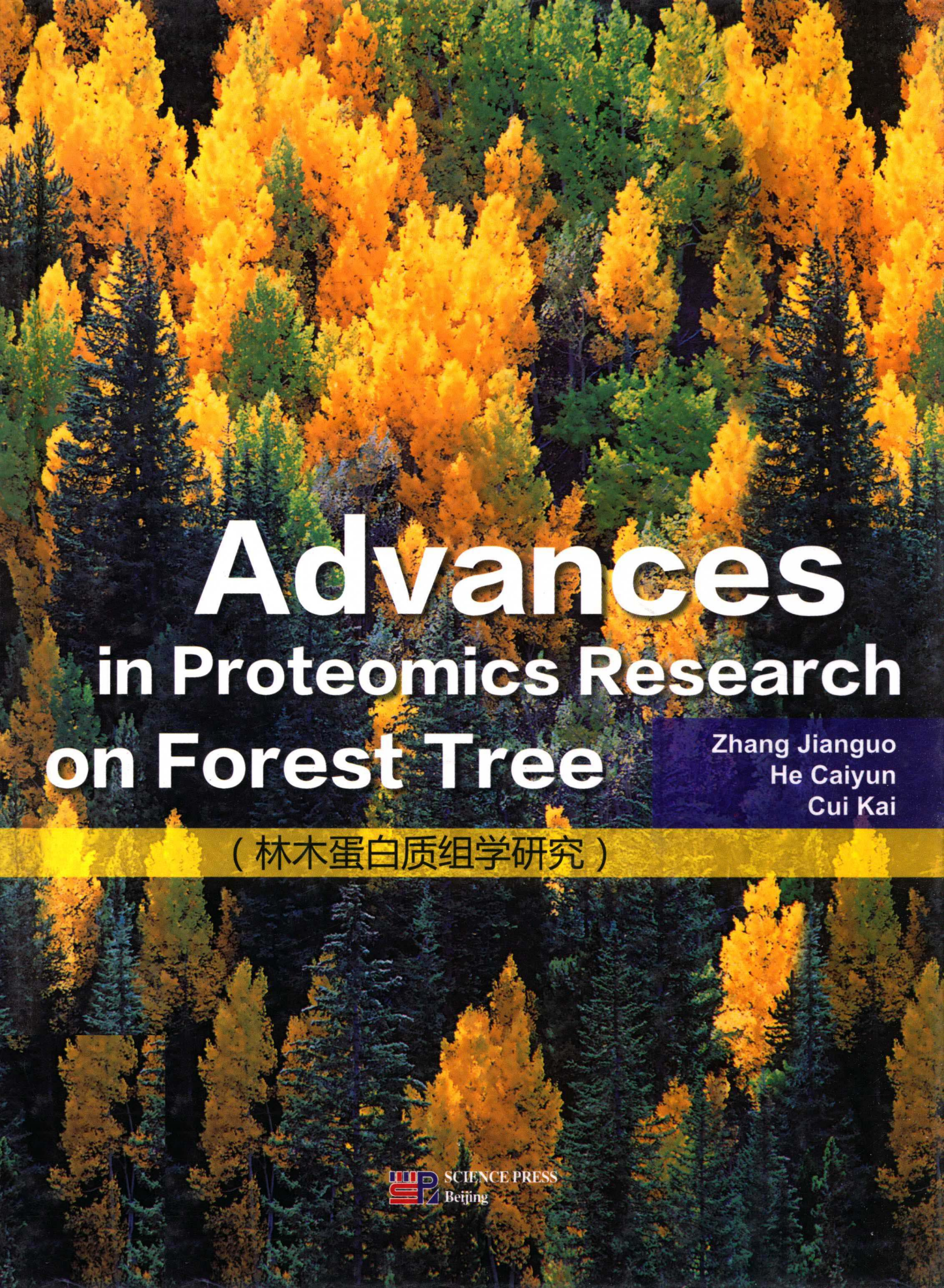

Advances in Proteomics Research on Forest Tree

Zhang Jianguo
He Caiyun
Cui Kai

（林木蛋白质组学研究）

SCIENCE PRESS
Beijing

Advances in Proteomics Research on Forest Tree

（林木蛋白质组学研究）

Zhang Jianguo　He Caiyun　Cui Kai

SCIENCE PRESS
Beijing

Advances in Proteomics Research on Forest Tree

Zhang Jianguo

He Caiyun

Cui Kai

ISBN 978-7-03-041967-5

Science Press Beijing

Responsible Editors: Wang Jing, Wang Hao

PREFACE

Nowadays, within the omics techniques, proteomics constitutes a priority research for any organism and configures a fundamental discipline in the post-genomic era. Forest trees cover one third of the global land surface, constitute many ecosystems, and play an important role in the world economy. The objectives of proteomics of forest trees, ranged from the study of biological processes such as growth and development, responses to stresses, embryogenesis, organogenesis, heredity, to practical aspects, including biodiversity and the identification of proteins characterizing natural variability and phylogeny or to be used as markers in breeding programs. Proteomics research in woody plants is limited to a reduced number of genera, including *Populus*, *Pinus*, *Picea*, *Eucalyptus*, *Fagus*, and *Phyllostachys*, mainly using the two-dimensional electrophoresis approaches coupled to mass spectrometry.

The contribution of proteomics to the knowledge of forest tree biology is being reviewed and discussed, based on our own research work and other researchers' papers. This book is organized in eight chapters starting with the review about advances in proteomics research on forest tree (Chapter 1), the comparison of methods for protein extraction from pine needles (Chapter 2), temporal and spatial profiling of internod elongation-associated protein expression in rapidly growing culms of bamboo (Chapter 3), physiological and protein responses to drought in four pine seedlings (Chapter 4), proteins responding to drought and high-temperature stress in *Pinus armandii* Franch (Chapter 5), proteins responding to drought and high-temperature stress in *Populus* ×*euramericana* cv. '74/76' (Chapter 6), physiological and protein response to drought stress in *Hippophae rhamnoides* (Chapter 7), and clonal reproduction and natural variation of *Populus canescens* patches (Chapter 8). These studies contain partially biological processes and practical aspects of common forest trees, and which provides a solid molecular basis for understanding the growth and development, stress responses and natural variation in some woody plants.

All of this content was researched, written and created by us over a period of several years, and many people and several institutions have supported this work in various ways. This book was supported by the construct project of science research and graduate teaching from Beijing Municipal Education Commission.

Zhang Jianguo, He Caiyun, Cui Kai

Sep. 2014

CONTENTS

Chapter

1 Advances in Proteomics Research on Forest Tree

Abstract: Forest plays an important role in maintenance of ecosystem equilibrium and provides material energy. It has a set of mechanism of its own growth, development, and metabolism. Proteomics is a crucial means to reveal the material life phenomenon in biology nowadays. In this chapter, the trend of technical system of proteomics, such as extraction, separation, identification method of protein, was introduced and existing problem was indicated. In addition, different research direction of forest proteomics (i.e. tress response, wood formation, developmental regulation, pest control) were discussed respectively combined with overseas research. Finally, the prospect to its future development was given.

