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Review

Proteomic analysis of Korean ginseng (*Panax ginseng* C.A. Meyer)

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Abstract

Although many reports have been published regarding the pharmacological effects of ginseng, little is known about the biochemical pathways operant in ginsenoside biosynthesis, or the genes involved therein. Proteomics analysis is an approach to elucidate the physiological characteristics and biosynthetic pathways of ginsenosides, main components of ginseng. In this review, we introduced the recent progress in proteomics studies of ginseng (*Panax ginseng C.A. Meyer*). We briefly reference the genomic analyses of *P. ginseng*, without which proteomics approaches would have been impossible. Functional genomics studies regarding secondary metabolism in *P. ginseng* are also introduced here, in order to introduce possible future prospects for further study.

Keywords: *Panax ginseng*; Proteomics; *Araliaceae*

Contents

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1. Introduction

Ginseng is a perennial herb of the family *Araliaceae*. Seven major species of ginseng are distributed throughout East Asia, Central Asia, and North America [1,2]. Most studies of ginseng have been carried out in *Panax ginseng* (Asian ginseng), *Panax quinquefolius* (American ginseng), or *Panax japonicus* (Japanese ginseng). The roots of *P. ginseng* have been commonly used in the formulation of tonics in Eastern Asia for over 2000 years. It is believed that the root of *P. ginseng* is a panacea, i.e., it is both a universal cure and it also promotes longevity. Various clinical and pharmacological effects associated with its use have been reported, such as anti-cancer activity, anti-circulatory shock effects, promotion of hematopoiesis, and modulation of immune functions and cellular metabolic processes on carbohydrates, fats and proteins [3–5].

It is believed that the pharmacological effects of *P. ginseng* vary according to the age and species of *P. ginseng* [6]. For example, it was reported that aged *P. ginseng* had a more pronounced anti-carcinogenic effect on lung tumors in mice than the unaged plant [7]. It is also believed that different parts of *P. ginseng* exhibit different properties and medical values [8].

Because *P. ginseng* must grow for at least 3 years before it begins to exert medically valuable effects, the productivity of *P. ginseng* can be significantly affected by various environmental factors such as temperature, condition of soil, light intensity, content of water and disease. Therefore, extensive studies have been carried out to elucidate the physiological responses of *P. ginseng* to environmental changes [9–12]. Methods for the differentiation and authentication of *P. ginseng* species and products have been developed in response to commercial demand. Those methods include product analysis using HPLC/mass spectrometry (MS) [6], PCR-restriction fragment length polymorphism [13–17], and proteome analysis [8].

Ginsenosides are considered to be the main active pharmacological compounds in *P. ginseng*. Thirty-one ginsenosides have, so far, been isolated from natural and processed *P. ginseng* roots, and novel ginsenosides continue to be reported [18,19]. The distribution of ginsenosides varies from species to species [2]. Each ginsenoside has different pharmacological effects, and even one single ginsenoside has been demonstrated to produce multiple effects in the same tissue [1,20,21].

Although many reports have been published regarding the pharmacological effects of ginsenosides, little is known about the biochemical pathways operant in ginsenoside biosynthesis, or the genes involved therein. In the effort to delineate these biosynthesis pathways and their regulation, a variety of analytical techniques have been employed, including *P. ginseng* EST sequence analysis [18].

Though there are many technical challenges and pitfalls, proteomics is becoming an essential technology in the field of biological science. Recent technical improvements in two-dimensional polyacrylamide gel electrophoresis (2-DE) and MS have made it possible to rapidly identify hundreds of proteins and investigate levels of protein expression, subcellular localization, and post-translational modification. Proteomics is applicable to the characterization of individual cells, estimation of genetic variability, phylogeny, characterization of mutants, screening of useful proteins, and the determination of the effects of different factors (e.g., light, heat, cold, and hormones) on plant growth [22,23].

In this review, we will discuss the proteomics challenges regarding *P. ginseng*, and recent proteomics studies of *P. ginseng*. For this purpose, we shall also briefly refer to the genomic analyses of *P. ginseng*, without which proteomics approaches would have been impossible. Functional genomics studies regarding secondary metabolism in *P. ginseng* are also introduced here in order to introduce possible future prospects for further study.

