydroxide (KOH)–based matrix in the ICP-OES. The results of these analyses suggest there is agreement between quantification methods, and EBA has a lower detection limit of about 300 mg kg^{-1} dry weight of Si. Several new floriculture species (zinnia, impatiens, verbena, and calibrachoa) were identified that take up and accumulate Si in significant concentrations.

Keywords: Colorimetry, EBA, ICP-OES, plant tissue analysis, SEM, silicon

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INTRODUCTION

Silicon (Si) is not considered to be an essential plant nutrient because most plant species can complete their life cycle without its presence (Marschner 1995). Still, some plant species can accumulate Si at concentrations higher than many essential macronutrients (Epstein 1999). Many investigations have shown a positive growth effect if Si is present, including increased dry mass and yield (Ma, Nishimura, and Takahashi 1989), enhanced pollination (Korndorfer and Lepsch 2001), and most commonly, increased disease resistance (Belanger et al. 1995; Bowen et al. 1992; Datnoff and Rodrigues 2005; Gillman, Zlesak, and Smith 2003; McAvoy and Bible 1996; Rodrigues et al. 2004). Some beneficial effects of Si, such as reduced incidence of micronutrient and metal toxicity (Britez et al. 2002, Cocker, Evans, and Hodson 1998; Horiguchi and Morita 1987; Horst and Marschner 1978), may occur even if Si is not taken up in appreciable amounts (Voogt and Sonnenfeld 2001). Silicon can also alleviate imbalances between zinc and phosphorus supply (Marschner et al. 1990).

Several methods for detecting and quantifying Si in plants have been reported including electron beam analysis (EBA), which is the use of scanning electron microscopy with energy dispersive X-ray analysis, colorimetric determination of Si after autoclave-induced digestion (Elliot and Snyder 1991), and inductively coupled plasma–optical emission spectroscopy (ICP-OES), which relies on similar digestion approaches as the colorimetric method (Snyder 2001). These tools enable detection of localized, Si-based depositions on or within specific plant anatomical structures and quantifying total Si with excellent detection limits and relatively little processing.

However, the EBA and ICP-OES methods are expensive and not readily available to most laboratories. Even if access to the equipment is unlimited, digestion techniques for Si-containing plant tissue use extremely caustic acids [hydrofluoric acid (HF)] or bases [18.5 M sodium hydroxide (NaOH)], which can corrode ICP-OES components and digestion vessels. Because of the different scales of analysis, it is not known how detection with one technique (EBA) would relate to quantification by another technique (ICP-OES or colorimetric techniques).

In this study, we report the correlations between Si detection with EBA and two different quantification methods (ICP-OES and colorimetric). We also report simplifications in using these techniques so that there are fewer steps needed for the EBA, less caustic chemicals in the tissue digestion, and less damage to vessels and ICP-OES components. During these tests, we evaluated Si content in 14 economically important floriculture species, some of which have never been reported to take up or accumulate Si. In doing so, easier, safer, and less expensive procedures were developed, and floriculture species were identified that may utilize Si to enhance resistance against both abiotic and biotic stresses.

MATERIALS AND METHODS

Plant Culture

Seedlings or cuttings were initially germinated or rooted using foam cubes (15 mm × 15 mm × 30 mm each; LC1-type, Smithers-Oasis, Kent, Ohio). Because the plant species have different developmental rates, the time required to produce suitable test material differed for the different species (Table 1). When the seedlings or rooted cuttings were large enough, they were transplanted into the lids of opaque plastic 5-L buckets containing aerated hydroponic solution, six plants per tub, and placed on a greenhouse bench. The solution was a modified Hoagland's solution containing 2.5 mM potassium nitrate (KNO_3), 2.5 mM calcium nitrate [$Ca(NO_3)_2$], 0.5 mM potassium phosphate (KH_2PO_4), 1.0 mM magnesium sulfate ($MgSO_4$), 70 μM iron (Fe) as Fe-diethylenetriamine-pentaacetic acid (DTPA), 4.5 μM manganese chloride ($MnCl_2$), 0.75 μM zinc chloride ($ZnCl_2$), 0.75 μM copper chloride ($CuCl_2$), 22.5 μM boric acid (H_3BO_3), and 0.05 μM sodium molybdate (Na_2MoO_4) (verbena contained half this rate for macronutrients because of their salt sensitivity) with or without 2.0 mM potassium silicate (K_2SiO_4). Potassium silicate was synthesized with fumed silica (SiO_2, 0.007 μm particle size) dissolved in 0.1 M potassium hydroxide (KOH). Ten tubs were used for each species, with half the tubs containing Si and the other half without Si. No glassware was used in making the nutrient solution, and 18 mega-ohm purified water was used exclusively during the course of the trial to minimize Si contamination. The pH was adjusted to 5.7 with sulfuric acid (H_2SO_4) or KOH before nutrient solutions were added to the hydroponic containers. Flowers were routinely removed to maintain vegetative growth. Temperature was measured with a thermocouple (Type K, Omega Engineering, Stamford, Conn.) with daytime temperature between 26 and 30°C, and night temperatures between 16 and 22°C. Relative humidity ranged from 25 to 50%, as measured with a solid-state humidity sensor (model CS500, Campbell Scientific, Logan, Utah).

