Effects of Variable Calcium and Manganese on the Enzyme, Nucleotide, and Nutrient Constituents of Sugarcane Grown by Sand Culture

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INTRODUCTION

Until recently the roles of calcium (Ca)[2] and manganese (Mn) in sugarcane culture have been studied from the soils standpoint. Low available Ca and high Mn is normal for many acid tropical soils (*1*).[3] Lime applied to such soils usually suppresses uptake of Mn, plus iron and aluminum, by raising soil pH. Thus the application of Ca as lime to acid soils has increased the Ca content of cane, while the Mn content declined (*15,17*). A significant question is whether Ca itself can suppress Mn uptake independently of pH changes.

More difficult to define are the effects of Ca and Mn which involve more than changes in elemental content of the plant. Ca-deficient plants have been found to contain significantly more sucrose than those given adequate Ca in sand culture (*14,22*). Application of Ca-compounds to cane fields has led to greater sugar yields, apparently by increased tonnage rather than higher sucrose values (*15,20*). Recently the sucrose-hydrolyzing enzyme invertase was shown to be activated *in vitro* by Mn (*7*). Later work revealed that amylase also is activated by Mn, thereby confirming an unusually significant role of this element in the regulation of carbohydrate-hydrolyzing reactions (*8*).

It has been shown that acid phosphatases and invertases are markedly suppressed in cane by high Mn supplied in sand culture (*23*). The same study showed that tyrosinase, a probable terminal oxidase of cane, may be either retarded or activated by high Mn, depending upon concurrent supplies of Si. With regard to Ca, a Ca × Mo interaction was discovered which blocks the normal capacity of Mo to suppress phosphatases (*6*). As a consequence of high Ca, enzymes such as ATP-ase and glucose-1-phosphatase can more readily hydrolyze essential intermediates of sucrose synthesis. Such nutrient functions cannot be discussed in terms of elemental content since the quantities actually involved with enzymes are but a tiny fraction of the total absorbed.

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[2] Abbreviations: Molar, M; Uridine diphosphate glucose, UDPG; ultraviolet; U.V.; trichloroacetic acid, TCA.

[3] Italic numbers in parentheses refer to Literature Cited pp. 250–1.

We suspect, however, that Ca and Mn affect sucrose production by means other than nutrient balance and enzyme regulation. This belief was recently strengthened when Mn and Fe were found to alter the number plus the concentration of rare sugars in cane-leaf extracts[4]. It is also felt that nucleotide content might be a significant factor in determining sucrose potential. To this end, nucleotide studies are in progress here (*12,13*) and have been extended to the present investigations of Ca and Mn roles.

The work reported herein had three objectives: 1, To determine whether Ca could modify the rate of Mn uptake; 2, to determine the main effects and interactions of Ca and Mn on specific enzymes; and 3, to study the effects of variable Ca and Mn on the sugar and nucleotide content of sugarcane.

MATERIALS AND METHODS

GROWTH AND PREPARATION OF PLANT MATERIALS

All plants were grown in the greenhouse using HCl-washed silica shot in glazed, 2-gallon containers. One liter of a balanced nutrient solution[5] was supplied daily to each container until the plants were 16 weeks of age. Treatments were initiated at this time and continued for 14 weeks. Two levels of Ca and three of Mn were given in factorial combination. Calcium was provided at rates of 0 and 10 meq./liter, and Mn at 0, 10, and 100 p.p.m. The treatments were replicated three times in a completely randomized greenhouse design.

A single harvest was taken when the plants were 30 weeks of age. Leaves +1 to +4 were frozen, lyophilized, and ground to pass a 60-mesh screen. Enzyme and sugar extracts were prepared in accordance with methods previously described (*2*).

LABORATORY ANALYSES

Sucrose was determined in water extracts by the method of Cardini *et al.* (*16*). Phosphatase was assayed according to methods described earlier (*2*), as was amylase (*8*), peroxidase (*10*), and tyrosinase (*11*). Protein was measured colorimetrically by the technique of Sutherland *et al.* (*25*), and enzyme activity was then computed as specific activity, *i.e.*, activity units per milligram of protein.

