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Exo-O-Glycosylhydrolases in Korea Ginseng Roots

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Abstract : We were screening the stele and the cortex of the ginseng roots (*Panax ginseng* C.A.Meyer) on the exo-O-glycosylhydrolase activities during vegetation period of 1999 year. The following p-nitrophenylglycosides were used to test exo-O-glycosylhydrolase activities: α- and β-D-galactopyranosides, α- and β-D-glucopyranosides, α- and β-D-mannopyranosides, N-acetyl-β-D-glucosaminide, α- and β-D-xylopyranosides α-L-rhamnopyranoside, β-D-glucuronide, β-D-galacturonide, β-L-, α-L- and β-D-fucopyranosides, α-L-arabinopyranoside. Only β-D-galactosidase, α-L-mannosidase, N-acetyl-β-D-glucosaminidase, α-D-galactosidase, α-L-arabinosidase, and β-D-fucosidase were found in both parts of ginseng roots. Their contents during the vegetation period were shown to differ considerably, being dependent not only on plant development stage but on plant tissue and environmental conditions too.

Key words: exo-O-glycosylhydrolase, β-D-galactosidase, α-L-mannosidase, N-acetyl-β-D-glucosaminidase, α-D-galactosidase, α-L-arabinosidase, β-D-fucosidase, seasonal variation, heat-shock stress, *Panax ginseng*.

INTRODUCTION

Despite of the long history of ginseng cultivation, the biochemical events carrying out in this plant are not elucidated yet. The aim of our investigation was to study seasonal variation in the activity of enzymes involved in metabolism of carbohydrates, whose conversions are fundamental in supporting the life of plants because sugars feed into essentially all aspects of plant metabolism.

Plenty of different enzymes are incorporated in the process of carbohydrate metabolism. But in the living cell all enzymes for which genes are presented are not being synthesized with maximum velocity all the time. On the contrary, the rate of production of the various enzymes is under powerful control in accordance with the metabolic needs and state of development of cell and organism. The mode of operation of this control is a question of the greatest biological importance.

In the current paper we report the study on the seasonal variations of the exo-O-glycosylhydrolases in stele and cortex of the 6th-growing year ginseng roots.

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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 in Taejon).

For enzyme activity assay only the root stem was used. Freshly sampling roots were washed under tap water. Side roots were removed. Inside (xylem and pith) and outside (phloem and cambium layer) parts of roots were studied separately.

2. Extraction procedure

Extraction procedure was described in detail in previous paper.[1)]

3. Enzyme activity assay

Reaction mixture (100 μl of 0.1% substrate, 100 μl of 0.2 M Na acetate buffer, pH 4.5, and 50 μl of extract) was incubated at 30°C for 15 min (for β-D-galactosidase, α-D-mannosidase and N-acetyl-β-D-glucosaminidase) or 60 min (for the rest enzymes). Neutral α-D-galactosidase was assayed at pH 7.0. The reaction was stopped by adding 1 mL of 0.5 M Na_2CO_3, and liberated p-nitrophenol was measured

Table 1. Harvesting dates of ginseng plants of 6th year growing period

Year	1998			1999							
Month	October	November	December	March	April	May	June	July	August	September	October
Day	29	26	28	4, 23	23	10, 25	23	14, 28	13, 23	9	8

at 400 nm.

All assays were performed in triplicate and readings were reproducible to within 10%.

One unit of enzyme activity was defined as the amount that hydrolyzed 1 nanomole of p-nitrophenyl glycoside per minute at the reaction conditions. Total activity was expressed in units per gram of fresh tissue.

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

RESULTS AND DISCUSSION

Two classes of enzymes are primarily involved in carbohydrate metabolism. Glycosylhydrolases participate in the catabolism of glycoconjugates, and glycosyltransferases are involved in the biosynthesis of oligosaccharides. Exo-O-glycosylhydrolases are more commonly named as glycosidases, referring to enzymes that progressively hydrolyze principally mono- or disaccharides from the non-reducing terminal of oligo- or polysaccharides.