EBA Analysis

At the end of the growth period, control and treatment leaves were harvested, washed with deionized water, and sampled for analysis with two scanning electron microscopes (SEM) under high-pressure vacuum mode (Hitachi Cold-Field Emission SEM, Model S-4700 and Hitachi Variable Pressure, Model S-3500, Hitachi Corp., Pleasanton, Calif.). Both young and mature, fully expanded leaves were used in the analysis.

Table 1. List of species tested, propagation method, establishment period in days (d), and period of Si exposure[a]

Genus/species	Cultivar	Common name	Starting material	Establishment period (days)	Period of exposure to Si (days)
Pelargonium × *hortorum*	Maverick white	Geranium	Seed	31	35
Dianthus spp.	Floral lace white	Dianthus	Seed	32	34
Tagetes erecta L.	African atlantis primrose	Marigold	Seed	32	40
Zinnia elegans L.	Oklahoma white	Zinnia	Seed	12	25
Antirrinum majus L.	Rocket white	Snapdragon	Seed	24	24
Begoniaceae	Prelude white	Begonia	Seed	13	24
Verbena × *hybrida* Voss	Tukana white	Verbena	Cutting	32	44
Catharanthus spp.	Pacifica	Vinca	Seed	42	44
Impatiens wallerana Hook.f	Super elfin white	Impatiens	Seed	64	25
Impatiens hawkeri Bull.	Sonic light lavendar	New Guinea impatiens	Cutting	49	30
Euphorbia pulcharima	Freedom red	Poinsettia	Cutting	30	32
Petunia × *hybrida*	White madness	Petunia	Seed	43	28
Calibrachoa × *hybrida*	Colorburst violet	Calibrachoa or trailing petunia	Cutting	30	30
Salvia divinorum	Vista white	Salvia	Seed	26	24

[a]The establishment period is the plant age prior to Si exposure in hydroponics, and the exposure period is the length of time the plants were allowed to be in contact with Si.

Beam voltage was 20.0 kV at a magnification between 30 and 500 × and a working distance of 12 mm (S-4700) or 15 mm (S3500) for EDXA. This voltage was also used for the X-ray microanalysis (Thermo Noran) for the respective images. Fresh cuttings of the edge and middle of the plant leaf were mounted on aluminum tabs with carbon sticky dots and coated with gold (2 nm) to prevent charging and distortion artifacts from evaporating water when viewed in the SEM. A total of at least eight samples were used for each treatment per species.

Colorimetric Analysis

Plants were harvested and divided into leaves, stem, and roots, washed in 0.1N HCl, and rinsed in deionized (18-mega-ohm purity) water. Samples were then placed in a paper bag and dried at 55 °C in a forced-air oven for at least 3 days. Dried tissue was weighed and processed according to the methods described by Elliot and Snyder (1991). Briefly, 0.1 g of tissue was ground into a fine powder (at least 0.05 mm particle size) either by hand using a Si-free mortar and pestle or with a motorized, stainless steel grinder (model BCG100WH1; Kitchen Aid, St. Joseph, Mich.) and placed into a polyethylene tube. Five milliliters of NaOH solution (1 g NaOH / mL H_2O) were added to the tissue in the tube and shaken to mix thoroughly. The capped tube was then placed in an autoclave and heated for 30 min, then allowed to cool to room temperature. After cooling, 2 mL of hydrogen peroxide (H_2O_2) were added to each tube and reheated in the autoclave for an additional 30 min. After cooling, 43 mL of distilled water were added to each tube.