Acid-soluble nucleotides were extracted from powdered leaf tissue with

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[4] Unpublished data.

[5] Nutrient concentrations, expressed as milliequivalents per liter, were provided as follows: Nitrate, 10; phosphate, 6; potassium, 5; calcium, 3; magnesium, 2; and sulfate, 2. Micronutrients, 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.

10-percent TCA at 22° C. Tissue debris was removed by centrifuge and washed with one bed-volume of 10-percent TCA. TCA was removed by repeated extraction with ethyl ether, the latter containing a small amount of water to retard peroxide formation. Residual ether was eliminated under reduced pressure at room temperature. The extract was then chromatographed on anion-exchange columns, employing the resin Dowex 1-X4, 50–100 mesh, prepared by the formate method of Hurlbert, et al. (19).

A 4-ml. sample was slowly washed on to the column with water and eluted with 1M sodium formate in 2N formic acid, pH 2.9, and 6N formic acid, pH 0.6, in that order. One-milliliter fractions were collected and appropriate dilutions were analyzed at 260 and 275 mμ with a Beckman Model DB spectrophotometer. Peak U.V.-absorbing fractions were combined and dried under reduced pressure, or frozen in thin layers and lyophilized. Samples of the U.V.-absorbing solutions were assayed for total reducing sugar by the dinitrosalicylic acid method of Summer (24), following a 100-minute hydrolysis in 6N HCl at 100°C. Total nucleotide content was estimated in terms of O.D.$_{260}$ units. A reference solution was prepared containing the monophosphates of adenosine, uridine, and cytidine, each at the rate of 0.01 μmole per milliliter. One O.D.$_{260}$ unit gave an O.D. reading of 0.22 at 260 mμ.

Paper chromatographic studies were conducted both with clarified leaf extracts and concentrated U.V.-absorbing samples. The descending technique was run in one dimension with Whatman No. 1 chromatography paper, employing the solvents ethanol-0.3M ammonium acetate, 2.5:1, or butanol-pyridine-water, 6:4:3. Ten to twenty λ (μliters) of cane preparations were usually spotted plus 2-λ volumes of 0.10-percent solutions of reference nucleotides. Dried chromatograms were scanned with a Chromato-Vue apparatus equipped with both long-wave and short-wave U.V. lamps. Sugars were located by staining with the silver nitrate dip technique (18).

RESULTS AND DISCUSSION

EFFECTS OF CA AND MN ON NUTRIENT UPTAKE

Leaf analyses for N, P, K, and Fe revealed no differences between treatments, and, as expected, Ca was significantly higher in plants receiving the higher Ca supply (table 1). To explain the fact that the Ca content did not vary greatly between the 0 and 10 meq./liter treatments, one must remember that all plants had received adequate Ca levels during their first 4 months of growth, and none was considered to be Ca-deficient. Manganese content increased with progressively higher Mn supply. More important is the fact that high Ca (10 meq./liter) enhanced Mn uptake,

giving almost 100 p.p.m. more Mn than the zero-Ca treatment (table 1). It therefore appears likely that lower Mn from soils is attributable to pH factors rather than the Ca of lime or silicon-bearing materials.

It is interesting to note that Si content was suppressed by high Mn (100 p.p.m.), and that this occurred only when Ca was low. Si suppression by Mn appears at a glance to be at variance with well-defined previous observations (23) which showed that increasing Mn supply caused significant Si increases in the leaf blade. We therefore feel that Ca is the critical factor which may have interfered with a Mn × Si relationship.

TABLE 1.—*Effects of variable calcium and manganese on the leaf-nutrient and silicon content of sugarcane grown in sand culture*[1]

Treatment— Ca meq./liter × Mn p.p.m.		N	P	K	Ca	Si	Mn	Fe
		Percent	Percent	Percent	Percent	percent	P.p.m.	P.p.m.
0	0	1.06	0.28	3.6	0.23	0.49	23	70
0	10	1.02	.26	3.6	.21	.42	135	66
0	100	1.08	.26	3.6	.21	.28	680	86
	Mean	1.05	0.27	3.6	0.22	0.40	279	74
10	0	1.02	0.25	3.4	0.33	0.51	135	88
10	10	.92	.27	3.4	.31	.32	156	85
10	100	1.10	.25	3.2	.30	.48	825	69
	Mean	1.01	0.26	3.3	0.31	0.44	372	81

[1] Each figure represents the computed mean of 3 replicates.

