Effects of Foliar Combinations of Gibberellic Acid and Silicon on Sucrose Production by Sugarcane

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INTRODUCTION

Recent findings have shown that both gibberellic acid (GA) and silicon (Si) affect sucrose production by sugarcane. Workers in Australia[2] and Hawaii[3] found that GA treatment of sugarcane increased sugar yields. It is generally believed that by increasing internode elongation during early stages of growth, more stalk tissue is made available for sugar storage at a later period. Applications of Si-containing materials to cane fields have improved sugar yields in Hawaii (*11*)[4] and Mauritius (*15*), apparently by a suppression of manganese toxicity.

However, more recent studies in Puerto Rico suggest direct relationships of GA and Si with sucrose synthesis. GA, while unquestionably stimulating internode elongation, also caused sucrose increases per unit of leaf tissue[5] (*9*). Si was found to increase leaf sucrose while markedly affecting hydrolytic and oxidative enzymes (*2,13*). It was proposed that GA increased sucrose by stimulating both sugar-forming and growth-promoting reactions, while Si increased sucrose by retarding inversion and terminal oxidation reactions. This theory was strengthened when Si, added to nutrient solutions, greatly enhanced the sucrose-promoting effects of foliar GA.

The present study was initiated to explore further the combined effects of GA plus Si upon sugar synthesis and storage. It was felt that if Si were acting as an enzyme regulator, then it might be possible to achieve this effect with much smaller quantities than had previously been supplied via the roots. The massive Si storage areas of the stalk might be bypassed with foliar application. Furthermore, if the foliar treatment could be combined with GA, then both GA and Si effects might be achieved with a single, inexpensive foliar application. Thus there were four objectives of

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[2] Report of Gibberellic Acid Field Trials on Sugarcane, 1963–64. Personal communication with Dr. P. Robinson.

[3] Conference on Coordination of Basic Research Programs of Sugarcane Agriculture, David North Research Centre, Queensland, Australia. Personal communication with Dr. A. Vlitos.

[4] Italic numbers in parentheses refer to Literature Cited, pp. 225–6.

[5] Unpublished data.

the present experiments: 1, To reaffirm the sucrose-stimulating effects of GA and Si; 2, to determine whether sufficient Si can be absorbed via the foliage to accomplish its sugar-promoting role; 3, to determine whether Si can accomplish its functions when combined with GA in a single application; and 4, to evaluate growth and enzyme effects of combined GA plus Si.

MATERIALS AND METHODS

One-eye cuttings of the variety P.R. 980 were planted in an HCl-washed quartz sand, "silica shot", contained in glazed, 2-gallon pots with glass wool over the drainage outlets. All seedlings received daily 1 liter of a complete nutrient solution[6] plus about 1 liter of tapwater.

Foliar treatments were applied at 12 weeks. Three levels of GA and four levels of Si were given in a 3 x 4 fractorial design. GA was supplied as the 10-percent potassium salt in amounts equal to 0, 0.01-, and 0.10-percent solutions of the pure acid. Sodium metasilicate[7] was given in amounts equal to 0, 100, 1,000, and 10,000 p.p.m. of elemental Si. All combinations of GA and Si formed true solutions. There were no precipitations, although high Si required about an hour to acheive solution. Tween-20 was used as wetting agent at the rate of 1 ml./liter of solution. All above-sand portions of the plants were sprayed until runoff had begun.

A single harvest was made 5 weeks following treatment. Leaves +1 to +4 and immature storage tissue (meristem) were frozen in a mixture of Dry Ice and acetone, lyophilized, and ground to a fine powder in accordance with procedures described previously (*1*).

Clarified water extracts of the plant powder were analyzed for total ketose by the method of Roe (*12*), and for sucrose by the modification of Cardini, *et al.* (*10*). Fructose was estimated by subtracting sucrose values from those of total ketose. Protein was precipitated from water extracts with solid ammonium sulfate, as described earlier (*1*), and employed for enzyme assay without dialysis. Phosphatase and ATP-ase was measured by techniques described previously (*3*), as was amylase (*5*), invertase (*4*), polyphenol oxidase (*7*), and peroxidase (*6*). Protein content of the enzyme preparations was determined by the method of Sutherland *et al.* (*14*) and enzyme action was recorded as specific activity (activity units per milligram of protein).

[6] Nutrient concentrations, expressed as milliequivalents per liter, were provided as follows: Nitrate, 10; phosphate, 6; potassium, 5; calcium, 3; magnesium, 2, and sulfate, 2; microelements, expressed as parts per million, were given as follows: Boron, 0.05; copper, 0.02; manganese, 0.50; zinc, 0.05; molybdenum, 0.01, and iron, 1.0.