2. Genomics of *P. ginseng*

Genomic studies of *P. ginseng* were initiated by the Plant Diversity Research Center (PDRC), which was established in 2000 by the Ministry of Science and Technology in Korea. The PDRC aims at the promotion of systematic management and utilization of plant resources indigenous to the Korean peninsula. *P. ginseng* has been selected as one of Korea's most important plant resources due to the richness and utility of its secondary metabolites like ginsenosides. However, the amount of genetic information on *P. ginseng* remains quite limited. Studies on *P. ginseng* genomics have been conducted within the framework of the PDRC's signature projects. The Center made funds available for the construction of bacterial artificial chromosome (BAC) library and sequence analyses of expressed sequence tag (EST) of *P. ginseng*.

2.1. BAC-end sequencing project

A BAC library of Korean *P. ginseng* was constructed and BAC-end sequences were obtained [24] which were then used to provide an initial view of the make-up of the

P. ginseng genome, with respect to the frequency and nature of protein-coding regions, transposable elements, and repetitive DNA. Of the 2167 BAC clones sequenced, 3127 ends generated 2492 non-redundant BAC-end sequences based on a phred quality value of 20. The average length of the BAC-end sequences was approximately 400 bp, with a total read length of 0.99 Mb. BAC-end sequence analysis revealed 10.2, 20.9, 3.8, and 65.1% of the sequence contributed to putative protein-coding regions, transposable elements, microsatellites and unknown genomic sequences, respectively.

The protein-coding regions found in the BAC-end sequences are useful in the identification of BAC clones encoding for proteins, which have been previously identified in other organisms. Comparison of the 2492 BAC-end sequences with the NR and EST databases of GenBank, and the *Arabidopsis* protein database of TAIR using BLAST (with a cut-off value of $\leq 10^{-6}$) revealed 254 protein-coding regions. Of these, 167 exhibited homology with known proteins which had been identified in other organisms. These protein-coding regions were categorized into different functional classes. Comparison of the functional categories of protein-coding regions, and their relative abundance, with those of *Arabidopsis* revealed that proteins related to metabolism, cell cycle and DNA processing, systemic regulation of interaction with the environment, development, and control of cellular organization were underrepresented in the *P. ginseng* sample, compared to *Arabidopsis*. On the other hand, proteins pertaining to energy, protein synthesis, cofactor requirement, subcellular localization, and transport facilitation were comparatively over-represented in the sequence sample from Korean ginseng.

A comparison of the BAC-end sequences with the EST database of Korean ginseng (http://plant.pdrc.re.kr/genepool/PG/ginseng.html) detected sequence homology with 254 ESTs. Of these, only 96 protein-coding regions exhibited a high degree of homology (*e*-value $\leq 10^{-4}$) with known proteins. The lower number of protein-coding regions identified in the BAC-end sequences may be attributable to the absence of introns in the ESTs. Using this EST database, BAC-end sequences were also analyzed for homology with the genes involved in the biosynthesis of ginsenoside, the active compound of medicinal importance [24].

2.2. EST sequencing project

An annotated list of approximately 26,000 ESTs of *P. ginseng* sequenced are available at http://plant.pdrc.re.kr:7777/index.html. For the purposes of EST sequencing, eight cDNA libraries were constructed from different organs, including the taproot, rhizome, developing seed, in vitro cultured seedling, soil-grown seedling shoot [18], hairy root produced by *Agrobacterium rhizogenes* infection, and jasmonate-treated hairy root. Only 59% of the unique sequences exhibited significant homology with previously known polypeptide sequences in the GenBank database.

One specific application of EST is the isolation of relevant genes. Via a keyword search of BLASTX results and a domain search of *P. ginseng* ESTs, several genes involved in ginsenoside biosynthesis could be identified. These include four oxidosqualene cyclase candidates involved in the cyclization reaction of 2,3-oxidosqualene, 9 cytochrome P450s, and 12 glycosyltransferase candidates that may be involved in modification of the triterpene backbone [18].

