Plant Omics Journal POJ

POJ 4(6):288-294 (2011) ISSN:1836-3644

Invited Review Article

Tree Omics and Biotechnology in China

Yanfang Yang[1], Qi Tang[2], Hongwei Liu[1], Deyou Qiu[1*]

[1]State Key Laboratory of Tree Genetics and Breeding, The Research Institute of Forestry, Chinese Academy of Forestry, China
[2]Guangxi Branch Institute, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, China

***Corresponding author: qiudy@caf.ac.cn**

Abstract

Omics is becoming a comprehensive approach to study the molecules in living organisms. In this paper, the progress of research in tree genomics, transcriptomics, proteomics, metabolomics and other biotechnologies in China is summarized. Genomics, including functional genomics, structural genomics and comparative genomics, have been studied since the 1990s and some important achievements have been made by Chinese scientists. The hottest research area already has changed from structural genomics to functional genomics, as more genes have been isolated and their functions explored. Transcriptomics, proteomics and metabolomics work are carried out in Chinese trees in recent years and some useful results have been obtained. As more data from transcriptomics, proteomics and metabolomics are obtained, much more bioinformatics and investigative work will be needed to infer the functions of genes, to further elucidate key genes in responsive pathways, as well as to understand metabolic networks. Compared to research in crop plants, more efforts in the omics and biotechnology of trees need to be made in the future in China.

Keywords: Omics, Genomics, Transcriptomics, Proteomics, Metabolomics, Transgenic, Trees, China.
Abbreviations: 2DGE: two-dimensional gel electrophoresis, AFLP: amplified fragment length polymorphism, ISSR: inter-simple sequence repeat, LC-ESI-MS: liquid chromatography coupled with electrospray ionization tandem mass spectrometry, QTL: quantitative trait loci, SSH: suppressive subtractive hybridization, RAPD: random-amplified polymorphic DNA, RFLP: restriction fragment length polymorphism, SAGE: serial analysis of gene expression, SNP: single nucleotide polymorphism, SSR: simple sequence repeat.

Introduction

Trees play important roles in people's lives. They are essential components of the natural landscape, and play crucial roles in global carbon maintenance, response to global climate change and conservation of biodiversity (Cervera et al., 2000). Trees provide structural and functional habitat for two-thirds of the Earth's terrestrial species and contain greater than 90% of all terrestrial biomass (Bradshaw et al., 2000; Taylor, 2002). Because of the important functions of trees, and with biotechnology rapidly improving, the molecular breeding studies of trees also have entered the 'omics' era. Completion of the *Haemophilus influenzae* genome sequence in 1995 marked a significant transition in the history of biological research (Fleischmann et al., 1995). Whole-genome sequencing and other high-throughput experimental technologies provide a huge amount of biological information for modern day scientists. New biological phenomena will be discovered and better understood through analyses of transcriptional regulation (transcriptomics), the genes terminal products (proteomics) and the metabolic products (metabolomics) (Kandpal, 2009). Huge bioinformatics database from a number of tree species have been rapidly accumulated, and molecular analyses of wood formation, secondary metabolism, flower development, and abiotic stress tolerance have been studied by Chinese tree scientists. We present here a brief review of trees genomics, some of its other omics offshoots, and some related biotechnologies in China.

Trees genomics research in China

In modern molecular biology and genetics, genome is defined as the entirety of an organism's hereditary information, and genomics is the discovery and study of many genes and their roles in establishing an organism's structure and function on a genome-wide scale. Genomics usually comprises functional genomics, structural genomics and comparative genomics (Michelmore, 2000; Chawla, 2002). Forestries entered the genomic era in the early 1990s, with the advent of forward and reverse genetic approaches, such as genetic mapping for detection of quantitative trait loci (QTL) (Cervera et al., 2000; Sewel and Neale, 2000), as well as gene knockout and over-expression (MacKay et al., 2004). From the early 1990's, rapid advancements in trees genomics have been made by many Chinese researchers. In recent years Chinese scientists have isolated many functional genes and constructed genetic maps for various trees.

Functional genomics

Functional genomics, or so-called post-genomics, is applied systematically and at the genome level to analyze the function of genes. The methods usually used in functional genomics include cDNA library construction, EST sequencing, DNA chip and microarray fabrication, as well as suppression subtractive hybridization (SSH). All these approaches are suitable and useful for studying the genomes of trees. The large-scale sequencing and analyses of ESTs is a fundamental part of genomics research in most forest tree species. Because sequencing technology is improving rapidly, trees functional genomics has entered a dynamic new era, and a number of achievements in tree functional genomics have been obtained

Table 1. Functional genes of trees isolated by Chinese scientists (Partial list).

