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Sucrose metabolism at three leaf development stages in bean plants

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Abstract

Sucrose metabolism was studied at three leaf development stages in two *Phaseolus vulgaris* L. cultivars, Tacarigua and Montalban. The changes of enzyme activities involved in sucrose metabolism at the leaf development stages were: (*1*) Sink (9-11 % full leaf expansion, FLE): low total sucrose phosphate synthase (SPS) activity, and higher acid invertase (AI) activity accompanied by low sucrose synthase (SuSy) synthetic and sucrolytic activities. (*2*) Sink to source transition (40-47 % FLE): increase in total SPS and SuSy activities, decrease in AI activity. (*3*) Source (96-97 % FLE): high total SPS activity, increased SuSy activities, decreased AI activity. The hexose/sucrose ratio decreased from sink to source leaves in both bean cultivars. The neutral invertase activity was lower than that of AI; it showed an insignificant decrease during the sink-source transition.

Additional key words: acid and neutral invertases; hexose/sucrose ratio; *Phaseolus vulgaris*; sucrose phosphate synthase; sucrose synthase.

Introduction

The leaf is an organ of limited life span. The ontogeny of individual leaves is often shortened (Dale 1988). The saccharide metabolism of leaves changes profoundly during development. Young leaves depend in part on saccharides imported from other regions of the plant; mature leaves produce photosynthates and are the major sources of transport sugars. The early growth of a leaf is supported by saccharides, and during leaf lamina expansion the rate of photosynthesis increases (Turgeon 1989).

During their development the leaves progressively acquire their photosynthetic capacity by differentiation of proplastids into functional chloroplasts (for reviews see

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Abbreviations: AI, acid invertase; BSA, bovine serum albumin; FLE, full leaf expansion; NI, neutral invertase; SPS, sucrose phosphate synthase; SuSy, sucrose synthase.

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Šesták 1985). Simultaneously, and at appropriate time, the sucrose import process must be halted while the sucrose synthesis and export start (Nguyen-Quoc *et al.* 1990, Sharma and Sengupta 1997). As a young leaf matures, its status changes from importer to exporter of photosynthates. In many dicot species, import-export transition occurs when the leaf is 40 to 50 % expanded (Giaquinta 1978). Transition of young leaves from net importers to exporters of assimilates occurs in a progressive "wave" from the tip to the base of the leaf blade (Fellows and Geiger 1974, Pitcher and Daie 1987, Turgeon 1989).

At some stage of the ontogeny, a leaf is partially sink and partially source: the transitional leaf. In regard to carbon balance and ability to export sucrose, transitional leaf contains a complete age sequence of cells in a developmental and functional gradient so that the tip functions as a source, the middle segment is transitional, and the basal portion as sink tissue (Turgeon 1989, Pitcher and Daie 1991). The ability of a young sink leaf to import is a function of its ability to metabolise incoming photosynthates that arrive mainly in the form of sucrose (Geiger and Fondy 1980, Ho and Baker 1982). Sucrose is the preferred form of carbon that is exported *via* the phloem to sink tissue. Rates of sucrose export from source leaves have been linked principally to the activity of sucrose phosphate synthase (SPS; EC 2.4.1.14) (Stitt 1994). At the sink, sucrose may be cleaved hydrolytically by invertase (EC 3.2.1.26) or non-hydrolytically by sucrose synthase (SuSy; EC 2.4.1.13) The products of sucrose cleavage are converted to hexose phosphates and can enter the respiratory pathways *via* glycolysis to provide substrates and reducing power for growth and storage product synthesis (King *et al.* 1997).

The aim of the present work was to study the leaf sucrose metabolism at three different development stages in two bean cultivars to understand the role of enzyme activities and their integrated significance in the sink to source process.

