

Genome-wide Identification and Expression Analysis of the Eyloglucan Endotransglucosylase/hydrolase Gene Family in Poplar

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Abstract

Background: Xyloglucan endotransglucosylase/hydrolase (XTH) plays an important role in the process of plant cell wall reconstruction, and also involved in plants stress resistance. However, its characteristics of XTH family genes have not been reported in poplar.

Results: In this study, we found 43 *PtrXTH* genes from *Populus simonii* × *Populus nigra*, and most of them contain two conserved structures (Glyco_hydro_16 and XET_C domain). The promoter regions of the *PtrXTH* genes contain many cis-acting elements related to growth and development and adverse stresses responses. Collinearity analysis revealed that the XTH family from poplar have an evolutionary relationship with other five species, including *Eucalyptus robusta*, *Solanum lycopersicum*, *Glycine max*, *Arabidopsis*, *Zea mays* and *Oryza sativa*. Through RNA-Seq analysis, we found that the *PtrXTH* genes have different expression patterns in the roots, stems and leaves, and many of them are highly expressed in the roots. In addition, we found 11 differentially expressed *PtrXTH* genes in the roots, 9 in the stems, and 7 in the leaves under salt stress, and verified the accuracy of RNA-Seq analysis by RT-qPCR.

Conclusion: All the results indicated that XTH family genes may play an important role in tissue specificity and salt stress response. This study laid a theoretical foundation for further study on the functions of XTH genes in poplar.

Introduction

As the external supporting structure of cells, cell wall largely determines the shape and size of cells in the process of plant growth and development. The main components of plant cell wall are cellulose, lignin, hemicellulose and pectin (Cosgrove 2005). Xyloglucan is the most important hemicellulose in the primary cell wall of dicotyledons (Cosgrove and Jarvis 2012). Xyloglucan endotransglucosylase/hydrolase (XTH) is widely present in plant cells, which can catalyze the cleavage and polymerization of xyloglucan molecules, and achieve cell remodeling by regulating the elasticity and ductility of cells (Eklof and Brumer 2010). It is a key enzyme in the process of plant cell wall reconstruction

XTH family genes contain a typical catalyze enzymatic reaction motif (HDEIDFEFLG), in which the first glutamic acid residue (E) is affinity site and the second is proton donor (Nishitani 1997; Van Sandt et al. 2006). XTHs can be used to form a covalent glycosylase intermediate, which can be decomposed by water to carry out hydrolysis reaction, or enter the sugar substrate to produce transglycosylation (Eklof and Brumer 2010). According to the conserved motifs, the XTH family can be divided into three groups (I, II, and III), and the group III is divided into IIIA and IIIB group (Campbell and Braam 1999). It has been found that XTHs in groups I, II and IIIB have significant xyloglucan endohydrolase (XET) activity, while XTHs in IIIA show xyloglucan endoglucosidase (XEH) activity (Baumann et al. 2007). And a small part of outlier group (early diverting) is associated with the root development of trees. In summary, XTH family is divided into I / II, IIIA, IIIB and the early diverting groups (Michailidis et al. 2009; Baumann et al. 2007).

There are many XTH family genes, whose expression characteristics are different in the different tissues during different developmental stages. Poplar *PtxtXET16-34* was specifically expressed in the developing wood, and overexpression of the gene could increase xyloglucan content in the xylem of the primary wall and promote the growth of vessel element (Nishikubo et al. 2011). In Arabidopsis, *AtXTH17*, *AtXTH18*, *AtXTH19* and *AtXTH20* were specifically expressed in roots and play an important role in root elongation and root hair initiation (Yokoyama and Nishitani 2001; Osato et al. 2006). *AtXTH21* participates in the growth of primary roots by changing the cellulose deposition and the extensibility in the cell wall (Liu et al. 2007). *AtXTH31* can encode genes of heel extension region expression and participates in root elongation and growth (Zhu et al. 2012). In addition, some XTH genes are active in the development of leaf (Matsui et al. 2005), fruit (Miedes and Lorences 2009) and flower (Harada et al. 2011). Many studies have also indicated that the expression of XTHs was regulated by plant hormones. For example, the expression of rice *OsXTH8* (Jan et al. 2004) and Arabidopsis *AtXTH21* (Liu et al. 2007) was up-regulated by gibberellin treatment. The expression of Banana *MA-XET1* was induced by ethylene and participates in the ripening and softening of peel and pulp (Lu et al. 2004). Under the treatment of brassinosteroid (BR), the expression of *AtXTH22* and *AtXTH24* genes increased significantly in Arabidopsis, thereby promoting the elongation of cell walls (He et al. 2003).