1.1 Introduction

With the advent of the post-genome era, proteomics have been a rapid development. Compared with genomics, proteomics can provide some unparalleled advantages. Firstly, it more accurately identifies the proteins and obtains related functions. Secondly, the "one gene, one protein (or function)" hypothesis is thus not applicable at present time, to a certain extent, proteomics can detect post translational modifications (PTMs) (Agrawal and Rakwal, 2006). Although proteomics research has been used on a large number of species, it mainly concentrated on model species and animals. About less than 5% of species was plant which focused on model plants such as *Arabidopsis* (*Arabidopsis thaliana*), wheat (*Triticum aestivum*), rice (*Oryza sativa*) and maize (*Zea mays*), poplar (*Populus trichocarpa*), etc. (Jorrín-Novo et al., 2009; Oeljeklaus et al., 2009). The species involved in the forest were less, which is disaccord with forestry importance in the ecosystem. Due to global climate change, energy shortages, the contradictions between human and nature will stand out in the future. Forestry role in human life will strengthen increasingly. So, it is a very meaningful work to spread proteomics research on forest trees to solve production problems. In this section, the research progress of proteomic technology system and hot research topics are being reviewed. Finally, the prospect of proteomics research in the forestry is discussed.

1.2 Research progress on proteomic technology system

1.2.1 Protein extraction method

Extraction of high concentration and high purity protein is the most important in the process of

the whole proteomics research. Because proteins include many complex biochemical properties, such as charge number, molecular weight, hydrophobicity, hydrophilicity, post-translational modification, and interaction with other molecules, and these properties are changed along with the species difference, such as developmental stages, cell and tissue types, and different growth conditions, many protein extraction methods were established. Harvesting the maximum amount of protein to match protein analysis (such as liquid chromatography and mass spectrometry) and protein identification is the most perfect method (Sheoran et al., 2009). It is a challenging to extract protein from plant tissues or cells, because of abundant metabolites and complex cell structure, for example the presence of plant cell walls. Usually, the protein content in plant cell is relatively low, and most of these proteins are proteases and oxidative enzymes (except dormant seeds and pollen) (Jamet et al., 2006), the metabolites of plant cells (such as pigments, phenolic mixture, lipids, polysaccharide) has a wide spectral range, these metabolites can cause pollution during protein extraction, which will impact protein separation and following analysis (Jamet et al., 2006). So far, a lot of protein extraction methods have been reported, phenol method and trichloroacetic acid-acetone precipitation method (TCA method) are commonly used in many plant tissues (Carpentier et al., 2005; Delaplace et al., 2006; Giavalisco et al., 2003).

