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THE ROLE OF STARCH, SUCROSE AND TRIOSE PHOSPHATES IN THE SOURCE - SINK RELATIONSHIP OF SOYBEAN (*Glycine max* L. Merrill)

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ABSTRACT

In a determination of starch, sucrose and triose phosphate in a whole cell homogenate sample and nonaqueously isolated chloroplast sample from plants of different source - sink ratios, the amounts of starch in chloroplasts, the number of starch granules per chloroplast and their size in the sink limited treatment were significantly higher than in the source limited and normal treatments. Thus it could be concluded that starch acts as a messenger from sink to source and thereby act as a regulator of source - sink relationships. Although there are some indication of regulation by triose phosphate and sucrose their role is less clear.

INTRODUCTION

Plants are subjected to environmental fluctuations during their entire cycle. They tend to adjust to these fluctuations by increasing or decreasing their yields, depending on the situations. The reason for this adjustment remains unclear, but plant physiologists believe it is because plants have the ability to maintain a certain source - sink balance. A source is any tissue that produce more carbon than it utilizes, while a sink is any tissue that utilizes more carbon than it produces.

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Experimental evidence indicates that in general most plants will adjust their long term photosynthetic rate to sink demand. It has been reported (King *et al.*, 1967) that at least in the wheat flag leaf, photosynthesis in the flag leaf was regulated by demand for assimilates. Whether altered sink demands have a direct effect on the adjustment of the source photosynthetic rate, or whether it is an incidental effect of accompanying changes in hormone synthesis and action is not known. Several observation have been made to support the postulate that sink demand has a direct effect on the photosynthetic rate (Peet and Kramer, 1980), and some metabolites in the leaf have been suggested as possible regulators of the photosynthetic rate (Mondal *et al.*, 1978; Thorne and Koller, 1974). Starch which accumulates in the chloroplasts when the photosynthetic supply exceeds the demand (Benjamin *et al.*, 1981), and sucrose in the cytosol which is higher when the sink demand exceeds the supply (Silvius *et al.*, 1981) are two of these metabolites.

Triose phosphates (TP), which are the early products of carbon fixation in the chloroplasts and the principal form by which carbon crosses the chloroplast membrane could also be possible regulators of the source - sink interactions (Heldt, 1976). It has also been shown that TP and 3-PGA are the only phosphorylated metabolites that fully permeate the chloroplast membrane (Heldt *et al.*, 1978).

Since starch, sucrose and TP are possible candidates for the role of messenger from sink to source, a study of the distribution of these metabolites between the chloroplast and the surrounding cytosol may contribute to our knowledge of the source - sink regulation. Thus in this study it was proposed that by adjusting the source - sink ratio of soybean plants, and then determining the different compartmentation pattern of these metabolites in the leaves it would be possible to better understand their role in the regulation of source - sink interaction in plants.

MATERIAL AND METHODS

An indeterminant soybean variety was grown under greenhouse conditions. Fifteen weeks after seeding, plants were treated to create three source - sink ratios: sink limited, normal and source limited. This was achieved by depodding and defoliation of the plants. Sink limited plants were adjusted to 30 pods per plant and all leaves were left intact. For the normal treatment, each plant was adjusted to 90 pods and all leaves left intact. For the source limited treatment, each plant was adjusted to 90 pods and the two side leaflets on all leaves were removed. The source - sink adjusted plants were then arranged in a randomized complete block design with five plants per replicate and three replicates per treatment. Two weeks after adjusting the source - sink ratio, the terminal leaflet from each leaf in the upper half of the canopy was harvested.

The leaves were washed in distilled water, blotted, deveined and frozen in Freon 12 (CCl_2F_2) cooled to its freezing point in liquid nitrogen. The leaves were then freeze dried (5-10μm Hg vacuum) and ground in a Cyclone Sample Mill (UD Corporation, Boulder, CO, USA). The ground leaves were then sifted through a 60μm mesh. The leaf powder was then used to obtain a whole cell homogenate sample and a nonaqueously isolated chloroplast sample employing the procedure described earlier using 5:5 (v/v) mixture of glycerol and polyethylene glycol (PEG) (molecular weight3 300).

The whole cell homogenate sample and the chloroplast pellet sample were diluted with two volumes of dry absolute methanol and centrifuged 20 min at 2000 x g to remove glycerol and PEG. The washed pellets were extracted with 13:4:3 (v/v/v) methanol/chloroform/0.2M formic acid (MCF) once and with 13:4:3 (v/v/v) methanol/chloroform/water (MCW) four times until the pellets were no longer green. These pellets were used for starch determination. Starch in the pellets remaining after MCF extraction was measured as reducing sugar following complete hydrolysis with glucoamylase (Libby, 1970).

