Seasonal Changes in Photosynthetic Enzyme Activity in Relation to Malate Content of Sugarcane Leaf

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This paper describes the seasonal changes in weight and malate content of two varieties of sugarcane, ‘N:CO 310’ (N:CO) and ‘Chikusha’ in relation to malate dehydrogenase, phosphoenolpyruvate carboxylase, phosphoenolpyruvate carboxykinase and malic enzyme activity. The stalk weight of ‘N:CO’ is apt to be higher than that of ‘Chikusha’. Since the difference could be attributed to photosynthetic and malic acid enzymes, these enzymes were analyzed in the two varieties during the growing period. The highest malic acid content in the basal leaf of ‘N:CO’ was shown in August, whereas that of ‘Chikusha’ was in July. The phosphoenolpyruvate carboxylase (PEPC) and phosphoenolpyruvate carboxykinase (PEPCK) in the middle and bottom leaves of ‘N:CO’ showed higher activities than those of ‘Chikusha’ in August and the malate dehydrogenase (MDH) and malic enzyme (ME) in the middle leaf of ‘N:CO’ showed higher activities than those of ‘Chikusha’ in August. The difference in weight of the two varieties might depend on these enzyme activities during the growing period.

Introduction

Two varieties of sugarcane, Chikusha and N:CO 310 have been used as the raw material for making the traditional commercial sugar, "Wasanbon". ‘Chikusha’ has higher amino acid content and grows faster but weighs less than N:CO 3101-4. In general, sugarcanes which belong to C-4 group of plants are known to be superior to C-3 plants in photosynthetic ability. The difference in weight in the two va-
rieties is thought to depend on the differences in photosynthetic ability. The seasonal changes in photosynthesis and TCA-cycle in relation to malate and fresh weight were investigated. Phosphoenolpyruvate carboxylase (PEPC) acts on CO₂ entering the stomata. The enzyme is localized in chloroplasts of the mesophyll cells and it produces oxaloacetate. The oxaloacetate is reduced to malate by malate dehydrogenase (MDH). C-4 plants utilize malate as a carrier of CO₂. NADP-malic enzyme catalyzes the formation of CO₂ from malate and it is localized in the bundle sheath chloroplasts. Phosphoenolpyruvate carboxykinase (PEPCK) in mitochondria catalyses the formation of phosphoenolpyruvate from oxaloacetate.

Materials and Methods

Materials

Sugarcane varieties Chikusha and N:CO were harvested from the field of Kagawa University located in Nagao, Kagawa Prefecture. The fertilization and planting operations are described in the previous paper(4). Samples were collected on the 25th of each month from May to November and the 10th of December. Three sugarcanes of each variety were harvested at random every month.

Methods

Enzyme extraction: Two g of a costa-removed leaf was cut into fine pieces and ground in a cooled mortar and pestle with 10 ml of 0.1 M Tris-HCl buffer (pH 7.5) containing 10 mM L-cystein and 2 g of sea sand. The resulting homogenate was filtered through 4 layers of cotton cloth and the filtrate was centrifuged at 10,000 × g for 10 min. The supernatant was filled up to 10 ml with 10 mM of the same buffer (pH 7.5) and the mixture was used as the source of crude enzyme after passing through Sephadex G-25. Two ml of the enzyme solution was applied to a Sephadex G-25 fine column (1.5 i.d. × 15 cm) which had been equilibrated with 10 mM Tris-HCl buffer (pH 7.5). The column was eluted with the same buffer at a flow rate of 12 ml per h. Each 2 ml fraction was collected and was monitored by quantitative absorbance at 280 nm for protein. The fractions combined from tubes 3 and 4 were used for enzyme assays and investigations of their properties.

Enzyme assay (Malate dehydrogenase): The formation of malate was assayed spectrophotometrically by measuring the decrease in NADH at 340 nm, pH 7.5 and 30 °C(5) after a 3 min preliminary incubation. The assay mixture contained 50 mM potassium phosphate buffer (pH 7.5), 10 mM NADH, 5 mM oxaloacetate and 2 µl crude enzyme. Distilled water was used in the blank experiment instead of oxaloacetate and the other conditions were the same as described above.

