Enzyme-Sugar Relationships in Immature Sugarcane Treated With Ascorbic Acid, Cysteine, Hydroxylamine, Cyanide, Silicon, and Iron

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INTRODUCTION

The behavior of cane oxidases and certain hydrolytic enzymes is greatly modified by *in vitro* exposure to chemical additives (*8,9,1,2,3*).[2] Much work has been done at this Station to determine whether such *in vitro* responses of basic catalysts can be duplicated in living plants (*4,5,6*). The most recent investigations are summarized in this paper.

Among chemicals studied were ascorbic acid, cysteine, hydroxylamine, and cyanide. Each of these is an effective oxidase inhibitor in the test tube. Silicon was included primarily to reaffirm its invertase- and oxidase-inhibitory roles (*18*). Application of these materials to the living cane was accomplished both as foliar sprays and nutrient-solution supplements.

MATERIALS AND METHODS

All plants were grown in the greenhouse using a sand-culture technique with controlled nutrient supply. One-eye cuttings were planted in glazed, 2-gallon pots containing quartz sand ("silica shot"). The sand was previously treated for 12 hours with 0.01 *N* HCl, and thoroughly leached with tapwater. Seedlings were watered daily until 3 weeks of age. Nutrient solutions were then applied and these were continued daily until harvest at about 12 weeks.[3]

Two groups of uniform plants were selected for treatment. The first set received silicon (Si) and cyanide (CN) as supplements to the nutrient solution, each at rates of 20 and 200 p.p.m. Iron (Fe) was provided at rates of 10 and 100 p.p.m. Additive sources were A.C.S.-grade sodium silicate, ferrous ammonium sulfate, and potassium cyanide. Control plants continued to receive the standard nutrient solution.

The second group was treated with foliar sprays at 11 weeks. Ascorbic acid, cysteine, hydroxylamine, and cyanide were applien by a Hudson

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 53–4.

[3] 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 supplied as follows: Boron, 0.05; copper, 0.02; manganese, 0.50; zinc, 0.05; molybdenum, 0.01; and iron, 1.0.

hand-sprayer with the nozzle adjusted for a fine-mist pattern. Tween-20 was added to facilitate coverage of all above-sand areas. Control plants received water plus Tween-20. Each experiment consisted of four replicates distributed within a randomized block design.

Plants receiving foliar sprays were harvested twice; once just prior to treatment, and again 3 days later. Only one harvest was made for the nutrition-supplement study. Both leaf and meristem samples were taken from four uniform plants of each replicate. These were frozen, lyophilized, extracted, and prepared for sugar and enzyme assay in accordance with procedures described earlier (*5,6*).

Hydrolytic and oxidative enzymes were assayed during the present study. Phosphatase activity was measured against β-glycerophosphate, ATP, and glucose-1-phosphate. Amylase and invertase were assayed using 1-percent solutions of soluble potato starch and sucrose, respectively. Tyrosinase (polyphenoloxidase) and peroxidase were also measured. Phosphatase procedures were described previously (*1*) as were those for invertase (*2*), amylase (*3*), tyrosinase (*9*), and peroxidase (*8*). Enzyme action was computed in terms of specific activity (units/milligram of protein). Protein was measured by the technique of Sutherland *et al.* (*19*).

Total ketose was measured by the resorcinol technique of Roe (*17*), and sucrose by the modification of Cardini *et al.* (*10*). Fructose was estimated by subtracting sucrose values from those of total ketose.

RESULTS AND DISCUSSION

EFFECTS OF SI, FE, AND CN AS NUTRIENT SUPPLEMENTS

The only plants showing visual effects of the nutrient-solution supplements were those receiving 200 p.p.m. of CN. These exhibited extreme stunting plus excessive yellowing and drying of older leaves. This condition was reflected by low total ketose and sucrose values in both leaf and meristem tissues (table 1). Fructose was increased in leaves by the high CN treatment. The low, or 20-p.p.m. CN treatment had no apparent effect on the young cane.

Both leaf and meristem enzymes appeared to be quite sensitive to the feeding-solution additives. Tyrosinase was 3 times more active in the stunted plants receiving high CN (table 2, item 7). It is interesting that tyrosinase should behave thus in the affected canes, for other workers with other plants have reported abnormally high oxidase activity among, or adjacent to infected tissues (*13,16*). Increased phenol content is even more widely recognized among infected plants (*11,12,14,15*), presumably as a function of general disease resistance. This might reflect a decrease in oxidase activity or synthesis. Tyrosinase action was suppressed in cane

TABLE 1.—*Mean values for leaf and meristem sugars of immature sugarcane supplied with variable silicon, iron, and cyanide in sand culture*[1]

Item No.	Treatment (p.p.m.)	Leaf sugars—			Meristem sugars—		
		Total ketose	Sucrose	Fructose	Total ketose	Sucrose	Fructose
1	Control	95.8	90.1	5.7	347	175	174
2	Si, 20	98.2	94.4	3.8	323	162	161
3	Si, 200	100.5	98.1	2.7	356	200	156
4	Fe, 10	98.2	94.3	4.1	330	169	161
5	Fe, 100	97.1	93.6	3.6	351	182	168
6	CN, 20	101.2	99.4	2.0	349	203	147
7	CN, 200	62.6	49.4	13.2	241	145	96
Mean		93.4	88.5	5.0	328	177	152

[1] Figures indicate milligrams per gram of dry weight.

