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RESEARCH ARTICLE

Proteomic Changes Between *Populus* Allotriploids and Diploids Revealed Using an iTRAQ-based Quantitative Approach

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Abstract: ***Background*:** Polyploid breeding is a powerful approach for Populus genetic improvement because polyploid trees have valuable characteristics, including better timber quality and a higher degree of stress resistance compared with their full-sib diploids. However, the genetic mechanism underlying this phenomenon remains unknown.

***Objective*:** To better understand the proteomic changes between Populus allotriploids and diploids, we examined the proteomic profiles of allotriploid and diploid Populus by iTRAQ labeling coupled with two-dimensional liquid chromatography and MALDI-TOF/TOF mass spectrometry (MS).

***Method*:** iTRAQ labeling coupled with two-dimensional liquid chromatography and MALDI-TOF/TOF mass spectrometry (MS).

***Results*:** Between the *Populus* allotriploid and the full-sib diploid, 932 differentially expressed proteins (DEPs) were identified. These DEPs were primarily involved in stress, defense, transportation, transcriptional and/or translational modification, and energy production. The pathway analysis indicated that most of the DEPs were implicated in carbohydrate transport and metabolism, nitrogen metabolism and glycolysis, and the ribosome assembly pathway. These data suggest high protein divergence between *Populus* allotriploids and diploids, and rapid changes during hybridization.

***Conclusion*:** The results provide new data for further understanding of the mechanisms of polyploid trees that generally display increased height growth compared with their full-sib diploids.

Keywords: iTRAQ, *Populus,* allotriploid, diploids, proteome, polyploid.

1. INTRODUCTION

Polyploidy, common in angiosperms, is a powerful mechanism for evolutionary changes in higher plants that can be induced by stress and defense responses [1-3]. There are two types of polyploidy: autopolyploid and allopolyploid. An autopolyploid is formed by intraspecific genome duplication, whereas an allopolyploid is derived from hybridization between different species that involves the merging and doubling of diverged genomes [4, 5]. A previous study showed structural and regulatory divergence between parental genomes and the duplication of genetic materials contributed to growth vigor and better fitness in allopolyploids [6-8].

Polyploid induction is an important mechanism for tree breeding. In *Populus* breeding, polyploidy has played an important role because the *Populus* allotriploid has valuable characteristics, including better timber quality and a higher degree of stress resistance compared with its full-sib diploid [9, 10]. Newly formed allopolyploids undergo widespread changes at the genetic level, causing phenotypic diversity [11, 12]. These changes in genomic composition and gene expression have been observed in *Arabidopsis* allopolyploids [7], *Brassica* hexaploids [13], cotton allopolyploids [5], *Tragopogon* allopolyploids [14], and wheat allohexaploids [15]. Unlike changes in gene expression, changes in protein levels in polyploids and their progenitors are rarely examined in trees.

As a large-scale study of proteins, proteomics is an important complement to genomics and transcriptomics because proteins are more directly related to biological function and phenotype. However, a limited correlation between protein and transcript expression levels has been reported [13, 16]. To enhance our understanding of gene expression in allopolyploids, proteomic approaches were applied to *Populus* polyploids. However, the traditional two-

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dimensional gel electrophoresis analysis has limitations, including gel-to-gel variation and issues with quantification based on spot intensity. Isobaric tags for relative and absolute quantitation (iTRAQ) is a suitable approach for the investigation of proteomic changes in plants because of this technique's high sensitivity for measuring protein abundance in related species when coupled with mass spectrometric (MS) analysis [17].

In the current study, we investigated proteome divergence between *Populus* allotriploid and the corresponding diploid. In previous studies from our laboratory, three groups of triploid hybrids were obtained from the first-division restitution (FDR) and second division restitution (SDR) 2n megaspores of the same poplar line (P. pseudo-simonii × P. nigra 'Zheyin3#') [10, 11, 18]. This synthesized Populus allotriploid does not exist in nature, and its genome shows a higher level of heterozygosity [18, 19]. Here, using iTRAQ-based proteomic analysis, we investigated the effects of polyploidization and hybridization on the proteome of the *Populus* allotriploid compared with the full-sib diploid. As a model tree in plant molecular biology, analysis of the *Populus* species will offer us the opportunity to determine the relative contribution of hybridization and genome doubling to changes in expression at the proteomic level in woody plants.

2. MATERIALS AND METHOD

2.1. Plant Materials and Growth Conditions

Plant material was harvested from three groups: FDR, SDR, and full-sib diploids. The allotriploid plants used in this study were full-sib progeny produced by mating of a maternal clone (ZY3) of P. pseudo-simonii × P. nigra and a male (BJY) P.pseudo-simonii × P. nigra (11). According to the previous studies from our lab [11, 20], ZY3 buds were exposed to 41 °C for 4h at suitable stages during megasporogenesis to induce first-division restitution (FDR) and second division restitution (SDR) 2n megaspores. When the stigmas of the ZY3-treated buds were receptive, they were pollinated with fresh BJY pollen. In the offspring seedlings, triploids were detected by flow cytometry measurement and were performed using a flow cytometer (BD FACSCalibur, San Jose, CA, USA) [10]. A known diploid plant derived from the same cross was used as a control.

All plants used in this study were cuttings from 90 hybrid plants in three groups (FDR, SDR and full-sib diploids), 30 genotypes in each group. One-year-old branches were used, and shoots approximately 15 cm in length were cut in April. The cuttings were planted in peat soil in plastic pots (27 cm in diameter and 27 cm in depth). All the plant materials were grown in the greenhouse of the National Engineering Laboratory for Tree Breeding (Beijing, China) under natural light and temperature conditions. In the group of FDR, 5 high (F1) and 5 low (F2) growth vigor allotriploid genotypes with significant differences in plant height were selected from 30 genotypes after 3 months of growth. The same method was used in the SDR and diploid groups. The mature leaves from the selected 3-month-old plants of *Populus* allotriploids and diploids were collected and frozen in liquid nitrogen for further analysis.

