Sucrose Synthase, UDP-glucose pyrophosphorylase and ADP-glucose Pyrophosphorylase in Korea Ginseng Roots

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Abstract : The seasonal variation in the activity of sucrose synthase, ADP-glucose pyrophosphorylase and UDP-glucose pyrophosphorylase in roots of *Panax ginseng* C.A.Meyer have been studied. It was revealed that sucrose synthase and ADP-glucose pyrophosphorylase are adaptive enzymes and can serve as markers of sink strength, while UDP-glucose pyrophosphorylase is the maintenance enzyme. The average day temperature exceeded 24°C appeared to cause the disturbance in refilling process, affecting the starch synthesis. Study on the dependence of oxygen consumption in stele tissue with temperature revealed the sharp accelerating of this process after 24°C.

Key words : Sucrose synthase, ADP-glucose pyrophosphorylase, UDP-glucose pyrophosphorylase, oxygen consumption, seasonal variation, heat-shock stress, *Panax ginseng*.

INTRODUCTION

Growth and development of plants are dependent upon the energy gained by fixing carbon dioxide into carbohydrates during photosynthesis and the translocation of newly fixed photoassimilates from their site of synthesis to regions of utilization and/or storage. Carbohydrate conversions are fundamental in supporting the life of plants because sugars feed into essentially all aspects of plant metabolism.

Plant organs can be divided into two kinds: (a) photosynthetically active source organs (defined as net exporters of photoassimilates), represented mainly by mature leaves, and (b) photosynthetically inactive sink organs (defined as net importers of fixed carbon). Sinks can be divided into at least two different classes[1]: (a) utilization sinks, highly metabolically active, rapidly growing tissues like meristems and immature leaves, and (b) storage sinks such as tubers, seeds, or roots, which deposit the imported carbohydrates in the form of storage compounds (e.g. starch, sucrose, fatty acids, or proteins).

To increase the amount of photoassimilates allocated to harvestable organs such as seeds, tubers or roots, a better understanding of sink-source interaction is needed.

It is also important to understand the environmental factors affecting plant productivity. To this reason, the monitoring of the enzymes metabolizing carbohydrates in the 6th-year ginseng roots had been carried out.

MATERIALS AND METHODS

All reagents used were supplied by Sigma (U.S.A.)

1. Plant material

Ginseng (*Panax ginseng* C.A. Meyer) plants used were in the sixth growing year in fields plots (KGTRI experimental field Eumseong).

For enzyme activity assay only the root body was used. Freshly sampling roots were washed under tap water. After remove the lateral roots, root body was cut and separated with two part, stele, inside from cambium, and cortex, outside from cambium, and enzymes activity were assayed by the part.

2. Extraction procedure

All procedures were conducted at 4°C.

For protein extraction 5 g to 10 g of tissues were powdered in liquid nitrogen with a mortar and pestle. The tissue powder was homogenized in extraction solution con-

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tained 200 mM HEPES/NaOH (pH 7.5), 3 mM magnesium acetate, 5 mM dithiothreitol (DTT), 2% (w/v) insoluble polyvinylpyrrolidone. The ratio of tissue weight (g) and extraction solution volume (mL) was 1:5. The homogenate was passed through two layers of Miracloth and centrifuged at $48000 \times g$ (Beckman L7-35, U.S.A.) for 40 min at 4°C. Precipitate was discarded, and solid $(NH_4)_2SO_4$ was added slowly to the supernatant with constant stirring to obtain 80% saturation. After standing overnight, the pellet was collected by centrifugation at $48,000 \times g$ for 30 min at 4°C and dissolved in a minimum volume of a solution of: 10 mM HEPES/NaOH (pH 7.5), 2 mM magnesium acetate and 2 mM DTT.

Crude protein extracts were used for determination of enzyme activity after desalting on Sephadex G-25 column. Assays were conducted immediately after the extract was desalted.

3. Enzyme activity assay

Assay of UD-Pglucose-pyrophosphorylase (UGPase) was conducted as follows[2] : The 1 mL reaction mixture contained 100 mM HEPES/NaOH (pH 7.5), 2 mM Mg acetate, 5 mM DTT, 0,02 mM glucose 1,6-diphosphate, 0.5 mM NAD, 1 mM UDPglucose, 2 U of phosphoglucomutase, 2 U of *Leuconostoc* glucose 6-P dehydrogenase (G6DH), and 50 mL of solution under study. After 3 min of preincubation at 30°C the reaction was initiated by addition of PPi (1 mM). The glucose 1-phosphate produced was coupled by phosphoglucomutase and GDH to yield 6-phosphogluconate and NADH. The production of NADH was monitored spectrophotometrically at 340 nm during 1 min with a Uvikon 941 plus (Kontron Instruments, Italy).