Extraction of membrane proteins, low abundance proteins and hydrophobic proteins is the main bottleneck of conventional extraction methods (Lilley and Dupree, 2006). Thus, many researchers have improved on it, such as Bio-rad company developed ProteoMiner low-abundance protein enrichment kits (Walton and Jayaraman, 2009); a cysteine shotgun method was used to probe the protein structure (Johnson et al., 2007; Tsai et al., 2008); microfluidic devices were used to separate and concentrate proteins in the bacterial cells (Bao and Lu, 2008), which is based on the principles of physics rather than chemical and biological reactions. Electrophoresis and microfluidic technology is also been used for sample preparation (Walton and Jayaraman, 2009). The general principle is as follows: firstly, preconcentrate low-abundance proteins using polydimethylsiloxane equipment, then isoelectric focusing (IEF) electrophoresis separates proteins, finally SDS-PAGE separates proteins based primarily on molecular weight, which is the three-dimensional gel electrophoresis (3-DE).

1.2.2 Protein separation technology

1.2.2.1 The gel-based protein separation techniques

Two-dimensional polyacrylamide gel electrophoresis (2-DE), mass spectrometry (MS) and bioinformatics technology are known as three supporting proteomics technology. 2-DE technology has been introduced 30 years from appearance (O'Farrell, 1975), although the progress of gel-free protein separation techniques (including protein microarrays and multidimensional liquid chromatography combined with stable isotope labeling) has been impressive in recent years, 2-DE technology still plays an irreplaceable role (Roe and Griffin, 2006). A major limitation of 2-DE

technology is that it is incompatible with the hydrophobic membrane proteins, which is an important component of proteins, and likely play a key role in cell (Lilley and Dupree, 2006). Two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) is a protein separation technique developed in recent years. The label protein mixture used a fluorescent dye, and scan proteins through a multi-channel laser (Heinemeyer et al., 2009). Compared with conventional 2-DE, the method eliminates the need for staining, protein separation speed is greatly improved, and has high repeatability. It has been reported that, in electrophoretic and microfluidic techniques, the detection of a compacted film of microbubbles produced due to the electrolysis of water was used for detecting electrophoretically-captured charged analyses. A key feature of this approach is that the microbubbles are visible under white light and enable detection of proteins in solution without the use of labels or dyes (Walton and Jayaraman, 2009). Kinetically stable proteins (KSPs) are trapped by an energy barrier in a specific state, unable to transiently sample other conformations. Xia et al. (2007) show the application of a diagonal 2D SDS/PAGE assay to identify KSPs in complex mixtures.

1.2.2.2 The gel-free protein separation techniques

With the utilization of spectroscopy in peptide sequencing, it is possible to identify large-scale proteins (Aebersold and Mann, 2003; Glinski and Weckwerth, 2006). Compared with 2-DE, the gel-free protein isolation techniques can much better dissolve proteins (Mitra et al., 2007), and the representative technology is shotgun proteomics (Lilley and Dupree, 2007). The general workflows are as follows: a proteolytic digest of the protein sample is analyzed by multidimensional liquid chromatography and mass spectrometer (LC-MS/MS) while the MS is operated in DDA (data dependent acquisition) mode. Combination with techniques of shotgun, stable isotope labeling and non-labeling, researchers can quantify the proteins. Tagging technique made the ^{15}N stable isotope penetration *in vivo* or *in vitro* target at the early stage (Benschop et al., 2007; Nelson et al., 2007), and then multiple isotopes (such as ^{16}O and ^{18}O) labeling method, the more precise one, has been used. Isobaric tags for relative and absolute quantitation (iTRAQ) is the frequently-used method at present (Ross et al., 2004). Non-label quantitative techniques quantity protein abundance according peptide peak intensity and peptide ion in MS (Kislinger et al., 2006).