EFFECTS OF CA AND MN ON SUCROSE CONTENT AND ENZYME ACTIVITY

As anticipated from earlier work (13,21), plants receiving zero Ca bore the highest leaf-sucrose content (table 2). This was true when Mn was supplied at 0 or 10 p.p.m., but 100 p.p.m. Mn eliminated the sucrose differences. High Mn accomplished this by suppressing sucrose to a level comparable to that obtained with high Ca. Since high Mn did not increase Ca content (table 1) we suspect that Mn has substituted for high Ca participating in reactions less directly related to sugar formation.

In order to study more closely the leaf-sugar constituents, extracts were chromatographed and stained. Figure 1,A illustrates chromatographed samples from one complete Ca × Mn replicate. Clearly evident for each treatment are masses of glucose and fructose, with sucrose obviously varying in response to Ca and Mn. Trace amounts of the trisaccharide raffinose (glucose-glucose-fructose) appear in each extract. No evidence

of melezitose (glucose-fructose-glucose) was found, nor was ribose or UDPG present in any preparation.

The enzymes amylase and peroxidase were strikingly affected by varying Ca and Mn levels (table 2, and Fig. 1,B). Mn suppressed amylase, but

TABLE 2.—*Mean values for leaf-sucrose content and enzyme activity of sugarcane supplied with variable calcium and manganese in sand culture*[1]

	Sucrose				Amylase			
Treatment	Mn_0[2]	Mn_{10}	Mn_{100}	Mean	Mn_0	Mn_{10}	Mn_{100}	Mean
Ca_0	103	117	84	101	45.3	37.9	23.4	35.5
Ca_{10}	73	79	84	79	19.6	20.4	22.4	20.9
Mean	88	98	84		32.4	29.1	23.1	

	Peroxidase				Tyrosinase			
Treatment	Mn_0	Mn_{10}	Mn_{100}	Mean	Mn_0	Mn_{10}	Mn_{100}	Mean
Ca_0	47.8	42.9	50.0	46.9	10.2	10.2	9.2	9.9
Ca_{10}	84.7	83.7	66.0	78.1	11.6	11.7	10.7	11.3
Mean	66.2	63.3	58.0		10.9	10.9	10.0	

	Phosphatase			
Treatment	Mn_0	Mn_{10}	Mn_{100}	Mean
Ca_0	7.3	5.9	4.9	6.0
Ca_{10}	4.5	5.5	4.6	4.8
Mean	5.9	5.7	4.8	

[1] Each value represents the computed mean of 3 replicates. Sucrose is recorded as milligrams per gram of dry weight, and enzymes are expressed as specific activity (activity units per milligram of protein).

[2] The numerals 0, 10, and 100 with Mn indicate treatments of 0, 10, and 100 p.p.m., respectively. With Ca, the numbers 0 and 10 signify treatments of 0 and 10 meq./liter, respectively.

only when Ca was low. Since high Mn also suppressed sucrose at low-Ca levels, these findings add support to the theory that maximum sucrose formation requires a highly active amylase for destruction of polysaccharides (*4,5,8,9*).