Sucrose synthase (SS) was assayed by the multi-enzyme method.[2] The 1 mL reaction mixture contained 100 mM HEPES/NaOH (pH 7.5), 2 mM Mg acetate, 5 mM DTT, 0.02 mM glucose $1,6-P_2$, 0.5 mM NAD, 2 U of phosphoglucomutase, 2 U G6DH, and 50 μL of solution under study. After 3 min of preincubation at 30°C the reaction was initiated by addition of sucrose (50 mM), UDP (1 mM) and PPi (1 mM). The production of NADH was monitored spectrophotometrically at 340 nm during 5 min with a Uvikon 941plus.

ADP-glucosepyrophosphorylase (AGPase) was assayed as followes[3]: The 1mL reaction mixture contained 100 mM HEPES/NaOH (pH 7.5), 2 mM Mg acetate, 5 mM DTT, 0.02 mM glucose $1,6-P_2$, 0.5 mM NAD, 1 mM ADP- glucose, 2 U of phosphoglucomutase, 2 U G6DH, and 50 mL

of solution under study. After 3 min of preincubation at 30×C the reaction was initiated by addition of PPi (1 mM). The production of NADH was monitored spectrophotometrically at 340 nm during 5 min with a Uvikon 941plus.

Enzyme activities were expressed as nanomoles of product formed per mg of total protein per minute. All assays were performed in triplicate and readings were reproducible to within 10%.

4. Assay of content of water-soluble proteins and carbohydrates

The protein concentration was determined using the Bio-Rad DC protein assay kit with bovine serum albumin as standard.

Carbohydrate content was assayed by phenol-sulfuric acid method.[4]

5. Oxygen consumption

Oxygen consumption was measured on polarograph (model Gilson 5/6H) using Clark type oxygen electrode. The processing chamber was covered with silicon, fitted with a magnetic stirrer, and temperature-controlled with bath circulator BL-720 (Yin Der, Taiwan). The basic line for the oxygen consumption was obtained under continuous stirring.

Immediately after being cut from a fresh sampling ginseng root, stele disks (2 mm thick, 8 mm in diameter) were transferred into the measuring chamber containing 1 mL of buffer solution (10 mM sodium phosphate, pH 6.5) to measure oxygen consumption at 15, 18, 21, 24, 27, 30, 33, and 37°C.

Oxygen consumption was expressed as the change of O_2 content in nanomoles per min per gram of fresh tissue.

RESULTS

During the vegetation period the content of water-soluble proteins and carbohydrates in ginseng roots were varying considerably (Fig. 1), but there were no differences in their contents between stele and cortex. Maximal protein contents were noted at the end of April (the sprouting time), and at the end of August (Fig. 1A), while the curve of change in content of water-soluble carbohydrates had three peaks: at the end of April, at the end of July and at the end of November (Fig. 1B).

The specific activity of UGPase was notably changing similarly both in stele and in cortex of roots during the vegetation period (Fig. 2A), having maximum at the end
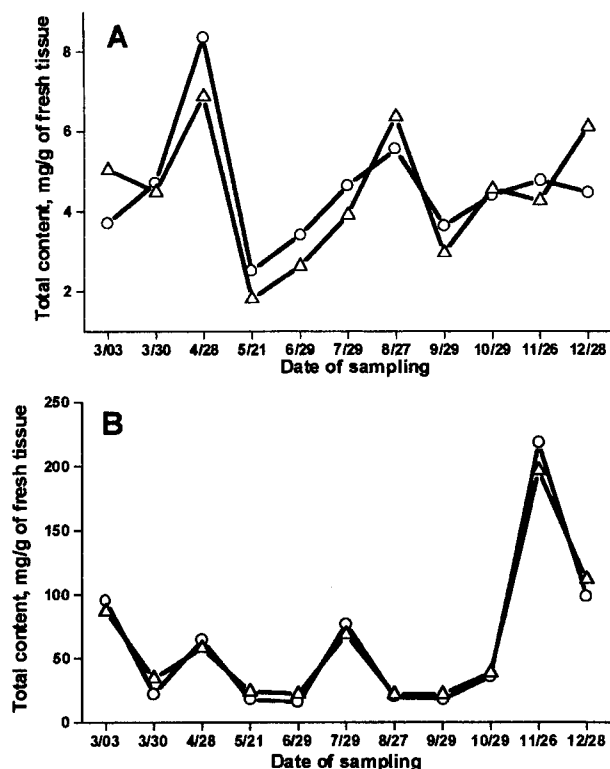
Fig. 1. Variation in the water-soluble protein (A) and carbohydrate (B) contents in extracts from stele (–○–) and cortex (–△–) of ginseng roots.