After additional cooling, 0.1 mL of the digested plant material mixture was added to 10 ml of distilled water. Hydrochloric acid (0.25 mL of 1:1 mixture of HCl and H_2O) was added along with an ammonium molybdate solution (0.5 mL, 100 g L^{-1} at pH of 7.0), shaken, and allowed to stand for up to 10 min. Tartaric acid (0.5 mL, 200 g L^{-1}) was added, shaken, and allowed to sit for an additional 3 min. Sodium bisulfate (0.7 mL, 50 g / 400 ml) was added and mixed. The blue color that developed was measured between 10 and 30 min at 650 nm. Finally, the absorbance was compared to a standard calibration curve of known Si concentrations prepared with soluble Si combined with the reagents as described previously.

ICP-OES Analysis

For digestion, 0.1 g ground tissue was placed in a 55-mL Teflon® vessel, and 3.0 mL of either 18.5 M NaOH or 7.5 M KOH were added. This mixture was heated in a programmable microwave (MARS Express; CEM Corp., Matthews, N.C.) by ramping the temperature up to 200 °C for 15 min, then

maintaining that temperature for 15 min. After the digested material cooled to room temperature, 2 mL of H_2O_2 were added, and the samples were heated again by ramping the temperature back to 200 °C over 15 min and held at 200 °C for an additional 5 min. The solution was cooled again and filtered (Whatman's no. 2). One ml of the filtrate was diluted with 9 ml deionized water (18-mega-ohm purity) and injected into the ICP-OES (Model IRIS Intrepid II; Thermo Electron Corp., Waltham, Mass.).

The initial calibration and wavelength selection was determined by using several lines identified by the ICP-OES manufacturer as being sensitive to Si: 212.415 nm (157 and 158), 250.6 nm (133 and 134), 251.612 nm, and 288.158 nm. These wavelengths were calibrated against 10 known Si concentrations of between 0 and 10 ppm. These same calibration standards were run as unknowns, and the output of each wavelength was compared to the known value. This resulted in selecting line 212.412 (158) for any additional analyses based on correlation (r^2), accuracy based on relative standard deviation (standard deviation divided by average value), and sensitivity (slope) of the wavelength (Table 2). The settings for the ICP-OES were flush and analysis pump rate: 130 rpm; RF power: 1150 W; nebulizer pressure: 32.1 PSI; and auxillary gas: 1.0 L min^{-1}.

An additional test was run with all of these wavelengths to determine the sensitivity to interferences caused by materials and compounds within digested plant tissue. Two plant tissue standards (tomato and spinach NIST standards 1573a and 1570a, respectively) were digested using this method, and then spiked with a known amount of Si (K_2SiO_4). The spiked standards were then analyzed as unknowns and measured with all wavelengths to determine if any plant compounds interfered with Si detection (Table 3). Again, wavelength 212.412 nm (158) was determined to be the most sensitive to Si and insensitive to interferences.

The ICP-OES was calibrated with a blank and high standard solution of 9.985 mg L^{-1} with a background matrix of 1.85 M NaOH or 0.75 M KOH. Quality control (QC) was immediately run with the high standard. If the QC failed ($\pm$ 10%), the instrument was recalibrated. An

Table 2. Wavelengths used and resulting calibration equation parameters in the ICP-OES detection methods for the original calibration curves over a range of Si concentrations of 0 to nearly 10 mg kg^{-1}

Parameter	Wavelength (nm)					
	212.412 (157)	212.412 (158)	250.690 (133)	250.690 (134)	251.612	288.158
r^2	0.3705	0.9995	0.0007	0.3656	0.9363	0.7809
Avg. RSD	−1.034	0.043	−0.930	1.349	0.596	0.914
Slope	0.049	23.7	−0.0014	0.0422	0.236	0.229
Intercept	0.0001	1.70	0.009	0.0006	0.0177	0.0627

Table 3. Tomato and spinach standards were spiked with two known concentrations of Si, and amount recovery was determined

Plant	Predicted value ($mg\,kg^{-1}$)	Wavelength (nm)					
		212.412 (157)	212.412 (158)	250.690 (133)	250.690 (134)	251.612	288.158
Tomato	0.166	−0.1909[a] (−115)	0.168 (101)	−0.300 (−180)	0.611 (368)	0.256 (154)	0.258 (155)
	1.065	−0.0002 (−0.019)	1.063 (99.8)	−0.1804 (−16.9)	−0.1102 (10.3)	0.71 (66.7)	0.7755 (72.8)
Spinach	0.145	0.3334 (230)	0.151 (104)	−0.2997 (−207)	0.3708 (256)	0.1157 (79.8)	0.1148 (79.2)
	1.044	0.02857 (2.74)	1.038 (99.4)	0.5388 (51.6)	−0.1971 (−18.9)	0.7823 (74.9)	0.8441 (80.9)

Note. Negative values indicate the sample was less than detection limit. Based on these tests, the wavelength of 212.412 (158) nm was used in subsequent tests.