1.3 The question in current proteomics research

Gene sequence—the research object of genomics, is relatively static, while the quantity, composition and interaction of proteomics is dynamic. Compared with the DNA or RNA, protein research is very difficult. On the one hand, there is no technique to amplify low-abundance proteins comparable to the polymerase chain reaction for nucleic acids. On the other hand, the different cells from one species have the same genomics, but have different protein constitutions. The difference changes along with the timing change.

Compared with current transcriptomics technology, the major drawback of proteomics is the low flux, which is determined by the inherent characteristics of 2-DE technology, such as low recovery from gel and unrepresentative result (Lester and Hubbard, 2002; Molloy et al., 2001). It is less effective to proteomics technology in the separation of highly hydrophobic protein and low-abundance protein (Görg et al., 2000), which limits the development of subcellular proteomics, especially membrane proteomics (Hesse and Hoefgen, 2006). Many techniques have been used to identify proteins, for example matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). Electrospray ionization (ESI) mass spectrometry is more used than MALDI-TOF MS because of more accurate. To increase flux of proteomics research, technique of multidimensional LC (cation exchange adsorption column) combined with ESI-MS has been successfully introduced (Link et al., 1999; Wolters et al., 2001). Due to the rapid generation of proteomic data, how can we solve data analysis problem? We could only rely on the development of bioinformatics, which will provide the appropriate tools to deal with these data (Thomas and Shevchenko, 2008).

1.4 Proteomics applications on forestry

1.4.1 Research on stress-response proteins in tree species

Normally, under natural conditions, plants are subjected to multiple abiotic and biotic stresses (Qureshi et al., 2007). Abiotic factors usually include temperature, climate, chemical composition and human activity, and biotic factors generally include bacteria, fungi, algae and viruses. Plants will induce specific protein expression under these stresses, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT), salt overly sensitive (SOS) pathway proteins, methyl jasmonate (MeJA) signaling pathway proteins, abscisic acid (ABA) signaling pathway proteins, reverse osmosis protein (Osmotin), mitogen-activated protein kinase (MAPK) signaling pathway proteins, calcium signaling pathway proteins, gamma amino butyric acid (GABA) proteins. In addition, plants subjected to specific abiotic stress (including water stress, low temperature stress, high temperature stress, heavy metal stress, ozone stress, ultraviolet stress, visible light stress, nutrient stress) will induced specific proteins, such as heat shock proteins (HSPs), chaparonins, phytochelatins (PCs), metallothioneins (MTs) and pathogen-related proteins (PRPs) and so on, will overexpress.

In the temperate climate of the northern hemisphere, winter survival of woody plants is determined by the ability to acclimate to freezing temperatures and to undergo a period of dormancy. Cold acclimation in many woody plants is initially induced by short photoperiod and low, non-freezing temperatures. These two factors were used to study changes in the proteome of bark tissues of peach trees. Using gel-based approach, it was found that the most significant factor affecting the proteome appeared to be low temperature, while the combination of low temperature and short photoperiod was shown to act either synergistically or additively on the expression of

some proteins. Fifty-seven protein spots on gels were identified by mass spectrometry, which involved in carbohydrate metabolism (e.g., enolase, malate dehydrogenase), defense or protective mechanisms (e.g., dehydrin, HSPs, and PR-proteins), energy production and electron transport (e.g., adenosine triphosphate synthases and lyases), and cytoskeleton organization (e.g., tubulins and actins) (Renaut et al., 2008).

Seasonal evaluation of total soluble protein fractions extracted from cortical parenchyma cells of mulberry (*Morus bombycis* Koidz.) tree identified a predominant 18kDa (WAP18) protein that was directly correlated to periods of cold acclimation. The WAP18 protein increased from September to December and then gradually decreased until June of next year. The maximum levels of WAP18 were detected in mid-winter, which corresponds to the maximum freeze tolerance in cortical parenchyma cells of mulberry tree. 2-DE confirmed that WAP18 consists of at least three proteins that range between an isoelectric point of 5.0 and 6.0. N-terminal amino acid sequence analysis demonstrated that all three proteins contain high sequence similarity to each other and high homology to pathogenesis-related (PR) –10/Bet v 1 protein families. The purified WAP18 exhibited *in vitro* cryoprotective activity (Ukaji et al., 2004). Sea buckthorn (*Hippophae rhamnoides*) is uniquely capable of growing well under extreme environmental conditions such as water deficit, low temperature, and high altitude. Such tolerance invokes much interest in understanding the biology of this plant species and its utilization potential. Drought stress-responsive proteins in *Hippophae rhamnoides* were analyzed using 2-DE and MALDI-TOF MS, 55 proteins exhibited changes in abundance under stress. Of these, 13 proteins were identified, including three that disappeared under drought, seven that were up-regulated, and three that were only detected under drought, four proteins were deemed as new discoveries in higher plants (Xu et al., 2009).