Plant cell wall is complex carbohydratebased dynamic interface that lies between the plant cytoplasm and its external environment and participates directly in cellular responses to exogenous stimuli.[2,3] Plant development involves a coordinated series of biochemical processes that, among other things, result in the biosynthesis and degradation of cell wall components. The controlled breakdown of polymers within the wall by endogenous cell wall-degrading enzymes has been proposed to play a role in ripening, abscission, cell division, growth, respiration, signal transduction, and pollen development.[4,5] But the role of specific plant cell wall-degrading enzymes in cell wall metabolism during growth and development remains unclear.[5]

Some exo-O-glycosylhydrolases may trim particular nonreducing terminal sugar residues off cell wall polysaccharides, oligosaccharides, and glycoproteins. Nonreducing terminal of side chains of branched polysaccharides seem to be major target of exo-O-glycosylhydrolases *in vivo*.

We were screening the ginseng roots on the exo-O-glycosylhydrolase activities during vegetation periods of 1998-1999 years. The following p-nitrophenylglycosides were used to test exo-O-glycosylhydrolase activities: α- and β-D-galactopyranosides, α- and β-D-glucopyranosides, α- and β-D-mannopyranosides, N-acetyl-β-D-glucosaminide, α- and β-D-xylopyranosides α-L-rhamnopyranoside, β-D-glucuronide, β-D-galacturonide, β-L-, α-L- and β-D-fucopyranosides, α-L-arabinopyranoside. Data for 1998 year were published early.[6] Dates of root sampling in 1999 are presented in Table 1.

Only β-D-galactosidase (EC 3.2.1.23), α-D-galactosidase (EC 3.2.1.22), α-L-arabinosidase (EC 3.2.1.55), β-D-fucosidase (EC 3.2.1.28), α-D-mannosidase (EC 3.2.1.24), and N-acetyl-β-D-glucosaminidase (EC 3.2.1.30) were detected in the roots at the reaction conditions.

During all period under study, content of β-D-galactosidase, α-L-arabinosidase, and β-D-fucosidase was higher in the cortex in comparison with the stele (Fig. 1), while tendency of changes in each enzyme content was almost identical for both root parts.

β-Galactosidase is the most abundant exo-O-glycosylhydrolase both in stele and cortex of ginseng roots. A cell wall β-galactosidase was shown to involve in the removal of galactose from cell wall components of different plants.[7-11] Some authors had reported α-L-arabinosidase related to the elongation process and cell wall autolytic processes.[12,13] There are no some clear data about the function of β-D-fucosidase in plants. The potential substrates for these enzymes are hemicellulose, pectic polysaccharides and arabinogalactan proteins. Small pectic fragments released by the action of these hydrolases act as signals to induce expression of other pectolytic enzymes.[14]

The above mentioned exo-O-glycosylhydrolases are typical acid hydrolases having pH optimum of action around 4.0. At pH 7.0 their activities reduced 2-3 times (data not displayed). They are located in vacuoles. But some isoforms of α-galactosidase are known to be intracellular enzymes with pH optimum of action about 7.0. So, we checked up α-galactosidase activity at two different pH-4.5 and 7.0. During the sprouting period, contents of both α-galactosidases were almost identical in both parts, but there was difference in the time of maximum content between acid and neutral forms of enzyme (Fig. 2). During the roots refilling period (June-August),