It is probable that Ca inhibition of amylase at low and medium Mn

levels may represent a protective casing of the enzyme by Ca, with subsequent minimization of potential activator-inhibitor effects. Again, although 100 p.p.m. Mn is unusually high for nutrient solutions, it is evident that high Mn produces a strong calciumlike effect upon amylase
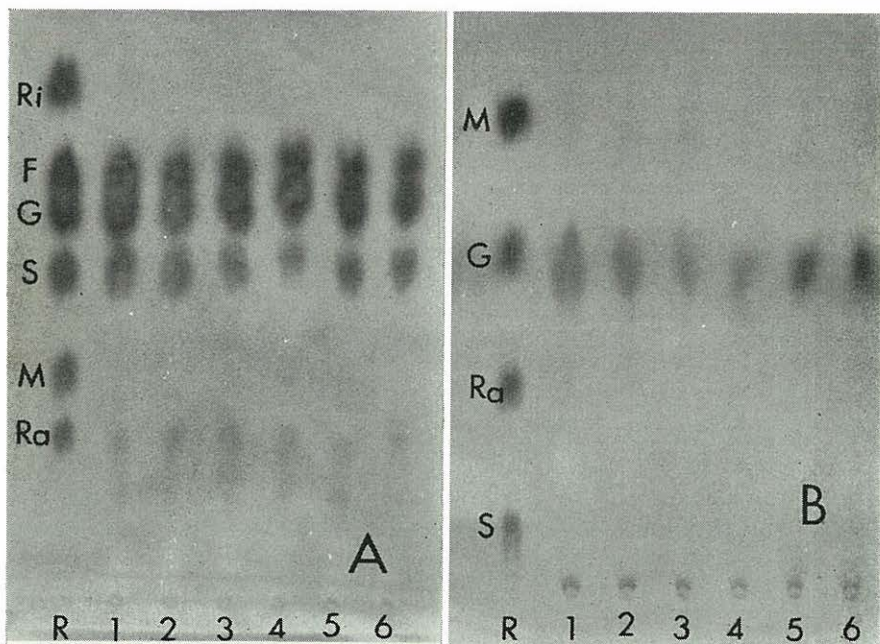

FIG. 1.—A, Paper chromatogram illustrating sugar of cane-leaf extracts derived from plants supplied with variable Ca (meq./liter) and Mn (p.p.m.) in sand culture. Numerals 1 through 6 represent the following treatments: 1, $Ca_0 \times Mn_0$; 2, $Ca_0 \times Mn_{10}$; 3, $Ca_0 \times Mn_{100}$; 4, $Ca_{10} \times Mn_0$; 5, $Ca_{10} \times Mn_{10}$; and 6, $Ca_{10} \times Mn_{100}$; letters signify the following reference sugars: Ri = ribose; F = fructose; G = glucose; S = sucrose; M = melezitose; and Ra = raffinose. B, Paper chromatogram illustrating variable glucose produced by action of amylase from plants receiving Ca and Mn in sand culture; numerals represent the same treatments listed above; the letters M and S represent maltose and stachyose, respectively; soluble potato starch was employed as substrate.

without increasing Ca content. An enzyme such as amylase might therefore provide a link between Ca and Mn content on one hand and sucrose content on the other.

Peroxidase was very active in high-Ca plants, as compared with those from which Ca was withheld (table 2). High Mn moderately retarded peroxidase at the high-Ca level, which verifies a similar Mn effect noted at

different levels of silicon (23). The high peroxidase action in tissues containing less sucrose also verifies earlier observations (2,3), although *in vitro* peroxidase experiments showed a moderate suppression of the enzyme by Ca (10). The relationship between peroxidase action and sucrose level remains obscure. It is suggested that this catalyst serves as a terminal oxidase in cane (2), and that it may take part in regulation of auxin level (23).

Phosphatase was inhibited by increasing Mn at the zero-Ca level, but Mn had no consistent effect when Ca was high. The enzyme, acting upon β-glycerophosphate, was already retarded by high Ca, so that Mn in effect induced a high-Ca type of response. This again was accomplished without altering Ca content.

EFFECTS OF CA AND MN ON NUCLEOTIDE CONSTITUENTS

Four of the six Ca $\times$ Mn treatments were selected for nucleotide analysis: $Ca_0 \times Mn_0$; $Ca_0 \times Mn_{100}$; $Ca_{10} \times Mn_0$; and $Ca_{10} \times Mn_{100}$. Samples were taken for TCA extraction from composites of the three replicates.