Materials and methods

Seeds of Tacarigua (T) and Montalban (M) bean (*Phaseolus vulgaris* L.) cultivars supplied by FONAIAP (Venezuelan Fund for Agriculture and Husbandry, Maracay, Venezuela) were germinated in trays on towel paper. 7-d-old seedlings were transferred to 4 000 cm^3 plastic pots containing a mixture of clay and sand (3 : 2). These plants were watered daily and maintained in a greenhouse under 31.0±2.5/20.5 ±1.5 °C maximum/minimum average temperature, 94/42 % maximum/minimum relative humidity (RH), and 662 $\mu mol\ m^{-2}\ s^{-1}$ average sun irradiance. After 46 d, leaves were then selected from four replicates and studied at three different developmental stages: (*1*) sink leaves 2 to 6 d-old, 9 % full leaf expansion (FLE) for T and 11 % FLE for M; (*2*) 6 to 8 d-old sink to source transition leaves, 40 % FLE for T and 47 % for M; (*3*) 8 to 12 d-old source leaves, 96 % FLE for T and 97 % FLE for M. Full expansion leaves had an area of 82 cm^2 for T and 87 cm^2 for M.

Four replicates (*i.e.*, from 4 plants) were used for the extractions and assays. One gram of leaf was ground 1 : 5 (m : v) in 50 mM HEPES-NaOH, pH 7.5, 5 mM $MgCl_2$, 1 mM EDTA, 0.5 kg m^{-3} BSA; 2 % (v/v) glycerol, 0.05 % (v/v) *Triton X-*

100, 2.5 mM dithiothreitol, 2 % (m/v) polyvinylpyrrolidone. The homogenate was centrifuged at 10 000×*g* during 30 s. The supernatant was desalted and concentrated using *Centricom 10* (*Amicon*, GB) tubes. The desalted and concentrated extracts were used in enzymatic assays. The activity of SPS (total and P_i-insensitive), SuSy in the synthesis way, and soluble neutral and acid invertases were assayed as reported by Castrillo *et al.* (1992). The SuSy in the sucrolysis way was assayed as reported by Nguyen-Quoc *et al.* (1990). The dinitrosalicylic acid method was used for hexose determination (Sturgeon 1990), the anthrone method for sucrose determination (Avigad 1990).

Results and discussion

Total SPS activity increased in both cultivars from sink to source leaf (Table 1). The SPS P_i-insensitive activity showed slightly higher values in the sink leaves (2-6-d-old) within the limits of experimental error (Table 1). These results agree with the increased SPS activities reported for the transition sink to source leaves. SPS is considered to be of primary importance in regulating sucrose synthesis (Stitt *et al.* 1988). This enzyme has been also detected in young importing leaves (Pollock 1976). Zimmerman *et al.* (1995) found in eelgrass that the leaf transition from sink to source was accompanied by a doubling in SPS activity which links the development of capacity to export carbon compounds in maturing leaves to an increase in SPS. However, Giaquinta (1978) found in sink and source leaves of sugar beet similar activities of AI and SuSy (sucrose cleavage) and detected SPS only in the source leaves.

The activities of SuSy in the synthesis and sucrolysis increased from sink to source leaves (Table 1). Claussen *et al.* (1985) concluded that in growing leaves a close relationship might exist between the activity of SuSy and the import of sucrose from source leaves. Schmalstig and Hitz (1987) reported that in very young soybean leaves all sucrose cleavage was carried out by SuSy, invertase contribution increased and remained by 42 % for the remainder of the import phase; in sugar beet sink leaves the invertase contribution to sucrose metabolism in expanding leaves was about 58 %. Nguyen-Quoc *et al.* (1990) studying the sucrose import to export transition at the cellular level in maize leaves, found a high SuSy activity in the basal part of young leaf.

The activities of AI and NI declined from sink leaves to source leaves (Table 2). The activity of NI was always lower than that of AI. These results confirm those for *P. vulgaris*, that the major sucrose-hydrolysing enzyme is a readily soluble invertase that is most active during early stages of leaf growth. The high activity of the enzyme correlates with a high hexose/sucrose ratio at the same period of development (Table 2); the high concentrations of hexoses fall as the leaves change to the source stage. Morris and Arthur (1984) reported that the high specific activity of soluble AI found in these leaves correlates with rates of leaf and epidermal cell enlargement. A similar correlation of AI and growth was found in *Citrus* (Schaffer *et al.* 1987), *Lolium* (Pollock and Lloyd 1977), and oat (Greenland and Lewis 1981). We found higher AI

Table 1. Average values and standard deviations for activities of total sucrose phosphate synthase (SPS), P_i-insensitive SPS, sucrose synthase (SuSy) synthesis and SuSy sucrolysis [μmol m^{-2} s^{-1}] for three leaf development stages in Tacarigua (T) and Montalban (M) cultivars.