Fig. 2. Variations in the specific activity (A) and total content (B) of UDP-glucose pyrophosphorylase in stele (–○–) and cortex (–▲–) of ginseng roots during the vegetation period. (---) : temperature

of May. But its total content was dropping during vegetation period (Fig. 2B). These marks for stele and cortex did not differ considerably.

The specific activity of SS had raised 10-fold during period since the end of April (sprouting) till the end of May (full foliage), then had returned to the initial value to the end of July (Fig. 3A). Total content of this enzyme had increased 3-times in stele and 2-times in cortex, following the change tendency in activity (Fig. 3B). Both specific activity and total content of sucrose synthase were higher in stele of roots.

The AGPase activity in stele was not detected at the end of April, while its highest value was observed both in stele and in cortex at the end of May (Fig. 4). After that it declined continuously till the end of October. Total content of AGPase was higher in stele than in cortex. But at the end of July, its activity was not detected in stele. At that time, the average day temperature was higher than 24°C (Fig. 4). Thus, starch synthesis in stele, the main sink organ of ginseng roots, was disturbed during 1-1.5 months the period when the environmental average temperature was higher than 24°C.
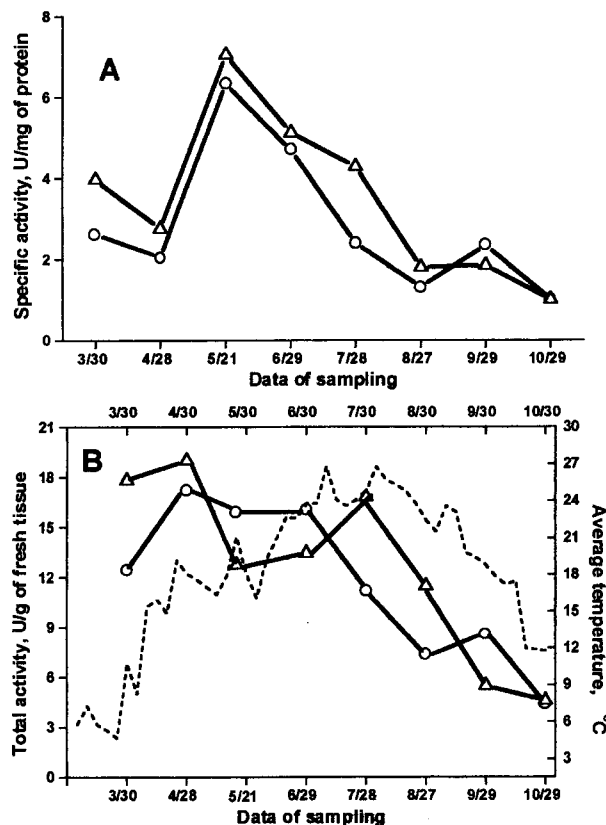
To estimate effect of temperature on respiration process in stele we used independent approach in which oxygen electrode was used to measure oxygen consumption in freshly cut stele discs at different temperatures-15, 18, 21, 24, 27, 30, 33, and 37°C. Oxygen consumption raised in a linear manner at temperature from 18 to 24°C, then sharply increased and dropped after 33°C (Fig. 5).

DISCUSSION

Growth and development of plants are dependent upon the energy gained by fixing carbon dioxide into carbohydrates during photosynthesis and the translocation of newly fixed photoassimilates from their site of synthesis to regions of utilization and/or storage. Carbohydrate conversions are fundamental in supporting the life of plants because sugars feed into essentially all aspects of plant metabolism.