2.2. Protein Extraction, Digestion, and iTRAQ Labeling

Total protein was extracted according to the method previously described by Qin *et al*. [21] with minor modifications. Pooled samples, representing 30 leaves (5 leaves from each of the 6 plant groups: F1, S1, N1, F2, S2 and N2) were ground into a fine powder in liquid nitrogen and transferred to an acetone solution containing 10% trichloroacetic acid (TCA) and 0.07% 2-mercaptoethanol. The resulting mixture was vortexed and sonicated for 10 min. The suspension was incubated for 1 h at −20 °C, vortexed every 15 min, and centrifuged at 9,000 × *g* for 20 min at 4 °C. The supernatant was discarded without disturbing the pellet. To reduce the acidity, the pellets were resuspended in cold acetone and centrifuged again at 20,000 × *g* for 30 min at 4 °C. The acetone wash was repeated 3 times. Each pellet was resuspended in 1 mL of protein extraction reagent [8 M urea, 4% (w/v) CHAPS, 30 mM HEPES, 1 mM PMSF, 2mM EDTA, and 10 mM DTT] with sonication. The samples were centrifuged at 20,000 × *g* for 30 min at 4 °C, and the pellets were discarded. The Bradford method was used to determine the protein concentration of the supernatant [22], and the samples were stored at −80 °C.

Protein samples were reduced with 10 mM DTT, alkylated with 55 mM iodoacetamide, digested with sequencing grade trypsin (Promega) at a ratio of 1:10 (w:w) for 12 h at 37 °C, and labeled using iTRAQ 8-plex kits (AB Sciex Inc., Framingham, MA, USA) according to the manufacturer's protocol. The four allotriploid samples were labeled with iTRAQ tags 113, 114, 119, and 121, and the diploid samples were labeled with tags 115 and 116.

2.3. Strong Cation Exchange Fractionation of the iTRAQ-labeled Peptides

For strong cation exchange (SCX), the peptide mixtures were loaded in solvent A (25% (v/v) acetonitrile, 10 mM ammonium formate, pH 2.8) onto a polysulfoethyl A column (2.1 × 100 mm, 5 μm, 300 Å; PolyLC, Columbia, MD, USA) and separated through a 0–20% linear gradient of solvent B [25% (v/v) acetonitrile, 500 mM ammonium formate] for 50 min, and then 100% solvent B for 15 min [Agilent 1100 High Performance Liquid Chromatography (HPLC) System]. The flow rate was 200 μL/min.

2.4. Reverse Phase LC-ESI-MS/MS

For each fraction, 10 μL was injected for nanoLC-MS/MS analysis using a Q-Exactive MS (Thermo Finnigan) equipped with Easy-nLC (Proxeon Biosystems, now Thermo Fisher Scientific). The iTRAQ-labeled peptides were loaded on the column (75 μm internal diameter, 15 cm; L-Column, CERI, Auburn, CA, USA) using a Paradigm MS4 HPLC pump (Michrom BioResources Inc., Auburn, CA, USA) and an HTC-PAL autosampler (CTC analytics, Zwingen, Switzerland). Buffers were 0.1% (v/v) acetic acid and 5% (v/v) acetonitrile in water (A) and 0.1% (v/v) acetic acid and 90% (v/v) acetonitrile in water (B). A linear gradient from 5% to 45% B was applied for 70 min, and peptides eluted from the column were introduced directly into an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) with a flow rate of 200 nL/min and a spray voltage of 2.0 kV. The range of the MS scan was the mass-to-charge ratio

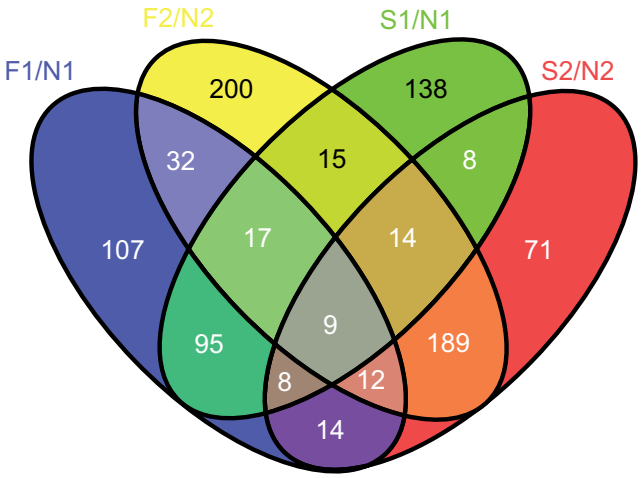


Fig. (1). Venn diagram showing differentially expressed protein among high growth vigor FDR (F1) and SDR(S1) compared with the high growth vigor diploids; low growth vigor FDR (F2) and SDR(S2) compared with the low growth vigor diploids (N2).

of 350 to 1,800, and the top three peaks were subjected to tandem MS analysis.

2.5. iTRAQ Protein Identification and Data Analysis

Protein identification was performed using the Mascot search engine against the NCBI(NCBInr) non-redundant sequence database for *Populus*(http://www.ncbi.nlm.nih.gov/protein?term=txid3694[populus]). For protein identification, a mass tolerance of 20 ppm was permitted for intact peptide masses and 0.05 Da for fragment mass tolerance, with allowance for one max missed cleavage in the trypsin digests. Proteins identified with a 1% false discovery rate (FDR) as determined by the Pro Group algorithm were used for further analysis. Conversion of glutamate to pyroglutamate (Gln->pyro-Glu; N-term Q), oxidation (M), and iTRAQ8plex (Y) are the potential variable modifications, while carbamidomethyl (C), iTRAQ8plex (N-term), and iTRAQ8plex (K) are fixed modifications. Proteins with a fold change of ≥1.5 or ≤0.667 and a *p*-value less than 0.05 were considered to be significantly differentially expressed. The Gene Ontology (GO) database (http://www.geneo- ntology.org/) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.ad.jp/kegg/) were used to annotate proteins using corrected *p*-values <0.05 as the threshold. Gene ontology (GO; http://www.geneontology.org) is an international standardization of gene function classification. It provides a set of dynamically updated controlled vocabulary to describe genes and gene product attributes in organisms. GO classifies functions according to three categories: molecular function, cellular component, and biological process.