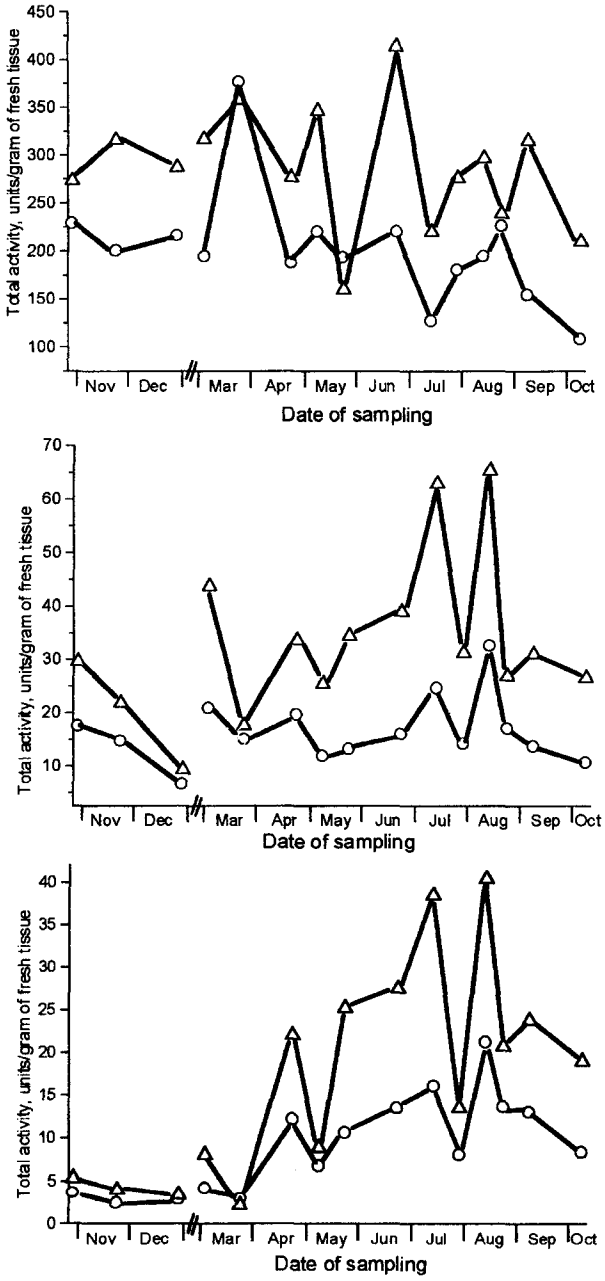

Fig. 1. Variation in the contents of β-D-galactosidase (A), α-L-arabinosidase (B), and β-D-fucosidase (C) in extracts from stele (–○–) and cortex (–△–) of ginseng roots during the vegetation period.

their contents were higher in the cortex in comparison with the stele (Fig. 2). Moreover, their expression in the cortex and the stele of ginseng roots during heat stress differed.

So, in the roots sampled July 14 and August 13, when average day temperature was higher than 24°C during several days (Fig. 4), the contents of both a-galactosidase

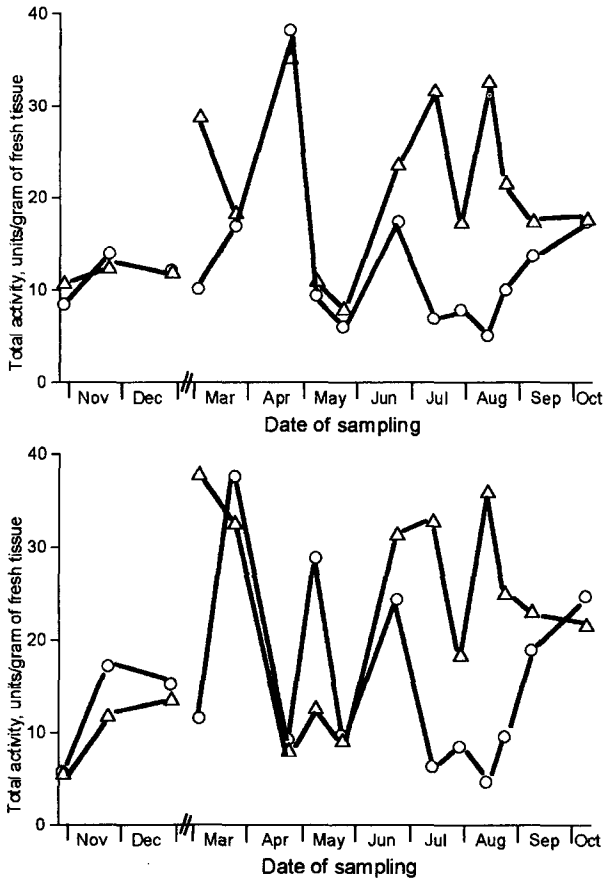

Fig. 2. Variation in the contents of acid (A), and neutral (B) α-D-galactosidase in extracts from stele (–○–) and cortex (–△–) of ginseng roots during the vegetation period.

