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Effect of Gibberellic Acid on Silica Content and Distribution in Sugarcane[1, 2]

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ABSTRACT

The effect of gibberellic acid on the content and distribution of silicon in the stem, leaf sheath, and leaf lamina of sugarcane was analyzed in relation to the effect of gibberellic acid on stem growth. Silicon content was measured by neutron activation analysis, and its distribution was followed by scanning electron microscopy and X-ray analysis.

Foliarly applied gibberellic acid increased stem length and fresh weight and decreased silicon content. Gibberellic acid treatments had little or no effect on growth or silicon content of leaf laminae or sheaths. The close correlation between increase in growth of an internode in response to gibberellic acid and the decrease in silicon content of that internode indicated a dilution effect of growth on the amount of silicon rather than a direct effect of gibberellic acid on silicon deposition. This conclusion was supported by scanning electrom microscopy, X-ray map photos, and counts of silica cells per unit of epidermis area.

Large amounts of silicon are deposited in the cell walls of grasses (*Poaceae*), particularly in the walls of specialized epidermal cells of the shoot (11, 12). Greatest concentrations of silicon in sugarcane (*Saccharum* spp.) occur in the inner tangential walls of the root endodermis (15) and in the silica cells of the leaf and stem epidermis (2, 18, 19). The percentage of silicon in shoot tissue is relatively high in sugarcane and is known to vary with plant age (3), season (7), soil silicate content (8), and species or cultivar (2).

In sugarcane, low silicon has been associated with deficiency symptoms, primarily leaf freckling (4, 18, 19), reduced photosynthesis (18, 19), and reduced yields (5, 8). Silicate amendments to the soil decrease leaf freckling (4, 18, 19) and increase tissue silicon (7) and yields (5, 16). The association of increased tissue silicon with increased enzyme activities, photosynthetic rates, and sugar yields prompted some investigators to propose essentially biochemical roles for silicon in sugarcane metabolism (1, 18, 19). Other workers consider the precipitation of certain soil solutes to be injurious to the roots, and the shoots of sugarcane to be the primary site of action of silicon (5, 16).

GA_3 increases growth and yields but decreases the percentage of silicon in affected tissues (13). GA_3 also reduces the percentage of silicon in *Avena* internodes, probably as a consequence of the increase in cell volume (17). In contrast, Neumann and Jańossy (14) found that GA_3 significantly increased the silicon content of cell walls in d_1 dwarf mutant of corn (*Zea mays*). When silicon in GA_3-treated sugarcane is low, it is not known if the decrease is the result of dilution due to GA_3-stimulated growth, reduction in the number of silica cells per organ, or a reduction in the concentration of silicon per cell. The purpose of the experiments reported here was to determine the effect of GA_3-stimulated growth on the quantity of silicon in vegetative shoot organs (leaf laminae, leaf sheaths, and internodes) and on the distribution of silicon in the shoot epidermis of sugarcane.

MATERIALS AND METHODS

Plant Material. Tissue processed for silicon analyses was collected from sugarcane plants growing in an Ultisol soil containing about 50 μg/g extractable silicon. Six months before harvest, 4-month-old primary and secondary stalks were selected for uniformity in appearance and marked on the 10th internode below the leaf spindle. This reference mark served to locate tissues known to respond to GA_3.

Stalks were treated by pipetting a 0.5-ml aqueous solution containing 0.0, 1.0, 2.5, or 5.0 mg GA_3 into the leaf whorl surrounding the spindle. Six months after treatment, stalks were excised below the reference internode, trimmed of attached leaf blades, weighed, and measured. The 8th internode above the reference internode was excised between leaf scars of successive leaves, weighed, measured, and set aside for processing for silicon analysis. Leaves 12 and 14 ranks above the reference internode were used for neutron activation analysis for silicon, while the intervening leaf, 13, was used for x-ray SEM[4] analysis.

Four commercial cultivars known to differ in response to exogenously applied GA_3 were compared. Data were compiled as the means or composite values for three plants for each of the four treatments. For neutron activation analysis, equal amounts of tissue from each plant within a treatment were pooled and processed as a composite sample. For the x-ray SEM analysis, the three were processed separately to give a mean for each cultivar.

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[2] Silica refers to $SiO_2 \cdot nH_2O$ or silica gel, the deposition form of silicon (Si) in grasses and other living organisms which accumulate silica.

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[4] Abbreviation: SEM, scanning electron microscopy.

Neutron Activation Analysis for Silicon. The 12th and 14th leaves above internode 10 (numbers negative 2 and negative 4 at the time of GA_3 application) were divided into sheath and laminae (blades) samples. The middle 12-cm segments were excised, frozen in liquid N_2, lyophilized, and pulverized into a fine powder. For stem tissue, the 8th internode above internode 10 (number 3 at a time of GA_3 application) was used. Longitudinally excised stem rind segments were frozen, lyophilized, and ground. The powders were then packed into polyethylene capsules, weighed, and analyzed for silicon.

