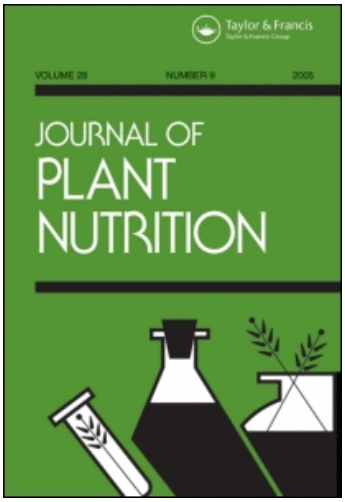

Journal of Plant Nutrition
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597277

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Online publication date: 01 December 2010

To cite this Article Kamenidou, Sophia , Cavins, Todd J. and Marek, Stephen(2011) 'CORRELATION BETWEEN TISSUE AND SUBSTRATE SILICON CONCENTRATION OF GREENHOUSE PRODUCED ORNAMENTAL SUNFLOWERS', Journal of Plant Nutrition, 34: 2, 217 — 223
To link to this Article: DOI: 10.1080/01904167.2011.533323
URL: http://dx.doi.org/10.1080/01904167.2011.533323

Journal of Plant Nutrition, 34:217–223, 2011

ISSN: 0190-4167 print / 1532-4087 online
DOI: 10.1080/01904167.2011.533323

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CORRELATION BETWEEN TISSUE AND SUBSTRATE SILICON CONCENTRATION OF GREENHOUSE PRODUCED ORNAMENTAL SUNFLOWERS

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□ *The beneficial effects of the "nonessential" plant nutrient, silicon (Si), are well documented for several agricultural crops. Soilless growth media used in greenhouse production provides only limited amounts of available Si to container grown plants compared to plants grown in mineral-derived soils. Si supplementation is documented to increase resistance to biotic and abiotic stresses in greenhouse crops, which accumulate Si in their tissues. However, optimum Si fertilization rates and acceptable Si levels in tissues and substrate have not been established for floriculture greenhouse production. For this study ornamental sunflower (*Helianthus annuus *L. 'Ring of Fire') was used to investigate the relationship between substrate Si and accumulation of Si in the tissues of plants grown in a peat-based media. Weekly substrate drenches of potassium silicate ($KSiO_3$), substrate incorporation of $KSiO_3$ hydrous powder, or rice husk ash were used as Si supplements. Overall, leaf, stem, and flower Si concentrations of Si-supplemented plants increased compared to nonsupplemented controls. A positive correlation was observed between substrate Si concentration and leaf Si concentration for all three Si sources used in this study. Therefore, leaf tissue is the most appropriate tissue to sample in order to determine the availability of Si in a substrate and could be used to establish acceptable Si levels for soilless greenhouse floriculture.*

Keywords: *Helianthus annuus*, silicon supplements, beneficial elements, saturated media extract (SME)

INTRODUCTION

The greenhouse floriculture industry predominantly uses peat-based substrates for ornamental plant production. Plants grown in such soilless substrates, or organic histosols, are often deficient for the "nonessential" plant

Received 21 January 2009; accepted 18 February 2009.
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nutrient, silicon (Si) (Voogt and Sonneveld, 2001). Though nonessential for growth and development, supplemental silicon improves greenhouse-grown plants' ability to cope with physical stresses and disease and can improve plants' aesthetic qualities (Belanger et al., 1995; Epstein, 1994; Kamenidou et al., 2008). Silicon's beneficial effects have been well-studied in several greenhouse vegetable crops and grasses (Datnoff et al., 2001; Ma, 2004), but poorly studied in floricultural crops. In addition, optimal fertilization rates for Si and guidelines for acceptable tissue and substrate concentrations have not been established for floriculture greenhouse production.

There is very limited information about Si deposition in greenhouse-produced ornamental crops. It is not known if the saturated media extract (SME) method, commonly used in the U.S. floriculture industry to monitor substrate electrical conductivity (EC), pH, and nutrients samples, could be useful to monitor Si uptake. Leaf tissues are frequently analyzed to determine Si and other nutrient concentrations. However, Si uptake and accumulation depends strongly on plant species, and whether Si concentration is higher in leaf, stem, root, or bracts depends on the mode of Si uptake (Ma and Takahashi, 2002).