[a]Actual recovered in $mg\,L^{-1}$. Numbers in parentheses indicate percentage recovered.

additional QC was run every 10 samples, and if it failed (± 10%), the ICP-OES was recalibrated. Every 20 samples, a rice standard containing 0.44% Si was run that had been digested in a manner similar to the test species. Detection limits are determined as described by Corre (undated) wherein a blank is run with similar test matrix 10 times, and the average, standard deviation, and relative standard deviation are calculated.

RESULTS AND DISCUSSION

EBA Identification of Si

The EBA system allowed for fairly rapid and specific determination of Si distribution and deposits in many of the floriculture species evaluated during this study (Table 4). Only geranium, snapdragon, petunia, and salvia did not contain Si in appreciable amounts in the areas investigated to result in detection with EBA. When present, Si was identified most commonly around the base of trichomes and along the leaf margins (Figure 1A and B). This is similar to what Dayanandan and Kaufman (1976) and Dengler and Lin (1980) found wherein a broad variety of species have been noted to accumulate Si at trichome bases or wherever the xylem ends. Si deposits were easily identified as areas associated with the lack of wrinkled epidermal cells (Figure 2A and B), suggesting that the cells were able to maintain structural integrity under the vacuum of the EBA.

Occasionally, similar surface features appeared in tissue from plants with Si and those without Si. In these cases, the morphology of the epidermis provided no clues about Si's presence (Figure 3A and B). In those instances, the presence of Si was only determined after X-ray analysis.

ICP-OES Analysis of Si–NaOH Digestion

Processing the plant material with NaOH along with H_2O_2 rapidly digested the samples into solution. There was a small amount of white precipitate in each digestion that required filtration prior to injecting into the ICP-OES. Some deterioration was observed at the bottom of many of the Teflon® tubes following digestion with the NaOH, occasionally resulting in the need for replacement of the tubes. This represents a large additional cost ($250 to $500 per tube) to this processing method. After calibrating the ICP-OES with suitable standards containing the same NaOH-based matrix, the color of the flame, which is typically green, was orange (Figure 4A). However, the flame did not extinguish, calibrated properly, and the excess sample properly drained. There was concern that the NaOH matrix would etch or corrode the torch as the samples were

Table 4. Si detection, distribution, and concentration in mature leaves using EBA, ICP-OES, and colorimetric methods

Plant	EBA		ICP-OES		Colorimetric	
	Si Detected	Location	Leaf Si content + Si ($mg\,kg^{-1}$)	Leaf Si content control ($mg\,kg^{-1}$)	Leaf Si content + Si ($mg\,kg^{-1}$)	Leaf Si content control ($mg\,kg^{-1}$)
Geranium	No	N/A	539.1 (one sample)	range: nd to 32.0	465.3 (one sample)	199.9 ± 93.8
Dianthus	Yes	Throughout leaves	362.0 ± 94.0	nd	286.9 ± 134.9	58.6 ± 26.0
Marigold	Yes	Throughout leaves	330.4 ± 39.4	range: nd to 20.3	486.0 ± 185.5	128.5 ± 94.6
Zinnia	Yes	Leaf trichomes	12,682.7 ± 615.0	nd	11,749.7 ± 1274.9	31.02 ± 35.8
Snapdragon	No	N/A	501.6 ± 67.5	nd	258.5 ± 149.1	nd
Begonia	Yes	Leaf trichomes	649.2 ± 128.0	191.9 ± 112.9	472.2 ± 83.6	265.4 ± 194.6
Verbena	Yes	Leaf trichomes & margins	8417.0 (one sample)	137.7 (one sample)	8225.5 ± 2080.1	160.3 ± 103.2
Vinca	Yes	Leaf trichomes	330.82 ± 36.32	93.12 ± 57.66	341.2 ± 113.7	72.38 ± 37.3
Impatiens	Yes	Leaf margins	2008.23 ± 131.29	79.82 ± 52.16	923.7 ± 70.4	180.5 ± 85.7
New Guinea impatiens	Yes	Leaf trichomes & margins	2314.3 ± 255.0	141.7 ± 40.6	1457.9 ± 298.3	221.0 ± 111.9
Poinsettia	Yes	Leaf surface	465.8 ± 213.5	nd	941.1 ± 667.6	299.9 ± 179.4
Petunia	No	N/A	197.3 ± 11.6	nd	237.8 ± 17.9	nd
Calibrachoa	Yes	Leaf trichomes	1536.48 ± 50.11	129.94 ± 17.28	1338.5 ± 166.1	241.2 ± 123.8
Salvia	No	N/A	529.7 ± 97.8	range: nd to 41.1	358.9 ± 94.9	246.1 ± 171.8