TABLE 2.—*Mean specific-activity values for leaf and meristem enzymes of immature sugarcane supplied with variable silicon, iron, and cyanide in sand culture*

Item No.	Treatment (p.p.m.)	β-GP-ase	ATP-ase	G-1-P ase	β-Amylase	Peroxidase	Tyrosinase	Grand mean
Values for leaf enzymes								
1	Control	29.0	35.6	12.0	289	73.7	15.5	75.8
2	Si, 20	29.7	35.6	13.0	392	79.1	15.0	94.1
3	Si, 200	23.4	27.5	10.3	311	75.2	9.8	76.2
4	Fe, 10	23.8	26.3	8.8	247	63.1	12.2	63.5
5	Fe, 100	23.7	27.5	11.4	256	91.5	22.4	72.1
6	CN, 20	19.0	20.6	9.0	185	66.3	11.5	51.9
7	CN, 200	30.1	31.6	12.7	237	62.0	50.2	70.6
Mean		25.5	29.2	11.0	274	72.9	19.5	72.0

Values for meristem enzymes

								Invertase	Grand mean
8	Control	17.8	18.3	7.6	382	135	51.3	3.3	87.9
9	Si, 20	16.1	16.2	7.4	353	159	43.1	2.6	85.3
10	Si, 200	16.0	16.0	7.1	372	77	35.3	1.6	75.0
11	Fe, 10	13.7	12.7	5.1	303	97	36.6	1.9	67.1
12	Fe, 100	17.2	16.2	6.3	339	110	58.5	2.7	78.5
13	CN, 20	18.5	16.5	6.6	336	87	46.6	2.2	73.3
14	CN, 200	26.4	23.9	7.8	387	125	35.1	2.8	86.9
Mean		17.9	17.1	6.8	353	113	43.8	2.4	79.1

meristem by the high CN treatment (table 2, item 14). Perhaps abnormal oxidase activity is a characteristic of all plants under stress.

High Si (200 p.p.m.) suppressed tyrosinase activity in leaves, while both the 20- and 200-p.p.m. treatments lowered tyrosinase activity in meristem (table 2, items 3, 9, and 10). Invertase was far less active when either high or low Si levels were supplied. Both the tyrosinase and invertase inhibition by Si conform to earlier observations (*18*), and significance of this Si function is discussed in the earlier paper.

Iron at the rate of 10 p.p.m. suppressed each enzyme measured in both leaf and meristem samples (table 2, items 4 and 11). Meristem peroxidase and invertase were particularly affected, even though Fe is an essential component of the peroxidase system. We feel that the Fe effects are indirect, most likely dependent upon availability or content of other elements in the plant tissues. Earlier studies showed that invertase was suppressed by 6 p.p.m. of Fe supplied in sand culture, but only when molybdenum and calcium were in low supply (*5*). The same quantity of Fe stimulated invertase when molybdenum was high (1 p.p.m.).

EFFECTS OF FOLIAR ADDITIVES

Application of ascorbic acid, cysteine, and hydroxylamine to cane foliage had no appreciable effect on leaf and meristem sugar content. The high CN treatment (1,000 p.p.m.) caused increased sucrose in both tissues (table 3, item 12). None of the additives induced visible changes in the treated plants.

Each of the leaf enzymes assayed was somewhat suppressed by the high CN treatment (table 4, item 12). Again we feel that this is an indirect response. Only amylase and tyrosinase was severely affected by CN. Cysteine at both the 50- and 1,000-p.p.m. levels caused marked increases in leaf amylase activity (table 4, items 5 and 6). Although hydroxylamine and ascorbic acid are excellent oxidase inhibitors *in vitro* (*8,9*), neither peroxidase nor tyrosinase were appreciably affected by the foliar treatments. Ascorbic acid was probably oxidized[4] or metabolized, but the fate of hydroxylamine is totally obscure.

Meristem amylase and invertase were severely inhibited by the 1,000-p.p.m. CN treatment (table 5, item 12). This invertase suppression may account for the high meristem sucrose values associated with 1,000 p.p.m. CN (table 3, item 12).