Extracts passed through Dowex-1 formate invariably yielded two U.V.-absorbing peaks with pH 2.9 formic acid-sodium formate, and a third peak when the column received $6N$ formic acid, pH 0.6. Analyses of peak fractions for each of the four treatments are summarized by table 3. As anticipated, most of the reducing sugar was retained in the initial U.V.-absorbing peak for each sample. By far the greatest amount was yielded by the high Ca $\times$ high Mn sample.

Phosphorus was concentrated in the second peak of each sample, with the low Ca $\times$ low Mn treatment predominating. It is important to note that P content of every third peak, that representing each treatment's highest nucleotide concentration, was more than twice as high in the low-Ca samples than in the high-Ca samples. Although table 1 indicates that total leaf P was not affected by Ca supply, the P from U.V.-absorbing effluents includes that retained by sugar phosphates or nucleotides before acid hydrolysis. Some of these are precursors of sucrose, such as fructose-6-phosphate, glucose-1-phosphate, UDPG, etc. Their vast biochemical importance underscores a serious limitation of the total P data of plant tissues, which, in itself, tells nothing of the form assumed by P after absorption. We would now suspect that Ca has some influence over the interconversion of organic and inorganic P.

Dried chromatograms of concentrated U.V.-absorbing fractions revealed several unknown compounds when scanned with U.V. lamps. Figure 2,A illustrates fluorescent areas derived by $6N$ formic acid elution of each Ca $\times$ Mn treatment sample. Of interest is the apparently low concentration of fluorescent materials from the low Ca $\times$ low Mn samples. One of the materials is absent entirely from this treatment. Attempts to identify

the unknown compounds were not successful. On the basis of earlier meristem studies we had anticipated adenosine, uridine, cytidine, and uracil (*12*), but Rf. values for leaf constituents gave no more than tentative identification of adenosine, uridine, and cytidine.

Figure 2,B presents the two initial U.V.-absorbing peaks obtained from each treatment sample and chromatographed with a series of known com-

TABLE 3.—*Analytical data for peak U.V.-absorbing effluent fractions obtained by anion-exchange chromatography of sugarcane leaf preparations*[1]

Treatment		U.V. Peak No.	Data classification—			
Ca × (meq./liter)	Mn (p.p.m.)		Total reducing sugar[2]	Total P[3]	Total nucleotide[4]	$\dfrac{E_{275}}{E_{260}}$
0	0	1	6.00	40	3.2	0.86
		2	1.14	680	3.6	.88
		3	1.18	104	63.2	1.04
0	100	1	[5]	[5]	2.7	.83
		2	[5]	[5]	4.1	.78
		3	1.14	98	66.0	1.03
10	0	1	2.88	60	2.2	.80
		2	1.18	480	4.2	.78
		3	1.16	44	53.6	1.03
10	100	1	11.50	50	2.7	.67
		2	1.58	420	4.1	.69
		3	1.06	43	63.0	1.03

[1] The sugarcane had been supplied with variable Ca and Mn in sand culture. All analyses were conducted with the milliliter giving maximum absorption at 260 mμ within each peak.

[2] Milligrams/milliliter.

[3] Milligrams × 10^3 per milliliter.

[4] O.D.$_{260}$ units.

[5] Data not available.

pounds. Samples from each of the three illustrated treatments contained a pair of strongly fluorescent compounds which almost superimposed upon one another. Of particular interest was a third fluorescing substance of varying spot size and mobility (spots a, b, and c). Component a, least mobile and most difficult of the three to detect, was produced in the low-Ca × low-Mn plants, whereas component c, extracted from high Ca × high-Mn plants was highly mobile and presented a large, vivid spot. Very likely a single compound, perhaps a free base, is represented in varying combination with Ca or Mn, or in combination with unknown products