Tree species	Gene name	Function	Reference
Ginkgo biloba	*Gbchs*	Chalcone synthase	Pang et al., 2004
Ginkgo biloba	*GbGGPPS*	Geranylgeranyl diphosphate synthase	Liao et al., 2004
Ginkgo biloba	*GbAsr*	Stress and ripening	Shen et al., 2005a
Ginkgo biloba	*GbANS*	Anthocyanidin synthase	Xu et al., 2008a
Ginkgo biloba	*Gbd*	Defensin gene	Shen et al., 2005b
Ginkgo biloba	*GbDXR*	1-deoxy-D-xylulose 5-phosphate reductoisomerase	Gong et al., 2005
Ginkgo biloba	*GbANR*	Anthocyanidin reductase gene	Shen et al., 2006
Ginkgo biloba	*GbTPS*	Trehalose gene	Wu et al., 2006
Ginkgo biloba	*GbCHS2*	Chalcone synthase	Xu et al., 2007
Ginkgo biloba	*GbGSTs*	Glutathinone S-transferase gene	Liu et al., 2007
Dendrocalamus latiflorus	*DlMADS8, DlMADS18*	MADS-box gene	Tian et al., 2005, Tian et al., 2006
Phyllostachys praecox	*PpMADS1, PpMADS2*	FUL3 and FUL1 clade of Poaceae AP1/SQUA-like genes	Lin et al., 2009
Taxus chinensis	*DXR*	1-deoxy-D-xylulose 5-phosphate reductoisomerase	Zheng et al., 2004
Taxus chinensis	*TCH1, TCH2*	Hydroxylase homologies, related to the biosynthesis of taxol/taxoids	Tu et al., 2004
Taxus chinensis	*TS1*	Related to the biosynthesis of taxol	Hu et al., 2004
Taxus chinensis	*14OH*	14 β-hydroxylase gene	Hu et al., 2006
Taxus media	*TmTXS*	Taxadiene synthase	Kai et al., 2005
Populus euphratica	*PeNHX 1-6*	Na(+)/H(+) exchanger genes	Ye et al., 2009
Populus euphratica	*Alfin-1*	Cys(2)/His(2) Zinc finger proteins	Wang et al., 2005
Populus euphratica	*PeNHX2*	Na +/H + Antiporter	Zhang et al., 2006
Populus euphratica	*PSTZ*	Zinc-finger protein gene	Wang et al., 2008
Populus suaveolens	*G6PDH*	Glucose-6-phosphate dehydrogenase	Lin et al., 2005
Populus deltoides	*PdPI*	MADS-box gene	Zhang et al., 2008a
White poplar	*RGAs*	Resistance gene analogues	Zhang et al., 2008b
White poplar	*PtDrl02*	TIR-NBS-encoding gene	Zheng et al., 2010
Pinus bungeana	*PbDHAR*	Dehydroascorbate reductase	Yang et al., 2009
Ammopiptanthus mongolicus	*AmCIP*	A. mongolicus cold-induced protein	Liu et al., 2006
Ammopiptanthus mongolicus	*CBL1*	Calcium sensor gene	Guo et al., 2010
Tamarix hispida	*ThLTP 1–14*	Lipid transfer proteins	Wang et al., 2009a
Tamarix hispida	*bZIP*	Basic leucine zipper proteins	Wang et al., 2010a
Tamarix androssowii	*MnSOD*	Superoxide dismutase	Wang et al., 2010b
Tamarix androssowii	*CAP*	Cold acclimation protein	Lin et al., 2006
Jatropha curcas	*accA*	Acetyl-coenzym A (acetyl-CoA) carxylase	Xie et al., 2010

by Chinese researchers. cDNA libraries have been constructed for tissues from many trees species, including ripe seed of *Camellia oleifera* (Shi et al.., 2004), *Eucommia ulmoides* (olive) bark (Zhou et al., 2004), poplar leaves from clones that are, respectively, susceptible and resistant to black spot disease (Zeng et al., 2004), *Populus euphratica* under salt stress (Zhu et al., 2007), tender tea shoots (Chen et al., 2004; Li et al., 2009) and tender tea roots (Zhao et al., 2008). DNA microarrays are a new technology that allows the whole genome to be monitored on a single chip so that a picture of interactions among thousands of genes can be observed simultaneously (Brazam et al., 2000). DNA microarray technology is a powerful tool for high throughput gene expression analysis in functional genome research. In tree plants, for example, cDNA microarrays were developed for tea plant, with a total of 1680 genes selected from the cDNA library of clone Longjing 43 (Zhao et al., 2006). Efforts currently focus on species that are important in Chinese forests and play significant roles in Chinese forest industry. The main forest trees include *Populus*, *Ginkgo biloba*, *Taxus chinensis, Eucalyptus* and *Tamarix.* As a key economic plant, a number of Bamboo GSS, EST and cDNA sequences also have been developed. Several functional genes relating to flower development of bamboo (*Dendrocalamus latiflorus*) were isolated, such as *PpMADS1/2* gene (Lin et al., 2009). Because of the important medicinal functions of secondary metabolism products, some trees, for example, *Ginkgo biloba* and *Taxus chinensis*, have been studied in recent years in China. In addition, a growing number of functional genes, which mainly relate to flower development, disease or stress resistance, and metabolism, have been isolated from trees in recent years by Chinese researchers (Table 1). Gene sequences identified through large-scale EST sequencing using targeted cDNA discovery methods such as suppressive subtractive hybridization (SSH), or based upon the poplar genome sequence, now are being used in several laboratories to characterize gene families and develop microarrays for comprehensive gene expression profiling. The application of genomics to tree species will help to understand the genetic and molecular basis of wood and cork production, as well as maturation and responses to environmental factors. Studies of such fundamental problems will be greatly facilitated by current developments in genomic technologies.

Structural genomics

Structural genomics involves mapping the genome, and ultimately producing a complete DNA sequence for any particular organism; however, recently the term often has been

Table 2. Genetic linkage maps of trees reported since 2000 by Chinese scientists (Partial list).