2.6. Western Blot Analysis

The samples used in the iTRAQ analysis were also used for Western blot analysis. Five differentially expressed proteins (Rubisco large subunit form I and form II, Pathogenesis-related protein 1, Ferritin(plant), calcineurin/EF-hand motif(calcineurin), Fructose-bisphosphate aldolase) were selected, and equal amounts of protein from the *Populus* allotriploid and diploid were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a PVDF membrane (Millipore Corporation, Bedford, MA, USA) at 100 V for 60 min. The membrane was immersed in 5% BSA in Tris-buffered saline and Tween 20 (TBS-T; 0.2 M Tris–HCl pH 7.6, 1.37 M NaCl, and 0.1% Tween-20) for 1 h at room temperature. The proteins were incubated with the corresponding polyclonal antibodies in 5% BSA in TBS-T for 3 h at room temperature and washed 3 times for 5 min with TBS-T. The membrane was incubated with a horseradish peroxidase-conjugated goat anti-rabbit antibody (Beijing Protein Innovation, Beijing, China) for 1 h at room temperature and washed 3 times for 5 min with TBS-T. The blot was developed with the Super ECL Plus Kit (Applygen, Beijing, China) and the signal was detected using X-ray film.

3. RESULTS

3.1. Proteome Divergence in *Populus* Allotriploid and Full-Sib Diploid

We determined proteomic variations between the *Populus* allotriploid and full-sib diploid using iTRAQ labeling and LC-MS/MS. A total of 7,381 common proteins were identified in all samples (Table **S1**). Among them, 929 were differentially expressed in the allotriploid and diploid *Populus* with fold changes ≥1.5 or ≤0.667.

When we compared the expression levels of the high growth vigor FDR (F1) and the high growth vigor SDR (S1) with the *Populus* diploids with high growth vigor, 294 and 304 proteins were differentially expressed, respectively (Fig. **1**). In the low growth vigor compared groups, 488 and 324 proteins were differentially expressed between the low growth vigor allotriploids (F2 and S2) and the low growth vigor diploids, respectively.

To obtain functional information about the 929 differentially expressed proteins, we used the Blast2GO program to determine the relevant biological processes and molecular functions. The results from the biological process categories showed that the differentially expressed proteins were mainly involved in apoptotic processes (0.60%), response to stimulus (5.6%), developmental processes (1.1%), cellular processes (11.1%), metabolic processes (61.7%), biological regulation (3.3%), and cellular component organization in biogenesis (3.3%) (Fig. **2A**).

According to the molecular function analysis, these differentially expressed proteins were classified into transporter activity (64%), translation regulator activity (1.7%), enzyme regulator activity (1.2%), catalytic regulator activity (39.9%), receptor activity (1.7%), nucleic acid binding transcription factor activity (0.6%), antioxidant activity (0.6%), structural molecular activity (19.1%), and binding (27.7%) (Fig. **2B**).

3.2. Protein Expression Patterns in the Allotriploid and Diploid *Populus*

Examination of the 932 differentially expressed proteins between allotriploid and diploid *Populus* aids in the understanding of the relevance of protein expression profiles and agronomic traits. A cluster analysis of the proteins identified

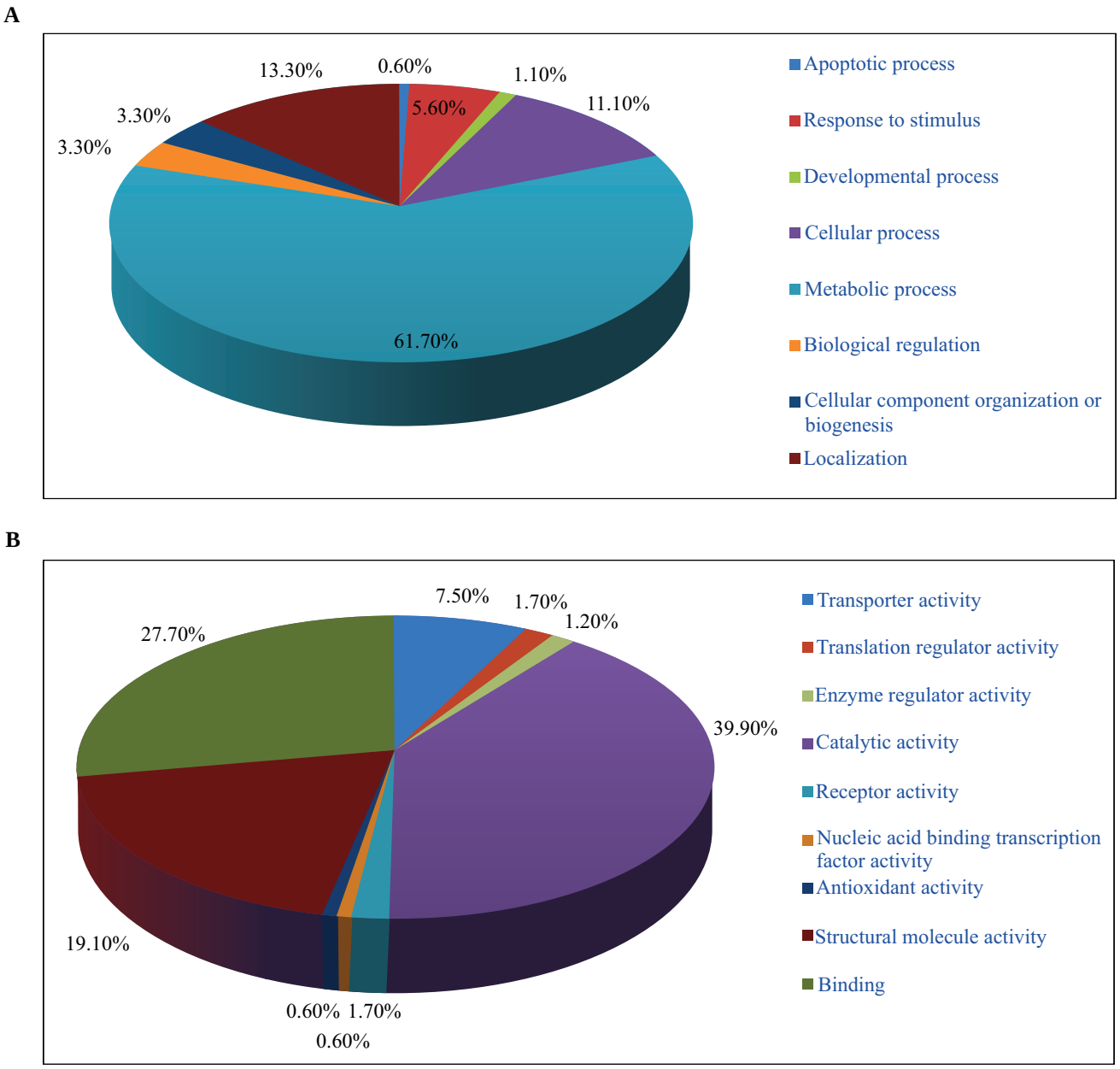


Fig. (2). (A) The Biological Process distribution of the identified differentially expressed proteins between the *Populus* allotriploid and full-sib diploids; **(B)** The Molecular Founction distribution of the identified differentially expressed proteins between the *Populus* allotriploid and full-sib diploids.

