Research Note

DIURNAL BEHAVIOR OF SUGARCANE ACID INVERTASE[1]

Unpublished data from the sugarcane variety P.R. 980 (*S. officinarum* × *S. spontaneum* × *S. sinense*) have shown large diurnal activity variations for the acid invertase localized in immature storage tissue. Although the data could not be analyzed statistically, they were consistent with the findings of Slack[2] in Queensland using the variety Pindar (*S. officinarum* × *S. spontaneum*). Rapid turnover of this enzyme could have decisive physiological importance owing to its roles in sugar storage,[3] sugar remobilization,[4] and growth processes.[5, 6] Moreover, its site of action, as a metabolic sink, is increasingly implicated in feedback control of source reactions,[7, 8] including photosynthesis. For these reasons supplemental experiments were performed for statistical analysis. In addition, diurnal acid invertase behavior was examined in pure *S. spontaneum* clones.

Three experiments were performed in the greenhouse using immature sugarcane propagated in sand culture with water and nutrient supplies held constant, as previously described.[9] P.R. 980 was used in each instance. In addition, clones of *S. spontaneum* (U.S. 56-14-4, U.S. 56-19-1, SES 84-A, and Djatiroto) were used in experiment 3. Immature storage tissue samples were harvested initially at 7:00 or 8:00 a.m. and at intervals of 3 to 4 hours thereafter. Tissues were either quick-frozen and lyophilized prior to ex-

[1] Manuscript submitted to the Editorial Board June 1, 1972.

[2] Slack, C. R., The physiology of sugarcane. VIII. Diurnal fluctuations in the activity of soluble invertase in elongating internodes, Aust. J. Biol. Sci. 18: 781–88, 1965.

[3] Glasziou, K. T., Accumulation and transformation of sugars in stalks of sugarcane. Origin of glucose and fructose in the inner space, Plant Physiol. 36 (2): 175–9, 1961.

[4] Hatch, M. D., Sacher, J. A., and Glasziou, K. T., Sugar accumulation cycle in sugarcane. I. Studies on enzymes of the cycle, Plant Physiol. 38 (3): 338–43, 1964.

[5] Hatch, M. D., and Glasziou, K. T., Sugar accumulation cycle in sugarcane. II. Relationship of invertase activity to sugar content and growth rate in storage tissue of plants grown in controlled environments, Plant Physiol. 38: 344–8, 1963.

[6] Glasziou, K. T., and Bull, T. A., The relation between total invertase activity and internode expansion in sugarcane stalks, Proc. Int. Soc. Sugar Cane Technol. 12: 575–81, 1967.

[7] Beevers, H., Metabolic sinks. Chap. 8 in: Physiological Aspects of Crop Yield, Am. Soc. Agron., Madison, Wisc., 1969.

[8] Neales, T. F., and Incoll, L. D., The control of leaf photosynthesis rate by level of assimilate concentration in the leaf. A review of the hypothesis, Bot. Rev. 34: 107–25, 1968.

[9] Alexander, A. G., and Montalvo-Zapata, R., Evaluation of chemical ripeners for sugarcane having constant nitrogen and water regimes, Part I, Tropical Agr. (in press).

traction,[9] or harvested fresh and blended immediately in distilled water chilled to about 2° C. Invertase preparation and assay methods are detailed elsewhere.[10] Briefly, the assay is based upon the quantity of reducing sugar released enzymically in a standard sucrose solution in one hour at 37° C and pH 5.5 (acetate buffer 0.05M). Invertase product was computed on the basis of fresh tissue weight (experiment 1), lyophilized tissue weight

TABLE 1.—*Diurnal acid invertase activity in sugarcane immature storage tissue. Variety P.R. 980* (S. officinarum × S. spontaneum × S. sinense)

Hour	Experiment 1	Hour	Experiment 2	
	Mg. product/g. fr. wt./hr.		Mg. product/g. dry wt./hr.	
			Greenhouse	Darkroom
8:00 a.m.	1.88 a*	7:00 a.m.	28.4 a	27.9 a
11:00 a.m.	2.82 b	11:00 a.m.	30.2 a	29.3 a
2:00 p.m.	1.10 c	3:00 p.m.	16.4 b	25.4 a
5:00 p.m.	0.72 c	7:00 p.m.	10.1 c	13.0 b
		11:00 p.m.	17.9 bd	8.1 bc
		3:00 a.m.	21.7 d	5.2 c

* Mean values in the same column bearing unlike letters vary significantly ($P < .05$).

TABLE 2.—*Diurnal behavior of acid invertase in* Saccharum *spp. immature storage tissue*

Saccharum clones	Mg. product mg. protein/hr., at hour—					
	8:00 a.m.	10:00 a.m.	12:00 noon	2:00 p.m.	4:00 p.m.	6:00 p.m.
S. spontaneum (Average of 4 clones)	1.51 a*	2.26 a	2.77 a	1.75 a	1.40 a	1.22 a
P.R. 980 (Interspecific)**	0.55 b	0.55 b	0.76 b	0.68 b	0.51 b	0.33 b

* Mean values in the same column bearing unlike letters vary significantly ($P < .05$).

** *S. officinarum* × *S. spontaneum* × *S. sinense*.

(experiment 2) and as mg. product per mg. of protein (experiment 3). Randomized block designs with three replicates of each treatment were used throughout the study. Data were submitted to statistical analyses in accordance with Student's *T* test or the Duncan New Multiple Range test.

Results from experiment 1, performed with natural illumination, showed significantly higher invertase activity at 11:00 a.m. than at 8:00 a.m., 2:00 p.m. and 5:00 p.m. (table 1). Lowest activity was at 5:00 p.m. In ex-

[10] Alexander, A. G., Hydrolytic proteins of sugarcane: The acid invertases, J. Agr. Univ. P.R. 49 (3): 287-307, 1965.

periment 2, naturally illuminated plants showed highest activity at 11:00 a.m. and lowest at 7:00 p.m. The two values differed significantly by a factor of 3. Additional samples taken at 11:00 p.m. and 3:00 a.m. revealed a significant nocturnal trend toward invertase recovery. On the other hand, plants excluded from light by transfer to a darkroom continued to lose invertase activity throughout the night (table 1). Nocturnal recovery was therefore not a function of darkness but of a normal day-night illumination sequence. The third diurnal experiment included invertase from pure *S. spontaneum* clones as well as P.R. 980 (table 2). In each instance highest activity was recorded at 12:00 noon and lowest at 6:00 p.m. The extreme levels differed by a factor of 2 to 3, even though absolute activity was in the order of 2.5 to 4 times higher in the *S. spontaneum* forms.

It is concluded that major activity changes occur on a diurnal basis and are not artifacts of sampling or analytical procedure, or of a single *Saccharum* genotype. Maximal and minimal periods range approximately from 11:00 to 12:00 a.m., and from 5:00 to 7:00 p.m., respectively.

Alex G. Alexander
Rafael Montalvo-Zapata
María G. Justiniano
Agronomy and Soils Department