[7] $Na_2SiO_3 9H_2O$.

RESULTS AND DISCUSSION

GROWTH EFFECTS OF GA AND SI

Plants receiving 10,000 p.p.m. of Si began wilting within 8 hours of treatment and various degrees of foliar yellowing appeared during the first 3 days. A few plants died. The majority remained alive, but stunted throughout the study. Fresh weights recorded at the final harvest indicate that only the 10,000-p.p.m. Si treatment severely retarded growth (table 1). Fresh weights were slightly increased by medium GA, but this effect was not acheived by high GA. Curiously, medium GA was most effective in promoting growth in the presence of high Si, thereby greatly alleviating the apparent toxicity of excessive Si.

TABLE 1.—*Mean values for fresh weights of immature sugarcane treated with foliar combinations of gibberellic acid and silicon*[1]

GA (percent solution)	Fresh weight (g./plant)—				Mean
	Si_0	Si_{100}	$Si_{1,000}$	$Si_{10,000}$	
0	238	217	217	84	189
.01	252	245	189	161	212
.10	217	203	210	98	182
Mean	236	222	205	114	

[1] Each figure represents the computed mean of 4 replicates; 4 uniform plants were cut within each replicate.

GA AND SI EFFECTS ON SUGARS

Leaf sucrose content was increased both by GA and Si as main effects, and maximum sucrose was obtained with combinations of the two factors (table 2). In the absence of GA, Si at 100 and 1,000 p.p.m. increased sucrose, yet the greatest increase was gained with 100 p.p.m. Si combined with 0.10 percent GA.

Sucrose content of immature storage tissue (meristem) reflects the GA and Si effects even more clearly (table 2). Both GA and Si significantly increased sucrose in the absence of the other, but a combination of 100 p.p.m. Si and 0.10-percent GA gave by far the highest yield recorded. For both leaf and meristem tissues the 10,000-p.p.m. Si treatment greatly retarded sugar production. Sucrose variations generally reflected concurrent changes in total ketose synthesis. Leaf fructose was usually suppressed by high GA and high Si applications.

The results confirm that both GA and Si can increase sucrose production as independent entities, and that when combined they can further increase sucrose to levels unattainable by either factor acting alone. It is evident,

however, that the range of Si action is reduced by GA. More specifically, as much as 1,000 p.p.m. Si still increased sucrose production when GA was absent, but when GA was included the 1,000 p.p.m. Si treatment clearly suppressed sucrose in both tissues (table 2).

It is significant that relatively small amounts of Si were needed to raise sucrose levels. Actually, only about 220 ml. of the 100-p.p.m. Si solution was received per pot of plants, and that only once. This contrasts sharply with the previous study in which each pot received about 1,000 ml. of a

TABLE 2.—*Mean values for leaf and meristem sugars of immature sugarcane treated with foliar combinations of gibberellic acid and silicon*[1]

GA (percent solution)	Leaf sugars (mg./g. of dry weight)—														
	Total ketose					Sucrose					Fructose				
	0	100	1,000	10,000	Mean	0	100	1,000	10,000	Mean	0	100	1,000	10,000	Mean
0	88	104	108	48	87	52	62	81	29	56	35	43	26	19	31
.01	100	124	82	60	92	63	84	51	51	62	37	40	30	10	29
.10	78	119	79	32	77	64	90	46	46	56	15	28	30	11	21
Mean	89	116	89	47		59	79	59	59		29	37	29	13	
	Meristem sugars (mg./g. of dry weight)—														
	Total ketose					Sucrose					Fructose				
0	239	239	284	211	243	48	58	125	58	72	191	181	159	154	171
.01	272	306	229	205	253	70	155	56	49	82	202	151	173	156	171
.10	220	284	230	200	234	32	130	62	18	61	188	155	168	182	173
Mean	244	276	248	205		50	114	81	42		194	162	167	164	

[1] Each figure represents the computed mean of 4 replicates. Silicon treatments are expressed as parts per million.

500-p.p.m. Si solution each day for 5 weeks (*8*). It is suggested that only a small fraction of Si entering through the root system ever takes part in sugar-regulating reactions. Rather, most of it must be stored or utilized as skeletal material in stalks and sheaths, a function more or less bypassed by direct application to foliar areas of sugar synthesis.