in the high growth vigor FDR (F1), the high growth vigor SDR (S1), the high growth vigor diploids (N1), low growth vigor FDR (F2), low growth vigor SDR (S2), and the low growth vigor diploids (N2) was conducted using Cluster 3.0 software (Michael Eisen, Stanford University), and the proteins were grouped according to their expression level. The protein expression patterns of low growth vigor group comparison sets (F2 *vs*. N2, S2 *vs*. N2) shared a major change in protein expression compared with those of the high growth vigor group comparison sets (F1 *vs*. N1, S1 *vs*. N1) (Fig. **3**).

3.3. Effect of Genome Dosage and Hybridization on Proteome Divergence in *Populus*

Using GO term enrichment analysis, we categorized the differentially expressed proteins between the sets of high growth vigor comparison (F1 *vs*. N1) and between the sets of low growth vigor comparison (F2 *vs*. N2) according to the classification of GO terms. The GO terms were summarized into three categories: biological process, molecular function, and cellular component (Fig. **4**). The terms nucleotide binding, carbohydrate metabolism, DNA methylation, cell differentiation and division, meristem initiation, and maintenance and development were dominant in the molecular function and biological process categories. In the cellular component category, ribosome, chloroplast, cell wall, and chloroplast stroma functional groups were significantly enriched in the differentially expressed proteins between the pairs of groups.

To understand the effect of hybridization on proteome divergence in *Populus*, we analyzed the differentially expressed proteins between allotriploids of high growth

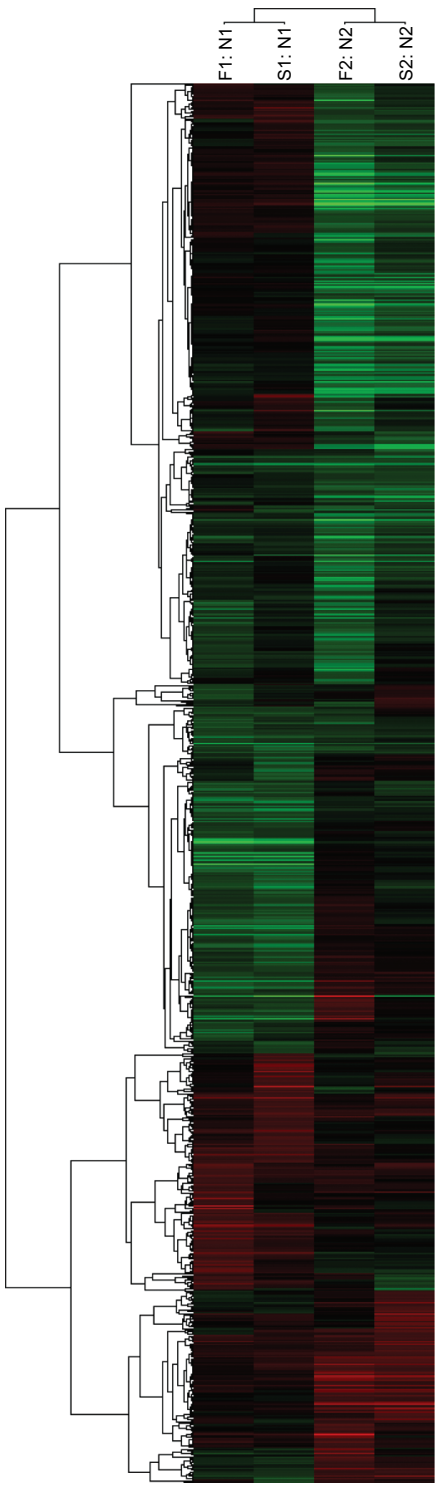


Fig. (3). Cluster map comparing the protein expression pattern of FDR,SDR,and the diploids. F1, high growth vigor FDR; S1, high growth vigor SDR; N1, high growth vigor diploids; N2, high growth vigor diploids; F2, low growth vigor FDR; S2, low growth vigor SDR. Red indicates higer expression,green indicates lower expression,and black indicates the same expression levels in the two strains.

vigor FDR (F1) and high growth vigor SDR (S1). The differentially expressed proteins were summarized into three categories: biological process, molecular function, and cellular component (Fig. **5**). These proteins covered a significant range of molecular functions, including catalytic activity, binding, structural molecule activity, transporter activity, receptor activity, translation regulator, enzyme regulator, and antioxidant activity (Fig. **4C**).

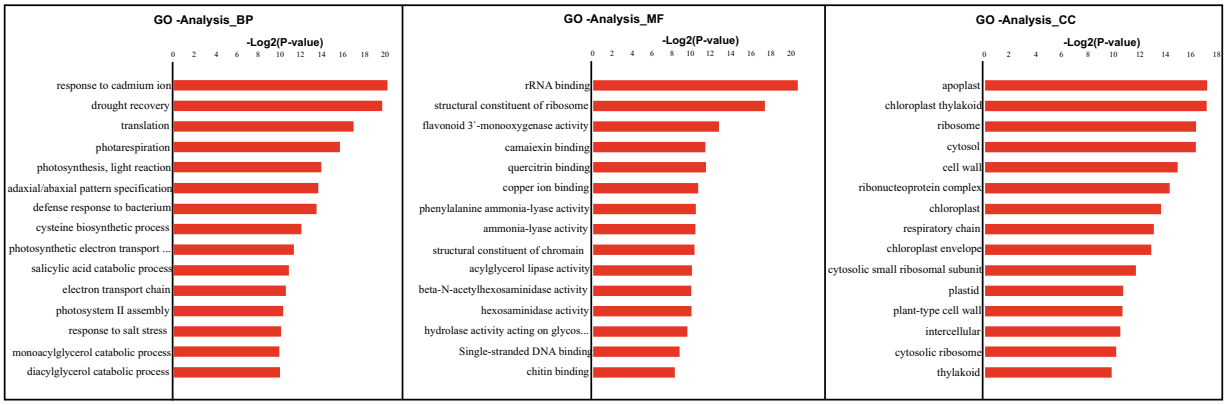
3.4. KEGG Pathway Analysis of Allotriploid and Diploid *Populus*