In particular, XTH genes play an important role in abiotic stress in plants. The content of xyloglucan was reduced in *AtXTH31* mutant of *Arabidopsis thaliana*, which leads to a decrease in the content of absorbed AL^{3+} , thereby improving the aluminum stress tolerance (Zhu et al. 2012). Similarly, Arabidopsis *XTH17* and *XTH15* mutants also had higher aluminum tolerance compared with wild-type plants (Zhu et al. 2013; Zhu et al. 2014). *CaXTH3* gene in pepper can be induced by drought, high salt and cold, and overexpression of the gene lead to severe folds of Arabidopsis leaves, however, which improves the drought and salt tolerance of transgenic Arabidopsis (Choi et al. 2011). Under low temperature, the expression of persimmon *DkXTH6* gene was inhibited, while *DkXTH7* showed a high level of transcription (Han et al. 2016). Overexpression of *DkXTH1* enhanced the tolerance of transgenic Arabidopsis to salt, ABA and drought stress, and affected the growth and survival of roots and leaves (Han et al. 2017).

The first XTH gene was discovered in cowpea (Nishitani and Tominaga 1992), then the XTH family genes were established in other species. Among them, a number of 33, 29, 35, and 25 XTH genes were identified in Arabidopsis (Yokoyama and Nishitani 2001), rice (Yokoyama et al. 2004), sorghum (Rai et al. 2016), and tomatoes (Miedes and Lorences 2009), respectively. In poplar, Geisler-Lee (Geisler-Lee et al. 2006) found 41 genes, and Yuan (Ye et al. 2012) found 42 XTH genes, but they all did not systematically analyze the gene expression patterns of XTH genes under salt stress in poplar. In the present research, we found 43 XTHs family genes in poplar. We determined the evolutionary relationship of poplar XTH family by gene structure analysis and homologous collinearity analysis. Meanwhile, the function of XTH family genes was identified by the gene expression pattern analysis among different tissues under salt stress. This study provides a new idea for the cultivation of high-quality salt-tolerant poplar varieties.

Results

Identification and phylogenetic of XTH family genes

In this study, 43 XTH members were found in poplar genome and named as *PtrXTH1* - *PtrXTH43* according to their position in the chromosome. The evolutionary relationship of XTH family genes from poplar and Arabidopsis was constructed by MEGA6. As shown in the Fig. 1, the evolutionary tree could be divided into 4 groups (Group 1, Group 2A, Group 2B and Early diverging group), which was similar to *Brassica rapa* (Wu et al. 2020). We found that the Group 1 contained the most members with 33 members. Group 2A had 6 members, Group 2B had 3 members, and group 4 had only one member (Figure 2a).

The physicochemical properties of XTH proteins were also analyzed (Supplementary Table 1). We found that *PtrXTH2* had the largest number of amino acids (351) with the molecular weight (MW) of 40.77 kDa, while *PtrXTH11* had the smallest number of amino acids (117) with the MW of 13.44 kDa. The theoretical isoelectric point (PI) ranged from 4.53 to 9.67, while the instability coefficient ranged from 27.44 to 55.83. In addition, we found that only one protein had grand average of hydropathicity (GRAVY) greater than 0, which was expressed as hydrophobic protein, and the others were less than 0, which were expressed as hydrophilic protein. For subcellular localization, there were 8 genes located in the extracellular, and the other 35 proteins were located in the plasma membrane, and 80% of XTH proteins had signal peptides.