The MCF and MCW extracts were combined and phase separated with chloroform and water. The aqueous phase was flash evaporated and used for TP, P_i and sucrose analyses. Dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P) were assayed following the oxidation of NADH at OD 340 after adding α-glycerophosphate dehydrogenase and triose phosphate isomerase respectively (Michal and Beutler, 1974). Glycerate-3-phosphate (3PGA) was assayed following the oxidation of NADH at OD 340 resulting from the addition of glyceraldehyde-3-phosphate dehydrogenase and 3-phosphoglyceric phosphokinase (Czok, 1974).

For sucrose determinations, the samples were heated in 0.2N H_2SO_4 in a boiling water bath for 15 min and then the total reducing sugars were determined (Hodge and Hofreites, 1962). Sucrose was calculated from the difference between the total reducing sugars after hydrolysis and reducing sugars before hydrolysis. P_i was determined colorimetrically (Murphy and Riley, 1962).

RESULTS AND DISCUSSION

Of the total amount of starch in the whole cell homogenate, 98.2% and 98.4% and 96.4% were recovered in the chloroplasts of the sink limited, normal and source limited treatment respectively. These observations support the earlier theory that starch was produced in the chloroplasts of leaves (Shannon, 1982). Only very small amounts of starch were measured in the cytosol of all three treatments. The level of starch in the chloroplasts of the sink limited treatment was significantly higher than the starch in the chloroplasts of the other two treatments (Table 1). Thus. compared to 636.8 and 529.1 pmoles of glucose equivalents per 1000 chloroplasts in the normal and source limited treatment respectively, the chloroplasts of the sink limited treatment contained 1100.9 pmoles of glucose equivalents per 1000 chloroplasts. The measured amount of starch in the chloroplast was also in agreement with treatment effect on starch granule number per chloroplast and size of starch granules in that there was a higher number of larger starch granules in the sink limited treatment (Fig. 1).

It is reported that in source leaves of unshaded plants (high source to sink ratio), starch concentration remained high compared to plants where all but one leaf was shaded (low source to sink ratio) (Thorne and Koller, 1974). Also starch concentration of 145 to 80 mg/dm^2 were observed for continuously and recently depodded plants compared to 50 mg/dm^2 in control plants (Mondal *et al.*, 1978). When the sink - source ratio was increased or decreased in cucumber plants, there was a respective decrease or increase in starch content (Mayoral *et al.*, 1985).

In this study, the chloroplasts of the sink limited treatment were found to contain approximately twice the amount of starch compared to chloroplasts of the source limited treatment, and also it was significantly higher than in both the normal and source limited treatments. It has been shown that depodded plants had a photosynthetic rate of 10 mg CO_2/dm^2/h compared to 15 mg CO_2/dm^2/h in

Table 1. The effect of adjusted source-sink ratios on metabolites in the chloroplasts and cytosol of soybean leaves

Metabolite		Sink-limited	Normal	Source-limited
		PMOLE/1000 chloroplasts		
Starch	Chloroplast	1100.90 ± 52.74 a	636.83 ± 27.946	529.11 ± 33.006
	Cytosol	19.41 ± 115.30	10.32 ± 74.13	18.97 ± 54.46
DHAP	Chloroplast	1.46 ± 0.06	1.20 ± 0.08	1.35 ± 0.12
	Cytosol	2.81 ± 0.88	1.72 ± 0.39	2.86 ± 0.65
G-3-P	Chloroplast	1.64 ± 0.05	1.28 ± 0.14	1.56 ± 0.10
	Cytoplast	1.83 ± 0.31	1.56 ± 0.41	2.59 ± 0.67
3-PGA	Chloroplast	0.15 ± 0.02	0.09 ± 0.05	0.0
	Cytosol	0.46 ± 0.32	1.05 ± 0.19	0.58 ± 0.36
Pi	Chloroplast	47.36 ± 2.79 a	22.09 ± 0.72 b	23.70 ± 1.03 b
	Cytoplast	18.39 ± 4.31 a	5.80 ± 10.39 b	13.27 ± 4.49 a
Sucrose	Chloroplast	0.69 ± 0.02	0.99 ± 0.90	0.52 ± 0.05
	Cytoplast	1.50 ± 0.35	0.40 ± 1.01	2.21 ± 0.24

Mean separation among treatments by Walter Duncan's Bayesian K-ratio + Test, K = 100
Starch data are presented as pmoles of glucose equivalents per 1000 chloroplasts.

control plants at 50 days from bloom (Mondal *et al.*, 1978). Although the photosynthetic rates of the plants with adjusted source - sink ratios were not measured in this experiment, it could be speculated that the high concentration of starch in the limited treatment would be accompanied by a high photosynthetic rate.