GA AND SI EFFECTS UPON CANE ENZYMES

In general, enzyme behavior verified earlier observations (*8*) and supported the thesis that small amounts of Si act through enzymes to bring about sugar changes. In particular, the Si- and GA-induced sucrose accumulation in immature storage tissue can be attributed to invertase

suppression. It may be seen from table 3 that raising Si from zero to 100 p.p.m. generally retarded invertase activity, and that GA enhanced the Si effect. In fact, invertase values reflect clearly the sucrose variations noted in table 2. In the absence of GA, increasing Si to 1,000 p.p.m. continued to suppress invertase and this treatment increased sucrose. Yet in the presence of GA, 1,000 p.p.m. Si caused a revival of invertase, and sucrose content subsequently declined. Earlier work at this Station revealed invertase suppression by Si supplied in nutrient solutions (*2,13*). The present data not only verifiy earlier work, but also show that suf-

TABLE 3.—*Mean specific-activity values for meristem enzymes of immature sugarcane treated with foliar combinations of gibberellic acid and silicon*[1]

GA (percent solution)	Phosphatase					ATP-ase					Amylase				
	0	100	1,000	10,000	Mean	0	100	1,000	10,000	Mean	0	100	1,000	10,000	Mean
0	18	15	18	22	18	18	15	17	22	18	41	33	42	59	44
.01	15	14	21	17	17	15	15	22	17	17	34	32	46	43	39
.10	11	16	20	16	16	12	16	21	17	17	29	43	58	40	43
Mean	15	15	20	18		15	15	20	19		35	36	49	47	
	Invertase					Peroxidase					Polyphenoloxidase				
0	3.9	3.7	2.1	4.8	3.6	20	19	15	18	18	13	12	14	23	16
.01	6.2	2.2	5.4	4.0	5.5	15	18	17	22	18	9	12	20	20	15
.10	5.0	1.7	4.4	5.9	4.3	13	15	17	19	16	7	14	19	16	14
Mean	5.0	2.5	3.9	4.9		16	17	16	19		10	13	18	20	

[1] Each figure represents the computed mean of 4 replicates.

ficient Si can be introduced through the leaves to accomplish the enzyme inhibition.

Our present thinking holds that plants subjected to severe physiological stress, such as those receiving 10,000 p.p.m. Si in the present study, will make a major effort to replace destroyed or inactivated enzymes. Thus, in the absence of GA, 10,000 p.p.m. Si yielded an increased invertase content (table 3). The presence of GA may have increased the plants' biochemical potential and sensitivity so that they could respond more quickly to the Si stress, *i.e.*, at 1,000 rather than 10,000 p.p.m. Si. The 100-p.p.m. Si treatment was presumably sufficient to retard invertase without causing undue physiological "alarm" within the plant, and without triggering the synthesis of new invertase.

Earlier work has shown that leaf phosphatase, amylase, peroxidase, and

polyphenol oxidase can all be suppressed by Si given continuously via nutrient solution (*8*). These data led to the thesis that Si can cause a general slackening of sugar utilization. During the present investigations, high Si again succeeded in retarding leaf phosphatase, ATP-ase, and the oxidases (table 4). However, the Si treatment which most effectively increased sugar content was only 100 p.p.m., and this concentration did not greatly affect enzymes other than invertase. While it is possible that direct sugar synthesizing reactions were affected by Si, at the moment we would have to conclude that invertase was the primary system responsible for sucrose variations.

TABLE 4.—*Mean specific activity values for leaf enzymes of immature sugarcane treated with foliar combinations of gibberellic acid and silicon*[1]

GA (percent solution)	Phosphatase					ATP-ase					Amylase				
	0	100	1,000	10,000	Mean	0	100	1,000	10,000	Mean	0	100	1,000	10,000	Mean
0	8.0	9.1	7.4	7.5	8.0	10.3	10.7	9.7	8.7	9.8	31	37	26	48	35.5
.01	10.1	8.1	9.4	5.6	8.3	11.9	9.6	11.7	7.8	10.2	52	35	35	28	37.5
.10	8.1	8.5	9.3	5.7	7.9	9.6	9.7	10.9	6.7	9.2	39	36	45	42	40.5
Mean	8.7	8.6	8.7	6.3		10.6	10.0	10.8	7.7		41	36	35	39	
	Peroxidase					Polyphenol oxidase					Protein (mg./g. of dry weight)				
0	58	80	38	29	51	9.4	10.1	10.2	9.0	9.7	7.1	6.9	7.5	8.7	7.5
.01	58	50	45	19	43	10.4	9.2	13.2	7.0	10.0	6.3	7.9	6.3	8.9	7.3
.10	80	50	48	24	51	8.4	9.4	10.8	7.0	8.9	7.9	8.0	7.1	8.5	7.9
Mean	65	60	44	24		9.4	9.6	11.4	7.7		7.1	7.6	6.9	8.7	

[1] Each figure represents the computed mean of 4 replicates.