For this study ornamental sunflower was used, since it has been reported as one of the dicotyledonous plants able to accumulate phytoliths (Sangster et al., 2001). Our objective was to investigate whether various substrate Si concentrations correlate with the resulting tissue Si concentrations of greenhouse produced ornamental sunflowers.

MATERIALS AND METHODS

Plants and Experimental Design

Helianthus annuus 'Ring of Fire' seeds were sown into 0606 (75 mL per cell) bedding flats using BM2 Germinating Mix (Berger Peat Moss, St. Modeste, Quebec, Canada) and transplanted to 20.3 (1.8 L) cm pots when 4–6 true leaves were present, to ensure developmental uniformity of replicates. Once transplanted, plants were grown in a polycarbonate covered greenhouse with night/day set temperatures of 15/18°C and fertilized with 150 mg·L^{-1} nitrogen (N) from 21N-2.5 phosphorus (P)-16 potassium (K) (The Scotts Co., Marysville, OH, USA).

The substrate was 4:1 peat:perlite (v:v) with 875 g·m^{-3} Micromax Granular (The Scotts Co.) and 3.5 kg·m^{-3} dolomitic limestone. The sources, rates and method of Si application used were: potassium silicate ($KSiO_3$) (0, 25, 50 and 75 mg·L^{-1} Si) weekly drenches, hydrous $KSiO_3$ (0, 140, 190 and 240 g·m^{-3} Si) substrate incorporation, and ashed rice husk (0, 90, 130 and 170 g·m^{-3} Si) substrate incorporation. The rice husk ash, was a natural by-product with high Si content (Riceland Industries, Jonesboro, AR, USA),

while both forms of $KSiO_3$ were from PQ Corporation (Valley Forge, PA, USA).

A randomized complete block design included three Si sources at four rates each with 12 replications per source/rate combination. Data collections included weekly pH and EC values, height (measured from pot rim to the tallest point), flower diameter (measured at the widest point), dry weights for the stem and the first fully expanded flower for each replicate, and stem diameter at the base of the main stem. Tissue and SME samples for all Si treatments and nonsupplemented controls were collected one week after the last application of $KSiO_3$ substrate drenches. Samples collected for tissue analyses included recently mature leaves, main stems and the first fully expanded flowers from each treatment and untreated controls. Substrate solution samples were collected using the SME method of Warncke (1986). Tissue and substrate Si analyses for each treatment were analyzed in triplicate and trend analysis (SAS Institute, Cary, NC, USA) was used for the collected data ($P \leq 0.05$).

Silicon Extraction

A modified Si extraction procedure (Novozamsky et al., 1984) was used. Leaf, flower and stem samples were dried at 100°C and ground using a Wiley mill equipped with an 850 μm (20-mesh) screen. Then, in 50 mL polycarbonate tubes, 100 mg of ground tissue sample or 1 mL of SME sample was mixed with 10 mL 1M hydrochloric acid (HCl) and 20 mL 2.3M hydrofluoric acid (HF). After shaking at 280 RPM overnight on an orbital shaker, samples were filtered (Whatman #41 ashless, coarse filter paper) and the filtrate containing solubilized Si retained for silicon quantification.

Silicon Analysis

To determine the Si concentration of each extract, a modified blue silicomolybdous acid procedure (Taber et al., 2002) was used. In a polycarbonate test tube, 1.0 mL Si extract was mixed by inversion with 3.0 mL 2.5% boric acid. To this suspension, 1.0 mL ammonium molybdate (5.4 g L^{-1}, pH = 7) was added, mixed again and incubated stationary for 5 minutes. Next, 0.5 mL 20% tartaric acid was added and mixed. Finally, 0.5 mL reducing solution (1-amino-2-sulfonic acid solution for silica, Fisher reagent) was added and the mixture inverted three times and incubated stationary for 30 minutes. A 1.5 mL aliquot of each sample was transferred to a disposable polystyrene cuvette and the absorbance at 650 nm measured using a UV-Visible Recording Spectrophotometer (UV-265, Shimadzu, Columbia, MD, USA). A standard curve was prepared using a 1 mg·ml^{-1} Si standard solution [in 2% sodium hydroxide (NaOH); Acros Organics, Geel, Belgium] diluted to 3, 6, 9, 12, or 24 mg Si L^{-1} ($r^2 > 0.99$).