We compared the 929 differentially expressed proteins (DEPs) with *Arabidopsis thaliana* proteins. *A. thaliana* is a model species for molecular biology research in plants; its genome has been sequenced, and extensive research has been conducted on its molecular pathways. KEGG pathway enrichment analysis for the DEPs revealed significant enrichment of specific pathways compared with distribution of the entire proteome. Among the DEPs in the high growth vigor group of FDR allotriploids (F1) and full-sib diploids (N1), 294 proteins had a KEGG pathway annotation, and the significantly enriched pathways ($p \leq 0.05$) were ribosome, glycosaminoglycan degradation, glycosphingolipid biosynthesis, photosynthesis, pantothenate and CoA biosynthesis, nitrogen metabolism, and the flavonoid biosynthesis pathway (Fig. **5A**). Of the proteins that were differentially expressed between low growth vigor group of FDR allotriploids (F2) and the full-sib diploids (N2), 488 were significantly enriched ($p \leq 0.05$) in ribosome, alanine, aspartate and glutamate metabolism, butanoate metabolism, glycolysis and gluconeogenesis, nitrogen metabolism, beta-alanine metabolism, fatty acid degradation, and arginine and proline metabolism pathways (Fig. **5B**).

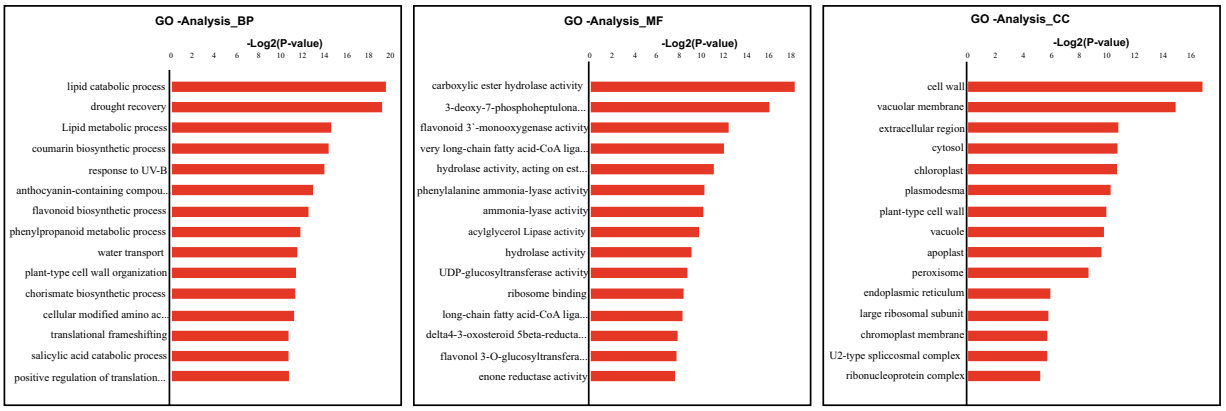
Between high growth vigor SDR allotriploids (S1) and full-sib diploids (N1), 304 proteins were differentially expressed, and the significantly enriched pathways ($p \leq 0.05$) were flavone and flavonol biosynthesis, phenylalanine, tyrosine and tryptophan biosynthesis, flavonoid biosynthesis, glycosaminoglycan degradation, biosynthesis of secondary metabolites, peroxisome, nitrogen metabolism, fatty acid degradation, and alpha-linolenic acid metabolism. Of the proteins that were differentially expressed between the low growth vigor group of SDR allotriploids (S2) and the full-sib diploids (N2), 325 were significantly enriched ($p \leq 0.05$) in ribosome, arginine and proline metabolism, nitrogen metabolism, alanine, aspartate, and glutamate metabolism. Therefore, the DEPs between the *Populus* allotriploid and the full-sib diploid are involved in a wide range of plant physiological processes after the formation of allopolyploids that may be essential for the differences in morphology between the *Populus* allotriploid and diploid.

3.5. Validation of iTRAQ Proteomic Data

Western blotting was performed to verify the expression of targeted proteins identified by the iTRAQ analysis of the *Populus* leaf. We selected five differentially expressed proteins, Rubisco large subunit form I and form II (RbcL), Pathogenesis-related protein1(PR-1), Ferritin(plant), calcineurin/EF-hand motif (calcineurin), Fructose-bisphosphate aldolase(FBA), and subjected the same protein samples that were used for iTRAQ analysis to Western blotting. The stability of expression of these proteins was analyzed using geNorm and Microcal Origin 6.0 software. The results of the



(a)



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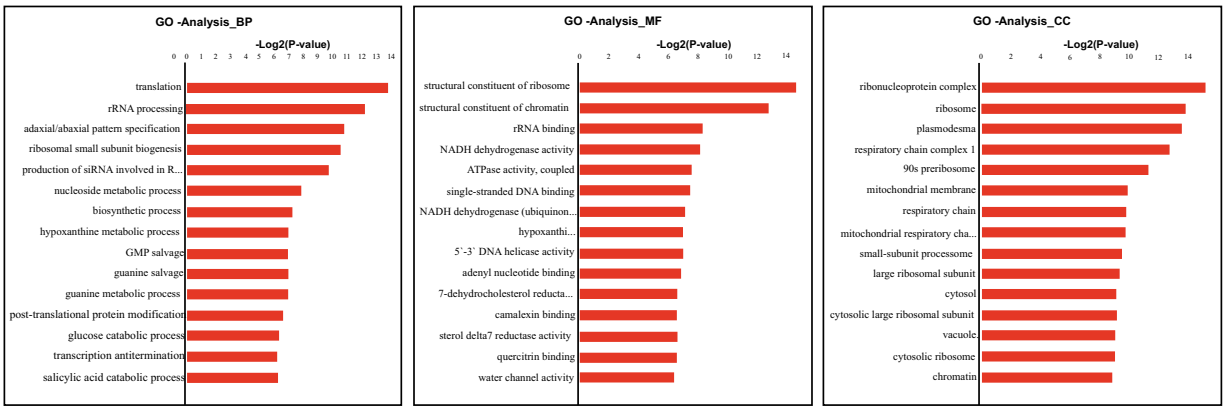

(c)

Fig. (4). (A) GO term enrichment analysis between high growth vigor FDR(F1) and high growth vigor diploids(N1); **(B)** GO term enrichment analysis between high growth vigor SDR(S1) and high growth vigor diploids(N1); **(C)** GO term enrichment analysis between high growth vigor FDR(F1) and high growth vigor SDR(S1).

western blots for RbcL , PR-1, Ferritin, calcineurin and FBA were consistent with the iTRAQ data (Fig. **6**). These results demonstrate the satisfactory quality of our experimental procedures and data.