Notes. Average values are shown, plus or minus one standard deviation. When at least one of the replicates had nondetectable concentrations (nd), only a range is shown. When all three replicates were nd, only nd is shown. All plants were replicated three times except the +Si geranium (n = 1) and both ± Si marigold (n = 6).

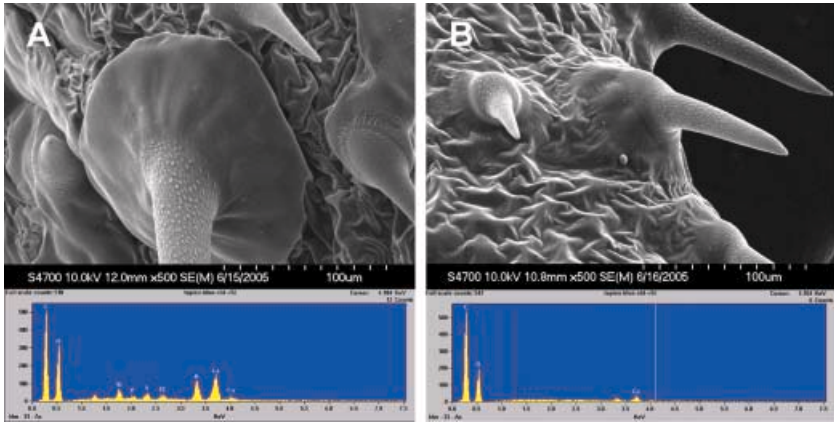

Figure 1. Scanning electron micrographs (top) and the corresponding spectrographs (bottom) of verbena trichomes with (A) and without (B) Si treatment. The Si-fed verbena contained Si and other elements within the base of each trichome. Si-fed verbena contained about 0.8% dry weight of Si based on both ICP-OES and colorimetric determinations.

run and cause an overestimate of Si in solution. Therefore, additional blanks were run during the analyses using NaOH to account for Si contamination from the torch or nebulizer.

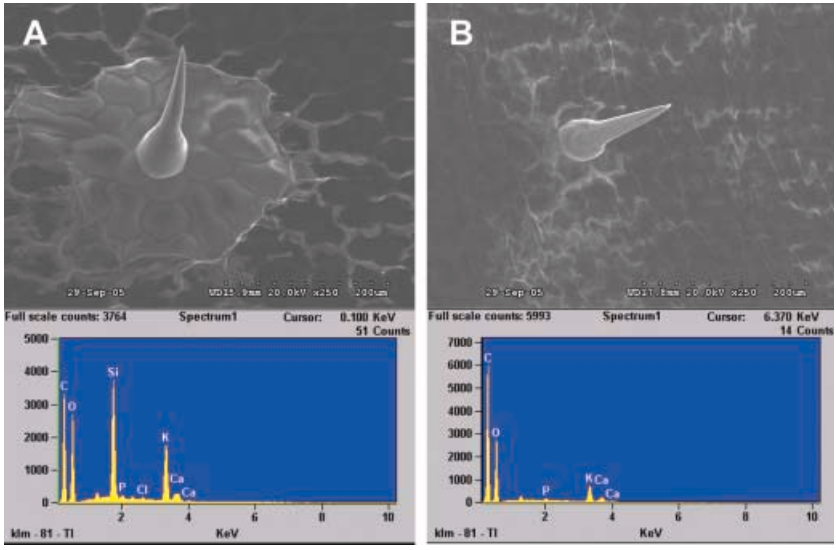

Figure 2. Scanning electron micrographs (top) and the corresponding spectrographs (bottom) of a zinnia trichome with (A) and without (B) Si treatment. Si-fed zinnia contained over 1% dry weight of Si based on both ICP-OES and colorimetric determinations.