3. Proteomics of *P. ginseng*

3.1. Proteomic challenges of P. ginseng

3.1.1. Sample preparation for 2DE

Recent technical improvements in proteomic analysis have made it possible to identify hundreds of proteins, and thus provide information regarding protein expression and cellular regulation. But the application of proteomic analysis to plant samples still has some limitations. Acquiring a smart and reproducible 2-DE gel image remains a difficult proposition, largely due to the interference of compounds such as lignins, polyphenols, tannins, alkaloids and pigments [25]. Therefore, the removal of these compounds is crucial to the acquisition of a clear 2-DE gel image. For the extraction of proteins from *P. ginseng* roots and leaves, we attempted two general methods for the preparation of plant samples: homogenization [26] and grinding with liquid nitrogen [27]. Via comparisons of 2-DE gel image analysis from differently prepared samples, we determined that grinding was a more efficient method for the removal of interfering compounds [28]. Ground samples were incubated in sample buffer containing detergent (0.3% SDS), reducing agent (200 mM DTT) for complete solubilization, and with DNase and RNase for the removal of nuclei acids. However, treatment with protease inhibitors had no effect in terms of improving the 2D image. Finally, the supernatant of the sample was prepared by TCA precipitation for the removal of contaminants. On the other hand, Lum et al. [8] employed a homogenization with extraction buffer containing urea, CHAPS, and DTT.

3.1.2. Databases

Proteome identification by mass spectrometry relies on genome sequence information from a database. Proteomic research on *P. ginseng* is, therefore, quite limited, as the full genome has not been sequenced. Although some plant protein databases are publicly available, such as for *Arabidopsis thaliana*, *Oryza sativa*, *Pinus pinaster*, and *Zea mays* [22], the use of these sequences for *P. ginseng* proteome analysis is limited. It is especially difficult to use these marginally related databases for the identification of specific proteins in *P. ginseng*. Several reports have, however, suggested that EST databases could be used for protein identification [29,30]. The total of 16,500 *P. ginseng* EST sequences compiled so far by Biopia (http://www.ibiopia.com/; Kyunggi-do, Korea), and 11,600 by Eugentech (http://www.eugentech.com/;

Daejeon, Korea). Some *P. ginseng* EST sequences exhibit a significant degree of homology with previously-known polypeptide sequences, but many portions displayed no homology whatsoever to previously-known sequences [18]. EST sequences are invaluable for use in the identification of genes involved in secondary metabolite biosynthesis [31].

The typical shortcomings of EST databases are that, firstly, many proteins are not represented and, secondly, the error rate in the EST sequences is often significant. Peptide mass fingerprinting alone may not result in the successful identification of proteins from EST databases as ESTs are generally too short to ensure significant protein coverage and sufficient number of matching peptides [32,33].

When the *P. ginseng* EST database was employed to match the MS/MS spectra produced by ESI-Q-TOF mass analysis, more proteins were identified than when using the NCBI nr protein databases. We tested the accuracy of this method by comparing the peptide sequence identified by database search with the results of de novo *sequencing*. The consistency of database search results was dependent on both the databases used, and the database search programs employed (Table 1). The accuracy of identification was increased when proteins were identified by more than two combinations of databases and/or search programs. By combining search results importing the EST database or NCBI nr database of green plants and MASCOT or SEQUEST search programs, more abundant and accurate peptide identification was achieved [34].

3.1.3. Selection of appropriate tissue

According to Korean and Chinese traditional medical knowledge, different species, age and parts of *P. ginseng* are believed to embody different properties and medical values [7,8]. This means that the physiological properties and the composition of active components of *P. ginseng* may differ by age, species, and part. Therefore, which *P. ginseng* tissue is employed in an experiment takes on increased importance.