RESULTS AND DISCUSSION

All three sources of Si tested resulted in increased sunflower stem diameters (Table 1). Stem dry weights of Si-treated *Helianthus* plants increased up to 28% and 15% with weekly $KSiO_3$ drenches and hydrous $KSiO_3$ substrate incorporation, respectively. These stem measurement increases, linearly correlated with increasing Si media supplementation rates. Though rice husk ash increased stem diameters and dry weights, stem dry weight increases did not correlate with the quantities of ash incorporated into media. This lack of correlation may be due to the inherent heterogeneity of available silicon in rice husk ash (Ma and Takahashi, 2002). Flower diameters increased linearly using weekly $KSiO_3$ soil drenches and rice husk substrate incorporation, or curvilinearly (quadratic) using hydrous $KSiO_3$ substrate incorporation (Table 1). However, even though flower diameter increased slightly with Si supplementation, flower dry weight was not affected (data not presented).

Increased dry weight production is often a plant response to Si supplementation due to stimulated photosynthesis, reduced transpiration rate and increased tissue strength, especially for Si accumulators like rice (Ma and Takahashi, 2002). In rice, Si deficiency reduced grain yield, while Si supplementation increased dry weight, grain yield and stem number. The

TABLE 1 Correlation of silicon supplementation rate with tissue measurements of *Helianthus annuus* 'Ring of Fire'

Silicon source	Silicon concentration	Stem diameter (cm)	Stem dry weight (g)	Flower diameter (cm)
	$mg \cdot L^{-1}$			
$KSiO_3$ five weekly drenches	0	1.46	14.9	11.3
	25	1.56	16.1	11.5
	50	1.55	16.6	11.6
	75	1.63	20.8	12.6
	Linear	**	**	**
	Quadratic	NS	*	NS
	$g \cdot m^{-3}$			
Hydrous $KSiO_3$ substrate incorporation	0	1.48	15.4	10.6
	140	1.66	18.3	12.0
	190	1.67	17.4	11.8
	240	1.65	17.7	11.5
	Linear	**	*	NS
	Quadratic	**	NS	**
	$g \cdot m^{-3}$			
Rice husk ash substrate incorporation	0	1.42	15.4	11.2
	90	1.59	17.9	11.6
	130	1.57	16.7	11.8
	170	1.57	17.6	12.1
	Linear	**	NS	**
	Quadratic	**	NS	NS

Significant from the untreated control at the 5% level (*) or 1% level (**).

most common forms of silicon deposits include silica bodies in epidermal cells and cells surrounding stomata and silicified trichomes (Piperno, 1988). Silicification of plant tissues is a slow cumulative process that occurs only in fully expanded mature tissues and not in immature organs still undergoing cell expansion (Parry and Smithson, 1964). This may explain why Si-supplementation increased the dry weights of longer-lived stems, while the dry weights of shorter-lived flowers showed no increases.

Silicic acid is thought to be the chemical form of Si that plant roots take up from the substrate and, via the transpiration stream, deposit in aerial tissues with the greatest evapotranspiration rates, where, due to water loss, silicic acid polymerizes into silica gel (Hodson and Sangster, 1989). All Si supplements in this study resulted in increased levels of Si accumulating in the sunflower tissues tested (Table 2). Leaves had the highest Si concentrations, followed by flowers then stems, for both Si-treated and untreated plants.