Species	Marker type	Map length (cM)	Coverage (%)	Reference
Cunningghamia lanceolata (Lanth) Hook	AFLP	2282.6/2565.8	100	Tong and Shi; 2004
Eucalyptus urophylla S. T. Blake	RAPD	1504.6	94.9	Gan et al., 2003
Eucalyptus tereticornis Smith	RAPD	1035.7	68.7	Gan et al., 2003
Populus bolleana Lauche	AFLP	1956	77	Zhang et al., 2004
Populus deltoids × *Populus euramericana*	RAPD	1914.2	73.6	Zhang et al., 2000
Betula pendula Roth × *Betula platyphylla* Suk	RAPD	955.6/1545.8	——	Jiang et al., 2007
Betula platyphylla	ISSR, AFLP	694.2/949.6	43.1/42.7	Wei et al., 2010
Hevea brasiliensis	SSR	1937.06	——	Feng et al., 2010
Betula platyphylla Suk. and *Betula pendula* Roth	RAPD, AFLP	2864.5/2489.7	——	Jiang et al., 2011
Betula platyphylla Suk × *Betula pendula* Roth	RFLP	1296.1/1035.8	——	Gao and Jiang, 2009
Litchi chinensis Sonn.	RAPD, SRAP, AFLP	1096.59	——	Zhao et al., 2010

extended to studies of the three-dimensional molecular structures of nucleic acids and proteins. Several common types of molecular markers, including restriction fragment length polymorphism (RFLP), random-amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), inter-simple sequence repeat (ISSR), and amplified fragment length polymorphism (AFLP) have been widely used for genetics linkage map construction in various trees; there has been a special focus on *Populus* and *Eucalyptus*, using markers either individually or in combination. Some progress in structural genomics of Chinese trees is summarized in Table 2. High-density linkage maps in forest trees have been shown to be useful in locating genes, facilitating marker-assisted selection, and clarifying the biological basis of complex traits (Wei et al., 2010). Single nucleotide polymorphisms (SNPs), as a new marker system, have been applied to genetic and breeding studies in many tree species from the genera *Pinus*, *Populus*, *Pseudotsuga*, *Eucalyptus* and *Picea*. Association studies in *Eucalyptus spp*. and *Pinus taeda* L. found that some SNP sites in various genes are associated with distinct wood property traits. More than 30 commercially important QTLs have been detected, and comparative mapping has been done for a few forest tree species. Comparing the references we collected, the research focus has been moving from structural to functional genomics, with comparative genomics just recently being performed in trees research in China.

Tree transcriptomics research in China

The transcriptome represents a comprehensive set of transcribed regions throughout the genome (Zhang et al., 2010). Transcriptomics aims at quantifying the levels of expression of all or a selected subset of genes based on the amounts of RNA present in a sample (Zduńczyk and Pareek, 2008). The field of transcriptomics provides information about both the presence and the relative abundance of RNA transcripts. Transcriptome profiling generally involves DNA, cDNA, oligonucleotide arrays, as well as serial analysis of gene expression (SAGE) (Kim, 2003). Recently, with a variety of platforms, such as Illumina (company) Genome Analyzer platform, ABI Solid Sequencing and Life Science's 454 Sequencing, RNA-Seq is a developed approach to transcriptome profiling that uses deep-sequencing technologies (Wang et al., 2009b). To date, countless genome-wide studies have investigated gene expression in many plants using the well-established approaches of microarrays and serial analysis of gene expression. To identify differentially expressed genes in a spontaneous sweet orange [*Citrus. sinensis* (L.) Osbeck] bud mutation, which causes lycopene accumulation and low citric acid, microarray analyses were performed during fruit development to investigate the bud mutation responsible (Liu et al., 2009). In another example, wood formation was studied in Chinese fir (*Cunninghamis lanceolata*); in this case, transcriptome analysis was completed with SSH and macroarray methods (Wang et al., 2007), and 405 unique ESTs were obtained that are preferentially expressed in differentiating xylem. miRNAs are an extensive class of small regulatory RNAs, which play an important role in many biological and metabolic processes. In our lab, we employed high throughput Illumina sequencing to identify miRNAs from *Taxus chinensis* cells, to investigate the effects of the taxoid elicitor methyl jasmonate (MJ) on miRNA expression (Qiu et al., 2009). The results provided valuable insights into molecular mechanisms involved in the regulation of taxoid biosynthesis. In addition, RNA-seq analysis of *Jatropha Curcas* seed and *Euphorbia fischeriana* root is being carried out in our lab for the purpose of isolating genes involves in phorbol biosynthesis. *Arabidopsis* transcriptome analyses using microarrays revealed the importance of transcriptional regulation in plant growth and responses to environmental pressures. Understanding transcriptional regulatory networks is fundamental to investigating the metabolic systems that control plant functions. Although much transcriptome work on trees has been carried out by Chinese researchers, and some notable achievements made such isolating and identifying a number of interesting candidate regulatory genes, much more work is needed to elucidate the functions of these genes and to clarify which genes play key roles in response networks.