If the purpose of proteomic analysis is the identification of genes involved in the biosynthesis of active components of *P. ginseng*, the hairy root of ginseng would appear to be an excellent target. Hairy roots are induced by the integration of T-DNA of Ri-plasmid of *A. rhizogene*s into the plant genome. It has been demonstrated that hairy roots exhibit rapid growth rates and a high degree of genetic stability, and also that they do not require exogenous growth hormone [35–37]. The induction and establishment of the hairy roots of *P. ginseng* has been successfully performed using this method [38–41]. These transformed hairy root lines grow more rapidly, and produce higher levels of saponins. The quantity and quality of ginsenoside production appears to vary according to their morphology [8,42]. With such hairy root lines, culture conditions can be optimized more easily [43,44]. The addition of biotic and abiotic elicitors to hairy root culture medium can modulate the production of ginsenosides [45], which should be considered to be the result of a fundamental change in cellular regulation.

3.2. Proteomics studies in P. ginseng

3.2.1. Proteome analysis of wild root and cultured hairy root

As a first step toward a comprehensive *P. ginseng* proteome analysis, the proteins expressed in cultured hairy roots were analyzed for the purposes of constructing a 2-DE

Table 1

(a) The number of high score hits obtained from each DB search and the number of sequences that are consistent with the DNS results, using 176 MS/MS spectra [34]

Database	DB search methods	Results of database search		Results of sequence alignment	
		Number of hits with high score (A)	Hit ratio (A/176[a])	Number of peptides similar to DNS (B)	Consistency with DNS (B/A)
Ginseng	MASCOT	105	60%	96	91%
EST	SEQUEST	112	64%	94	84%
Green plant	MASCOT	50	28%	50	100%
Protein	SEQUEST	84	48%	55	65%

(b) Summary of peptide sequences found more than once among the four different combinations between Ginseng EST DB or NCBI nr protein DB and MASCOT or SEQUEST search programs

Database	DB search methods	Results of database search		Results of sequence alignment	
		Number of high score hits found more than once among the other methods (C)	Hit ratio (C/176[b])	Number of peptides similar to DNS among the (C) sequences (D)	Consistency with DNS (D/C)
Ginseng	MASCOT	104	59%	96	92%
EST	SEQUEST	110	63%	94	85%
Green plant	MASCOT	50	28%	50	100%
Protein	SEQUEST	76	43%	55	72%

[a] 176 is the total number of the MS/MS spectra sample set.
[b] 176 is the total number of the MS/MS spectra sample.

Table 2
Summary of protein analysis of *Panax Ginseng C. A. Meyer* proteome [46]

Steps	Protein number
2-DE analysis (pH 3–10, 4–7, 4.5–5)	~300
MADLI–TOF Mass spectrometer (peptide mapping)	159
Function-identified proteins	17 (4)[a]
Function-unidentified proteins	142
ESI-Q-TOF mass spectrometer (amino acid sequencing)	102
Function-identified proteins	87
Function-unidentified proteins	15
Total function-identified proteins	91

[a] Of the 17 proteins identified by peptide mapping, 13 proteins were included in the 87 proteins identified by amino acid sequencing and only four proteins were not included.

reference map. *P. ginseng* hairy roots were chosen because of the following advantages: (1) ease of sample preparation. Cultured hairy root grows rapidly in our culture conditions (doubling time is only 3 weeks). (2) EST database. More than 26,000 ESTs from cultured hairy root (strain KGHR-8) have been sequenced, and are currently available for protein identification. (3) From the 2-DE display, we found that more proteins are induced in cultured hairy roots. (4) Cultured hairy roots are useful in the large-scale production of secondary metabolites, most notably, ginsenosides. The understanding of *P. ginseng* metabolism in the cultured hairy roots is a prerequisite for the mass production of secondary metabolites. From the cultured hairy roots, approximately 280 protein spots were detected on a pH 3–10 gradient 2-DE gel [46] by silver staining. From the 159 cultured hairy root proteins, the putative functions of 91 proteins were ascertained (Table 2). More than 20% of the identified proteins were involved with energy metabolism and stress response. Interestingly, the glycolytic enzymes, such as enolase, glyceraldehyde 3-phosphate dehydrogenase, and aldolase were present as isotypes, which might be due to post-translational modification. Identified proteins did not include the enzymes involved in the production of secondary metabolites. To our knowledge, this is the first reference map for the *P. ginseng* proteome. The induced proteins in the wild *P. ginseng* roots were quite different from those isolated from the cultured hairy roots, although more than 90 protein spots were commonly expressed (Fig. 1). This suggests that different environments (culture conditions) may induce the expression of different proteins. Specifically, four abundant proteins (28, 26, 21 and 20 kDa) in wild *P. ginseng* root were exclusively induced as isoforms, having different p*I* values on 2-DE [47]. A 28 kDa abundant protein from the *P. ginseng* main root had been previously purified and designated GMP (ginseng major protein) but the function of GMP was, at that time, unidentifiable [48]. Using proteome analysis, we found that all of the abundant proteins originated from a common 28×10^3 kDa protein, and also that GMP exhibits a high degree of sequence homology with both plant RNases and RNase-like proteins. However, purified GMP exhibited no RNase activity, even though it contained conserved amino acid residues known to be essential in the active sites of