There were some other stress-response proteins in tree species, such as white pine blister rust fungus (*Cronartium ribicola*) stress in *Pinus lambertiana* (Ekramoddoullah and Tan, 1998), water-deficit stress in maritime pine (*Pinus pinaster*) (Costa et al., 1998), phosphinotricin (PPT) stress in scots pine (*Pinus sylvestris*) seedlings (Avila et al., 1998), copper stress in marijuana (*Cannabis sativa*) roots (Bona et al., 2007), heat stress in different Norway spruce (*Picea abies*) ecotypes (Valcu et al., 2008).

1.4.2 Proteomic studies on wood formation

Wood formation has been an important aspect in forestry research, which resulted from many complex metabolic activities, such as cell division, cell differentiation, cell elongation, tissue maturity and lignification. To determine the molecular mechanisms taking place at the top and base of the deformity stem in hybrid poplar (*Populus tremula* × *Populus alba*), after 45min or one week of inclination, the changes induced in protein accumulation were studied by 2-DE and quantitatively analyzed using image analysis software. About 300 protein spots were reproducibly detected and analyzed. Forty percent of these proteins showed significant changes after inclination.

MS analysis of 135 spots led to the identification of 60 proteins involved in a wide range of activities and metabolisms. Very different patterns of protein expression were obtained according to conditions tested, highlighting the complexity of gravitropic responses. These results suggested that primary and secondary tissues present specific mechanisms to sense reorientation and to respond to inclination (Azri et al., 2009). Celedon et al. (2007) described that the proteins participating in the processes involved in juvenile wood formation by isolating proteins from the cambial region of *Eucalyptus grandis*, at three ages of growth (6-month-old seedlings, 3- and 6-year-old trees), and also identify proteins differentially expressed. Using a 2-D-LC-MS/MS strategy, they identified a total of 240 proteins, with 54 corresponding spots being present in at least two ages. Overall, nine proteins classified into the functional categories of metabolism, cellular processes, and macromolecular metabolism showed significant changes in expression. Proteins were classified into seven main functional categories. Of which, metabolism representing 35.2% of the total proteins identified. Using 2-DE technology and microsequence identified analysis, the seasonal variation of lignin proteins in *Populus trichocarpa* was studied, and it was found that there were some specific protein expression at different times and different parts. Their data show that for the study of xylogenesis, two-dimensional protein gel comparisons combined with systematic protein sequencing may yield information complementary to that from EST sequencing strategies (Mijnsbrugge et al., 2000).

To better understand the molecular mechanisms underlying the process of cell wall development in flax, proteins of phloem fiber secondary cell wall were analyzed by fluorescent (DiGE) labels and 2D-gel electrophoresis, with identities assigned to some proteins by mass spectrometry. The abundance of many proteins in fibres was notably different from the surrounding non-fibre cells of the cortex, with approximately 13% of the 1,850 detectable spots being significantly enriched in fibres. Following mass spectrometry, they assigned identity to 114 spots, of which, 51 were significantly enriched in fibres. they observed that a K^+ channel subunit, annexins, porins, secretory pathway components, β-amylase, β-galactosidase and pectin and galactan biosynthetic enzymes were among the most highly enriched proteins detected in developing flax fibres, with many of these proteins showing electrophoretic patterns consistent with post-translational modifications (Hotte and Deyholos, 2008).

1.4.3 Proteomic studies on growth and development in tree species

Using proteomics technology, we can deeply understand regulatory mechanisms in plant growth and development from biological macromolecules level. For example, the holm oak leaf protein expression was assessed by 2-DE on pH 5 to 8 linear gradient immobilized pH gradient strips, and proteins were detected by Coomassie staining. Biological variance was determined for the same protein spots from independent tissue extracts corresponding to leaves from different trees, or the same tree at different orientations or sampling times during a day. Values of 26% for the analytical variance and 58.6% for the biological variance among independent trees were

obtained. A representative set of the major proteins were subjected to liquid chromatography-tandem mass spectrometry analysis (Jorge et al., 2005). And also the variation in the holm oak leaf proteome at different plant developmental stages, between provenances and in response to drought stress, have been analysed (Jorge et al., 2006). By comparative proteomic approach, the development, maturation, and germination of date palm zygotic embryos, have been studied. Proteins were resolved by 2-DE in the 5~8 pH range. The total protein content and the number of spots resolved increased from 12 weeks after pollination (WAP) to 17 WAP, decreasing upon 15 days after germination. Up to 194 spots showed qualitative or quantitative differences between stages. Samples were also clustered based on Pearson distance and Ward's minimum distance. Sixty-five variable spots were subjected to MS analysis, resulting in 21 identifications. The identified proteins belong to the following functional categories: enzymes of glycolysis, tricarboxylic acid cycle, carbohydrate biosynthesis, protein translation, storage (glutelin), and stress-related proteins. The evolution pattern of the functional groups was examined and discussed in terms of metabolism adaptation to the different embryogenic and germination stages (Sghaier-Hammami et al., 2009).