Gene structure analysis of poplar XTH family genes

In general, the genes that are clustered in one group have a similar structure. In this study, we found that Group 1 had 2 to 3 introns except *PtrXTH11* (1 member) and *PtrXTH1* (4 member), and Group 2A have 3 introns except *PtrXTH5* (1 member). In addition, there were 3 introns in Group 2B and Early diverging group, respectively (Fig. 2b). We used PFAM data to analyze the conserved domain of the 43 XTHs. We found that the *PtrXTH11*, *PtrXTH43* and *PtrXTH5* only had the Glyco_hydro_16 domain, and the other 40 genes had the 2 conserved domains (Glyco_hydro_16 and XET_C domain) (Fig. 2c).

In addition, we found 10 related motifs through the MEME online software (Fig. 2d and Supplementary Table 2). The longest one contained 50 amino acids (motif 3 and motif 4), and the shortest contained 7 amino acids (motif 7) (Fig. 2d). Glyco_hydro_16 contained the motifs 1, 4, 6, 7 and 8, but not all protein had the 5 motifs. XET_C contained the motif 5 and motif 10. This results were similar to the previous study (Wu et al. 2020).

Cis-acting elements in *PtrXTHs*

In this study, the 2000 bp upstream sequences of *PtrXTHs* were analysed by PlantCARE. As shown in the Fig. 3 and Supplementary Table 3, we found 16 types of cis-elements in the upstream promoter sequence of *PtrXTHs*. Among them, there were many elements related to abiotic stresses such as drought-inducibility, defense and stress responsiveness, low-temperature responsiveness, and wound-response.

The components including meristem expression, analytical induction, circadian control, seed-specific regulation, cell cycle regulation and light responsive were related to plant growth and development. The auxin responsiveness, MeJA-responsiveness, gibberellin-responsive, abscisic acid responsiveness and zein metabolism regulation were related to hormones response. In addition, there were multiple elements in response to a certain stress (Supplementary Table 4). For example, I-box, AE-box, and G-Box were involved in light responsive. P-box, TATC-box and GARE-motif were involved in gibberellin-responsive. Those result showed that the *PtrXTHs* can be regulated by the cis-elements in plant growth and stress response.

Chromosomal distribution and synteny analysis of *PtrXTHs*

We mapped the poplar XTH family genes to poplar chromosomes through genome annotation. As shown in the Fig. 4, the 43 *PtrXTHs* were not centrally allocated to a certain chromosomes, while they were randomly allocated to as many chromosomes as possible in a scattered manner. For example, the chromosomes 6, 10, 16, 19 and scaffold were allocated one member, the chromosomes 1, 3 and 5 were allocated two members, and the chromosome 6 had the most members (6 genes). Interestingly, we found the *PtrXTHs* were not located on the chromosomes 12, 15, and 17.

We analyzed the repetitive events of the *PtrXTHs* through MCscan. As shown in the Fig. 4a and Supplementary Table 4, there were 6 pairs of tandem repetitive events among 43 *PtrXTHs*, of which *PtrXTH18* shared two pairs of repetitive events. And 6 pairs of fragment duplication events were also identified in *PtrXTHs* (Figure 4b), which were distributed on the different chromosomes.

In order to further study the evolutionary relationship of *PtrXTHs*, we constructed systematic maps of the XTH genes between poplar and six other species, which including four dicotyledonous (*Eucalyptus robusta*, *Solanum lycopersicum*, *Glycine max* and *Arabidopsis*) and two monocotyledons (*Zea mays* and *Oryza sativa*). As shown in the Fig. 5 and Supplementary Table 5, there were 8 homologous pairs in *Arabidopsis*, 14 pairs in *Glycine max*, 12 pairs in *Eucalyptus robusta*, 11 pairs in *Solanum lycopersicum*, 1 pair in *Zea mays*, and 0 pairs in *Oryza sativa*. Among them, a total of 16 *PtrXTHs* showed collinearity relationship with other 6 species (7 in *Arabidopsis*, 8 in *Glycine max*, 9 in *Eucalyptus robusta*, 10 in *Solanum lycopersicum*, and 1 in *Zea mays*). In addition, the *PtrXTHs* shared homologous pairs in the different species (Figure 6). For example, *PtrXTH10* was homologous with the gene from *Eucalyptus robusta*, *Solanum lycopersicum* and *Zea mays*, and *PtrXTH7*, *PtrXTH15* and *PtrXTH37* were shared in *Glycine max*, *Arabidopsis* and *Solanum lycopersicum*.