SUMMARY

Variable combinations of gibberellic acid (GA) and silicon (Si) were applied to the foliage of 12-week-old sugarcane grown in sand culture. GA levels were equivalent to 0, 0.01-, and 0.10-percent solutions of the pure acid, and Si levels were 0, 100, 1,000, and 10,000 p.p.m. of elemental Si. A 3 x 4 factorial design was employed with four replicates. There were four objectives: 1, To reconfirm the ability of both GA and Si to stimulate sucrose formation; 2, to determine whether sufficient Si could be absorbed by the leaves to accomplish its sucrose-promoting effect; 3, to determine whether GA and Si can accomplish these functions when combined in a single application; and 4, to evaluate growth and enzyme responses to combined GA plus Si.

A single harvest of leaf and immature storage tissue was taken for analysis 5 weeks after treatment. The following results were obtained:

1. Fresh-weight data showed that 10,000 p.p.m. of Si severely retarded growth. GA increased internode elongation and moderately increased fresh weight at the medium level. Si toxicity was partly alleviated by medium GA.

2. Sucrose content of leaf and immature storage tissues was increased by both GA and Si as main effects. Maximum sucrose was achieved with a combination of 0.01-percent GA plus 100 p.p.m. of Si.

3. Sucrose increases were partially a result of stimulated total ketose production. Leaf fructose was generally lowered by high GA and Si treatments.

4. Enzyme behavior verified earlier observations. Phosphatase, ATPase, peroxidase, and polyphenol oxidase were all retarded by high Si. However, sucrose increases appear to have been due almost entirely to invertase suppression by Si and GA.

5. Results confirm that both GA and Si can increase sucrose production and storage as independent entities, and when combined they can further increase sucrose to levels unattainable by either factor acting alone. Relatively small amounts of the two constituents are needed and these can be combined readily within a single foliar application.

RESUMEN

Se aplicaron combinaciones diferentes de ácido giberélico (AG) y de silicio (Si) a hojas de caña de azúcar de 12 semanas de edad sembrada en arena. Las concentraciones de AG eran equivalentes a soluciones del ácido puro al 0, 0.01 y 0.10 por ciento, y las de Si eran 0, 100, 1,000 y 10,000 partes por millón de Si elemental. El diseño utilizado fue un factorial de 3 x 4, con cuatro replicaciones. Fueron cuatro los objetivos: 1, Comprobar nuevamente la habilidad para estimular la formación de sacarosa, tanto del AG como del Si; 2, determinar si las hojas podían absorber el Si necesario para producir este estímulo en la producción de la sacarosa; 3, determinar si el AG y el Si pueden realizar estas funciones cuando se combinan en una sola aplicación; y 4, evaluar los efectos de una combinación del AG y del Si sobre el crecimiento y las enzimas.

Se cosechó sólo una vez para hacer el análisis de la hoja y del tejido reservante tierno, 5 semanas después del tratamiento. Se obtuvieron los resultados siguientes:

1. Datos obtenidos del peso húmedo señalaron que las 10,000 p.p.m. del Si retrasaron grandemente el crecimiento. La aplicación de AG aumentó el crecimiento de los entrenudos y también moderadamente el peso húmedo

en la concentración intermedia. Los efectos tóxicos del Si disminuyeron en parte con la concentración intermedia del AG.

2. Como efectos principales, se observaron aumentos en el contenido de sacarosa de la hoja y del tejido reservante tierno, tanto por el AG como por el Si. El contenido más alto de sacarosa se obtuvo mediante la aplicación de una combinación de AG al 0.01 por ciento y 100 p.p.m. de Si.

3. Los aumentos en la sacarosa fueron el resultado parcial de haberse estimulado la producción de cuetosas totales. La fructosa en la hoja disminuyó con los tratamientos altos del AG y del Si.

4. El comportamiento de las enzimas verificó las observaciones anteriores. La alta concentración de Si disminuyó la actividad de la fosfatasa, la ATPasa, la peroxidasa y la oxidasa de polifenol. Sin embargo, parece que los aumentos en la sacarosa se deben, casi totalmente, a la supresión de la invertasa por el Si y el AG.

5. Los resultados comprueban que el AG y el Si pueden aumentar la producción y las reservas de sacarosa independientemente uno del otro, y que cuando se combinan pueden aumentar la sacarosa hasta niveles inalcanzables por cualquiera de los dos factores independientemente. Sólo son necesarias cantidades relativamente pequeñas de ambos constituyentes, las cuales pueden combinarse fácilmente en una sola aplicación foliar.

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