forms raised in cortex, being in good coincidence the increasing contents of water-soluble carbohydrates and proteins (Fig. 3). At the same time, in the stele their activities were suppressed. In previous work we had shown that the respiration processes in the stele tissue were sharply activated when temperature was higher then 24°C.[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.[15)] The initial step in catabolism of the oligosaccharides of raffinose family is the hydrolysis of galactosyl residues by a-galactosidase.[16)]

The metabolic processes in the stele and the cortex seem to be affected with high temperature stress in different way. In *Cucurbitaceae* two species of a-galactosidase

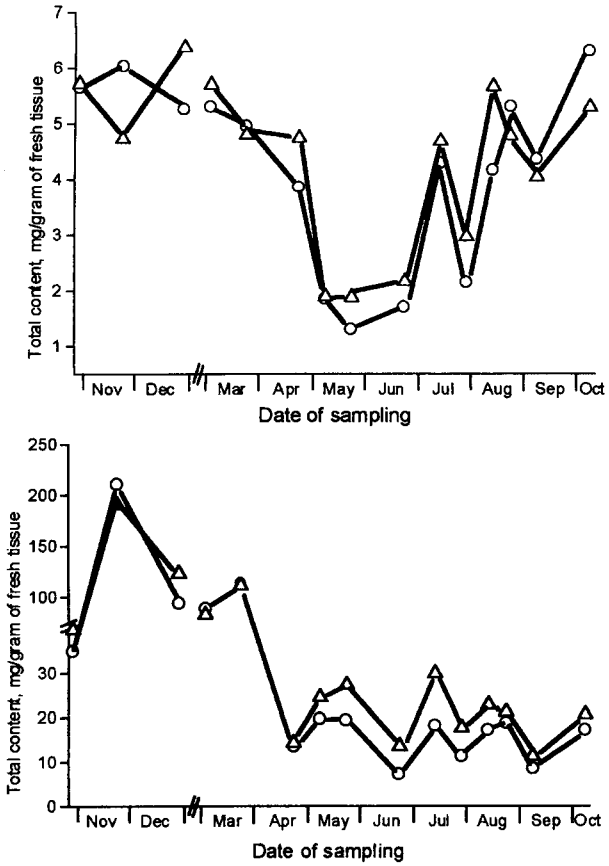

Fig. 3. Variation in the contents of the water-soluble protein (A) and carbohydrate (B) in extracts from stele (–○–) and cortex (–△–) of ginseng roots during the vegetation period.

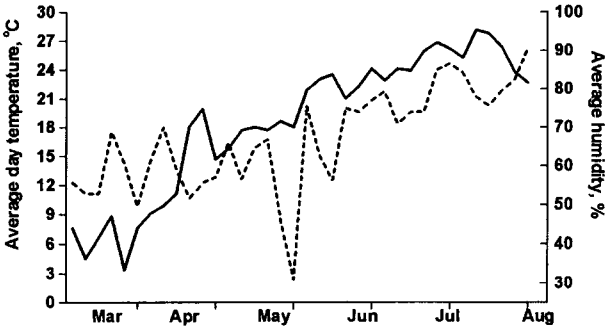

Fig. 4. Dynamics in changes of average day temperature (契) and average day humidity (-----) in Taejon during spring and summer 1999. (Data of Korea Meteorological Administration)

having distinct pH optimums acid and alkaline have been identified and dependence of their distribution and function on plant development and plant tissues was proposed.[17,18)]

The seasonal variations in contents of a-mannosidase and N-acetyl-β-D-glucosaminidase were in coincidence with