Tree proteomics research in China

The proteome is the complete set of proteins encoded by a genome. Proteomics is the study of those proteins, including the their functions, locations in various cellular compartments, times of expression, and the types and extent of post-translational modifications (Grant et al., 2001). Ultimately, proteomics aims to identify and quantify the cellular levels of each protein encoded by the genome (Andrew and Bernhard, 2006). A combination of techniques involving two-dimensional gel electrophoresis (2DGE) and mass spectrometry is the most

commonly used approach in proteomics. Recently, proteomics methods have gained widespread applications to tree studies in Chinese labs. Many investigators have reported advances based upon recent technological developments that identify proteins in trees. For example, using gel-free (in-solution) protein digestion and phosphopeptide enrichment, combined with a nanoUPLC-ESI-MS/MS strategy, six phosphorylation sites on eight P-proteins were identified from *Populus* dormant terminal buds (Liu et al., 2010). Another example, 244 proteins expressed in different regeneration stages were obtained and queried against public databases in combination with anatomical approaches (Du et al., 2006). With a shotgun proteomic method, a systematic proteomic study of *Populus* chloroplasts was initiated and 119 proteins were identified successfully (Yuan et al., 2011). To understand the mechanisms of bud-dormancy and bud-burst in *Pinus sylvestris* L. var. mongolica litv., 96 proteins with altered expression patterns were identified using NanoLC-ESI-MS/MS in the apical buds at four critical developmental stages (Bi et al., 2011). Pan et al.. (2009) employed a proteomic approach, 2-DE combined with MALDI-TOF-MS, to identify proteins differentially expressed, in comparison to WT, in the citrus mutant 'Hong Anliu' at four stages of fruit maturation. Mulberry dwarf (MD) is a serious infectious disease of mulberry caused by phytoplasma. To better understand the pathogen-stress response of mulberry (*Morus alba* L.) to MD phytoplasma, a comparative proteomic analysis was conducted with 2-DE of infected and healthy leaves. A model for the mechanism underlying mulberry dwarf was proposed based on observed physiological and biochemical changes in the diseased plants (Ji et al., 2009). Although there are several reports of using proteomics approach to analyze the proteins of some trees species, much work remains to be carried out for the more important tree resources. Additional recent effort has been devoted to developing next-generation technologies that will allow for the better characterization of proteome-phenotype relationships, by elucidating more fully the link between protein-expression profiles and distinct cellular processes or conditions (Kuster et al., 2005). Therefore, the proteins identified in the above studies should be investigated more thoroughly with other technologies to reach the essential aims of proteomics research.

Tree metabolomics research in China

Metabolomics is the study of global metabolite profiles in a system (cell, tissue, or organism) under a given set of conditions (Goodacre et al., 2004). Metabolomics (or metabonomics) has been labeled one of the new "omics", joining genomics, transcriptomics, and proteomics as a science employed toward the understanding of global systems biology (Rochfort, 2005). Metabolomics has developed slowly in China and has only recently become an area of major research interest. Nevertheless, metabolomics is fast becoming a popular tool for studying the cellular state of many systems, such as *Arabidopsis* (Ren et al., 2009) and *Artemisia annua* L. (Liu et al., 2011). In forest plants, for example, using liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESI-MS), a rapid method for analyzing small amounts of biological samples of taxanes was developed by Zhao et al.. (Zhao and Yu, 2005). Their results confirmed the feasibility of characterizing taxanes in biological samples by LC-ESI-MS analysis. The analytical methodology provides a rapid, conventional and reliable tool to study metabolic profiling of taxanes for structural elucidation in taxol biosynthesis.

Tree transgenic research in China

Transgenics is a relatively new approach to genetic improvement of trees. Transgenic tree research in China was initiated in late 1980s. Almost 20 species have been genetically transformed and are at different stages of research, such as *Populus* (He et al., 2008; Yang et al., 2010), *Betula* (Zeng et al., 2009) and *Pinus* (Tang and Tian, 2003). Transgenic tree studies focus primarily on resistance to insect, disease, and abiotic-stresses, as well as improving wood properties. For example, in 1996, insect-resistant poplar (*Populus nigra* L.) plants were developed with *Agrobacterium tumefaciens* strains carrying a binary vector containing different truncated forms of a *Bacillus thuringiensis* (*B.t.*) toxin gene (Wang et al., 1996). Stem segments from diseased *Paulownia tomentosa* ×*P. fortunei* hybrids, along with leaves from healthy control plants, were transformed with the expression vector p438PRSI using *Agrobacterium* (Du et al., 2005). Although transgenic technology has many advantages as a method to improve and breed trees, there are potential ecological risks to other crop plants once transgenic trees are released into natural environment. Using transgenic technology to improve characters of trees will also face problems such as reduction of the genetic diversity of the tree populations developed. Therefore, it is important to pay close attention to issues of ecological safety and biodiversity before large-scale transgenic tree plantations are established. To sum up, "omics" in trees lag behind those of important crop plants, and face other inherent problems such as long generation times, but that there have been promising initial efforts in all areas of modern "omics" research. It is anticipated that "omics" will become more and more important global systems biology tool and be applied in trees' all research areas.

Acknowledgements

We thank Dr. Baohong Zhang and Dr. John Stiller for their critical reading and editing the manuscript. This work is supported by a grant from The Research Institute of Forestry, Chinese Academy of Forestry (ZD200902).

References

Andrew RJ, Bernhard (2006) The model organism as a system: integrating 'omics' data sets. Molecular Cell Biology. 7: 198-210.

Bi YD, Wei ZG, Shen Z, Lu TC, Cheng YX, Wang BC, Yang CP (2011) Comparative temporal analyses of the *Pinus sylvestris* L. var. *mongolica litv*. apical bud proteome from dormancy to growth. Mol Biol Rep. 38(2): 721-729.Bradshaw HD, Ceulemans R, Davis J, and Stettler R (2000) Emerging model systems in plant biology: poplar (*Populus*) as a model forest tree. J Plant Growth Regul. 19(3): 306-313.