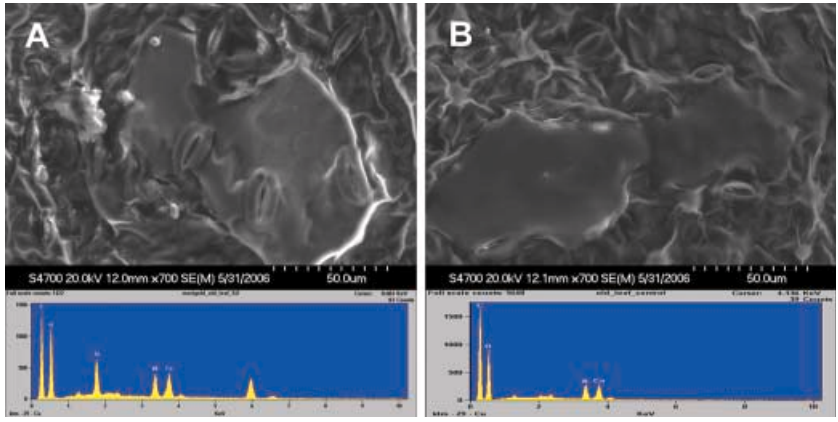

Figure 3. Scanning electron micrographs (top) and the corresponding spectrographs (bottom) of a marigold leaf surface with (A) and without (B) Si treatment. Marigold contained between 300 and 400 mg kg^{-1} dry weight of Si based on colorimetric and ICP-OES determinations. The swollen epidermal cells appeared in both Si-fed and control plants, making scanning electron detection of Si deposition difficult without a spectrograph.

At the end of the run after the torch was cooled, it was clear that the torch had become damaged, compared to an unused, clean torch, requiring replacement prior to another set of analyses (Figure 5A and B). Additional tests with new torches indicated that if the torch was heated with a NaOH solution matrix, the torch would need to be replaced after cooling regardless of the number of samples analyzed during a single run. Again, this was a substantial increase in cost for analyzing Si in ICP-OES with this method (torches are between $350 and $700).

Figure 4. An ignited ICP-OES torch had an orange flame while analyzing samples with NaOH-digested plant tissue for Si analysis (A) but had a more typical green flame (B) when analyzing samples with KOH-digested plant tissue for Si analysis.