4. DISCUSSION

Populus has been proposed as a model woody species and is now widely used to study tree-specific traits at the plant, organ, and tissue level [23]. Plant leaves are vital organs for photosynthesis, gas exchange, and water transpiration, which contribute to the biosynthesis of plant biomass and energy [24, 25]. Therefore, studies on the *Populus* leaf proteome will advance our understanding of the role of proteins in forest trees. Proteomics research in woody plants is primarily based on two-dimensional gel electrophoresis coupled to MS and is limited to a small number of woody spe-

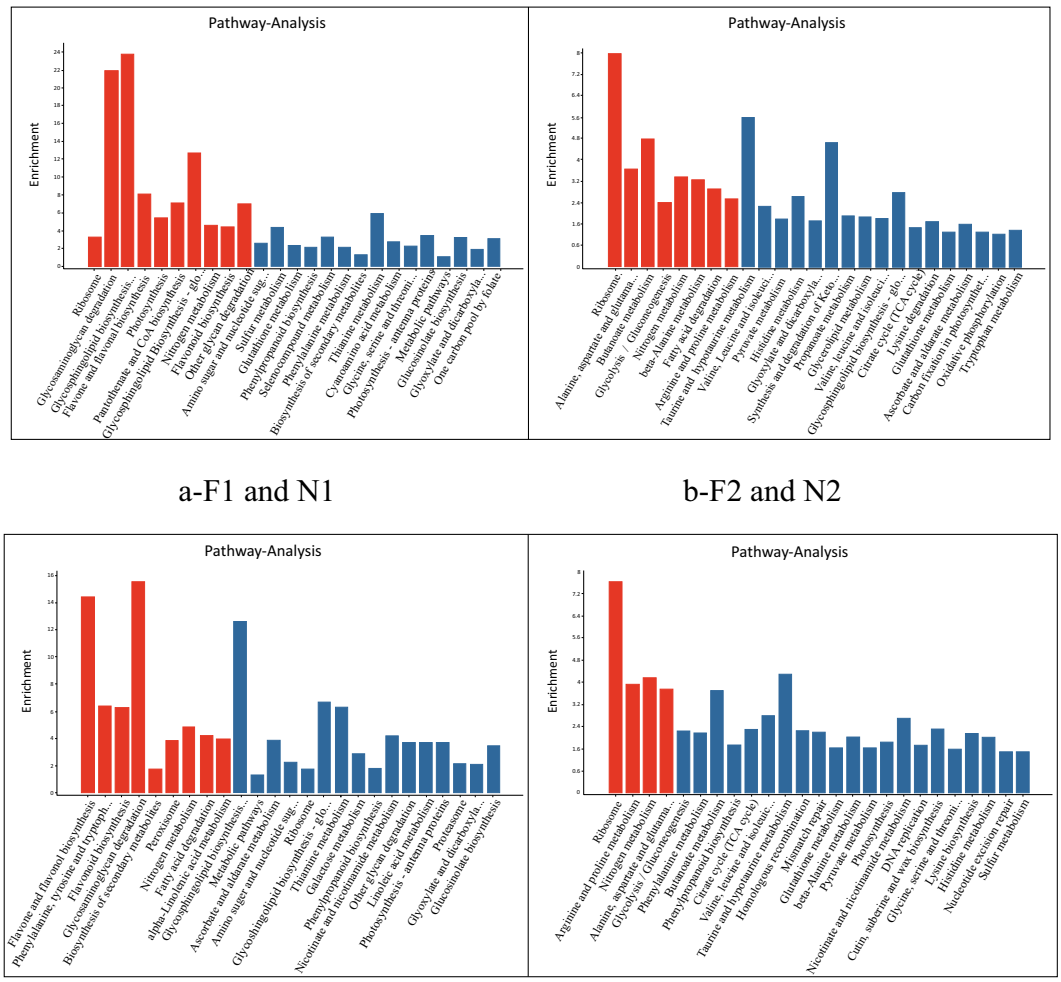

Fig. (5). KEGG pathway assignments for differentially expressed proteins(DEPs) between *Populus* allotriploid and diploid full-sibs: **(a)** high growth vigor FDR (F1) and high growth vigor diploids(N1); **(b)** low growth vigor FDR (F2) and low growth vigor diploids(N2); **(c)** high growth vigor SDR (S1) and high growth vigor diploids(N1); **(d)** low growth vigor SDR (S2) and low growth vigor diploids(N2). The x-axis indicates differentially expressed proteins enriched in KEGG pathways, and the y-axis indicates the extent of enrichment. The red bars represent significant differences ($P < 0.05$), and the blue bars indicate no significant difference.

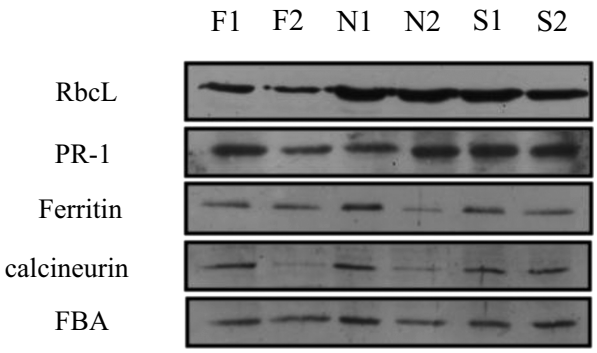

Fig. (6). Western blotting detection of *populus* proteins. F1, high growth vigor FDR; S1, high growth vigor SDR; N1, high growth vigor diploids; N2, high growth vigor diploids; F2, low growth vigor FDR; S2, low growth vigor SDR.

cies, including *Pinus*, *Picea*, *Eucalyptus*, *Populus*, and *Fagus* [23]. Proteomic analysis of the leaves of *Pinus* [26], *Picea* [27], *Eucalyptus* [28], *Populus* [29], and *Fagus* [30] were reported with two-dimensional gel electrophoresis coupled with MS, and 850, 676, 600, 730, and 140 protein spots were detected, respectively. We identified 7,381 proteins in the leaves of the *Populus* allotriploid and the corresponding full-sib diploid using the iTRAQ approach in this study and 929 differentially expressed proteins were identified, confirming the advantage of the iTRAQ approach in identifying low abundance and highly hydrophobic proteins.

