

EFFECT OF SPENTWASH AS FERTI-IRRIGATION ON THE ENZYMES OF SUCROSE METABOLISM IN SUGARCANE

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Enzymes of sucrose metabolism *viz.* sucrose synthase (SS), sucrose phosphate synthase (SPS) and invertase were studied in the leaves of sugarcane (*Saccharum officinarum* L.) var. 'Co 7527' under the influence of spentwash as ferti-irrigation with respect to crop age. The activity of SS increased with increasing concentrations of spentwash while higher concentration (1,500 ppm BOD) lowered the activity by 1.42% and 4.16%, respectively, in 9 and 12 months old crop. SPS exhibited higher activity in 9 months old sugarcane with stimulatory effect at 1,000 and 1,500 ppm BOD treatment while invertase showed higher activity in 9 months old crop and declined at 12 months by exhibiting stimulatory effect at 1,000 ppm BOD. The elevation in SPS/invertase ratio at higher concentration of spentwash treatment can be attributed to the contributory factor for maintenance of high sucrose level. The study indicated that use of spentwash up to 1,500 ppm BOD as ferti-irrigation does not affect sucrose metabolism.

Key words: enzymes, spentwash treatment, sucrose metabolism, sugarcane

INTRODUCTION

Utilisation of industrial effluent after proper treatment in agriculture has raised the hope of recycling the effluent in constructive way (Vaisman *et al.* 1981, Hegde and Patil 1983). The concept of ferti-irrigation using spentwash is advocated very recently for the disposal of distillery waste after the recovery of biogas. Thus, the land application of spentwash offers twin benefits of water pollution control and utilisation for agricultural production. Large area under sugarcane cultivation in Maharashtra has erected many sugar factories, which produce substantial amount of spentwash. Sugarcane being a major cash crop of Maharashtra its productivity is mainly confined to sucrose synthesis which is governed by the enzymes sucrose synthase, sucrose phosphate synthase while invertase cause its breakdown. In the present investigation therefore, an attempt has been made to score the activity of these enzymes under the influence of spentwash with respect to crop age.

MATERIAL AND METHODS

The seed sets with three eye buds of sugarcane (var. 'Co 7527') were pretreated with Bavistin to avoid fungal infection and space planted in an experimental plots (80 m × 7 m) supplied with farm yard manure and recommended doses of NPK. The sprouted buds were allowed to grow for one month by irrigating with river water. The plots were then irrigated with spent-wash having BOD values 100, 500, 1,000 and 1,500 ppm at an interval of ten days and continued up to the maturity of cane growth. The ferti-irrigation was given with gradual increase in BOD level to avoid shock treatment. The control plot was irrigated with river water.

The enzymes *viz.* sucrose synthase (UDP-D-Glucose: D-Fructose-2-Glucosyl-transferase EC 2.4.1.13), sucrose phosphate synthase (UDP-D-Glucose: Fructose-6-phosphate-2-Glucosyl transferase EC 2.4.1.14) and invertase (O-B-D fructo-turanocide-fructo hydrolyse EC 3.2.1.26) were extracted from the sugarcane leaves by the method of Nomura *et al.* (1969) with slight modification to suite our laboratory conditions.

Enzyme extraction and assay

One g fresh and cleaned leaf tissue was homogenised in 10 ml 10 mM tris-HCl buffer (pH 7.5) containing 10 mM 2-mercaptoethanol and 50 mg polyvinyl pyrrolidone (PVP M.wt 40,000). The slurry was filtered through 4 layered muslin cloth and centrifuged at 10,000 g for 10 min using refrigerated centrifuge. The supernatant was passed through Sephadex G-25 column (6 × 1 cm) pre-equilibrated with extraction medium. The eluted extract was used as an enzyme source. The activity of sucrose synthase and sucrose phosphate synthase was assayed by the method of Snell and Snell (1961) while invertase by the method of Nelson (1944). The enzyme protein was estimated using Folin-phenol reagent (Lowry *et al.* 1951).

RESULTS AND DISCUSSION

The values of enzyme activity studied in the leaves of sugarcane under the influence of spentwash treatment at 6, 9 and 12 months crop age are given in Table 1. The sucrose synthase (SS) activity was increased with increasing concentrations of spentwash while higher concentration (1,500 ppm BOD) lowered the activity by 1.42% and 4.16%, respectively, at 9 and 12 months crop age (Table 1a). Further, the elevation of SS with respect to crop age showed gradual increase in the activity both in spentwash treated and non-treated plants.