Brazma A, Robinson A, Cameron G, Ashburner M (2000) One-stop Shop for Microarray Data. Nature. 403(6771): 699-700.

Cervera MT, Plomion C, Malpica C (2000) Molecular markers and genome mapping in woody plants. In: Jain SM, Minocha, SC, eds. Molecular Biology of Woody Plants, Vol. 1. Dordrecht, the Netherlands: Kluwer Academic Publishers, 375-394.

Chawla HS (2002) Introduction to plant biotechnology (2nd ed). Enfield: Science Publishers, Inc.

Chen L, Zhao LP, Gao QK (2004) Contruction of tender shoots cDNA library and preliminary analysis of expressed sequence tags sequencing of tea plant. Journal of Tea Science. 24 (1): 18-22 (In Chinese).

Du J, Xie HL, Zhang DQ, He XQ, Wang MJ, Li YZ, Cui KM, Lu MZ (2006) Regeneration of the secondary vascular system in poplar as a novel system to investigate gene expression by a proteomic approach. Proteomics. 6(3): 881-895.

Du T, Wang Y, Hu QX, Chen J, Liu S, Huang WJ, Lin ML (2005) Transgenic *Paulownia* expressing *shiva-1* gene has increased resistance to *Paulownia* Witches' broom disease. Journal of Integrative Plant biology. 47(12): 1500-1506.

Feng SP, Li WG, Yu F, Wang JY, Wu YT (2010). Construction of genetic linkage map for rubber tree (*Hevea brasiliensis*) based on SSR markers. Yi Chuan. 32(8): 857-863 (In Chinese).

Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, Bult CJ, Tomb JF, Dougherty BA, Merrick JM, McKenney K, Sutton Granger, FitzHugh W, Fields C, Gocayne JD, Scott J, Shirley R, Liu LL, Glodek A, Kelley JM, Weidamn JF, Phillips CA, Spriggs T, Hedblom E, Cotton MD, Utterback TR, Hanna MC, Nguyen DT, Saudek DM, Brandon R, Fine LD, Fritchman JL, Fuhrmann JL, Geoghagen NSM, Gnehm CL, McDonald LA, Samll KV, Fraser CM, Smith HO, Venter JC (1995) Whole-genome random sequencing and assembly of Haemophilus influenzae Rd. Science. 269(5223):496-512.

Gan S, Shi J, Li M, Wu K, Wu J, Bai J (2003) Moderate-density molecular maps of *Eucalyptus urophylla* S. T. Blake and *E. tereicornis* Smith genomes based on RAPD markers. Genetica. 118: 59-67.

Gao FL, Jiang TB (2009). Construction of genetic linkage maps of silver birch based on AFLP markers. Yi Chuan. 31(2): 213-218 (In Chinese).

Gong Y, Liao Z, Chen M, Zuo K, Guo L, Tan Q, Huang Z, Kai G, Sun X, Tan F, Tang K (2005) Molecular cloning and characterization of a 1-deoxy-D-xylulose 5-phosphate reductoisomerase gene from *Ginkgo biloba*. DNA Seq. 16(2):111-120

Goodacre R, Vaidyanathan S, Dunn WB, Harrigan GG, Kell DB (2004). Metabolomics by numbers: acquiring and understanding global metabolite data. Trends in Biotechnology. 22(5): 245-252.

Grant SGN, Blackstock WP (2001) Proteomics in neuroscience: from protein to network. The Journal of Neuroscience. 21(21): 8315-8318.

Guo LL, Yu YH, Xia XL, Yin WL (2010) Identification and functional characterization of the promoter of the calcium sensor gene *CBL1* from the xerophyte *Ammopiptanthus mongolicus*. BMC Plant Biology. 10: 18.

He L, Lu H, Liu QL, Chen XM, Jiang XN (2008) Overexpression of *mtlD* gene in transgenic *Populus tomentosa* improves salt tolerance through accumulation of mannitol. Tree Physiol. 25(10): 1273-1281.

Hu G, Fan T, Mei X (2004) Identification of a cDNA clone specific for the taxol synthesis phase of *Taxus chinensis* cells by mRNA differential display. Nat Prod Res. 18(4): 365-371.

Hu XL, Xu MY, Liu DH, Qiu DY (2006) Cloing a DNA fragment of taxane 14 β-hydroxylase gene and construction of its plant expression vectors for plant system. Molecular Plant Breeding. 4(2): 243-250 (In Chinese).

Ji XL, Gai YP, Zheng CC, Mu ZM (2009) Comparative proteomic analysis provides new insights into mulberry dwarf responses in mulberry (*Morus alba* L.). Proteomics. 9: 5328-5339.

Jiang TB, Li SC, Gao FL, Ding BJ, Qu YJ, Tang XH, Liu GF, Jiang J, Yang CP (2007) Genetic linkage map of *Betula pendula* Roth and *Betula platyphylla* Suk based on random amplified polymorphisms DNA markers. Yi Chuan. 29(7): 867-873 (In Chinese).

Jiang TB, Zhou BR, Gao FL, Guo BZ (2011) Genetic linkage maps of white birches (*Betula platyphylla* Suk. and *B. pendula* Roth) based on RAPD and AFLP markers. Mol Breeding. 27: 347-356.

Kai GY, Zhao LX, Zhang L, Li ZG, Guo BH, Zhao DL, Sun XF, Miao ZQ, Tang KX (2005) Characterization and expression profile analysis of a new cDNA encoding taxadiene synthase from *Taxus media*. Journal of Biochemistry and Molecular Biology. 38(6), 668-675.