of the different nutritional regimes. The compounds seemingly superimposed are apparently the monophosphates of adenosine, cytidine, or glucose. The mobile compound (a,b,c) is possibly glucoseamine.
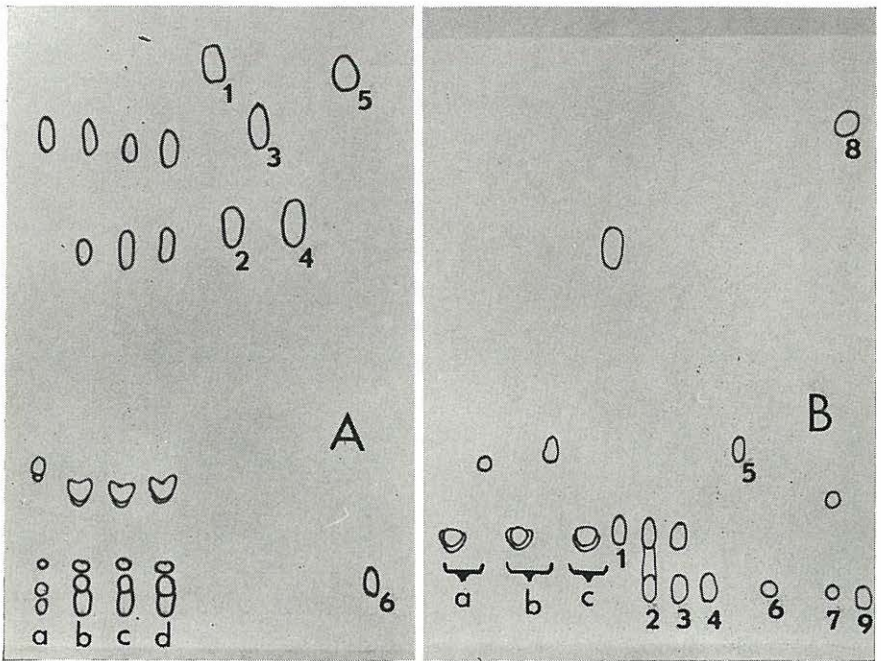

Fig. 2.—A, Paper chromatogram illustrating size and position of fluorescent spots which represent U.V.-absorbing materials of cane leaves; the plants had been subjected to variable Ca (meq./liter) and Mn (p.p.m.) in sand culture; small letters stand for $6N$ formic acid elutions from anion-exchange columns, with treatments represented as follows: a, $Ca_0 \times Mn_0$; b, $Ca_0 \times Mn_{100}$; c, $Ca_{10} \times Mn_0$; and d, $Ca_{10} \times Mn_{100}$. Numbers signify the following reference compounds: 1 = uridine; 2 = adenosine; 3 = cytidine; 4 = uracil; 5 = xanthine; and 6 = UDPG. B, Paper chromatogram illustrating fluorescent compounds obtained from cane leaves; each small letter stands for the initial pair of U.V.-absorbing peaks obtained with formic acid-sodium formate from anion-exchange columns, with treatments represented as follows: a, $Ca_0 \times Mn_0$; b, $Ca_{10} \times Mn_0$; and c, $Ca_{10} \times Mn_{100}$. Numbers signify the following reference compounds: 1 = adenosine monophosphate; 2 = cytidine monophosphate; 3 = glucose monophosphate; 4 = uridine monophosphate; 5 = glucoseamine; 6 = deoxyribose nucleic acid; 7 = flavin adenine dinucleotide; 8 = xanthine; and 9 = diphosphopyridine nucleotide.

Sugar components of peak U.V.-absorbing fractions were chromatographed with butanol-pyridine-water, dried, and stained. Figure 3 illustrates a typical chromatogram of sugars obtained from resin columns with

formic acid-sodium formate, plus several reference sugars commonly found in plants. From two to six distinct compounds were obviously present within each of the peak fractions. Several features are immediately evident, including at least two sugars present in the low-Ca × low-Mn peaks (No. 1) which are absent from the high-Ca × low-Mn peaks (No. 2). Considerably more sugar was present in the low-Ca sample as evidenced
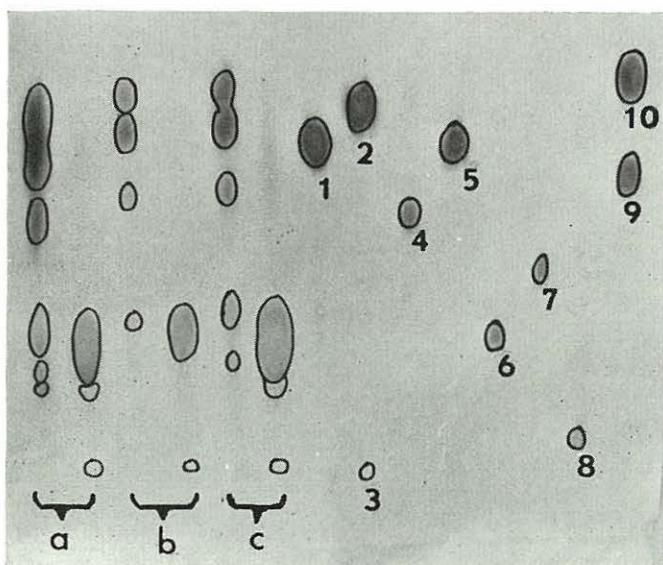