Xylem sap collected from *Populus trichocarpa* × *Populus deltoides* using root pressure was estimated to contain more than 100 proteins. Ninety-seven of these proteins were identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). These proteins were classified into 10 functional categories including metabolism, signaling, stress response and cell wall functions. The majority of xylem sap proteins were metabolic enzymes involved in processes including translation, proteolysis, and glycolysis. Stress-related proteins were also prevalent (Dafoe and Constabel, 2009). *Pinus radiata* is one of the most economically important forest tree species. To identify key proteins related to tree growth, productivity and responses to environmental factors, a proteomic approach is being utilized. In this paper, proteins were separated in 2-DE, 549 spots were detected in Coomassie-stained gels within the 5~8 pH and 10~100kDa M(r) ranges. After LC/MS/MS analysis of in-gel tryptic digested 150 spots, 115 proteins were identified, and these protein functions were mainly involved in carbohydrate metabolism and photosynthetic enzymes (Valledor et al., 2008). Furthermore, many proteomic analysis of differentially expressed proteins were expanded, such as growth and development in loblolly pine (*Pinus taeda*) (Groome et al., 1991), pollen tube development in *Pinus strobus* (eastern white pine) (Fernando, 2005), early somatic embryogenesis in white spruce (*Picea glauca*) (Lippert et al., 2005).

1.4.4 Proteomic studies on forest pest

Some specific genes will rapidly response in many forest tree species when they suffer pest infestation. Using of proteomics methods can effectively understand the related biological process. Citrus sudden death (CSD) is a disease of unknown etiology that greatly affects sweet (*Citrus sinensis*) oranges grafted on Rangpur lime rootstock. Cantú et al. (2008) performed a proteomic analysis to generate information related to this plant pathogen interaction. Protein profiles were

generated using 2-DE, the protein spots were well distributed over a p*I* range of 3.26 to 9.97 and a molecular weight (MW) range from 7.1kDa to 120kDa. These protein spots were identified as chitinases, miraculin-like proteins, and potential trypsin inhibitors. It suggested that down-regulation of chitinases and proteinase inhibitors in CSD-affected plants were relevant since chitinases are well-known pathogenesis-related protein, and their activity against plant pathogens is largely accepted. Long-lived conifer trees depend on both constitutive and induced defenses for resistance against a myriad of potential pathogens and herbivores. Lippert et al. (2009) used the advantages of Norway spruce (*Picea abies*) cell suspensions combined with chitosan elicitation to investigate the early proteome response in a conifer. iTRAQ labeling was used for protein separation. Comparison of elicitor-induced proteome and transcriptome responses in Norway spruce, cells consistently identified features associated with calcium-mediated signaling and response to oxidative stress that have not previously been observed in the response of intact trees to fungal attack. A comparative analysis of sweet cherry (*Prunus avium*) fruits proteome e induced by salicylic acid (SA) at different maturity stages was reported. The results demonstrated that SA enhanced the resistance of sweet cherry fruits against *Penicillium expansum*, resulting in lower disease incidences and smaller lesion diameters, especially at earlier maturity stage. Based on proteomics analysis, 13 and 28 proteins were identified after SA treatment at earlier (A) and later (B) maturity stage, respectively. 7 antioxidant proteins and 3 pathogenesis related-proteins were identified at both earlier and later maturity stages, while 5 heat shock proteins and 4 dehydrogenases were only detected at later stage (Chan et al., 2008). There have many similar studies about Conifer defense against insects, for example, proteome analysis of Sitka spruce (*Picea sitchensis*) bark induced by mechanical wounding or feeding by *Pissodes strobi* (Lippert et al., 2007).