Expression patterns of *PtrXTH* genes in different tissues

In order to parse the expression patterns of poplar XTH genes, we analyzed the expression patterns of *PtrXTHs* in the different tissues by RNA-Seq. As shown in the Fig. 7 and Supplementary Table 6, the XTH

genes were divided into 4 groups according to their expression patterns. Among them, the expression pattern of the genes in the first group was not obvious. However, the genes in the second group were mainly expressed in the leaves, and the third group and the fourth group were expressed in the roots and the stems, respectively. Similarly, some duplicate gene pairs had similar expression patterns. For example, *PtrXTH1* and *PtrXTH9* were expressed in the leaves, and *PtrXTH17* and *PtrXTH18* were expressed in the roots. However, some tandem gene pairs showed different expression trends, for instance, the *PtrXTH21* was expressed in the leaves, while the *PtrXTH24* gene was mainly expressed in the stems. The *PtrXTH26* gene was mainly expressed in the roots, while the *PtrXTH27* gene was mainly expressed in the leaves.

***PtrXTHs* expression analysis under salt stress**

We analyzed the gene expression patterns of *PtrXTHs* in different tissues under salt stress by RNA-Seq. As shown in the Fig. 8 and Supplementary Table 7, there were 7 differential expression genes (DEGs) in the leaves (4 up- and 3 down-regulated), 9 DEGs in the stems (4 up- and 5 down-regulated), and 11 DEGs in the roots (5 up- and 6 down-regulated). In addition, we analyzed the shared genes between different tissues. As shown in the Fig. 8d, there were 3 shared genes (0 up- and 3 down-regulated) was found in the roots and stems. Three shared genes (2 up- and 1 down-regulated) were found in the stems and leaves. Two shared genes (0 up- and 2 down-regulated) were found in the roots and leaves. Furthermore, one shared genes (0 up- and 1 down-regulated) were found in roots-stems-leaves.

To verify the accuracy of the RNA-Seq data, the DEGs in the roots, stems and leaves were selected for RT-qPCR. As showed in the Fig. 9, the RT-qPCR results were in consistent with the RNA-Seq analysis generally, which proved the accuracy of our data.

Discussion

XTH family genes play an indispensable role in plants growth and development. In this study, we found 43 XTH genes from *Populus simonii* × *Populus nigra*, which were named as *PtrXTH1* to *PtrXTH43* according to their location information on the chromosomes. The 43 *PtrXTHs* were divided into 4 subgroups compared with Arabidopsis, which was similar with rice and soybean. Most XTH genes contain two main conserved domains (Glyco_hydro_16 and XET_C domain). However, we found that *PtrXTH5* and *PtrXTH11* were lack of the XET_C domain. We speculate that there was a loss of XET_C domain during the evolution of the poplar XTH genes.

Poplar genome has experienced many whole-genome replication events in the process of evolution, including tandem repeat, fragment repeat and conversion events (Tuskan et al. 2006; Flagel and Wendel 2009). In this study, we analyzed the collinearity events of XTH family genes in poplar. Interestingly, we found that the number of tandem repeat gene pairs was same to that of fragment repeat gene pairs. We speculate that the two evolutionary mechanisms were co-regulated in the process of evolution. In addition, not all repetitive gene pairs had same expression patterns. We found that 7 out of 12 repetitive

gene pairs had different expression patterns. For example, the fragment repetitive gene pair *PtrXTH10* / *PtrXTH25* had same structure and conserved motif, however, they displayed obviously different expression patterns. *PtrXTH10* was mainly expressed in the roots, while *PtrXTH25* was mainly expressed in the leaves. Therefore, we speculate that *PtrXTH* genes participate in the replication process, which causes gene mutations, leading to the change of gene function and expression patterns. This phenomenon is also occurred in the ERF family (Liu et al. 2019b) and NAC family (Liu et al. 2019a). In addition, the collinear relationship between *PtrXTHs* and other six species were analyzed. We found that *PtrXTHs* had more collinear gene pairs with dicotyledons plants than that with monocotyledons plants.