Fig. 3.—Paper chromatogram illustrating sugar constituents of U.V.-absorbing preparations from sugarcane supplied with variable Ca and Mn in sand culture. Each small letter represents the initial pair (peak Nos. 1 and 2) of U.V.-absorbing peaks eluted from anion-exchange columns with formic acid-sodium formate; treatments are as follows: a, $Ca_0 \times Mn_0$; b, $Ca_{10} \times Mn_0$; and $Ca_{10} \times Mn_{100}$. Numerals signify the following reference sugars: 1 = Glucoseamine; 2 = maltose; 3 = UDPG; 4 = glucose; 5 = galactose; 6 = raffinose; 7 = melezitose; 8 = stachyose; 9 = turanose; and 10 = fructose.

by the relatively broad and heavy stains. Yet when both Ca and Mn were high (No. 3), one of the deleted sugars reappeared in conjunction with generally increased sugar content.

Specific identification was difficult, but the sugars glucose, maltose, and raffinose appeared in all treatment samples. In addition, peak fractions of sample No. 1 (low Ca × low Mn) apparently include glucoseamine and turanose. Two sugars appeared in effluents derived by $6N$ formic acid elution (not shown), one of which was identified as raffinose. The full significance of the sugar and nucleotide variations is not yet understood.

Undoubtedly some of these play important roles in sucrose production. In any event it is important to recognize the capacity for Ca and Mn to alter their number and content.

SUMMARY

Immature sugarcane was subjected to variable calcium (Ca) and manganese (Mn) in sand culture. Two levels of Ca were provided in factorial combination with three levels of Mn. Leaf samples were harvested for analysis after 3 months of treatment. There were three objectives: 1, To determine whether Ca could modify the rate of Mn uptake; 2, to determine main effects and interactions of Ca and Mn on specific enzymes; and 3, to study Ca and Mn effects on sugar and nucleotide content of leaves. The following results were obtained:

1. High Ca, 10 meq./liter, enhanced Mn uptake rather than cause Mn suppression.

2. Silicon content was decreased by high Mn (100 p.p.m.), but only when Ca was low. Ca thus is able to interfere with the Mn $\times$ Si interaction by which increasing Mn supply decreases Si uptake.

3. Leaf sucrose content was generally higher among plants receiving low Ca. This verifies observations by other workers.

4. High Mn appeared to substitute for high Ca in its influence upon sucrose content and amylase activity. A high-Ca effect upon amylase is induced by Mn without increasing Ca content.

5. High Mn greatly retarded amylase action when Ca supply was low, but had no effect when Ca supply was high.

6. Peroxidase action was relatively high among plants containing less sucrose, verifying earlier observations at this laboratory. High Ca greatly stimulated the enzyme.

7. Content of acid-soluble nucleotides did not vary greatly among treatments. Reducing sugar and total phosphorus content of nucleotide preparations varied strikingly. Sugar content was greatest in preparations from plants receiving both high Ca and high Mn. More than twice as much phosphorus was found in hydrolyzed, low-Ca nucleotide preparations than was present in those of plants receiving high Ca. Ca thus appears to affect the interconversion of organic and inorganic phosphorus.

8. Both Ca and Mn affected the number, concentration, and paper chromatographic properties of unknown fluorescing constituents obtained by anion-exchange chromatography. Adenosine, uridine, cytidine, and glucoseamine were tentatively identified.