Kandpal RP, Saviola B, Felton J (2009) The era of 'omics unlimited. BioTechniques. 46: 351-355.

Kim HL (2003) Comparison of oligonucleotide-microarray and serial analysis of gene expression (SAGE) in transcript profiling analysis of megakaryocytes derived from CD34+ cells. Experimental and Molecular Medicine. 35(5): 460-466.

Kuster B, Schirle M, Mallick P, Aebersold R (2005) Scoring proteomes with proteotypic peptide probes. Nature Rev Mol. Cell Biol. 6: 577-583.

Li DH, Yao LJ, Yu YB, Jiang CJ, Zhou TS, Wu L (2009) Construction of cDNA library of *Camellia sinensis* cv. Ziyang 1 and primary analysis of expressed sequence tags (ESTs). Journal of Anhui Agricultural University. 36(3): 347-350 (In Chinese).

Liao Z, Chen M, Gong Y, Guo L, Tan Q, Feng X, Sun X, Tan F, Tang K (2004) A new geranylgeranyl diphosphate synthase gene from *Gingko biloba*, which intermediates the biosynthesis of the key precursor for ginkgolides. DNA Seq. 15(2): 153-158.

Lin SZ, Zhang ZY, Liu WF, Lin YZ, Zhang Q, Zhu BQ (2005) Role of glucose-6-phosphate dehydrogenase in freezing-induced freezing resistance of *Populus suaveolens*. Journal of plant physiology and molecular biology. 31(1):34-40.

Lin EP, Peng HZ, Jin QY, Deng MJ, Li T, Xiao XC, Hua XQ, Wang KH, Bian HW, Han N, Zhu MY (2009) Identification and characterization of two bamboo (*Phyllostachys praecox*) AP1/SQUA-like MADS-box genes during floral transition. Planta. 23(1): 109-120.

Lin SJ, Jiang J, Wang YC (2006) The cold acclimation protein gene from *Tamarix androssowii*. Molecular Plant Breeding. 4(2): 299-300 (In Chinese).

Liu BY, Wang H, Du ZG, Li GF, Ye HC (2011) Metabolic engineering of artemisinin biosynthesis in *Artemisia annua* L. Plant Cell Rep. 30: 689-694.

Liu CC, Lu TC, Li HH, Wang HX, Liu GF, Ma L, Yang CP, Wang BC (2010) Phosphoproteomic identification and phylogenetic analysis of ribosomal P-proteins in *Populus* dormant terminal buds. Planta. 231(3): 571-581.

Liu M, Lu C, Shen X, Yin W (2006) Characterization and function analysis of a cold-induced *AmCIP* gene encoding a dehydrin-like protein in *Ammopiptanthus mongolicus*. DNA seq. 17(5): 342-349.

Liu Q, Zhu AD, Chai LJ, Zhou WJ, Yu KQ, Ding J, Xu J, Deng XX (2009) Transcriptome analysis of a spontaneous mutant in sweet orange [*Citrus sinensis* (L.) Osbeck] during fruit development. J Exp Bot. 60(3):801-813.

Liu XF, Deng ZX, Gao S, Sun XF, Tang KX (2007) Molecular cloning and characterization of a glutathione S-transferase gene from *Ginkgo biloba*. DNA Seq. 18 (5): 371-379.

MacKay J, Bérubé H, Regan S, Séguin A (2004) Functional genomics in forest trees: Application to the investigation of defense mechanisms and wood formation. In: Walter C, Carson M, eds. Plantation Forest Biotechnology for the 21st Century. Kerala, India: Research Singpost, 163-180.

Michelmore R (2000) Genomic approaches to plant disease resistance. Current Opinion in Plant Biology. 3: 125-131.

Pan ZY, Liu Q, Yun Z, Guan R, Zeng WF, Xu Q, Deng XX (2009) Comparative proteomics of a lycopene-accumulating mutant reveals the important role of oxidative stress on carotenogenesis in sweet orange (*Citrus sinensis* [L.] osbeck). Proteomics. 9: 5455-5470.

Pang Y, Shen GA, Liu C, Liu X, Tan F, Sun X, Tang K (2004) Molecular cloning and sequence analysis of a novel chalcone synthase cDNA from *Ginkgo biloba*. DNA Seq. 15 (4): 283-290.

Qiu DY, Pan XP, Wilson IW, Li FL, Liu M, Teng WJ, Zhang BH (2009) High throughput sequencing technology reveals that the taxoid elicitor methyl jasmonate regulates microRNA expression in Chinese yew (*Taxus chinensis*). Gene. 436(1-2): 37-44.

Ren Y, Wang T, Peng Y, Xia B, Qu LJ (2009) Distinguishing transgenic from non-transgenic *Arabidopsis* plants by (1) H NMR-based metabolic fingerprinting. J Genet Genomics, 36(10): 621-628.

Rochfort S (2005) Metabolomics reviewed: a new "omics" platform technology for systems biology and implications for natural products research. J Nat Prod. 68(12): 1813-1820.

Sewel MM, Neale DB (2000) Mapping quantitative traits in forest trees. In: Jain SM, Minocha SC, eds. Molecular Biology of Woody Plants Vol. 1. Dordrecht, the Netherlands: Kluwer Academic Publishers, 407-424.

Shen G, Pang Y, Wu W, Miao Z, Qian H, Zhao L, Sun X, Tang K (2005b) Molecular cloning, characterization and expression of a novel jasmonate-dependent defensin gene from *Ginkgo biloba*. J plant Physiol. 162(10):1160-1168.