XTH family genes are involved in the development of plant tissues and organs, and they have expression specificity of tissue and cell. In tobacco, *NtXTH4*, *NtXTH5*, *NtXTH12*, and *NtXTH19* had high expression levels in 19 tissues (Wang et al. 2018), and a similar situation occurred in barley (Fu et al. 2019). In our study, we found that the *PtrXTHs* in group 1 such as *PtrXTH16* and *PtrXTH31* were not expressed in tissues, which may play a redundant role in the process of evolution. The other three groups of *PtrXTHs* were expressed in different tissues. However, there were more *PtrXTHs* expressed in the roots and stems than that in the leaves, which indirectly proves that *PtrXTHs* may play an important role in the development of root and stem. There has been evidence to prove it. For example, Arabidopsis *XTH19* and *XTH23* (a homologous gene of *PtrXTH19*) were regulated by *BES1* to participate in the development of lateral roots through brassinosteroid signaling pathway, contributing to lateral roots adaptation to salt (Xu et al. 2020). The deficiency of *XTH9* (a homologous gene of *PtrXTH40*) in Arabidopsis regulated the secondary wall thickening by triggering integrity signal of cell wall, and stimulating the production of xylem cells (Kushwah et al. 2020).

During the process of growth and development, plants are subject to multiple abiotic stresses such as high temperature, salinity and drought (Ahuja et al. 2010). XTH genes play an important role in abiotic stress. In our study, we analyzed the expression pattern of poplar XTH family genes under salt stress by RNA-Seq. We found 11 differential expression genes in the roots, 7 in the leaves and 9 in the stems. In addition, RT-qPCR was used to verify the accuracy of RNA-Seq, which indicated the results were consistent in general. Phylogenetic analysis also showed that those genes play an important role in salt stress. For example, Arabidopsis gene *XTH30*, a homologous gene of *PtrXTH2* and *PtrXTH8*, was up-regulated under salt stress, and the lack of the gene slowed down the decrease of crystalline cellulose content and microtubule depolymerization under salt stress, which had a negative impact on salt tolerance (Yan et al. 2019). Overexpression of the *PeXTH* from *Populus euphratica* (a homologous gene of *PtrXTH28*) could increase water holding capacity and reduce salt concentration in fleshy tissues and mesophyll cells, which improved the salt tolerance of transgenic tobacco (Han et al. 2013). All evidences indicate that *PtrXTH* genes play an important role in regulating plant response to stress.

Conclusion

In this study, we identified 43 XTH genes from poplar and divided them into 4 groups. These genes were evenly distributed on the 16 chromosomes and 2 scaffolds in poplar. We identified 12 pairs of duplication

events among poplar XTH family genes, including 6 pairs of fragment duplication and 6 pairs of tandem duplication. In addition, we analyzed the homologous evolutionary relationship of XTH genes between poplar and other six species, which indicates the *PtrXTHs* had strong homologous relationship with dicotyledonous plants, compared to monocotyledonous plants. Furthermore, we profiled the expression pattern of *PtrXTHs* in different tissues under salt stress through RNA-Seq and RT-qPCR. There were more *PtrXTHs* expressed in the roots and stems than that in the leaves, which indicates *PtrXTHs* may play an important role in the development of root and stem. All the results in this study provide a theoretical basis for function identification of poplar XTH genes.

Materials And Methods

Identification of poplar XTH genes

All amino acid sequences of poplar XTH proteins were obtained from the Phytozome12 (<https://phytozome.jgi.doe.gov/pz/portal.html>). The hidden markov models of two typical XTH family protein structures (PF00722 and PF06955) were downloaded from the Pfam database (<http://pfam.xfam.org/>). The HMMER3.0 (Finn et al. 2011) was used to search for all potential XTH proteins in poplar. Then we used the SMART database (<http://smart.embl-heidelberg.de/>) to filter again. The ExpASy website (<http://web.expasy.org/protparam/>) was used to calculate the physical and chemical parameters. SignalP v4.1 server (<http://www.cbs.dtu.dk/services/SignalP/>) was used to predict the signal peptide cleavage site, and ProtComp 9.0 (<http://linux1.softberry.com>) was used to predict the subcellular localization.