(Phosphoenolpyruvate carboxylase): The formation of oxaloacetate was measured by coupling with added malate dehydrogenase(6). The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.5), 20 mM NaHCO₃, 2 mM MgCl₂, 0.25 mM phosphoenolpyruvate, 0.1 mM NADH, 1 mM mercaptoethanol, 2 units malate dehydrogenase and 0.2 ml of crude enzyme. Distilled water was used in the blank instead of phosphoenolpyruvate. PEPC activity was measured by following the rate of NADH oxidation, monitored at 340 nm and 30 °C.
PEPCK activity was measured by coupling with added malate dehydrogenase\(^7\). The PEPCK assay was carried out in the same way as PEPC except that 2 mM ADP was added to the reaction mixture. Soluble protein was determined according to the method of Lowry \textit{et al.}\(^8\) using bovine serum albumin as the standard. MDH, and PEPC and PEPCK activities were expressed as the amount of malate and oxaloacetate, respectively, liberated per min per mg of protein.

\textit{(Malic enzyme)}: Malate incorporation of NaH\(^{14}\)CO\(_3\) into acid-stable products was determined at 30 °C\(^9\). The reaction mixture contained 100 mM Tris-HCl buffer (pH 7.5), 25 mM NaHCO\(_3\), 8 mM MgCl\(_2\), 20 mM pyruvate, 2 mM mercaptoethanol, 0.15 mM NADPH, 2 \(\mu\)Ci NaH\(^{14}\)CO\(_3\) (20 \(\mu\)Ci/100 \(\mu\)l) and crude enzyme. After 10 min incubation, the reaction was stopped by adding 0.5 ml of 1.5 N HCl and incubation was continued for 90 min at 30 °C. Radioactivity of the reaction mixture was determined by a liquid scintillation counter. The activity was expressed as \(\mu\)mol CO\(_2\) incorporation per min per mg of protein.

\textit{Determination of malic acid by gas chromatography (G.C.)}. Sample preparation and G.C. condition were carried out in a similar manner as in the previous paper\(^3\).

\textbf{Results and Discussion}

\textit{Properties of malate dehydrogenase (MDH), phosphoenolpyruvate carboxylase(PEPC), and phosphoenolpyruvate carboxykinase (PEPCK)} in sugarcane leaves: The optimum pH for MDH in ‘Chikusha’ was 6.2, 6.4 and 8.0 when measured in glycine, Tris-HCl and potassium phosphate buffer, respectively, whereas that in ‘N:CO’ was 6.2, 7.0 and 8.0. The optimum temperature for MDH in two varieties was 45 °C in potassium phosphate buffer. The optimum pH for PEPC in ‘Chikusha’ was 7.0, 7.5 and 8.0 when measured in glycine, Tris-HCl and potassium phosphate buffer, respectively, whereas that in ‘N:CO’ was 8.5, 7.5 and 6.5. The optimum temperature for PEPC in two varieties was 40 °C in potassium phosphate buffer. The optimum pH for PEPCK in ‘Chikusha’ was 6.5, 7.5 and 8.0 when measured in glycine, Tris-HCl and potassium phosphate buffer, respectively, whereas that in ‘N:CO’ was 7.0, 7.5 and 7.5. The optimum temperature for PEPCK in the two varieties was 40 °C. The Km value for MDH, PEPC and PEPCK in the two varieties was 5.0, 12.5 and 4.0 mM, respectively.