Starch is synthesized in the chloroplasts of the plant cell. As proposed in the pathway for carbon fixation within the chloroplast and cytosol (Shannon, 1982), the 3-phosphoglyceraldehyde (3PGA) produced in the initial reaction of photosynthesis can either be transferred out of the chloroplast to the cytosol where it is converted to sucrose for export or it can remain in the chloroplast. Within the chloroplast the 3PGA can be converted to fructose-6-phosphate (F6P) which can be utilized for starch synthesis or be recycled to Ribulose bisphosphate (RuBP) by the Calvin cycle. When 3PGA is produced in excess of what is required in the cytosol for sucrose synthesis, more 3PGA remains in the chloroplast and starch production increases.

Thus by depodding plants the sink strength is limited and the above reactions take place in the leaf. The starch produced in this way could lower the photosynthetic rate so that production of 3PGA is reduced, thereby maintaining the source - sink balance of the plant. The reverse would occur in the case of the defoliated plants where the source is limited. More 3PGA would be transported out into the cytosol for sucrose synthesis by the limited number of leaves so that the sinks could be satisfied. As a consequence

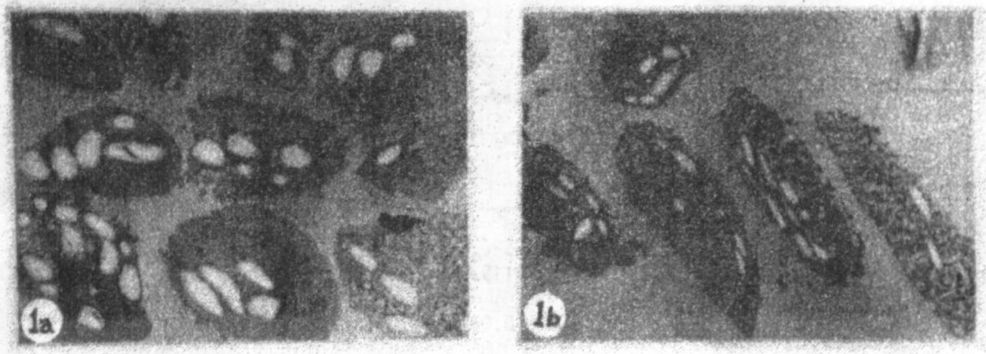

Fig. 1. Transmission electron micrographs of chloroplasts isolated from a. sink limited and b. source limited treatments, x 12,300. Note size difference in starch granules.

there will be no build up of starch in the chloroplasts. From the above results therefore, we could believe that starch in fact could act as a regulator of the source - sink relationship in soybean.

Except for 3PGA which was not detected in the chloroplasts of the source limited treatment, TPs were present in both chloroplasts and cytosol of all three treatments (Table 1). TPs and 3PGA were higher in the cytosol than in the chloroplasts. There was no apparent effect of the source - sink ratio on partitioning of the metabolites between the chloroplasts and cytosol.

The amount of P_i in the chloroplasts of all three treatments were much higher than the phosphorylated metabolites and the amount of P_i in the sink limited chloroplasts were significantly higher than the amount in the chloroplasts of the normal and source limited treatments (Table 1). The amount of P_i in the cytosol of the sink and source limited treatments were significantly higher than that in the cytosol of the normal treatment. However, the standard deviation of the normal cytosol was twice as high as the average.

Sucrose was measured in both the chloroplasts and cytosol and except for the normal treatment, it was higher in the cytosol than in the chloroplasts (Table 1). According to Shannon (1982), there no enzymes for sucrose synthesis or hydrolysis in the chloroplasts. The production of sucrose in the cytosol occurs when the TP is transported out of the chloroplasts and converted *via* the action of the enzymes of gluconeogenesis and sucrose synthesis (Shannon, 1982). Work on phloem translocation strongly indicates that sucrose is synthesized in the cytosol of mesophyll cells in C_3 plants from where it is stored in vacuoles (Giaquinta, 1978). Preparations of spinach chloroplasts, whether isolated from protoplasm or by conventional methods, contained sucrose or related oligosaccharides which did not seem to be derived from a cytoplasmic contamination and also these were hydrolyzed to glucose and fructose during incubation in the dark (Stitt and Heldt, 1981).

In this study too, the chloroplasts of all three treatments contained sucrose. Whether the sucrose was cytoplasmic and/or vacuolar contamination is difficult to determine since there was some contamination of the chloroplasts with cell wall debris that had cytosol attached. It is reported that large amount of sucrose accumulate in vacuoles of spinach leaves during the day (Heldt *et al.*, 1978) and also that vacuoles sedimented to the lowest layer during nonaqueous fractionation of the spinach leaves on a CCl_4-heptane gradient (Gerhardt and Heldt, 1984). Although a different nonaqueous isolation procedure was used in the present study, it is possible that some vacuoles or vacuolar content were pelleted with the chloroplasts. This could be an explanation for the higher than expected sucrose content of the chloroplast pellet.

From the above results it could be concluded that starch acts as a messenger from sink to source and thereby regulates source - sink interactions at least in soybean. Although there are some indication of regulation by TP and sucrose, their roles as messenger in source - sink relationships is not definite.

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