Phylogenetic analyses of *PtrXTH* proteins

The protein sequences of XTH family from poplar and *Arabidopsis* were downloaded from the Phytozome12 (<https://phytozome.jgi.doe.gov/pz/portal.html>) and TAIR online websites (<https://www.arabidopsis.org/>), respectively. ClustalW (Thompson et al. 2002) was used for multiple sequence alignment of the proteins, and MEGA6 (<http://www.megasoftware.net/mega6/>) was used to construct phylogenetic trees with the neighbor-joining (NJ) method (bootstrap analysis for 1000 repetitions). The evolutionary tree was visualized through EvolView online software (Zhang et al. 2012).

Gene structures and conserved motif analyses of *PtrXTHs*

The poplar genomic sequences were downloaded from the Phytozome12 database (<https://phytozome.jgi.doe.gov/pz/portal.html>). We used GSDS (<http://gsds.cbi.pku.edu.cn/>) online software to analyze the gene structure of XTH family genes. MEME (<http://meme-suite.org/tools/meme>) online software was used to analyze the conserved motifs with default parameters, and the motifs were visualized with TBtools software (Chen et al. 2020a).

Cis-acting element analysis of PtrXTHs

The upstream 2000 bp sequence of the XTH genes was obtained from the Phytozome12 database. The cis-elements in all the sequences were predicted through PlantCRAE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/> 559.html/), and the cis-elements were screened and visualized by TBtools software (Chen et al. 2020a).

Chromosomal locations, synteny and duplications analyses of PtrXTHs

We used the position information of the XTH genes in the poplar genome to map each gene to the corresponding chromosome position. The genomic data of the poplar was downloaded from phytozome12, and each gene was mapped to the corresponding chromosome position through the position information of the poplar XTH genes. We used the MCscan (Wang et al. 2012) to calculate the repetitive events among the *PtrXTHs*. Genomes comparison among different species was performed by Blatxp. Dual Synteny Plotter was drawn to calculate the repetitive events of *PtrXTHs* between poplar and the other six species (*Eucalyptus robusta*, *Solanum lycopersicum*, *Glycine max*, *Arabidopsis*, *Zea mays* and *Oryza sativa*) by TBtools software (Chen et al. 2020a).

Plant material and stress treatments

The experimental material used in this study was di-haploid *Populus simonii* × *Populus nigra*, which were provided by the experimental forest of Northeast Forestry University, Harbin, China. The poplar seedlings were grown in 1/2 MS medium, and one-month-old seedlings were stressed with 0 and 150 mM NaCl for 0 and 12 hours, respectively. The roots, stems and leaves at different time points were collected for RNA extraction. Meanwhile, the samples were sent to GENEWIZ Company for RNA-Seq with Illumina platform (Yao et al. 2020). The RNA-Seq data was used to profile the expression patterns of the *PtrXTH* genes in different tissues, and the DESeq2 (Love et al. 2014) was used to screen the differentially expressed genes (DEGs) with absolute \log_2 (fold change) ≥ 1 and the adjusted p-value ≤ 0.05 . The heatmap was drawn by TBtools (Chen et al. 2020b). In addition, we used RT-qPCR to verify the accuracy of DEGs screened by RNA-Seq. And the detailed information were referred to our previous studies (Zhang et al. 2019). All the samples were prepared with three biological replicates. All the primers were list in the Supplementary Table 8.

Abbreviations

Xyloglucan endotransglucosylase/hydrolase (XTH); xyloglucan endohydrolase (XET); xyloglucan endoglucosidase (XEH); molecular weight (MW); differentially expressed genes (DEGs).

Declarations

Ethics approval and consent to participate

The di-haploid *Populus simonii* × *Populus nigra* were provided by the State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, Harbin, China. The study complies with relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. The raw sequencing data used during the study have been deposited in NCBI SRA with the accession number SRP267437.

Competing interests

The authors declare that they have no conflict of interest.

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Author contribution

T J and B Z designed research. Z C conducted experiments, data analysis and wrote the manuscript. X Z, K Z, Y G and Q G performed in data analysis. W Y revised the manuscript. All authors read and approved the manuscript.