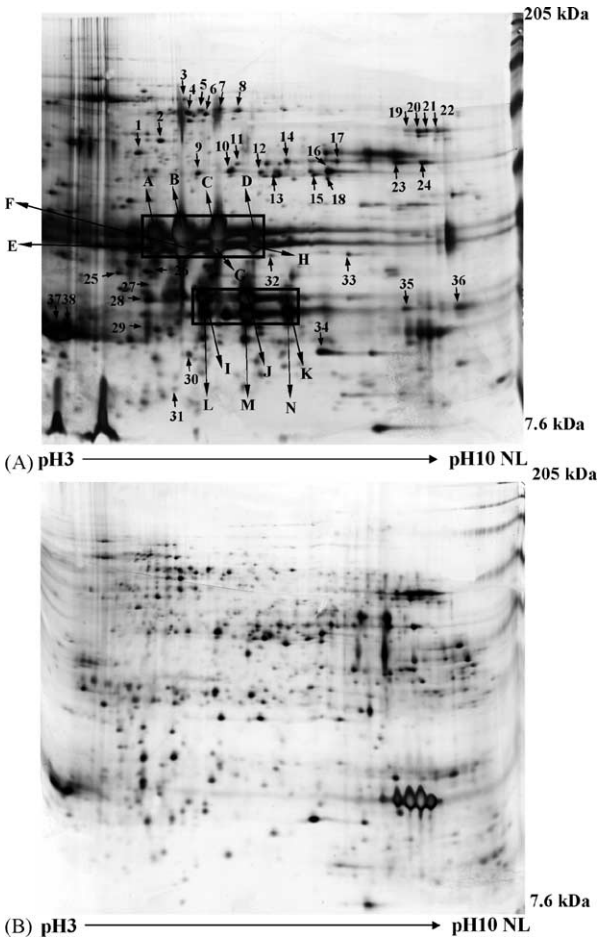

Fig. 1. 2-DE of wild main root (A) and cultured hairy root (B) of *P. ginseng*. Commonly induced and identified proteins from the wild main root and the hairy root are indicated by arrows and numbered. Ginseng major proteins (GMPs) are boxed and alphabetized [47].

RNase. Protein induction analysis in wild *P. ginseng* roots also revealed that the amounts of GMP fluctuated according to seasonal fluctuation. These results suggest that the GMPs are root-specific RNase-like proteins, which might function as vegetative storage proteins in wild *P. ginseng*, conferring survival advantages in the natural environment. Further studies are required for identifying the location of these storage proteins in the cell, and for explanation as to why there are so many variations of the isotypes. Proteome analysis will be useful in gaining a better understanding of the expression and functions of GMP in the wild *P. ginseng* roots.