The enzyme sucrose phosphate synthase (SPS) exhibited higher activity at 9 months crop age than that of 6 and 12 months. However, with increasing concentrations of spentwash it showed variation in the activity (Table 1b). Though the SPS was reduced in 100 and 500 ppm BOD treated plants of 6 months old, it was stimulated by 20.58% in 1,000 ppm BOD level of spentwash irrigation and even the higher concentration (1,500 ppm) did not exhibit any detrimental effect on SPS as compared to that of control. At 9 months crop age the SPS was stimulated by 8.66% and 5.69% in 1,000 and 1,500 ppm BOD, respectively, and reduced by 25.86% in 100 ppm and 12.6% in 500 ppm BOD treatment. More or less similar trend was noticed at 12 months crop age. The age-wise screening of SPS showed considerable decline at maturity of the crop obviously because the sugarcane possess a source sink relationship and the amount of sucrose synthesised was transported to internodal tissue and no more sucrose synthesis takes place in the matured leaf tissue. Even at maturity if sucrose tends to accumulate more in non-photosynthetic internodal tissue, it

Table 1

Effect of different concentrations of spentwash used as ferti-irrigation on enzymes of sucrose metabolism with respect to age in sugarcane (var. 'Co 7527')

Spentwash treatment BOD (ppm)	a: sucrose synthase		
	Crop age (months)		
	6	9	12
Control	0.51	0.70	0.72
100	0.56	0.75	0.77
500	0.58	0.80	0.83
1000	0.62	0.86	0.89
1500	0.55	0.69	0.69
	b: sucrose phosphate synthase		
Control	0.34	1.16	0.25
100	0.25	0.86	0.20
500	0.27	1.02	0.23
1000	0.41	1.27	0.29
1500	0.34	1.23	0.27
	c: invertase		
Control	0.11	0.24	0.05
100	0.10	0.21	0.05
500	0.11	0.23	0.05
1000	0.13	0.28	0.06
1500	0.11	0.23	0.05

The values are expressed in mg sucrose mg⁻¹ protein h⁻¹ and are mean of three determinations

can be attributed to the presence of SPS in internodal tissue. As such, the localisation of this enzyme not only confined to photosynthetic tissue but also occur in non-photosynthetic tissue actively engaged in sucrose biosynthesis (Huber and Huber 1996). Recently Frederik *et al.* (2000) reported that SPS remain at least three times higher than SS in sugarcane internodal tissue. In the present investigation we observed stimulatory effect of spentwash on SS and SPS activity in the photosynthetic leaf tissue which contribute towards the transport of sucrose from source to sink.

The enzyme invertase exhibited higher activity at 9 months crop age and declined sharply at 12 months with having stimulatory effect of spentwash treatment at 1,000 ppm BOD (Table 1c).

Overall stimulation in the activity of enzymes of sucrose metabolism can be attributed to more uptake of mono and divalent cations like Na^+ , K^+ , Mg^{2+} , Mn^{2+} and Ca^{2+} from the spentwash used as a ferti-irrigation for growing sugarcane (Bhosale 2000). As such Prasolova *et al.* (1976) noted that bivalent cations *viz.* Mg^{2+} , Mn^{2+} and Ca^{2+} stimulate the activity of SS. Work of Salerno and Pontis (1978) also indicated the regulatory effects of inorganic cations and anions on the activity of SPS. They noted that divalent cations Mg^{2+} and Mn^{2+} stimulate the activity of SPS. The findings of Slabnik *et al.* (1968) and Nomura and Akazawa (1974) supported the above observations.

The SPS/invertase ratio provides some sort of budgetary status of sucrose metabolism and was maintained even at higher concentration of spentwash (Table 2). This observation led us to conclude that the higher synthetic/hydrolysing enzyme ratio contribute for maintaining high sucrose level and under the light of this it can be said that spentwash treatment up to 1,500 ppm BOD can safely be used as ferti-irrigation for growing sugarcane.

Table 2

Ratio of sucrose phosphate synthase/invertase in sugarcane (var. 'Co 7527') under the influence of spentwash treatment

Spentwash treatment BOD (ppm)	Sucrose phosphate synthase/invertase ratio		
	Crop age (months)		
	6	9	12
Control	3.09	4.83	5.20
100	2.50	4.09	3.70
500	2.45	4.43	4.60
1000	3.15	4.53	4.46
1500	3.09	5.34	5.40

*

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