9. Paper chromatograms revealed that the sugars glucose, maltose, and raffinose appeared in all peak U.V.-absorbing fractions. Glucoseamine and

turanose were apparently present only in plants receiving both low Ca and low Mn.

Results of this study suggest that Ca interacts with Mn in mineral absorption and enzyme effects, that Ca can alter known Mn × Si relationships, and that the consequences of variable Ca and Mn content are felt by the plant over a broad range of biochemical reactions.

RESUMEN

Plantas inmaduras de caña de azúcar cultivadas en arena se sometieron a tratamientos variables de calcio (Ca) y manganeso (Mn). Se usaron dos niveles de Ca en un tratamiento combinado con tres niveles de Mn. Después de 3 meses de tratamiento se tomaron muestras de las hojas para analizarse. Tres fueron los propósitos del análisis: 1, Determinar si el Ca podía modificar el ritmo de absorción del Mn por la planta; 2, determinar los efectos e interacciones principales del Ca y el Mn sobre ciertas enzimas específicas; y 3, estudiar los efectos del Ca y el Mn sobre el contenido de azúcares y nucleótidos en la hoja. Los resultados fueron los siguientes:

1. Un alto nivel de Ca (10 miliequivalentes por litro) aceleró la absorción del Mn más bien que suprimirla.

2. El contenido de silicio disminuyó al aplicarse un alto nivel de Mn (100 partes por millón), pero sólo cuando el nivel de Ca era bajo. De modo que el Ca puede interponerse en una interacción de Mn × Si ya establecida en la que al aumentarse el Mn disminuye la absorción del Si.

3. El contenido de azúcar en la hoja generalmente aumentó en las plantas que recibieron poco Ca. Esto confirma las observaciones hechas por otros investigadores.

4. Un alto nivel de Mn aparentemente puede sustituir un alto nivel de Ca en cuanto a su efecto sobre el contenido de la sacarosa y la actividad de la amilasa. El Mn produce el mismo efecto de un alto nivel de Ca sobre la amilasa sin aumentar el contenido de Ca.

5. El Mn aplicado a un alto nivel demoró grandemente la acción de la amilasa cuando el suministro de Ca fue bajo, pero no tuvo efecto alguno cuando el suministro de Ca fue alto.

6. La acción de la peroxidasa fue relativamente intensa en las plantas que contenían poca sacarosa, confirmando las observaciones previas hechas en este laboratorio. El Ca a niveles altos estimuló grandemente la enzima.

7. El contenido de nucleótidos ácidosolubles no varió marcadamente entre los tratamientos. Los azúcares reductores y el contenido total de fósforo en preparaciones de nucleótidos variaron notablemente.

El contenido de azúcar fue mayor en las preparaciones de aquellas plantas que recibieron altos niveles de Ca y Mn. En las preparaciones

hidrolizadas de nucleótidos, bajas en calcio, se encontró más del doble de la cantidad de fósforo que en las plantas que recibieron un alto nivel de Ca. El Ca aparentemente afecta la interconversión de fósforo orgánico e inorgánico.

8. Tanto el Ca como el Mn afectaron el número, la concentración y las propiedades en papel cromatográfico de constituyentes fluorescentes desconocidos que se obtuvieron mediante la cromatografía de intercambio aniónico. La adenosina, la uridina, la citidina y la glucosamina se identificaron tentativamente.

Cromatogramas en papel revelaron que azúcares tales como la glucosa, maltosa y rafinosa aparecieron en todos los puntos cimeros de las fracciones de absorción ultravioleta. Aparentemente la glucosamina y la turanosa sólo se encontraban en las plantas que recibieron tratamientos bajos en Ca y Mn.

Los resultados de este estudio sugieren que existe una interacción entre el Ca y el Mn, en la absorción de minerales y en su efecto sobre las enzimas, que el Ca puede alterar las relaciones conocidas del Mn × Si, y que las consecuencias que puedan resultar de los contenidos variables de Ca y Mn se manifiestan en la planta en una amplia serie de reacciones bioquímicas.

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