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FRUCTOSE 2,6-BISPHOSPHATE-MODULATED PHOTOSYNTHESIS IN SORGHUM LEAVES GROWN UNDER LOW WATER REGIMES

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Key Word Index—*Sorghum bicolor*; Gramineae; leaves; photosynthesis; fructose 2,6-bisphosphate; water-stress.

Abstract—The influence of low water potentials on photosynthetic sucrose formation, fructose 2,6-bisphosphate (F2,6BP) content, partitioning of photosynthate and related enzymes was investigated in control and water-stressed leaves of *Sorghum bicolor* cv. CSH 5. The accumulation of F2,6BP in water-stressed plants reduced sucrose formation in the leaves. The activities of fructose 1,6-bisphosphatase and sucrose phosphate synthase were reduced in water-stressed plants. The distribution of ^{14}C into starch increased with increasing stress, while the incorporation into sucrose was drastically reduced in the stressed plants. The activity of fructose 6-phosphate 2-kinase was very high in stressed plants, while the levels of fructose 2,6-bisphosphatase were low. The results suggest that low water potentials result in F2,6BP accumulation, which modulates sucrose synthesis and carbon-partitioning in sorghum leaves. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Photosynthetic CO_2 assimilation per unit leaf area depends on the capacity of the plant's photosynthetic mechanism and on environmental factors, such as CO_2, radiation, temperature and water supply. Productivity of plants is greatly decreased by water-deficiency in most parts of the world and much emphasis is given to improving the drought-tolerance of varieties [1]. The main limitation to assimilation under water-stress, is suggested as inhibition of photophosphorylation, which decreases RuBP regeneration, rather than the enzymatic or light reactions [2].

Partitioning of photosynthetically fixed carbon between starch and sucrose in leaf mesophyll cells is biochemically controlled. The effects of fructose 2,6-bisphosphate (F2,6BP) in the control of partitioning of photosynthate between starch and sucrose has gained much attention in recent years [3–5]. F2,6BP has been shown to integrate the carbon metabolism in C_3 plants and in mesophyll and bundle sheath cells of C_4 plants [3, 6]. Despite several investigations on the role of F2,6BP in controlling sucrose synthesis, relatively little is known about its control over the carbon-flow in whole leaves under water-stress, where photosynthesis is limited. The work described herein, explores the relationships between photosynthetic sucrose formation and the effects of F2,6BP on the related enzymes in sorghum leaves under water limited conditions.

RESULTS AND DISCUSSION

Sucrose content and photosynthetic rates in sorghum leaves with increasing water-stress ranging from 0.25 MPa to 2.0 MPa are shown in Table 1. An inverse relationship was observed between the contents of leaf sucrose and F2,6BP (Table 2). F2,6BP accumulated to greater levels with increasing water-stress, while its content was low in well-watered plants. The distribution of ^{14}C into starch during photosynthesis increased with increasing water-stress, while the incorporation of ^{14}C into sucrose was drastically reduced in stressed plants (Table 3). The activities of sucrose phosphate synthase and fructose 1,6-bisphosphatase (F1,6BPase) declined very rapidly with decreasing leaf water potentials (Table 4). The activity of fructose 6-phosphate, 2-kinase (F6P,2-kinase) was extremely high in water-stressed leaves, while fructose 2,6-bisphosphatase (F2,6BPase) activities were very low in well-watered plants (Table 5).

The control of photosynthate-partitioning by F2,6BP under limited photosynthetic conditions has not been well established. During photosynthesis, the rate of conversion of triose-P into end-products, such as sucrose or starch, has to be balanced to the rate of CO_2 fixation for optimal maintenance of the photosynthetic apparatus in green leaves. Sucrose is the principal end-product of photosynthesis and, therefore, control of its synthesis is essential to maintain efficient carbon-partitioning [7]. The data obtained in the present study have shown that the sucrose contents in sorghum leaves were more sensitive to water-stress than was fixation of CO_2. Greater accumulation of F2,6BP in the leaves of stressed plants coincided with higher activities of F6P,2-kinase, indicating the accelerated activity of this

Table 1. Rates of photosynthesis and sucrose concentrations in sorghum leaves at different water-stress regimes. Sucrose content was measured at 1100. Photosynthetic rates between 1000 and 1400. Values are means of five independent determinations ± SE

Water potential (MPa)	Photosynthesis ($mg\ CO_2\ dm^{-2}\ h^{-1}$)	Sucrose ($\mu mol\ mg\ chl)^{-1}$
Control	45.9±5.6	14.6±1.2
0.25	40.9±4.8	13.8±1.2
0.50	35.2±3.5	11.5±0.9
1.0	30.6±3.1	9.7±0.6
1.5	25.3±2.6	7.5±0.3
2.0	21.5±1.9	6.6±0.3

Table 2. Sucrose and fructose 2,6-bisphosphate contents in sorghum leaves (at 1100) at different water-stress regimes. Values are means of five independent determinations ± SE