Sucrose is a major product of photosynthesis in the cytosol of source organs and the main carbohydrate translocated via
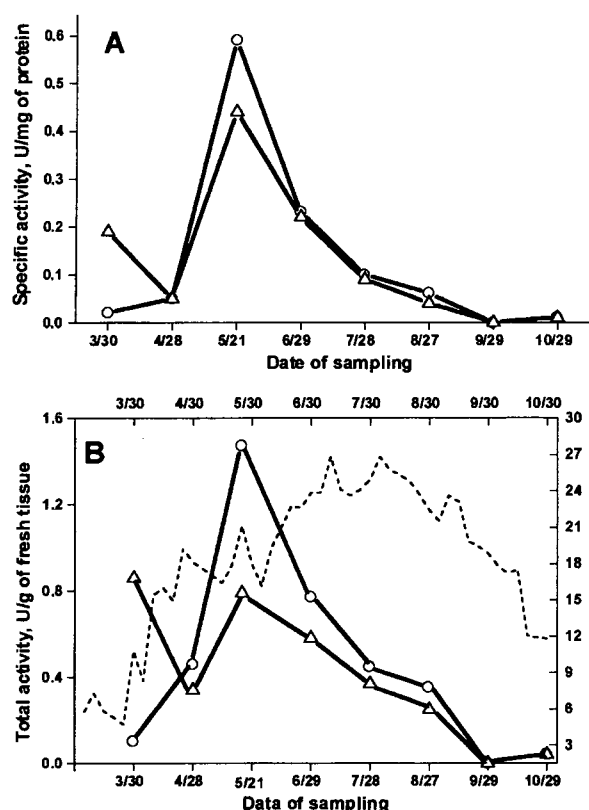
Fig. 3. Variations in the specific activity (A) and total activity (B) of sucrose synthase in stele (−O−) and cortex (−△−) of ginseng roots during the vegetation period.
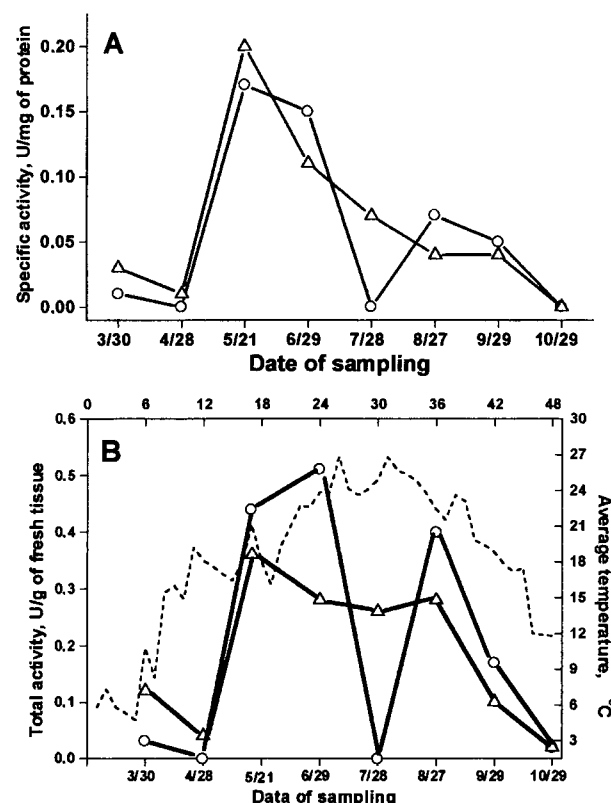

Fig. 4. Variations in the specific activity (A) and total content (B) of ADP-glucose pyrophosphorylase in stele (−O−) and cortex (−△−) of ginseng roots during the vegetation period. (Dash line designs the day average temperature).

the phloem to non-photosynthetic sink organs; it is the major form of carbon which plant sinks grow on. [5,6] In sink tissues, the sucrose is metabolized or deposited in the form of reserve compounds such a starch or lipids. It was demonstrated that sucrose synthase (UDP-D-glucose:D-fructose 2-α-glucosyltransferase, EC 2.4.1.13) has the predominant sucrose breakdown activity in the cytoplasm of actively filling sinks whereas its activity decreased more than fivefold in mature and quiscent sinks.[7-9] The UDP and PPi-dependent sucrose synthase pathway is used as a biochemical measurement of sink strength.

The major reserve carbohydrate in ginseng is starch. It is stored in the form of water-insoluble, osmotically inactive granules in amyloplasts and chloroplasts, which are the major if not the only subcellular organelles where starch biosynthesis takes place. The starch synthase and branching enzyme are responsible for the formation of amylose and amylopectin, by the addition of one molecule of ADP-glucose to an α-1,4-glucosyl chain and by cleavage of an α-1,4-glucan chain and religation via an α-1,6 linkage, respectively. ADP-Glucose is formed from

glucose-1-phosphate and ATP by the action of the enzyme ADPglucose pyrophosphorylase (ATP: α-glucose-1-P adenylyltransferase, EC 2.7.7.27), which catalyzes this important regulatory step in the biosynthesis of starch in plants.[10] In nonphotosynthetic storage organs the role of AGPase in starch deposition is controlled primarily at the transcriptional level and/or mRNA stability. Thus, the enzyme AGPase is thought to control the rate of starch synthesis and can be also used as a determinator of sink strength.