TABLE 2 Correlation of silicon supplementation rate with tissue silicon concentrations of *Helianthus annuus* 'Ring of Fire' and SME values

		Silicon concentration (μg/g)			
Si Source	Conc.	Leaf	Flower	Stem	SME
	$mg \cdot L^{-1}$				
$KSiO_3$ five weekly	0	4253	3652	2350	30
drenches	25	4816	4584	3198	33
	50	5208	4283	3000	33
	75	5882	5012	3247	66
	Linear	**	**	**	**
	Quadratic	NS	NS	**	NS
	r	0.86	0.78	0.55	
	$g \cdot m^{-3}$				
Hydrous $KSiO_3$ substrate	0	4253	3652	2350	30
incorporation	140	5015	4029	2411	54
	190	5663	4387	2558	62
	240	5859	4453	2238	43
	Linear	**	**	NS	NS
	Quadratic	**	NS	*	*
	r	0.66	0.64	−0.71	
	$g \cdot m^{-3}$				
Rice husk ash substrate	0	4253	3652	2350	30
incorporation	90	4638	3621	3425	33
	130	4561	4580	3009	42
	170	5829	3775	3077	43
	Linear	**	*	**	NS
	Quadratic	**	**	**	NS
	r	0.99	−0.15	−0.016	

NS, *, **, Non significant (NS), or significant at 5% (*), or 1% (**) level.

SME: saturated media extract soilless substrate samples.

r: coefficient of correlation for leaf, flower, and stem Si concentration vs. SME samples for each Si source.

Weekly $KSiO_3$ drenches and hydrous $KSiO_3$ substrate incorporation, but not rice husk ash substrate incorporation, correlated with higher Si concentrations in saturated media extracts (SME) (Table 2). The lower SME Si levels in rice husk ash treatments may have been due to the incomplete solubility of Si present in the burned rice husk-supplemented substrate (Ma and Takahashi, 2002).

Surprisingly high SME Si concentrations, 30 $\mu g \cdot g^{-1}$ Si, were measured in control plant substrates (Table 2). The Si concentrations in control samples were presumed to have been much lower than Si-supplemented plants. This is our first study using the blue silicomolybdous acid procedure to determine Si levels in SME samples and thus, may need to optimized to accurately determine Si concentrations present in SME samples, which may have contained interfering substances that absorbed red light (assay $\lambda =$ 650 nm) and artefactually inflated spectrophotometric determinations of Si. Alternatively, irrigation water contained 4 $\mu g \cdot ml^{-1}$ Si, according to the blue silicomolybdous procedure, and this Si 'contamination' may have accumulated in media and resulted in the observed high Si levels in the control SME samples. However, since Si levels accumulating in tissues correlated more frequently with supplement concentrations than SME concentrations, the Si in irrigation water may be in a chemical form that is less available to plants than the Si in media supplements.

Leaf Si concentrations positively correlated with SME Si concentrations samples for all three Si sources, while stem Si concentrations showed the weakest correlations with SME Si concentrations (Table 2). Flower Si concentrations positively correlated with SME Si values, when Si was supplied as either of the two forms of $KSiO_3$, but not when Si was applied as rice husk ash. Silica deposition in plants is affected by such factors as tissue age, type and location, as well as, the plant species' inherent rates of Si uptake by the roots and overall evapotranspiration (Sangster et al., 2001). In our study, the highest Si deposition occurred in leaf tissues, which also likely have the highest transpiration rates.

Elemental nutrients in substrates (or soils) can correlate with those in accumulating leaves (Jaszczolt, 1978; Johansson, 1979; Csatho, 1998). But, in many situations, the tissue content of an elemental nutrient does not correlate with that present in the growth medium. Thus, tissue nutrient analysis is not always a satisfactory indicator of a nutrient's availability in a substrate (Ulicevic et al., 1976; Parra, 1971). For example, *Spartina anglica*, a salt marsh grass which accumulates high levels of Si, showed no correlation between Si levels accumulated in shoot tissues and Si concentrations of the sediment porewater in which it grew (de Bakker et al., 1999).

Overall, a positive correlation between leaf Si concentrations and SME Si concentrations existed for all three Si sources tested in this study. Therefore, leaf tissues are likely the most appropriate tissue to sample to determine the

availability of Si in a substrate and could be used to establish acceptable Si levels for soilless greenhouse floriculture.

ACKNOWLEDGMENTS

This study was supported in part by funding from Oklahoma Agricultural Experiment Station Projects 2534 (TJC) and 2536 (SMM), the HORTECUS Exchange Program (SK), and the American Floral Endowment.

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