All of the meristem enzymes assayed revealed some degree of activation by the 50-p.p.m. cysteine treatment (table 5, item 5). Only tyrosinase was previously known to be affected by cysteine and this only as strong *in vitro*

[4] Unpublished experiments in Puerto Rico have revealed ascorbic acid oxidase in cane-leaf preparations.

inhibition. The 1,000-p.p.m. cysteine application did, in fact, cause a slight suppression of tyrosinase, yet so great a concentration should not have been required. Furthermore, each of the other catalysts stimulated by low cysteine was also retarded by the high cysteine treatment.

TABLE 3.—*Mean values for leaf and meristem sugars of immature sugarcane treated by foliar application of ascorbic acid, cysteine, hydroxylamine, and cyanide*[1]

Item No.	Treatment (p.p.m.)	Values for leaf sugars			Values for meristem sugars		
		Total ketose	Sucrose	Fructose	Total ketose	Sucrose	Fructose
	Ascorbic acid						
1	Control	95.3	85.1	10.3	311	42.8	268
2	50	99.5	90.7	8.9	309	58.2	251
3	1,000	98.7	91.8	6.9	307	45.5	261
	Cysteine						
4	Control	95.8	92.6	3.3	299	39.9	259
5	50	95.3	91.1	4.2	291	29.6	262
6	1,000	95.2	89.8	5.4	287	26.4	261
	Hydroxylamine						
7	Control	93.7	85.4	8.3	269	39.3	230
8	50	92.6	80.5	12.1	241	37.8	203
9	1,000	84.9	72.5	12.4	251	32.1	219
	Cyanide						
10	Control	90.4	64.0	26.5	300	54.6	245
11	50	99.9	76.8	23.1	267	39.9	227
12	1,000	101.1	89.5	11.6	312	93.3	219
Mean		95.2	84.2	11.1	287	45.0	242

[1] Figures represent milligrams per gram of dry weight. Each figure represents the computed mean of 4 replicates.

SIGNIFICANCE OF ENZYME REGULATION IN SUGARCANE

The experiments presented above constitute our latest effort toward enzyme regulation in sugarcane. While it is believed that many desirable changes can be induced in cane by control of critical enzymes, these studies have been only partly successful.

Certain chemicals have been shown to cause major enzyme changes in cane tissues, but with few exceptions these could not have been anticipated from *in vitro* findings, and the enzyme changes have not usually produced desirable results. Only the phosphatase inhibitors, molybdenum and tungsten (*4,6*), and to a limited extent the amylase inhibitor, mercury (*7*), have induced predictable results when supplied to living plants. We feel that the principle of enzyme control is sound, but few useful applications

will be found until biologists have more thoroughly bridged the void between test-tube reactions and those of living organisms.

SUMMARY

Immature sugarcane was treated with chemical additives to determine whether significant and predictable changes could be induced in enzyme behavior. All plants were grown in sand culture with controlled nutrient supply.

One group received foliar application of ascorbic acid, cysteine, hydroxylamine and cyanide; the other group received silicon, iron, and cyanide as nutrient-solution supplements. Enzymes assayed included acid phosphatases, invertase, amylase, peroxidase, and tyrosinase (polyphenoloxidase). Each of the chemicals tested was known to affect one or more enzymes *in vitro*.

Plants receiving 1,000 p.p.m. of cyanide as a foliar spray increased sucrose in leaves and meristem within 3 days. All enzymes measured were suppressed by CN. Amylase was markedly stimulated by 50 and 1,000

TABLE 4.—*Mean specific-activity values for leaf enzymes of immature sugarcane treated by foliar application of ascorbic acid, cysteine, hydroxylamine, and cyanide*[1]

Item No.	Treatment (p.p.m.)	Values for leaf enzymes—						Grand mean
		β-GP-ase	ATP-ase	G-1-P ase	Amylase	Peroxidase	Tyrosinase	
	Ascorbic acid							
1	Control	29.8	32.1	13.4	151	320	8.1	92.4
2	50	24.8	26.8	12.9	162	306	6.8	89.9
3	1,000	25.7	27.7	12.5	146	304	7.2	87.2
	Cysteine							
4	Control	26.6	28.8	11.8	140	375	10.1	98.7
5	50	30.5	32.4	12.1	199	359	10.1	107.2
6	1,000	29.2	30.6	11.4	226	405	7.0	118.2
	Hydroxylamine							
7	Control	26.4	31.1	11.0	219	288	12.4	98.0
8	50	25.5	31.0	12.1	173	281	5.5	88.0
9	1,000	27.5	33.1	11.1	243	311	9.3	105.8
	Cyanide							
10	Control	37.1	43.1	11.8	209	78	6.9	64.3
11	50	34.6	39.0	11.3	191	76	4.9	59.5
12	1,000	30.7	35.1	9.5	143	62	3.2	47.3
Mean		29.0	32.6	11.7	200	264	7.6	89.0

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

p.p.m. of cysteine. All the enzymes assayed were moderately stimulated by 50 p.p.m. of cysteine, whereas 1,000 p.p.m. caused general suppression.