1.5 Conclusion and prospect

Currently, the importance of systems biology has attracted wide attention (Friboulet and Thomas, 2005). It is generally considered isolated studies have not described the complex phenomenon of life. Combined with advantages of traditional biology, molecular biology, computer technology and systems engineering, the object of modeling systems biology is to solve biological problems on overall level using high-throughput method. In fact, this integration is beneficial to interdisciplinary development, and will promote the common progress of all disciplines. The most obvious example is the successful completion of the human genome sequencing (Venter et al., 2001). In recent years, many studies of high-throughput methods have been reported, and these advances introduced many new disciplines. According to the central criterion of biology, these disciplines were named as genomics, transcriptomics, proteomics and metabolomics. The principle of gene chip technology is to show the profile of the transcriptome on system level (Schena et al., 1995). Similarly, followed by the development of high-throughput technology, such as tandem mass spectrometry, proteomics was born and have made significant progress (Souchelnytskyi, 2005; Smith and Figeys, 2006). The progress in cyanobacteria study has testified advance in high-throughput proteomics technology (Ow and Wright, 2009). In the past few years, researchers

have studied several genera of cyanobacteria using shotgun technology (Barrios-Llerena et al., 2006; Anderson et al., 2006), the relative quantification of multiple isotopic labeling combined with the shotgun method (Ow et al., 2008), nitrogen labeled method (Pandhal et al., 2009; Pandhal et al., 2008), and metabolic network analysis (Pham and Wright, 2007). These studies involved in protein expression, metabolic pathways, non-coding genes. Their common goal is to gradually explore high-throughput strategies instead of the traditional 2-DE technology, which showing a development trend in proteomics.

The inherent characteristics of tree species, for example fewer sequencing genome, long growth cycle, imperfect transformation system, restrict proteomics progress on tree species. Currently, varieties of trees on forest proteomics research are less, and mostly focus on *Populus* and some conifers. Meanwhile, the study contents mostly concentrate on the stress response and wood property. In addition, these studies can't make transverse comparison because of different purposes and technical system. With an increase in number of tree species studied, as well as amplitude of research fields and upgrade of proteomics technology system, forest proteomic studies will rapidly develop in the future.

References

Aebersold R, Mann M. 2003. Mass spectrometry-based proteomics. Nature, 422(6928): 198–207

Agrawal G K, Rakwal R. 2006. Rice proteomics: a cornerstone for cereal food crop proteomes. Mass spectrometry reviews, 25(1): 1–53

Anderson D, Campbell E L, Meeks J C. 2006. A soluble 3D LC/MS/MS proteome of the filamentous cyanobacterium *Nostoc punctiforme*. Journal of Proteome Research, 5(11): 3096–3104

Avila C, Garcia-Gutierrez A, Crespillo R, et al. 1998. Effects of phosphinotricin treatment on glutamine synthetase isoforms in scots pine seedlings. Plant Physiology and Biochemistry, 36(12): 857–863

Azri W, Chambon C, Herbette S, et al. 2009. Proteome analysis of apical and basal regions of poplar stems under gravitropic stimulation. Physiologia Plantarum, 136(2): 193–208

Bao N, Lu C. 2008. A microfluidic device for physical trapping and electrical lysis of bacterial cells. Applied Physics Letters, 92: 214103

Barrios-Llerena M E, Chong P K, Gan C S, et al. 2006. Shotgun proteomics of cyanobacteria-applications of experimental and data-mining techniques. Briefings in Functional Genomics & Proteomics, 5(2): 121–132

Benschop J J, Mohammed S, O'Flaherty M, et al. 2007. Quantitative phosphoproteomics of early elicitor signaling in *Arabidopsis*. Molecular & Cellular Proteomics, 6(7): 1198–1214

Bona E, Marsano F, Cavaletto M, et al. 2007. Proteomic characterization of copper stress response in *Cannabis sativa* roots. Proteomics, 7(7): 1121–1130

Cantú M, Mariano A, Palma M, et al. 2008. Proteomic analysis reveals suppression of bark chitinases and proteinase inhibitors in citrus plants affected by the citrus sudden death disease. Phytopathology, 98(10): 1084–1092

Carpentier S C, Witters E, Laukens K, et al. 2005. Preparation of protein extracts from recalcitrant plant tissues: an evaluation of different methods for two-dimensional gel electrophoresis analysis. Proteomics, 5(10): 2497–2507

Celedon P A F, de Andrade A, Meireles K G X, et al. 2007. Proteomic analysis of the cambial region in juvenile *Eucalyptus grandis* at three ages. Proteomics, 7(13): 2258–2274

Chan Z, Wang Q, Xu X, et al. 2008. Functions of defense-related proteins and dehydrogenases in resistance response induced by salicylic acid in sweet cherry fruits at different maturity stages. Proteomics, 8(22): 4791–4807