Details of sample handling and instrument conditions are published elsewhere (9). The samples were first irradiated in the University of Michigan 2MW pool-type Ford Nuclear Reactor for 5 min, and their induced γ ray radioactivity was measured by using an Ortec Model 475 Matching Multiplexer Unit. Data were recorded and reduced with a computer programmed to tabulate masses of samples and standards, irradiation time, and isotope half-lives. Results are presented as the mean percentage of dry weight from two analyses on the same sample.

SEM and X-Ray Analyses. From laminae, sheaths, and internodes of each sugarcane cultivar, dry tissue pieces, 1.0 × 0.5 cm, were cut with a sharp razor blade, mounted with Tube Koat (Television Tube Koat, GC Electronics, Rockford, IL) carbon paint on 2.54-cm diameter carbon stubs, and coated with a fine dispersion of carbon, about 500 Å thick. Carbon, rather than gold, is used to coat the specimens, because gold interferes with the concurrent x-ray analysis for silicon with a SEM. Specimens were examined with a SEM (JEOL model JSM-U3; Japan Electron Optics Laboratory Co., Ltd., Tokyo 196, Japan) at an accelerating voltage of 15 Kev. Silicon x-ray maps were obtained by coupling the SEM to an x-ray detector and amplifier system (KEVEX) and to a multichannel analyzer (Northern Scientific Model 710; Tracor Northern Scientific Co., Middleton, WI). Selected areas were first photographed in the secondary electron emission mode, then mapped by x-ray analysis for silicon. These basic methods, with the SEM for silicon x-ray analysis as applied to sugarcane, are also cited in detail elsewhere (10, 11). The silicon x-ray maps were used for analyzing silicon distribution patterns and for determining frequency of silica cells in the epidermises of leaf sheaths, leaf laminae, and internodes.

RESULTS

The sugarcane cultivars used in this investigation all increased in stalk length and internode length and decreased in stem internode silicon percentage in response to GA_3 applications. In the segment of stem elongating at the time of GA_3 application, there was a 2- to 3-fold increase in internodal length and fresh weight elicited by GA_3 treatment. The increased growth was accompanied by a significant reduction in the percentage of silicon (as much as 50%) (Table I). For all cultivars, the amounts of GA_3 applied produced a somewhat bell-shaped dose-response curve with maximum growth increases occurring with the 2.5-mg GA_3 per stalk treatment. The percentage of silicon varied inversely with growth (fresh weight) (Fig. 1).

The percentage of dry weight of silicon in sugarcane showed minor differences among cultivars but large differences among organs. The leaf lamina and the leaf sheath contained approximately equal amounts of silicon, whereas the internodes contained one-tenth as much silicon as did either the leaf blade or the leaf sheath (Table II). The percentage of silicon of leaf blade and leaf sheath tissues did not appear to be affected by GA_3 treatment, but that of the internode tissue did. In all treatments, there was a reduction in the percentage of silicon in internode 3 as a result of GA_3 application. The least amount of silicon was seen in all cultivars when they received an application of 2.5 mg GA_3 per stalk (Fig. 1). In comparing the amount of silicon in internode 3 among the cultivars, cv. H64-848 always had the greatest percentage for each treatment. Cultivar H58-4392 had the lowest percentage of initial silicon, and it was changed the least by GA_3 treatment. H59-3775 showed the greatest reduction in silicon in response to GA_3 application.

Silicon x-ray distribution maps and frequency counts (Table III) of silica cells in epidermal surfaces of leaf sheaths, laminae, and internodes indicated that, for all GA_3 treatments, the number of silica cells per unit epidermal surface signficantly decreased in the internode, actually increased in the leaf sheath (with the exception of the 5.0 mg/stalk GA_3 treatment), and did not show any large increase or decrease in the leaf lamina. The result with the internode is most likely due to the greater amount of lengthening that takes place in the long epidermal cells that separate the silica cells as a consequence of GA_3 treatment.

DISCUSSION

The internodes with GA_3-stimulated growth showed a lower percentage of silicon than did the control, nonstimulated internodes. The percentage of silicon was more closely correlated with internode fresh weight than with amount of GA_3 applied. A linear regression between the percentage of dry weight of silicon and the fresh weight of these internodes gave a negative correlation coefficient of $r = 0.79$. Thus, the observed reduction in percentage of silicon in internodes of GA_3-treated plants appeared to result from an inverse relationship between growth and silicon content rather than from any direct effect of GA_3 on silicon deposition. This conclusion is supported by the leaf silicon data. The leaf area of sugarcane was not altered by GA_3, and we were unable to measure any effect of GA_3 on silicon content of leaf sheaths or leaf laminae. That silicon content of leaf sheaths is unaltered by GA_3 treatment is significant: the support provided by the sheaths for the internodes elongating inside them is still maintained and is especially important for internodes whose lengths are significantly increased by GA_3 while their percentage of silicon is decreased.