3.2.2. High light-induced proteome in leaves

The ability of *P. ginseng* to adapt to light intensity is generally restricted to a 5–20% range of full sunlight [49]. Exposure to excess light, greater than 500 μmol photons m^{-2} s^{-2}, resulted in characteristic photoinhibitory symptoms, including decreased photosynthesis, increased photorespiration and chlorosis. These processes accelerate at elevated temperatures [50–52]. Controlling light intensity is, therefore, important in terms of controlling the productivity

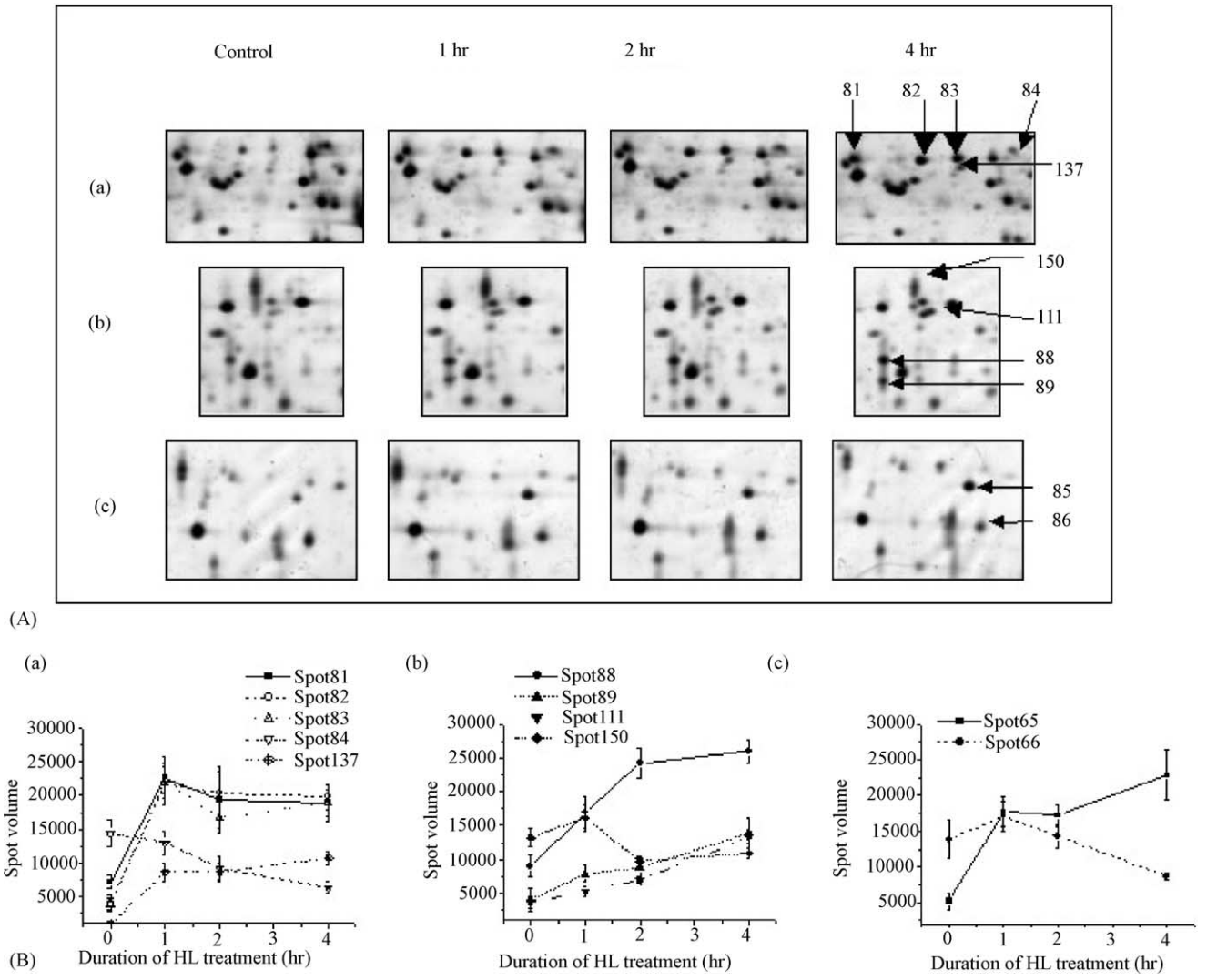

Fig. 2. HL-induced changes of protein spots. Intact plants were exposed to HL (1700 μmol photons m^{-2} s^{-2}) for 0, 1, 2, or 4 h. After HL treatment, proteins were extracted from leaves and separated by 2-DE (A). The spot volume is plotted in B [53].