Analysis of proteomic change was reported in several species, including *Brassica* [13], *Arabidopsis* [7], cassava [31], and wheat [32]. This research shows that the proteomic data suggest that genome divergence and hybridization may have profound effects on the proteome. Chromosomal rearrangements, including inversions, translocations, and fusions [33-35], may lead to differences in gene expression and the proteome in allopolyploids because allopolyploids are derived by hybridization of different species, from unreduced gametes. In our study, the differentially expressed proteins in

allotriploids and diploids were enriched in the defense response, metabolism, and ribosomal assembly pathways. Proteins involved in response to stress may contribute to the increased fitness and adaptation in allotriploids. Moreover, ribosomes are the sites of protein synthesis: the increased expression of these proteins may result in improved protein synthesis in allotriploids, and this may in turn lead to the high growth vigor of allotriploid *Populus*.

To understand the regulation of protein expression in Populus allotriploid, we also analyzed the differentially expressed protein profiles in the allotriploid and diploid *Populus* associated with growth vigor. Interestingly, the proteins related to metabolism were significantly enriched in high-growth allotriploid *Populus* relative to the full-sib diploid. Correlation of heterosis and enhanced metabolic activities was also reported in a study of diploid *A. thaliana* hybrids [36]. As a result, it has been proposed that metabolic activities are involved in the enhancement of some traits of *Populus* allotriploids, including faster growth.

CONCLUSION

This study suggested high protein divergence between *Populus* allotriploids and diploids, and rapid changes during hybridization. We analyzed differentially expressed proteins between the allotriploids and diploid hybrids using the GO and KEGG databases to identify candidate proteins that may contribute significantly to vegetative growth and development. Such differentially expressed proteins are involved in a wide range of plant physiological processes that may be essential for development of the differences in morphology and physiology evident between these *Populus* allotriploids and their full-sib diploids. The differentially expressed proteins between high and low growth vigor *Populus* allotriploids also participate in important biological processes, and these differentially expressed proteins were affected by hybridization and polyploidy. The results provide new data for further understanding of the mechanisms of polyploid trees that generally display increased vegetative growth compared with their full-sib diploids.

FUNDING

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AUTHORS' CONTRIBUTIONS

Xiangyang Kang and Yi Wang conceived and designed the experiments; Yi Wang, Yun Li, Yujing Suo, and Yu Min performed the experiments; Yi Wang analyzed the data; Yi Wang, Xiangyang Kang, and Yun Li wrote the paper.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

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ADDITIONAL FILES

Additional file 1: identification of the differentially expressed proteins.

Additional file 2: Blast2go analysis results of of the differentially expressed proteins.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

REFERENCES

[1] Leitch, A.R.; Leitch I.J. Genomic plasticity and the diversity of polyploid plants. *Science*, **2008**, *320*, 481-483.

[2] Jackson, S.; Chen, Z.J. Genomic and expression plasticity of polyploidy. *Curr. Opin. Plant Biol.*, **2010**, *13*, 153-159.

[3] Kong, F.; Mao, S.J.; Jiang, J.J.; Wang, J.; Fang, X.P.; Wang, Y.P. Proteomic changes in newly synthesized *brassica napus* allotetraploids and their early generations. *Plant Mol. Bio. Rep.*, **2011**, *29*, 927-935.

[4] Chen, Z.J. Molecular mechanisms of polyploidy and hybrid vigor. *Trends Plant Sci.*, **2010**, *15*(2), 57-71.

[5] Hu, G.; Koh, J.; Yoo, M.; Chen, S.; Wendel, J.F. Gene-expression novelty in allopolyploid cotton: a proteomic perspective. *Genetics*, **2015**, *200*, 91-104.

[6] Annett, P.; Anne, B.; Christian, B.; Annette H. Breeding of polyploid heather (*Calluna vulgaris*). *Euphytica*, **2014**, *199*, 273-282.

[7] DW-K, N.; Zhang, C.; Miller, M.; Shen, Z.; Briggs, S.P.; Chen, Z.J. Proteomic divergence in *Arabidopsis* autopolyploids and allopolyploids and their progenitors. *Heredity*, **2012**, *108*, 419-430.

[8] Ni, Z.F.; Kim, E.D.; Ha, M.S.; Lackey, E.; Liu, J.X.; Zhang, Y.R.; Sun, Q.X.; Chen, Z.J. Altered circadian rhythms regulate growth vigor in hybrids and allopolyploids. *Nature*, **2009**, *457*, 327-331.

[9] Zhu, Z.T.; Kang, X.Y.; Zhang, Z.Y. Studies on selection of natural triploids of *Populus tomentosa*. *Sci. Silave Sin.*, **1998**, *34*, 22-31.

[10] Lu, M.; Zhang, P.D.; Kang, X.Y. Induction of 2n female gametes in *Populus. adenopoda* Maxim by high temperature exposure during female gametophyte development. *Breed. Sci.*, **2013**, *63*, 96-103.

[11] Wang, J.; Li, D.L.; Kang, X.Y. Induction of unreduced megaspores with high temperature during megasporogenesis in Populus. *Ann. Foren. Sci.*, **2012**, *69*(1), 59-67.

[12] Chen, Z.J. Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annu. *Rev. Plant Biol.*, **2007**, *58*, 377-406.

[13] Shen, Y.Y.; Zhang, Y.; Zou, J.; Meng, J.L.; Wang, J.B. Comparative proteomic study on Brassica hexaploid and its parents provides new insights into the effects of polyploidization. *J. Proteomics*, **2015**, *112*, 274-284.

[14] Koh, J.; Chen, S.; Zhu N.; Yu, F.H.; Pamela, S.S.; Douglas, E.S. Comparative proteomics of the recently and recurrently formed natural allopolyploid *Tragopogon mirus* (Asteraceae) and its parents. *New Phytol.*, **2012**, *196*, 292-305.

[15] Qi, B.; Huang, W., Zhu, B.; Zhong, X.F.; Guo, J.H.; Zhao, N.; Xu, C.M.; Zhang, H.K.; Pang, J.S.; Han, F.P.; Liu, B. Global transgenerational gene expression dynamics in two newly synthesized al-

lohexaploid wheat (*Triticum aestivum*) lines. *BMC Biol.*, **2012**, *10*, 1-18.