Leaf age [d]	Total SPS		P_i-insens. SPS		SuSy synthesis		SuSy sucrolysis	
	T	M	T	M	T	M	T	M
2-6	2.90±0.28	1.10±0.31	0.90±0.30	0.91±0.31	0.15±0.05	0.15±0.05	0.10±0.04	0.12±0.04
6-8	4.00±1.12	4.30±1.40	0.70±0.24	0.75±0.30	0.20±0.07	0.18±0.06	0.15±0.05	0.20±0.06
8-12	5.00±1.40	5.52±1.50	0.75±0.29	0.80±0.32	0.25±0.09	0.22±0.07	0.20±0.07	0.22±0.07

activities in sink leaves but they were accompanied by increased SuSy activities.

Table 2. Average values and standard deviations for acid invertase and neutral invertase activities [μmol m^{-2} s^{-1}] and hexose/sucrose ratio [g kg^{-1}] for three leaf development stages in Tacarigua (T) and Montalban (M) cultivars.

Leaf age [d]	Acid invertase		Neutral invertase		Hexose/sucrose	
	T	M	T	M	T	M
2-6	3.20±1.00	3.30±1.10	0.35±0.10	0.33±0.11	10.45±3.45	9.70±3.00
6-8	1.20±0.42	1.50±0.46	0.30±0.10	0.29±0.12	5.68±1.60	4.64±1.50
8-12	0.64±0.28	0.49±0.16	0.20±0.07	0.22±0.08	1.12±0.47	1.07±0.35

The decline in hexose/sucrose ratio from sink to source leaves (Table 2) was due to the high AI activity. The ratio decreased because in the source leaves the sucrose synthesis was higher due to SPS. Leaves of dicotyledons stop importing and begin to export when they are 30-60 % fully expanded. Developing leaves continue to import photoassimilates from source leaves after they have begun to export their own products (Turgeon 1989 and references therein). The proportion of incoming carbon from import and own photosynthesis in young developing leaves changes dramatically as the leaf grows (Dickson and Larson 1975). Laminar expansion is the result of cell division and cell enlargement. Cell division is more prominent at the initial stage of growth, followed by a second stage in which cell enlargement occurs and intercellular spaces develop (Dale and Milthorpe 1983). In *Phaseolus* leaves, the values of wall plastic extensibility fall as the rate of leaf expansion goes down (Van Volkenburgh *et al.* 1985), the decline in leaf expansion coinciding with the period when maximum rates of photosynthesis are achieved. If high concentrations of locally produced assimilates enhance the rate of wall synthesis, it exceeds the rate at which wall loosening can occur; this may result in reduced extensibility (Dale 1988). According to Farrar (1992) it is unlikely that sucrose concentration alone controls export and the status of sugars mediates between import, growth, and metabolism as the ultimate controllers of sink status. Import into sinks is thus controlled in the short term by growth-related processes, apparently mediated by cytosolic sugar pool size.

This fine control works within a framework of maximum growth set by the sucrose-controlled expression of key gene coding for proteins central to the rate control of growth and respiration. Farrar (1992) proposed a hypothesis for the role of sucrose in controlling shoot : root ratio.

Pitcher and Daie (1991) working with sugar beet leaves at the sink-to-source transition indicated that transition, which was among 6-9-d-old leaves, is not light-regulated. Ability to load sucrose for export from leaves is light-dependent and the assimilate import may be terminated by interruption of phloem unloading into the mesophyll cells of developing leaves. Development of export ability is irreversible. These results indicated that transition is development regulated and not coupled to photosynthetic capacity. Harn *et al.* (1993) working with sugar beet leaves found a regulation of expression of genes encoding the two regulatory enzymes of sucrose biosynthesis in developing leaves and a molecular coordination of some of the regulatory enzymes of carbon metabolism during leaf transition.

My results show the change of enzyme activities involved in sucrose metabolism at three different bean leaf development stages: increase in total SPS activity from sink to source, tendency of P_i-insensitive SPS activity to decline towards source stage, increase in SuSy activities from sink to source accompanied by a decline in AI acitvity to source stage. The changes in NI invertase activity were not significant, showing only a declining tendency. The hexose/sucrose ratios were higher at sink stage and decreased to the source stage.

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