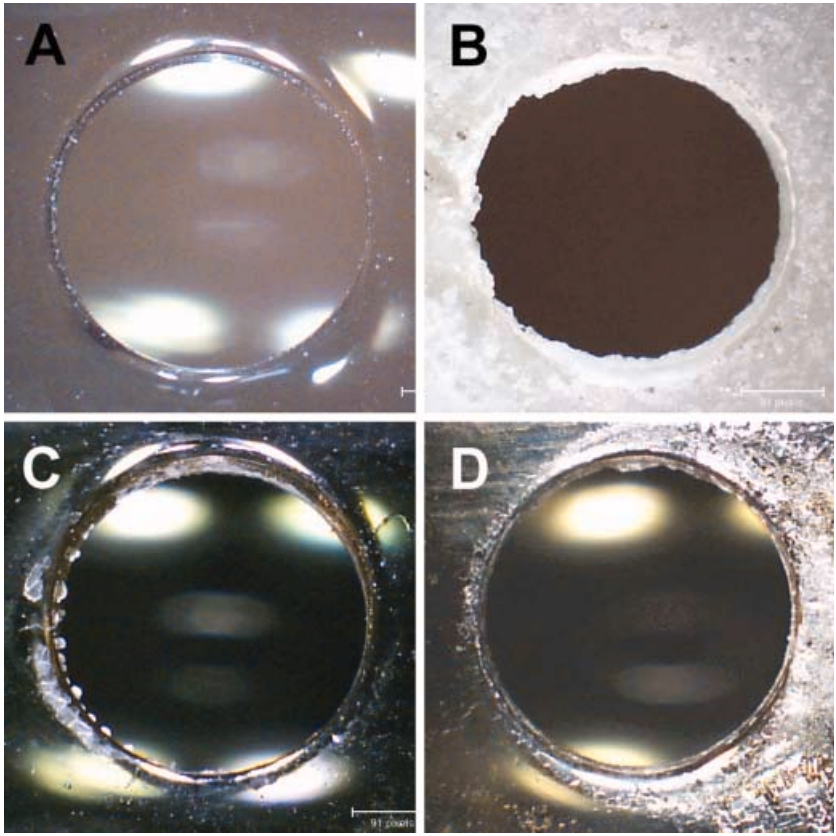

Figure 5. Radial view hole from a clean, unused ICP-OES torch (A). Radial hole from an ICP-OES torch after running about 50 samples with NaOH-digested plant tissue for Si analysis, cleaned with aqua regia and sonicated 3 h (B). Radial hole from an ICP-OES torch after running 90 samples with KOH-digested plant tissue for Si analysis (C). Radial hole from the same torch as in C after running 570 samples with KOH-digested plant tissue for Si analysis, cleaned with aqua regia and sonicated for 3 h (D).

ICP-OES Analysis of Si–KOH Digestion

It was determined that the use of KOH may minimize the damage to the Teflon® tubes and the torch because of its larger atomic size (MW of Na = 23, MW of K = 39). Furthermore, it was hypothesized that reducing the concentration of the base for digestion may minimize damage and the threat of Si contamination from the nebulizer or torch.

Three concentrations of KOH (3.25 M, 7.5 M, and 15 M) were used to digest rice plant tissue containing 0.44% Si (Datnoff, personal plant

standard) and analyzed in the ICP. Both the 7.5M and 15M digestions resulted in accurate quantification of Si (0.44% ± 0.007 vs 0.45% ± 0.009 respectively). For future digestions, 7.5M KOH was chosen to reduce the use of exceptionally strong bases.

Digesting with 7.5M KOH and H_2O_2 did not damage any of the Teflon® tubes, even after multiple digestions in the same tube. Analyzing samples with the KOH-based matrix in the ICP-OES also produced the characteristic green flame consistent with analyzing samples with an acid matrix (Figure 4B). After analyzing a number of samples, the torch was allowed to cool and was removed. The torch showed minimal visible damage, and we were able to make multiple runs without replacement (Figure 5C and D). All subsequent tissue analyses were performed using the KOH-digestion method.

ICP-OES Quantification of Si

The detection limits were calculated to be 2 ppb in solution, which would calculate to 3 ppm when tissue dilution factors are considered. Si was detected in all tissue samples derived from plants supplied with Si, but the value varied greatly among species. The highest amount was detected in zinnia (1.2% ± 0.06) followed by verbena (0.8%), impatiens (0.2% ± 0.01), New Guinea impatiens (0.23% ± 0.03), and calibrachoa (0.15% ± 0.005) (Table 4). The other treated species had Si concentrations between 0.05 and 0.02%. The coefficient of variation (CoV; calculated as the standard deviation divided by the average value) was typically between 10 and 20% of the average for the plants supplemented with Si with a range of 3% to 46%.

Si was not detected in some of the control leaves including dianthus, zinnia, snapdragon, poinsettia, and petunia, suggesting the concentration of Si was less than detectable limits in all three replicate samples. Only the range is reported if Si was not detected in at least one of the replicate samples from a treatment/species combination. The CoV was much higher for the control plants ranging from 13 to 65% with an average of 50% (Table 5). This average was based on only the samples that contained detectable levels of Si using this method.

Colorimetric Quantification of Si

Similar quantities of Si were observed using the colorimetric method as that of the ICP-OES method. Zinnia still contained the highest concentration (1.2% ± 0.13) followed by verbena (0.8% ± 0.2), New Guinea impatiens (0.15% ± 0.03), and calibrachoa (0.13% ± 0.02) (Table 4). Only a few species deviated substantially between ICP-OES

Table 5. Coefficient of variation (CoV) for ICP and colorimetric methods

Plant	ICP-OES		Colorimetric	
	+Si (%)	Control (%)	+Si (%)	Control (%)
Geranium	N/A	N/A	N/A	46.9
Dianthus	26.0	N/A	47.0	44.4
Marigold	11.9	N/A	38.1	73.6
Zinnia	4.8	N/A	11.1	115.4
Snapdragon	13.4	N/A	57.7	N/A
Begonia	19.7	58.8	17.7	73.3
Verbena	N/A	N/A	25.2	64.3
Vinca	11.0	61.9	33.3	51.5
Impatiens	6.5	65.3	7.6	47.4
New Guinea impatiens	11.0	28.6	20.4	50.6
Poinsettia	45.8	N/A	70.9	59.8
Petunia	5.8	N/A	7.5	N/A
Calibrachoa	3.2	13.3	12.4	51.3
Salvia	18.5	N/A	26.4	69.8
Average	15.2	49.8	28.9	62.4