Water potential (MPa)	Sucrose ($\mu mol\ mg\ chl^{-1}$)	F2,6BP ($pmol\ mg\ chl^{-1}$)
Control	13.5±2.1	78.2±6.1
0.25	11.2±2.0	98.6±8.6
0.50	9.5±1.8	125.8±11.7
1.0	7.9±1.5	190.2±15.6
1.5	6.2±1.1	208.9±16.1
2.0	4.5±0.8	279.6±16.9

Table 4. Activities of sucrose phosphate synthase and fructose bisphosphatase in leaf extracts of sorghum. Values are means of five independent determinations ± SE

Water potential (MPa)	SPsynthase ($\mu mol\ mg\ chl^{-1}\ h^{-1}$)	FBPase ($\mu mol\ mg\ chl^{-1}\ h^{-1}$)
Control	21.7±3.5	120.5±9.6
0.25	20.5±2.9	110.7±9.3
0.50	19.3±2.7	100.6±7.5
1.0	18.7±2.7	90.2±5.8
1.5	12.6±2.1	70.5±5.1
2.0	8.9±1.2	52.8±4.6

enzyme under water-limiting conditions. The increase in the ratio of F6P, 2-kinase/F2,6BPase with increasing water-stress, which was related to the starch/sucrose ratio, clearly indicates the altered photosynthate-partitioning, so that more starch is synthesized, instead of sucrose (Tables 3 and 5). The reduced levels of sucrose synthesis, presumably due to increased accumulation of F2,6BP, under water-stress resulted in more starch accumulation (Table 3). It is thought that the supply of Pi to chloroplasts is reduced under these conditions, which stimulate starch synthesis. Increased ADP glucose pyrophosphorylase activity by raising PGA/Pi in chloroplasts has been reported previously [8].

Effective regulation of photosynthetic sucrose formation will require the active coordination of FBPase and SPsynthase. Any imbalance between photosynthetic rates and the activities of these two key enzymes of sucrose synthesis will alter carbon-partitioning. The reduced synthesis of sucrose in water-stressed leaves was due to the inhibition of FBPase and sucrose phosphate synthase. The inhibition of these two enzymes by F2,6BP has been described previously [3, 9].

In conclusion, F2,6BP-modulated photosynthetic sucrose formation plays a crucial role in assimilate partitioning in sorghum leaves under water-limiting conditions.

EXPERIMENTAL

Materials. All biochemicals and enzymes were from Sigma (U.S.A.) or from Boehringer (Germany). Reagents were purchased from commercial sources and were of analytical grade. Seeds of *Sorghum bicolor* L. (cv. CSH-5) were sown in 5-l pots containing a mixt. of vermiculite and soil and chemical fertilizer (N:P:K = 1:1:1). All plants were established outdoors under full sunlight. Plants received light intensities (400–700 nm) of 1800 $\mu mol\ m^{-2}\ s^{-1}$ throughout the growth period.

Table 3. Distribution of ^{14}C between starch and sucrose fractions in sorghum leaves. Leaves were allowed to fix $^{14}CO_2$ for 10 min followed by photosynthesis in air for 5 min. Values are means of five independent determinations ± SE

Water potential (MPa)	Starch (% total ^{14}C incorporation)	Sucrose	Starch / Sucrose
Control	31.5±5.6	42.5±6.3	0.74
0.25	33.1±5.7	39.3±5.8	1.10
0.50	39.8±6.3	35.6±4.9	1.11
1.0	48.0±7.1	29.2±3.2	1.65
1.5	55.8±7.9	19.8±2.1	2.89
2.0	58.9±8.1	12.8±1.5	4.83

Table 5. Activities of fructose-6-phosphate, 2-kinase and fructose 2,6-bisphosphatase in leaf extracts of sorghum. Values are means of five independent determinations ± SE

Water potential (MPa)	F6P-2 kinase	F2,6BPase	F6P,2-kinase / F2,6BPase
	(pmol mg chl^{-1} h^{-1})		
Control	90.8±4.3	185.6±8.7	0.48
0.25	100.6±5.1	180.2±8.1	0.55
0.5	115.8±5.9	169.5±6.9	0.68
1.0	160.2±7.5	143.3±5.7	1.12
1.5	190.3±9.6	90.8±5.3	2.09
2.0	285.6±10.4	70.9±4.5	4.03

Mean maximum day/night temps were 35/20°. Four-week-old plants were used for experimentation. Leaves from the fourth and fifth position from the bottom of the plants were used. Water-stress was induced by termination of watering to the four-week-old plants. Control plants were maintained under the same conditions as the water-stressed plants, except that they were kept well-watered. Measurements of leaf water potentials were made psychrometrically on leaf discs at 30° using a thermocouple psychrometer. The time required for equilibrium between the H_2O vapour pressure of leaf samples and that of the psychrometer chamber varied from *ca* 45 min at −0.5 MPa to 120 min at 2.0 MPa.