Costa P, Bahrman N, Frigerio J M, et al. 1998. Water-deficit-responsive proteins in maritime pine. Plant molecular biology, 38(4): 587–596

Dafoe N J, Constabel C P. 2009. Proteomic analysis of hybrid poplar xylem sap. Phytochemistry, 70(7): 856–863

Delaplace P, van der Wal F, Dierick J F, et al. 2006. Potato tuber proteomics: comparison of two complementary extraction methods designed for 2-DE of acidic proteins. Proteomics, 6(24): 6494–6497

Ekramoddoullah A, Tan Y. 1998. Differential accumulation of proteins in resistant and susceptible sugar pine (*Pinus lambertiana*) seedlings inoculated with the white pine blister rust fungus (*Cronartium ribicola*).

Canadian journal of plant pathology, 20: 308–318

Fernando D D. 2005. Characterization of pollen tube development in *Pinus strobus* (Eastern white pine) through proteomic analysis of differentially expressed proteins. Proteomics, 5(18): 4917–4926

Friboulet A, Thomas D. 2005. Systems Biology—an interdisciplinary approach. Biosensors and Bioelectronics, 20(12): 2404–2407

Giavalisco P, Nordhoff E, Lehrach H, et al. 2003. Extraction of proteins from plant tissues for two-dimensional electrophoresis analysis. Electrophoresis, 24(1–2): 207–216

Glinski M, Weckwerth W. 2006. The role of mass spectrometry in plant systems biology. Mass spectrometry reviews, 25(2): 173–214

Görg A, Obermaier C, Boguth G, et al. 2000. The current state of two-dimensional electrophoresis with immobilized pH gradients. Electrophoresis, 21: 1037–1053

Groome M C, Axler S R, Gifford D J. 1991. Hydrolysis of lipid and protein reserves in loblolly pine seeds in relation to protein electrophoretic patterns following imbibition. Physiologia Plantarum, 83(1): 99–106

Heinemeyer J, Scheibe B, Schmitz U K, et al. 2009. Blue native DIGE as a tool for comparative analyses of protein complexes. Journal of proteomics, 72(3): 539–544

Hesse H, Hoefgen R. 2006. On the way to understand biological complexity in plants: s-nutrition as a case study for systems biology. Cellular & Molecular Biology Letters, 11(1): 37–56

Hotte N S C, Deyholos M K. 2008. A flax fibre proteome: identification of proteins enriched in bast fibres. BMC Plant Biology, 8(1): 52

Jamet E, Canut H, Boudart G, et al. 2006. Cell wall proteins: a new insight through proteomics. Trends in plant science, 11(1): 33–39

Johnson C P, Tang H Y, Carag C, et al. 2007. Forced unfolding of proteins within cells. Science, 317(5838): 663–666

Jorge I, Navarro R M, Lenz C, et al. 2005. The holm oak leaf proteome: analytical and biological variability in the protein expression level assessed by 2-DE and protein identification tandem mass spectrometry de novo sequencing and sequence similarity searching. Proteomics, 5(1): 222–234

Jorge I, Navarro R M, Lenz C, et al. 2006. Variation in the holm oak leaf proteome at different plant developmental stages, between provenances and in response to drought stress. Proteomics, 6(S1): S207–S214

Jorrín-Novo J V, Maldonado A M, Echevarría-Zomeño S, et al. 2009. Plant proteomics update (2007~2008): second-generation proteomic techniques, an appropriate experimental design, and data analysis to fulfill MIAPE standards, increase plant proteome coverage and expand biological knowledge. Journal of proteomics, 72(3): 285–314

Kislinger T, Cox B, Kannan A, et al. 2006. Global survey of organ and organelle protein expression in mouse: combined proteomic and transcriptomic profiling. Cell, 125(1): 173–186

Lester P J, Hubbard S J. 2002. Comparative bioinformatic analysis of complete proteomes and protein parameters for cross-species identification in proteomics. Proteomics, 2(10): 1392–1405

Lilley K S, Dupree P. 2006. Methods of quantitative proteomics and their application to plant organelle characterization. Journal of experimental botany, 57(7): 1493–1499

Lilley K S, Dupree P. 2007. Plant organelle proteomics. Current opinion in plant biology, 10(6): 594–599

Link A J, Eng J, Schieltz D M, et al. 1999. Direct analysis of protein complexes using mass spectrometry. Nature Biotechnology, 17(7): 676–682

Lippert D N, Ralph S G, Phillips M, et al. 2009. Quantitative iTRAQ proteome and comparative transcriptome analysis of elicitor-induced norway spruce (*Picea abies*) cells reveals elements of calcium signaling in the