of *P. ginseng*. In an attempt to elucidate the regulatory mechanisms *P. ginseng* to adapt to low light intensities, comparative proteomic analysis was performed [53]. The expression level of several proteins changed rapidly with exposure to different intensities of light (Fig. 2). The level of cytosolic ascorbate peroxidases proteins in leaves was increased rapidly in high light intensity. These results are consistent with the report on *Arabidopsis*, in which the mRNA levels of cytosolic ascorbate peroxidase underwent increases upon exposure to excess light [54]. The rapid increase (within 30 min) of cytosolic smHSP in *P. ginseng* leaves might constitute a protective mechanism, which guards the cells from damage inflicted by high light intensities, a system which is also operant in *Arabidopsis*. Light-induced damage of the thylakoid membrane proteins was also observed. It is expected that we could obtain more information regarding the light response of *P. ginseng* by comparing the light-responsive proteome changes occurring in *P. ginseng*, a less light-tolerant species, and *P. quinquefolius*, a more light-tolerant species.

3.2.3. Applications of proteome analysis

Lum et al. [8] utilized proteome analysis for the discrimination of different species (Oriental ginseng, American ginseng, and cultured ginseng), and different parts (main root, lateral roots, rhizome head and skin). They showed that 2-DE maps of different ginseng samples had sufficient differences to permit easy discrimination.

The lack of a genome database makes the process of protein identification a rate-determining factor in *P. ginseng* proteomics. Combined analysis of the EST database and peptide mass fingerprinting alone proved to be inefficient in *P. ginseng* proteome analysis (less then 10% identification rate). We determined, instead, that combined analysis, using both internal sequences from MS/MS spectra and an EST sequence database, was an efficient and accurate protein identification method for *P. ginseng* proteome analysis (more than 80% identification rate). Such analysis might also be applicable to other plants, from which genomic information is not available. Analysis of *P. ginseng* showed that many

induced proteins manifested as isoforms. These proteins can be useful samples for the analysis of post-translational modifications in plants. Further proteome analysis can be performed following pre-fractionation by chromatographic and electrophoretic methods such as free-flow electrophoresis (FFE), and multiple-dimensional liquid chromatography (MDLC) for the enrichment of low-copy proteins. Identification of these proteins will provide information regarding the enzymes involved in the production of secondary metabolites, such as in ginsenosides biosynthesis.

Some investigators reported applying proteomics in investigations of plant responses induced by environmental variations. By comparing proteins changed by anoxia with those induced by pretreatment at low oxygen levels in the root tips of maize, Chang et al. [55] suggested that many proteins might be involved in the acclimation induced by hypoxic treatment. Because of the long growth period for medical use, the productivity of *P. ginseng* is affected by many environmental factors. Comparative proteomic analysis can be a useful method for the identification of proteins involved in response to environmental stresses such as temperature, light, and fungal attack.

Various efforts have been exerted in order to find genes involved in ginsenoside biosynthesis. Suppression subtractive hybridization between the mRNAs of 4- and 1-year-old root tissue [56] and *P. ginseng* EST sequence search [18] are examples to achieve the objective. Proteome analysis can surely be used as an efficient and useful complementary method for the identification of related genes involved in ginsenoside biosynthesis.

4. Functional genomics for secondary metabolism analysis in *P. ginseng*

Functional genomic studies of *P. ginseng* have also been carried out in order to elucidate the genes involved with the secondary metabolism of saponin biosynthesis in *P. ginseng*. In order to select the hairy root lines of *P. ginseng* that exhibit better production of specific ginsenosides, DNA microarray and metabolic profiling have been undertaken. In parallel, several laboratories have studied the generation of *P. ginseng* transgenic plants through genetic manipulation.