Shen GA, Pang Y, Wu W, Liu X, Zhao L, Sun X, Tang K (2006) Isolation and characterization of a putative anthocyanidin reductase gene from *Ginkgo biloba*. J Plant Physiol. 163(2): 224-227.

Shen GA, Pang YZ, Wu WS, Deng ZX, Liu XF, Lin J, Zhao LX, Sun XF, Tang KX (2005a) Molecular cloning, characterization and expression of a novel *Asr* gene from *Ginkgo biloba*. Plant Physiology and Biochemistry. 43 (9): 836-843.

Shi MW, Tan XF, Wang YQ, Hu FM (2004) Question and solution of construction cDNA library from seed of *Camellia oleifera*. Nonwood Forest Research. 22(2):53-55 (In Chinese).

Tang W, Tian YC (2003) Transgenic loblolly pine (*Pinus taeda* L.) plants expressing a modified δ-endotoxin gene of *Bacillus thuringiensis* with enhanced resistance to *Dendrolimus punctatus* Walker and *Crypyothelea forosicola* Staud. Journal of Experimental Botany. 54(383): 835-844.

Taylor G (2002) Populus: Arabidopsis for forestry. Do we need a model tree? Ann Bot. 90: 681-689.

Tian B, Chen Y, Li D and Yan Y (2006) Cloning and characterization of a bamboo LEAFY HULL STERILE1 homologous gene. DNA Seq. 17(2): 143-151.

Tian B, Chen YY, Yan YX, Li DZ (2005) Isolation and ectopic expression of a bamboo *MADS-box* gene. Chin Sci Bull. 50: 217-224

Tong CF, Shi JS (2004).Constructing genetic linkage maps in Chinese fir F_1 progeny. Acta Genet Sin. 31(10): 1149-1156 (In Chinese).

Tu J, Zhu P, Cheng KD, Meng C (2004) Cloning and sequencing of hydroxylase genes involved in taxol biosynthesis. Z Naturforsch C. 59(7-8): 561-564.

Wang C, Yang CP, Gao CQ, Wang YC (2009a) Cloning and expression analysis of 14 lipid transfer protein genes from *Tamarix hispida* responding to different abiotic stresses. Tree Physiology.29(12):1607-1619.

Wang GF, Gao Y, Yang LW, Shi JS (2007) Identification and analysis of differentially expressed genes in differentiating xylem of Chinese fir (*Cunninghamia lanceolata*) by suppression subtractive hybridization. Genome, 50(12): 1141-1155.

Wang GJ, Castiglione S, Chen Y, Li L, Han YF, Tian YC, Gabriel DW, Han YN, Mang KQ, Sala F (1996) Poplar (*Populus nigra* L.) plants transformed with a *Bacillus thuringiensis* toxin gene: insecticidal activity and genomic analysis. Transgenic Research. 5(5): 289-301..

Wang JY, Xia XL, Wang JP, Yin WL (2008) Stress responsive Zinc-finger protein gene of *Populus euphratica* in tobacco enhances salt tolerance. Journal of Integrative Plant Biology. 50(1): 56-61.

Wang JY, Yin WL, Xia XL (2005) Cloing and structure analysis of Zinc finger protein gene in *Populus euphratica* Oliver. Yi Chuan. 27(2): 245-248 (In Chinese).

Wang Y, Gao C, Liang Y, Wang C, Yang C, Liu G (2010a) A novel *bZIP* gene from *Tamarix hispida* mediates physiological responses to salt stress in tobacco plants. J Plant Physiol. 167(3): 222-230.

Wang YC, Qu GZ, Li HY, Wu YJ, Wang C, Liu GF, Yang CP (2010b) Enhanced salt tolerance of transgenic poplar plants expressing a manganese superoxide dismutase from *Tamarix androssowii*. Mol Biol Rep. 37(2): 119-124.

Wang Z, Gerstein M, Snyder M (2009). RNA-Seq: a revolutionary tool for transcriptomics. Nature Rev. Genetics 10(1): 57-63.

Wei ZG, Zhang KX, Yang CP, Liu GF, Liu GJ, Lian L, Zhang HG (2010) Genetic linkage maps of *Betula platyphylla* suk based on ISSR and AFLP markers. Plant Mol Biol Rep. 28(1): 169-175.

Wu W, Pang Y, Shen GA, Lu J, Lin J, Wang J, Sun X, Tang K (2006) Molecular cloning, characterization and expression of a novel trehalose-6-phosphate synthase homologue from *Ginkgo biloba*. J biochem Mol Biol. 39 (2): 158-166.

Xie WW, Gao S, Wang SH, Zhu JQ, Xu Y, Tang L, Chen F (2010) Cloning and expression analysis of carboxyltransferase of acetyl-coA carboxylase from *Jatropha curcas*. Z Naturforsch C. 65(1-2): 103-108.

Xu F, Cheng H, Cai R, Li LL, Chang J, Zhu J, Zhang FX, Chen LJ, Wang Y, Cheng SH, Cheng SY (2008) Molecular cloning and function analysis of an anthocyanidin synthase gene from *Ginkgo biloba*, and its expression in abiotic stress responses. Mol Cells. 26 (6): 536-547.

Xu F, Cheng SY, Cheng SH, Wang Y, Du HW (2007) Time course of expression of chalcone synthase gene in *Ginkgo biloba*. Journal of plant physiology and molecular biology. 33(4): 309-317.