The close correlation between increases in internode growth and decreases in percentage of silicon in response to GA_3 treat-

Table I. *Sugarcane Stalk Growth, Internode Growth, and Silicon Content in Response to Application of GA_3*

Each value is the mean of 12 measurements (4 cultivars × 3 stalks each) with 1 SD from the mean.

GA_3 Application	Whole Stalk		Internode 3[a]			
	Length	Fresh Weight	Length	Fresh Weight	Silicon	Silicon
mg/stalk	*cm*	*g*	*cm*	*g*	*%*	*mg/internode*
0.0	177 ± 20	1190 ± 193	7 ± 1	46 ± 9	0.086	796
1.0	215 ± 16	1300 ± 194	19 ± 3	101 ± 21	0.053	1060
2.5	238 ± 26	1710 ± 211	22 ± 3	135 ± 3	0.039	1070
5.0	212 ± 26	1480 ± 112	15 ± 4	105 ± 26	0.070	1460

[a] Internode 3 is the internode attached to the 3rd leaf below the spindle leaf.

Table II. *Percentage of Dry Weight of Silicon in Tissues of Sugarcane Plants Treated with GA_3*

The data are on Internode 3 and laminae and sheaths of leaves 12 and 13. Each value is the mean of 12 measurements (4 cultivars × 3 stalks each) with 1 SD from the mean.

Tissue	Silicon of GA_3-Treated Plants			
	0.0 mg GA_3/Stalk	1.0 mg GA_3/Stalk	2.5 mg GA_3/Stalk	5.0 mg GA_3/Stalk
	% dry wt			
Leaf lamina	0.965 ± 0.034	0.936 ± 0.034	0.914 ± 0.033	0.984 ± 0.032
Leaf sheath	1.006 ± 0.039	1.082 ± 0.041	0.906 ± 0.037	0.985 ± 0.037
Internode	0.086 ± 0.008	0.053 ± 0.009	0.039 ± 0.014	0.070 ± 0.012

Table III. *Frequency of Epidermal Silica Cells in Sugarcane Treated with GA_3*

The data are the mean values for four cultivars.

GA_3 Application	Mean No. Silica Cells[a]						
	Leaf Sheath		Leaf Lamina		Internode		
	Adaxial	Abaxial	Adaxial	Abaxial	Top	Middle	Bottom
mg/stalk	*per 9.0 cm²*						
0.0	0	29	16	13	36	43	45
1.0	0	42	21	18	24	14	29
2.5	0	51	24	15	20	10	27
5.0	0	12	18	15	29	20	31

[a] Mean of three counts made at random by three individuals.

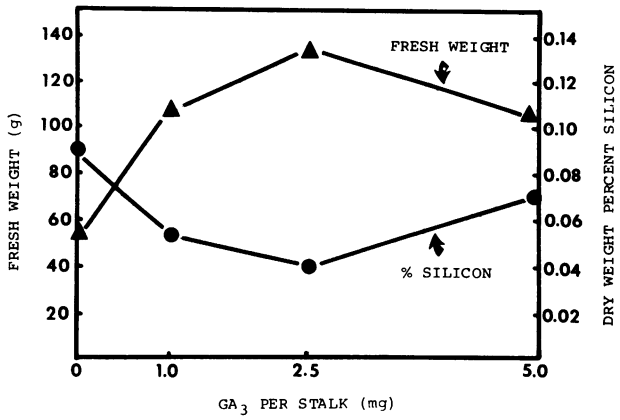

FIG. 1. Stalk internode 3 fresh weight and silicon content as a function of amount of GA_3 applied to the spindle leaves. Data points represent the mean of four cultivars.

ment indicated a dilution effect of growth on silicon content. A similar conclusion was reached by others who measured the effect of GA_3 on *Avena* silicon content (17) and is supported by the seasonal changes in growth and silicon content of sugarcane (3, 18, 19). Similarly, glyphosate decreased silicon content of *Agropyron repens* while increasing growth (6). These dilutions could result from a reduction in (a) the number of silica cells per unit area, (b) the number of silica cells per internode, (c) the amount of silicon per silica cell, or (d) a combination of these effects. Direct counts of silica cells in SEM x-ray map photos showed that GA_3 reduced the density of silica cells per unit area (Table III), thus supporting the first explanation.

Since tissue moisture was not affected by GA_3 treatment, fresh weights of internodes were converted to normal dry weights so that the amount of silicon per internode could be approximated from the percentage of dry weight of silicon in the internode samples. These calculations showed that the reduction in concentration of silicon in GA_3-enlarged internodes was not the result of a reduction in the total quantity of silicon per internode. On the contrary, there was a general increase in the amounts of silicon per internode as the internode size increased in response to GA_3 (Table I).

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