Notes. CoV was calculated as the standard deviation divided by the average (Table 3) for each tissue and method. N/A (not applicable) indicates either insufficient replicates (geranium) or one or more replicates were determined to be less than the detectable limits for that method. The quantification of Si was relatively more precise using the ICP-OES method. However, there is no NIST standard for Si in plant tissue, so it is currently impossible to determine one method's accuracy relative to another.

and colorimetric methods including snapdragon (0.05% vs 0.025%), impatiens (0.2% vs 0.09%), and poinsettia (0.046% vs 0.094%). The CoV was typically between 25 and 35% of the average for the plants supplemented with Si with a range of 8% to 70%.

In the non-Si-treated plants, only snapdragon and petunia had Si contents less than the detectible limits. In all control plants, Si concentrations were determined to be less than the Si-amended plants. The CoV was again much higher in the control plants, ranging from 44 to 115% of the average (Table 5). If only the samples are calculated from which the ICP-OES control samples were calculated, the range is 47 to 73%.

Si Uptake in Floriculture Crop Species

In a taxonomic review of Si uptake, Ma, Miyake, and Takahashi (2001) analyzed the mineral content of 500 species of plants and concluded that Si accumulation (more than 1% dry mass content Si) is not a universal characteristic in all higher plants but confined to monocots in the families

Graminaceae, Cyperaceae, and Erlocaulales. A relatively small proportion of horticulturally valuable crops have been investigated for their potential for Si uptake, accumulation, and deposition. Cucumber (Belanger et al. 1995) and other Cucurbits (Heckman, Johnston, and Cowgill 2003) are generally accepted to utilize Si to withstand some fungal pathogens. Among floricultural crops, *Rosa hybrida* was responsive to Si to combat black spot infection (*Diplocarpon rosae*) (Gillman, Zlesak, and Smith 2003) and development of powdery mildew (*Podosphaera pannosa*) (Datnoff et al. 2006). Bract necrosis in poinsettia was reduced when Si was applied either in the nutrient solution or as a foliar spray (McAvoy and Bible 1996). Shoot number, flower number, and dry weight were enhanced by 25, 62, and 66%, respectively, over the control for paper daisies (*Helichrysum adenohorum*) amended with silicon (Muir et al. 1999). In a similar study, open flowers per head were increased by 28% in snapdragons while bent/lodged stems were reduced by 33%. Frantz et al. (2005) reported Si deposits on the leaf margins of New Guinea impatiens (*Impatiens hawkeri* W. Bull). Voogt and Sonneveld (2001) and Voogt, Wubben, and Straver (2005) evaluated the floricultural crops gerbera (*Gerbera* spp.), carnation (*Dianthus caryophyllus*), heath aster (*Aster ericoides*), poinsettia (*Euphorbia pulcherrima*), African violets (*Saintpaulia ionantha*), and rose (*Rosa*) with aster, poinsettia, African violets, and rose containing appreciable amounts of Si (more than 25 mmol kg^{-1} dry mass). In the current study, zinnia accumulated Si to more than 1% dry weight, whereas verbena, impatiens, New Guinea impatiens, and calibrachoa accumulated Si from 0.2 to 0.8%. The biological effect of the relatively high Si contents in these species deserves further study. Interestingly, petunia and calibrachoa, both in the Solanaceae family, which includes the nonaccumulators tomato and sweet pepper (Voogt and Sonneveld 2001), accumulated extremely different amounts of Si in the leaf tissues.

CONCLUSIONS

As the use of Si increases to assist in ameliorating biotic and abiotic stress, reliable detection, distribution, and quantification methods will be increasingly important. The use of EBA was an effective tool for localization of Si, but its use required a tissue concentration greater than 200 to 300 mg kg^{-1} to easily locate Si deposits. This method is useful for some species that accumulate significant amounts of Si. The colorimetric method coupled with autoclave-assisted digestion with NaOH has been an inexpensive and available method for years. In cases where processing many samples requires the use of automated processing and analytical techniques, ICP-OES is a viable option. However, the reliance on the

same reagents used in the colorimetric methods resulted in this technique not being widely adapted, because of equipment failure from the reagents. Switching from NaOH-based matrices to KOH matrices reduced the equipment failure and still provided acceptable correlations with the colorimetric methods and excellent detection limits.

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