Yang HL, Zhao YR, Wang CL, Yang ZL, Zeng QY, Lu H (2009) Molecular characterization of a dehydroascorbate reductase from *Pinus bungeana*. J Inter Plant boil. 51(11): 993-1001.

Yang LY, Sun Y, Xie LQ, Liang AH (2010) A novel approach for in *situ* bud transformation of *Populus* by *Agrobacterium*. Scandinavian Journal of Forest Research. 25(1): 3-9.

Ye CY, Zhang HC, Chen JH, Xia XL, Yin WL (2009) Molecular characterization of putative vacuolar NHX-type Na+/H+ exchanger genes from the salt-resistant tree *Populus euphratica*. Physiologia Plantarum. 137(2): 166-174.

Yuan HM, Li KL, Ni RJ, Guo WD, Shen Z, Yang CP, Wang BC, Liu GF, Guo CH, Jiang J (2011) A systemic proteomic analysis of *Populus* chloroplast by using shotgun method. Mol Biol Rep. 38(5): 3045-3054.

Zduńczyk Z, Pareek CS (2008) Application of nutrigenomics tools in animal feeding and nutritional research. Journal of Animal and Feed Sciences. 17: 3-16.

Zeng FS, Zhan YG, Nan N, Xin Y, Qi FH, Yang CP (2009) Expression pf *bgt* gene in transgenic birch (*Betula platyphylla* Suk.). African Journal of Biotechnology. 8(15): 3392-3398.

Zeng YR, Huang MR, Wang MM (2004) Construction of cDNA Libraries with leaves of clones susceptible and resistant to black spot disease in poplar. Journal of Nanjing forestry university (Natural Sciences Edition). 28 (3): 83-85 (In Chinese).

Zhang B, Su X, Zhou X (2008a) A MADS-box gene of *Populus deltoides* expressed during flower development and in vegetative organs. Tree Physiol. 28(6): 929-934.

Zhang D, Zhang Z, Yang K, Li B (2004) Genetic mapping in (*Populus tomentosa* × *Populus bolleana*) and *P. tomentosa* Carr. using AFLP markers. Theor Appl Genet. 108(4): 657-662.

Zhang GJ, Guo GW, Hu XD, Zhang Y, Li QY, Li RQ, Zhuang RH, Lu ZK, He ZQ, Fang XD, Chen L, Tian W, Tao Y, Kristiansen K, Zhang XQ, Li SG, Yang HM, Wang J, Wang J (2010) Deep RNA sequencing at single base-pair resolution reveals complexily of the rice transcriptome. Genome Reserch. 20(5): 646–654.

Zhang Q, Zhang ZY, Lin SZ, Zheng HQ, Lin YZ, An XM, Li Y, Li HX (2008b) Characterization of resistance gene analogs with a nucleotide binding site isolated from a triploid white poplar. Plant Biol (Stuttg). 10(3): 310-322.

Zhang X, Zeng YL, Li JY, Zhao G, Zhang FC (2006) Cloning and sequence analysis of Na+/H+ antiporter (*PeNHX2*) from *Populus euphratica*. Biotechnology. 16(3): 9-13.

Zhang XY, Yin DM, Zhuge Q, Huang MR, Zhu LH, Zhai WX, Wu RL, Wang MX (2000) RAPD Linkage mapping in a *Populus deltoides* × *Populus euramericana* F1 family. Yi Chuan. 22(4): 209-213 (In Chinese).

Zhao CF, Yu LJ (2005). LC-ESI-MS metabolic profiling analysis of taxanes from the extracts of *Taxus chinensis* cell cultures. Acta Pharmaceutica Sinica. 40 (8): 734-739 (In Chinese).

Zhao LP, Gao QK, Chen L, Wang XC, Yao MZ (2006) Development and preliminary application of cDNA Microarray of Tea plant (*Camellia sinensis*). Journal of tea Science. 26(3): 166-170 (In Chinese).

Zhao LP, Ma CL, Chen L (2008) Construction and expressed sequence tags analysis of young roots cDNA library of tea plant. Molecular plant breeding. 6(5): 893-898.

Zhao YH, Guo YS, Hu YL, Zhang B, Liu R, Ouyang R, Fu JX, Liu CM (2010) Construction of a genetic linkage map of litchi with RAPD, SRAP and AFLP. Acta Horticulture Sinica. 37(5): 697-704 (In Chinese).

Zheng HQ, Lin SZ, Zhang Q, Lei Y, Hou L, Zhang ZY (2010) Functional identification and regulation of the *PtDrl02* gene promoter from triploid white poplar. Plant Cell Rep. 29(5): 449-460.

Zheng QP, Yu LJ, Liu Z, Li MY, Xiang F, Yang Q (2004) Cloning and analysis of cDNA encoding key enzyme gene (*dxr*) of the non-MVA pathway in *Taxus chinensis* cells. Chinese Journal of Biotechnology 20(4): 548-553 (In Chinese).

Zhou MB, Hou L, Zhu DX, Pei Y, Zhao DG (2004) Construction and analysis of a cDNA library in *Eucommia ulmoides* Olive. Journal of Mountain Agriculture & Biology. 23(1): 58-59 (In Chinese).

Zhu XL, Ma Y, Zhang FC (2007) Construction of a cDNA library and cloning of *nhx* cDNA from *Populus euphratica* under salt stress. Bulletin of Botanical research. 1: 82-88 (In Chinese).