It has been reported that seasonal variation in activity is greater in some enzymes of plant sugar metabolism than in others. Much of the variation occurs either during plant development or in response to rapid, stressful environmental changes. Thus it was suggested that there are maintenance and adaptive pathways for glycolysis and gluconeogenesis.[11,12]

Adaptive enzymes are characterized by large (5- to 10-fold or more) and rapid changes in specific activity from very low levels. Maintenance enzymes are characterized by relatively small (no more than 3-fold) and slow

changes in specific activity associated with development or in response to changing environmental conditions.

According to these criteria, we can identify UGPase of ginseng roots as maintenance enzyme, whereas sucrose synthase and AGPase can be identified as adaptive ones, appeared in connection with the refilling process in ginseng roots.

Temperature is one of the major environmental factors that effects many processes, including leaf photosynthesis, carbon partitioning in the leaf, its allocation to developing sinks, and acquisition of assimilates among different sinks.[13] Elevated temperatures is known to lead to (a) increased photorespiration and inhibition of net photosynthesis in leaves[14]; (b) increased respiration rates and therefore a considerable loss of photosynthate in growing sinks such a roots[15]; and (c) a reduction of sucrose import into storage sinks such as potato tubers.[16]

Ginseng cultivation involves many difficulties in practice due to the ginseng plant itself has botanical properties different from those of other plants. Wild ginseng plants have been found mostly in cool areas of the Northern hemisphere. The average air temperature of ginseng growth in the winter and the summer are 0.9~13.8°C and 20~25°C, respectively. The physiological disorders are considered to arise at temperatures above 35°C.

The most prominent feature observed in the period under review is the pronounced influence of the environmental average temperature on filling process. Namely, the AGPase activity was not observed in stele at the end of July, when the average temperature was higher than 24°C (Fig. 4). At the same time, the increased content of UGPase in cortex (Fig. 2B) was probably connected with the activation of respiration processes.

Study on the dependence of oxygen consumption in stele tissue with temperature revealed the sharp accelerating of this process after 24°C (Fig. 5). Since the most commonly used respiratory substrates are carbohydrates, rates of respiration must have a profound effect on carbohydrate metabolism.

It is interesting to note that the minimal content of water-soluble carbohydrates observed at the ends of May and June (Fig. 1B) clash with the highest activity of AGPase (Fig. 4). During heat-wave period the considerable increase of these carbohydrates and proteins was observed (Fig. 1). It is known that a general stress response in all kingdoms is expression of protective proteins and increased amounts of metabolites which are a part of normal metabolism and which are considered compatible solutes. Examples are sugars, sugar alcohols, low-complexity carbohydrates (e.g., fructans, raffinose series), tertiary amines, sulfonium compounds and amino acids.[17] The pronounced increasing of water-soluble carbohydrates was observed also at the end of November (Fig. 1B) that can be connected with the transition period to plant dormancy.

At present, interpretation of changes in sucrose, starch metabolism and respiration at elevated temperature in ginseng roots is limited by a lack of detailed information about changes in the levels of metabolites and nucleotides.

ACKNOWLEDGMENTS

요　약

6년생 고려인삼근(Panax ginseng C.A. Meyer) 중의 Sucrose synthase, UDP-glucose pyrophosphorylase 및 ADP-glucose pyrophosphorylase의 활성을 생육 시기별로 조사한 결과, Sucrose synthase 와 ADP-glucose pyrophosphorylase는 뿌리저장활성 지표로서 adaptive enzyme의 특성을 나타내는 반면, UDP-glucose pyrophosphorylase는 maintenance enzyme으로서 존재하였다. 평균기온이 24°C 이상일 때 전분합성이 저하되고 중심부의 산소소비량이 급격히 증가되었다.
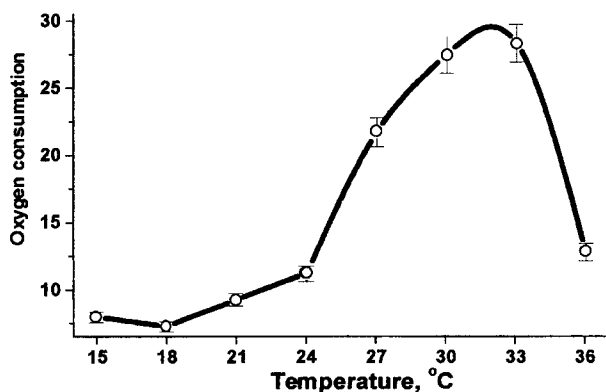

Fig. 5. Oxygen consumption in stele of ginseng root at different temperatures. (Oxygen consumption is expressed in nanomoles O2 per min per gram of fresh tissue.)

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