Photosynthesis. Leaf gas-exchange rates were monitored with single attached leaves enclosed in an acrylic plastic leaf chamber (6 × 10 × 0.5 cm); a 1-kW metal-halide lamp provided the light source. Light intensities were measured with a quantum sensor. The air-stream (CO_2 concn 330 μl l^{-1}) was passed into the leaf chamber at a constant rate of 3 l min^{-1}. CO_2 uptake was measured with an IR gas analyzer.

^{14}C-Distribution into sucrose and starch. Leaves were fed with $^{14}CO_2$ (0.1 m Ci m mol^{-1}) for 10 min followed by 5 min of $^{12}CO_2$. Leaves were quickly frozen in liquid N_2 and stored at 80°. Distribution of ^{14}C in starch and sucrose was determined according to ref. [10].

Enzyme extraction. After gas-exchange measurements, half of each leaf was frozen in liquid N_2. The frozen leaves (0.2 g) were rapidly homogenized in a chilled mortar with 3 ml extraction buffer containing 50 mM HEPES–NaOH (pH 7.5), 10 mM $MgCl_2$, 1 mM EDTA, 5 mM DTT, 0.5% BSA and 0.2% (v/v) Triton X-100. The extract was filtered through cheesecloth and an aliquot was collected for chlorophyll determination [11]. The homogenate was centrifuged at 30 000 *g* for 25 min and the supernatant passed through Sephadex G-25, which was pre-equilibrated with the extraction buffer.

Enzyme assays. Each enzyme assay was optimized with respect to pH and individual components, as detailed below. Nevertheless, enzyme activities were linear with respect to time. Great care was taken regarding the conditions (according to the standard procedures given for each enzyme) to avoid losses of activity during extraction and assaying. *Fructose 6-phosphate-2-kinase (F6P-2K)* was assayed essentially as described in ref. [12] by following F2,6BP formation enzymatically with pyrophosphate fructose 6-phosphate 1-phosphotransferase. The reaction mixt. contained 0.1 M HEPES (pH 7.6), 5 mM K-phosphate, 5 mM F6P, 2 mM ATP, 5 mM $MgCl_2$, 15 mM G6P, 4 units ml^{-1} creatine phosphokinase, 5 mM phosphocreatine and the enzyme extract. Aliquots were removed at 5 min intervals and measured for F2,6BP formation. Fructose 2,6-bisphosphatase (F2,6BPase) was estimated according to ref. [13]. The reaction mixt. (1 ml) contained 100 mM HEPES buffer (pH 7), 1 mM NADP, 1 mM EDTA, 1 unit of glucose-6-phosphate dehydrogenase, 1 unit of phosphoglucose isomerase, 25 μM F2,6BP and the enzyme extract. *Fructose 1,6 bisphosphatase* was assayed as described in ref. [14] with slight modifications. The reaction mixt. (1 ml) contained 100 mM Tris–HCl buffer (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 0.3 mM NADP, 0.25 mM F1,6BP, 4 units of phosphoglucoisomerase, 2 units of glucose-6-phosphate dehydrogenase and the enzyme extract. The reaction was followed spectrophotometrically by the increase in A at 340 nm. *Sucrose phosphate synthase* was assayed as described in ref. [15]. The assay mixt. (1 ml) contained 50 mM HEPES–NaOH (pH 7.5), 8 mM UDPG, 8 mM F6P, 10 mM $MgCl_2$ and the enzyme. The reaction was terminated by adding 0.1 ml 1 N NaOH. The tubes were heated at 100° for 10 min to remove unreacted F6P. After cooling, 0.5 ml of 0.1% (v/v) resorcinol in 95% EtOH and 1 ml of 30% HCl were added. The tubes were incubated at 30° for 10 min. The tubes were then cooled and the A at 520 nm was recorded. Sucrose contents were measured according to ref. [16]. F2,6BP and sucrose contents in leaves were measured routinely at 11 am.

Extraction and assay of fructose 2,6-bisphosphate. The liquid N_2-frozen leaf (100 mg fr. wt) was homogenized with 1 ml extraction medium containing 50 mM Tris–HCl (pH 8.2), 5 mM EDTA, 25% (v/v) $CHCl_3$ and 65% EtOH. The homogenate was centrifuged and the pellet re-extracted ×2 with 0.5 ml extraction soln. Supernatants were combined and evapd to dryness under N_2. Samples were made up to 150 μl with 50 mM Tris–HCl (pH 8) and then stored at −20°. Fructose 2,6-bisphosphate was assayed according to ref. [17]. The reaction mixt. (0.5 ml) contained 50 mM Tris–HCl (pH 8), 10 mM $MgCl_2$, 0.15 mM NADH,

3 mM EDTA, 2 mM DTT, 0.5 mM PPi, 1 mM F6P, 0.5 unit of α-glycerophosphate dehydrogenase, 0.25 unit of aldolase, 5 units of triose phosphate isomerase and 0.02 unit of pyrophosphate/F6P phosphotransferase. Aliquots (0.5–4 pmol F2,6BP) of samples were added and the rate of reaction compared to a standard curve with known quantities of F2,6BP. All reactions were run at 25°. Using this extraction and assay procedure, percentage recovery of F2,6BP added to the sample during homogenizing was 70–75.

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