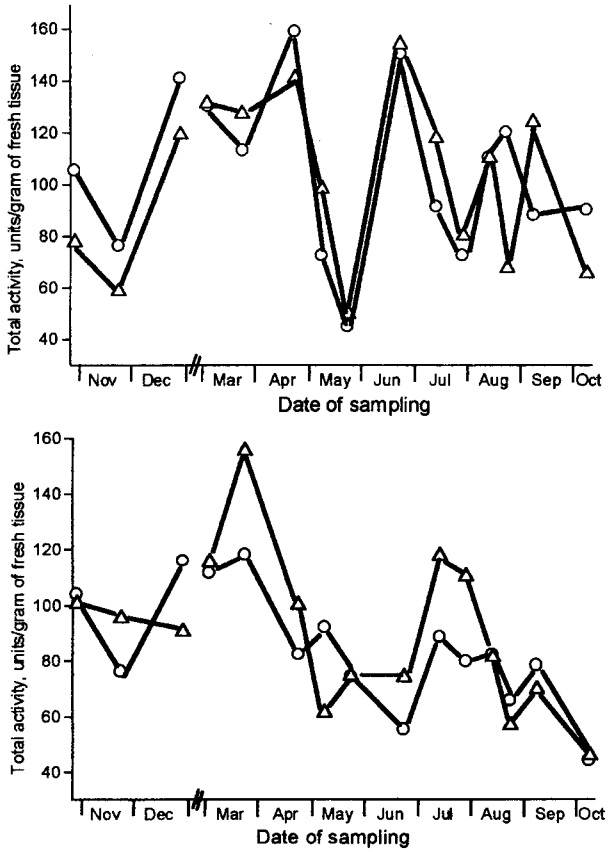

Fig. 5. Variation in the contents of α-L-mannosidase (A) and N-acetyl-β-D-glucosaminidase (B) in extracts from stele (–○–) and cortex (–△–) of ginseng roots during the vegetation period.

the development stage (Fig. 5). These enzymes are commonly encountered in plants and implicated in the N-linked glycosylation pathway.[19,20)] α-D-Mannosidase participates in the catabolism of cell-wall carbohydrates, too. The highest contents of these enzymes were observed during sprouting period. During refilling period the expression of N-acetyl-β-D-glucosaminidase (Fig. 5B) seemed to be connected with heat-shock proteins turnover (Fig. 3A). At the same time, dynamics of a-mannosidase content was more complex and resembled more the behavior of enzymes degrading the cell-wall polysaccharides (Fig. 1).

Our understanding of the effects of stress on carbon partitioning processes is still very poor. Extreme temperatures are known to be the adverse environmental factors commonly encountered by land plants. However, the adverse environmental factors are almost never present alone. It is necessary, therefore, to understand how plants respond to combined stress signals. An increasing number studies indicate cross-protection between different envi-

ronmental stresses. From our observation, the higher level of humidity seems to protect ginseng plant against heat-shock stress.[21] So, extracts from roots collected in July 14 and August 13 contained more water-soluble carbohydrates and proteins then extracts from roots harvested in July 29 (Fig. 3). The two groups also differed in expression of glycosidases (Fig. 1, 2, 5). That period was characterized with high average day temperatures exceeded 24°C. But at the end of July average humidity was considerably higher then in the middle of July and in the middle of August.

The precise function of exo-O-glycosylhydrolases has yet to be determined, but they are doubtless involved in numerous processes, from wall modification to metabolite transport and cell signaling. These enzymes must be characterized in terms of their substrate requirements, substrate affinities and spatial and temporal patterns of expression. The individual roles of such enzymes, as well as their cooperative interaction, is needed for understanding of roots carbohydrates metabolism which is still in its infancy and is a fertile area for future studies.

ACKNOWLEDGMENTS

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요 약

6년생 고려인삼근(*Panax ginseng* C.A. Meyer)중 수종의 exo-O-glycosylhydrolase 활성을 중심부와 주피부로 나누어 생육시기별로 조사하였다. α-D-galactosidase, β-D-galactosidase, α-L-mannosidase, N-acetyl-β-D-glucosaminidase, α-D-galactosidase, α-L-arabinosidase와 β-D-fucosidase는 중심부와 주피부에서 모두 활성이 있으나 β-L-mannosidase, α-D-xylosidase, β-D-xylosidase, α-D-rhamnosidase와 β-D-glucosidase의 효소활성은 검색되지 않았다. β-D-galactosidase의 활성은 연중 높게 유지되었고 α-L-mannosidase의 활성도 높은 경향이었다. 인삼근중 탄수화물 대사 효소의 활성은 생육시기와 환경조건 및 부위에 따라 매우 다른 양상을 나타내었다.

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