\textit{Seasonal changes in MDH, PEPC and ME activities in leaves}

\textit{Malate dehydrogenase (MDH)}: The activities of MDH in the leaves taken from three positions in the plant are shown in Fig. 1. In ‘Chikusha’, the activities of MDH increased in the three leaves until August and then decreased. The MDH activity in the top leaf was about 2.5 times as high as that in the basal leaf in August and it was higher than that of the middle leaves (significantly different at 1% level when calculated similarly to the previous paper\(^{10}\)). The profile of the MDH activity in ‘Chikusha’, was almost the same as that in ‘N:CO’ but there were no significant differences in the MDH activities in the three leaves of ‘N:CO’ harvested in August. The MDH activity in the leaves of ‘N:CO’ was about 1.7 times as high as that of ‘Chikusha’ in August. No data in the basal leaf for ‘Chikusha’ were obtained because leaves fall off in December.
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Fig. 1. Seasonal changes in malate dehydrogenase activity in sugarcane leaves cv. Chikusha and N:CO. □: top part, ■: middle part, ●: basal part. Values are means with S.E. (n=3).

Fig. 2. Seasonal changes in phosphoenolpyruvate carboxykinase activity in the leaves of two sugarcane varieties. Symbols as shown in Fig. 1.

**Phosphoenolpyruvate carboxylase (PEPC)**: The top and middle leaves of ‘Chikusha’ showed highest PEPC activity in August and the basal in September, whereas all three leaves in ‘N:CO’ showed highest activity in August. The PEPC activity in the leaves of ‘N:CO’ was about 1.7 times that of ‘Chikusha’ in August (Fig. 2). The phenomenon may be explained as follows: CO₂ entering the leaf of ‘N:CO’ was used more effectively than in ‘Chikusha’. ‘N:CO’ showed higher growing rate than ‘Chikusha’.

**Phosphoenolpyruvate carboxykinase (PEPCK)**: The highest PEPCK activity in ‘Chikusha’ was observed in the top and middle leaves in August and in the basal in September, whereas that in ‘N:CO’ was observed in the three leaves in August. The PEPCK activity in the leaves for ‘N:CO’ was about 1.5 times as high as that of ‘Chikusha’ in August (Fig. 3). The PEPCK activity was apt to be higher in ‘N:CO’ than in ‘Chikusha’ but there were no significant differences in the MDH, PEPC and ME activities between the two varieties.

**Malic enzyme (ME)**: The highest ME activity in ‘Chikusha’ was observed in the middle and basal leaves in August and the top in September, whereas that in ‘N:CO’ was observed in the top and basal leaves in September and in the middle of August.

No MDH, PEPC, PEPCK and ME activities were found in the stalk and root of sugarcane.

**Seasonal changes in weight and malic acid content of sugarcane leaves**: Fig. 4 and 5 show the seasonal changes in leaf weight and malic acid content of sugarcane leaves, respectively. Leaf weight increased rapidly in July to October in both varieties. In ‘Chikusha’ it increased in the middle and basal leaves in September and then decreased. In ‘N:CO’ it reached the highest value in November. The top and middle leaves of ‘N:CO’ weighed about two times that of ‘Chikusha’ and the basal leaf about 1.5 times. The stalk weight of ‘N:CO’ was about two times as large as that of ‘Chikusha’ at the harvest period. Fig. 6 shows the seasonal changes in malic acid content of sugarcane leaf. The highest malic acid content of the top
leaf of ‘Chikusha’ was observed in August and in the basal and middle in July, while in ‘N:CO’ it was observed in August in the middle and basal leaves and in July in the top leaf. Although the malic acid content in the two varieties was almost the same at harvest period, the leaf weight of ‘N:CO’ was higher than that of ‘Chikusha’. Therefore, the difference in weight of the two varieties is due to the differences in MDH, PEPC, PEPCK and ME activities. MDH, PEPC and PEPCK activities in the top and middle leaves of the two varieties peaked in August. As temperature and daylength during the developing period incre-
ased, photosynthesis and organic metabolism in the leaves appeared to increase. In general, the stalk weight of 'N:CO' is higher than that of 'Chikusha' and the growth rate of the stalk was faster during August and October (1-4). The PEPC and PEPCK activities in the middle and basal leaves of 'N:CO' were higher than those of 'Chikusha' and the MDH and ME activities in the same leaf were also higher than those of 'Chikusha' (significant difference at 5% level). It may be concluded that the difference in weight of the two varieties depends on these enzyme activities.

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References


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