[16] Anne, M.; Philippe, B.; Herve, T.; Karine, A. Analysis of gene expression in resynthesized Brassica napus allotetraploids: Transcriptional changes do not explain differential protein regulation. *New Phytol.*, **2010**, *186*, 216-227.

[17] Ross, P.L.H.Y.; Marchese, J.N.; Williamson, B.; Parker, K.; Hattan, S.; Khainovski, N.; Pillai, S.; Dey, S.; Daniels, S.; Purkayastha, S.; Juhasz, P.; Martin, S.; Bartlet-Jones, M.; He, F.; Jacobson, A.; Pappin, D.J. Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. *Mol. Cell Proteomics*, **2004**, *3*, 1154-1169.

[18] Dong, C.B.; Suo, Y.J.; Wang, J.; Kang, X.Y. Analysis of transmission of heterozygosity by 2n gametes in *Populus* (Salicaceae). *Tree Gen. Genom.*, **2015**, *11*(799), 1-7.

[19] Dong, C.B.; Suo, Y.J.; Kang, X.Y. Assessment of the genetic composition of triploid hybrid *Populus* using SSR markers with low recombination frequencies. *Can. J. For. Res.*, **2014**, *44*, 692-699.

[20] Wang, J.; Kang, X.Y.; Li, D.L. High temperature-induced triploid production during embryo sac development in Populus. *Silvae Genet*, **2012**, *61*(3), 85-93.

[21] Qin, J.; Gu, F.; Liu, D.; Yin, C.C.; Zhao, S.J.; Chen, H.; Zhang, J.N.; Yang, C.Y.; Zhan, X.; Zhang, M.C. Proteomic analysis of elite soybean Jidou17 and its parents using iTRAQ-based quantitative approaches. *Proteome Sci*., **2013**, *11*, 1-11.

[22] Bradford, M.A. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **1976**, *72*, 248-254.

[23] Abril N.; Gion, J.M.; Kerner R.; Gerhard M.S.; Rafael M.N.; Plomion C.; Renaut J.; Valledor L.; Jesus V.J. Proteomics research on forest trees, the most recalcitrant and orphan plant species. *Phytochemistry*, **2011**, *72*, 1219-1242.

[24] Evans, J.R.; Von Caemmerer, S. Enhancing photosynthesis. *Plant Physiol.*, **2011**, *155*(1), 19.

[25] Makino A. Photosynthesis, grain yield, and nitrogen utilization in rice and wheat. *Plant Physiol.*, **2011**, *155*, 125-129.

[26] Valledor, L.; Castillejo, M.A.; Lenz, C.; Rodriguez, R.; Canal, M.J.; Jorrin, J. Proteomic analysis of Pinus radiata needles: 2-DE map and protein identification by LC/MS/MS and substitution-tolerant database searching. *J. Proteome Res.*, **2008**, *7*, 2616-2631.

[27] Kjellsen, T.D.; Shiryaevab, L.; Schroderb, W.P.; Richard S.G. Proteomics of extreme freezing tolerance in Siberian spruce (*Picea obovata*). *J. Proteomics*, **2010**, *73*, 965-975.

[28] Fiorani Celedon, P.A.; De Andrade, A.; Meireles, K.G.; Gallo de Carvalho, M.C.; Caldas, D.G., Moon, D.H.; Carneiro, R.T.; Franceschini, L.M.; Oda S.; Labate C.A. Proteomic analysis of the cambial region in juvenile *Eucalyptus* grandis at three ages. *Proteomics*, **2007**, *7*, 2258-2274.

[29] Bonhomme, L.; Monclus, R.; Vincent, D.; Carpin, S.; Claverol, S.; Lomenech, AM.; Labas, V.; Plomion, C.; Brignolas, F.; Morabito, D. Genetic variation and drought response in two Populus×euramericana genotypes through 2-DE proteomic analysis of leaves from field and glasshouse cultivated plants. *Phytochemistry*, **2009**, *70*, 988-1002.

[30] Kerner, R.; Winkler, J.B.; Dupuy, J.W.; Jurgensen, M.; Lindermayr, C.; Ernst, D.; Muller-Starck, G. Changes in the proteome of juvenile European beech following three years exposure to free-air elevated ozone. *iForest*, **2010**, *4*, 69-76.

[31] An, F.F.; Fan, J.; Li, J.; Li, Q.X.; Li, K.M.; Zhu, W.L.; Wen, F.; Carvalho Luiz, J.C.B.; Chen, S.B. Comparison of leaf proteomes of Cassava (*Manihot esculenta* Crantz) cultivar NZ199 diploid and autotetraploid genotypes. *PLoS ONE*, **2014**, *9*(4), 1-13.

[32] Nazrul, I.; Hisashi, T.; Hisashi, H. Proteome analysis of diploid, tetraploid and hexaploid wheat: Towards understanding genome interaction in protein expression. *Proteomics*, **2003**, *3*, 549-557.

[33] Kuittinen, H.; de Haan, A.A.; Vogl, C.; Oikarinen, S.; Leppala, J.; Koch, M. Comparing the linkage maps of the close relatives *Arabidopsis* lyrata and *A. thaliana*. *Genetics*, **2004**, *168*, 1575-1584.

[34] Yogeeswaran, K.; Frary, A.; York, T.L.; Amenta, A.; Lesser, A.H.; Nasrallah, J.B. Comparative genome analyses of *Arabidopsis spp.* inferring chromosomal rearrangement events in the evolutionary history of *A. thaliana. Genome Res.*, **2005**, *15*, 505-515.

[35] Lysak, M.A.; Berr, A.; Pecinka, A.; Schmidt, R.; McBreen, K.; Schubert, I. Mechanisms of chromosome number reduction in Arabidopsis thaliana and related Brassicaceae species. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 5224-5229.

[36] Meyer, R.C.; Witucka-Wall, H.; Becher, M.; Blacha, A.; Boudichevskaia, A.; Dörmann, P.; Fiehn, O.; Friedel, S.; Von Korff, M.; Lisec, J. Heterosis manifestation during early *Arabidopsis* seedling development is characterized by intermediate gene expression and enhanced metabolic activity in the hybrids. *Plant J.*, **2012**, *71*, 669-683.