Plants receiving 200 p.p.m. of cyanide as a nutrient-solution supplement were greatly stunted and revealed low sugar content of leaf and meristem tissues. Tyrosinase was about 3 times more active in high-cyanide plants than in controls.

TABLE 5.—*Mean specific-activity values for meristem enzymes of immature sugarcane treated by foliar application of ascorbic acid, cysteine, hydroxylamine, and cyanide*[1]

Item No.	Treatment (p.p.m.)	Values for meristem enzymes—							Grand mean
		β-G-P-ase	ATP-ase	G-1-P ase	Amylase	Invertase	Peroxidase	Tyrosinase	
	Ascorbic acid								
1	Control	34.1	31.7	12.8	319	8.9	123	50.0	82.8
2	50	33.2	34.4	15.3	343	7.3	93	45.2	81.6
3	1,000	35.1	33.1	14.4	367	9.4	99	42.1	85.7
	Cysteine								
4	Control	37.4	35.1	13.7	302	13.6	114	44.2	80.0
5	50	51.1	45.2	19.3	495	17.6	137	54.3	68.3
6	1,000	34.8	32.5	13.1	370	14.3	76	36.1	82.4
	Hydroxylamine								
7	Control	26.0	26.9	8.0	269	9.3	86	33.9	65.6
8	50	20.4	21.4	7.3	220	6.0	61	26.9	51.9
9	1,000	26.0	25.3	9.1	281	7.1	93	37.5	68.4
	Cyanide								
10	Control	20.4	20.6	8.6	190	7.0	65	101	58.9
11	50	19.2	19.1	5.7	129	3.6	87	107	52.9
12	1,000	17.0	17.0	4.9	92	1.2	85	102	45.6
Mean		29.6	28.5	11.0	281	8.8	93	56.7	68.7

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

Silicon added to nutrient solutions at rates of 20 and 200 p.p.m. greatly retarded invertase and tyrosinase. This confirms similar observations recorded earlier, and it is suggested that enzyme inhibition is a physiological function of silicon in sugarcane. Iron added to nutrient solutions at the rate of 10 p.p.m. caused general enzyme suppression, particularly with regard to meristem peroxidase and invertase.

Significance of enzyme regulation in living cane is briefly discussed.

RESUMEN

Se trató caña de azúcar inmadura con aditivos químicos para determinar si es posible inducir cambios significativos que puedan predecirse respecto

a la acción de las enzimas. Todas las plantas se sembraron en arena, controlándose el suministro de nutrimentos.

A un grupo se le hizo aplicaciones foliares de ácido ascórbico, cisteína, hidroxilamina y cianuro; al otro se le suministró silicio, hierro y cianuro en forma de una solución de nutrimentos suplementarios. Las enzimas que se probaron fueron las fosfatasas ácidas, invertasa, amilasa, peroxidasa y tirosinasa (polifenoloxidasa). Se sabía que cada uno de los agentes químicos probados afectaba una o más de las enzimas *in vitro*.

Las plantas que recibieron 1,000 p.p.m. de cianuro en forma de aspersión foliar aumentaron el contenido de sacarosa en las hojas y el meristemo en un espacio de 3 días. Todas las enzimas que se calcularon fueron inhibidas por el CN. La amilasa fue marcadamente estimulada por 50 a 1,000 p.p.m. de cisteína. Todas las enzimas que se analizaron fueron estimuladas moderadamente por 50 p.p.m. de cisteína, mientras que 1,000 p.p.m. causó un efecto inhibitorio general.

Las plantas que recibieron 200 p.p.m. de cianuro en forma de solución suplementaria de nutrimentos apenas crecieron, revelando un bajo contenido de sacarosa en los tejidos de las hojas y el meristemo. La tirosinasa fue 3 veces más activa en las plantas que recibieron grandes cantidades de cianuro que en las plantas testigo.

El silicio añadido a las soluciones de nutrimentos a razón de 20 y 200 p.p.m. retardaron grandemente la acción de la invertasa y la tirosinasa. Esto confirma observaciones previas, y sugiere que la inhibición enzimática es una función fisiológica del silicio en la caña de azúcar. El hierro añadido a las soluciones de nutrimentos a razón de 10 p.p.m. causó una inhibición enzimática general, especialmente en la peroxidasa y la invertasa del meristemo.

Se discute brevemente lo que significa la regulación enzimática en la caña de azúcar.

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