4.1. DNA microarray

Approximately 3000 activation-tagged hairy root lines have been produced [57]. In order to gain an initial understanding of the correlation between hairy root morphology and ginsenoside production, genes which are differentially expressed in *P. ginseng* hairy roots, which exhibit a unique morphology were identified using a 5 K cDNA microarray. Among the 250 genes analyzed, 63 (including 14 that remain unclassified) were differentially expressed in a hairy root line containing a high level of ginsenosides. Of the genes that had been previously functionally categorized, 29% and 17% were active in metabolism and stress responses, respectively. Most of these were primarily associated with ribosomal proteins, thereby functioning in protein synthesis and destination. Their expression was down-regulated in the hairy roots with less lateral branching. It has been suggested that such phenotype may have resulted from the manipulation of metabolic activities by translational machinery.

4.2. Metabolite profiling

On the other hand, a majority of *P. ginseng* hairy root lines showed no overt phenotype (personal communication with D.W. Choi). Metabolite analysis should reveal a phenotype in many silent mutants, eventually leading to the elucidation of gene functions. To analyze a large number of cultures, a high throughput system for metabolic profiling is clearly required. GC/MS was used for the metabolic profiling of 151 *Arabidopsis* mutants, and 326 compounds were identified from each mutant [58]. Alternatively, crude extracts of cultures can be subjected to FTIR, ^{1}H-NMR, or ESI MS analysis, producing complicated overlapped profiles [59]. Comparisons between cultures can be made using multivariate analyses of metabolite profiling data, based on metabolite fingerprinting, as has been demonstrated at the individual plant level [60,61].

4.3. Transgenic plants

After identification of useful genes via functional genomics studies, such as DNA microarray, proteomics and metabolic profiling, genetic manipulation of *P. ginseng* plants has potential as a valuable tool in improving the production of novel ginseng plants. In *P. ginseng*, genetic transformation has been applied extensively for hairy root production [62–64]. Yang and Choi [65] reported the regeneration of transgenic plants, from the *Agrobacterium*-mediated transformation of the hairy roots of *P. ginseng*. Likewise, studies have been conducted regarding the genetic transformation of *P. ginseng* plants through the introduction of the GUS gene [66,67]. Choi et al. [68] reported the production of herbicide-resistant transgenic *P. ginseng*, via the introduction of the phosphinothricin acetyl transferase gene. The transfer of the transgenic ginseng plantlets to soil was successfully accomplished via acclimatization in autoclaved perlite. Not all of the plantlets survived in the soil that had not been autoclaved, due to largely fungal infections, which occurred particularly in the region between the roots and leaves.

5. Conclusions

Cultivation of ginseng is difficult, and requires a growth period of 4–6 years before roots can be harvested for the use as medicine. This plant should be cultivated under shadowy conditions, and consecutive cultivations in the same soil has to be avoided. Root rot disease caused by fungi, and red skin

disease of unknown etiology are serious constraints in the cultivation of *P. ginseng*.

The root of *P. ginseng* has been used for thousands of years as a folk medicine in Asian countries. The major active ingredients of ginseng have been demonstrated to be ginseng saponins, which are composed of various ginsenosides [69–71]. Pharmacological effects of ginseng have been demonstrated in the central nervous system, cardiovascular, endocrine and immune system. Ginseng and its constituents have been theorized to exhibit both anti-stress and anti-oxidant activity [1,72], and to exert various effects involving stress and the immune system [73–76]. However, neither the metabolic pathways nor the genes involved in ginsenoside biosynthesis have yet been established.

A number of proteomic studies of *P. ginseng* were conducted in order to improve the cultivation conditions of *P. ginseng*, gain better understanding of the ginsenoside biosynthesis pathway, and increase the production of ginseng plants. The lack of genomic DNA sequence information of *P. ginseng* constitutes a major bottleneck with regard to the identification of proteins in proteomics studies. Thus, as an alternative, genomics studies have been conducted using EST sequencing and BAC-end sequencing.

An integrated approach to functional genomics, featuring an interdisciplinary synergy between genomics, transcriptomics, proteomics, and metabolomics will usher in entirely new standards with respect to the identification of useful novel genes, and the elucidation of gene function and interaction between genes and/or proteins. Development of efficient methods for production of transgenic ginseng plants after genetic manipulation would pave the way for better understanding of the metabolic qualities inherent in ginsenoside biosynthesis, and provide us with the information necessary to establish better cultivation conditions for ginseng growth.

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