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Not applicable.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Figures

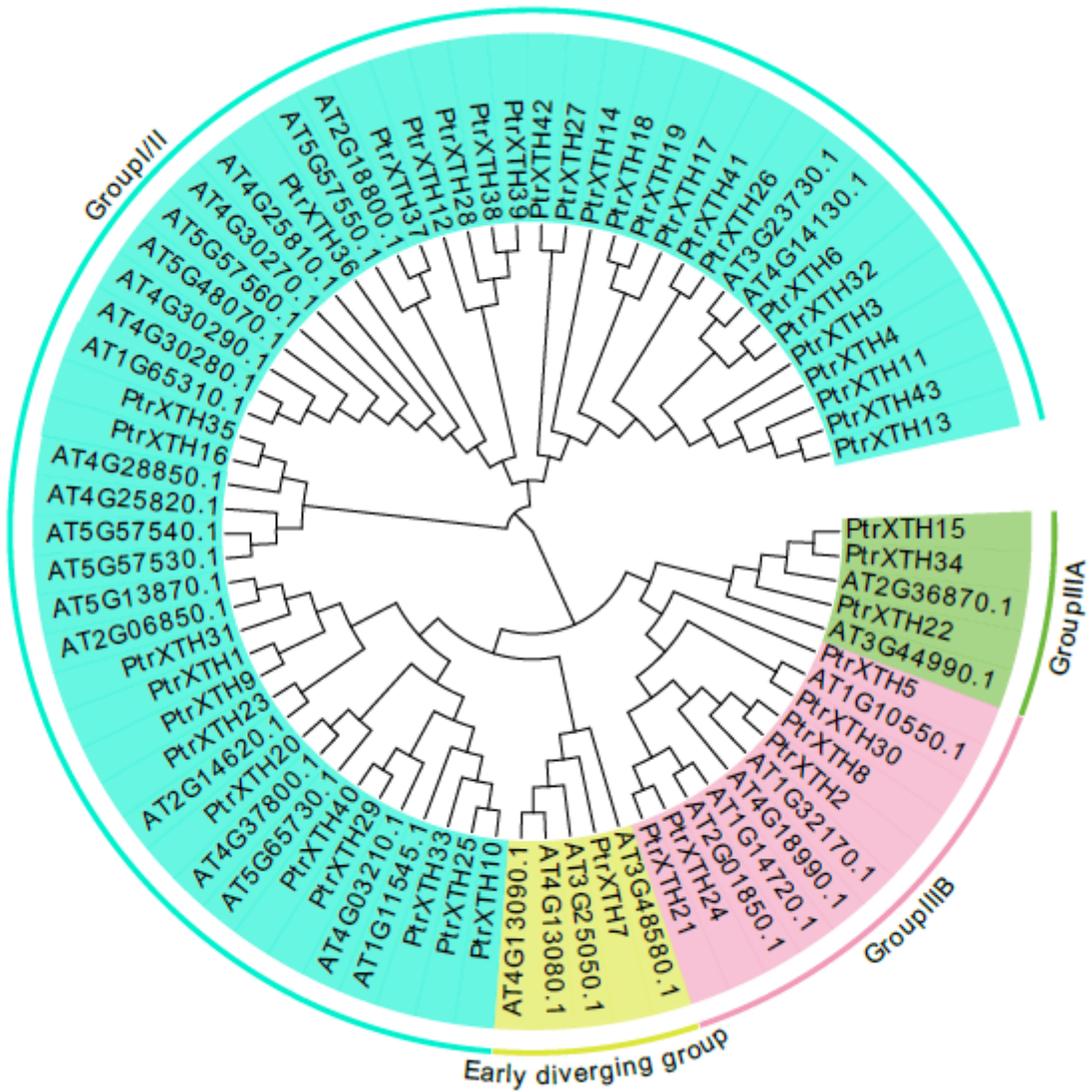


Figure 1

Phylogenetic tree of poplar and Arabidopsis. The phylogenetic was constructed by MEGA6 software, and the method was neighbor-joining with 1000 bootstrap replicates. The evolutionary tree was divided to 4 groups, each color represents a group.

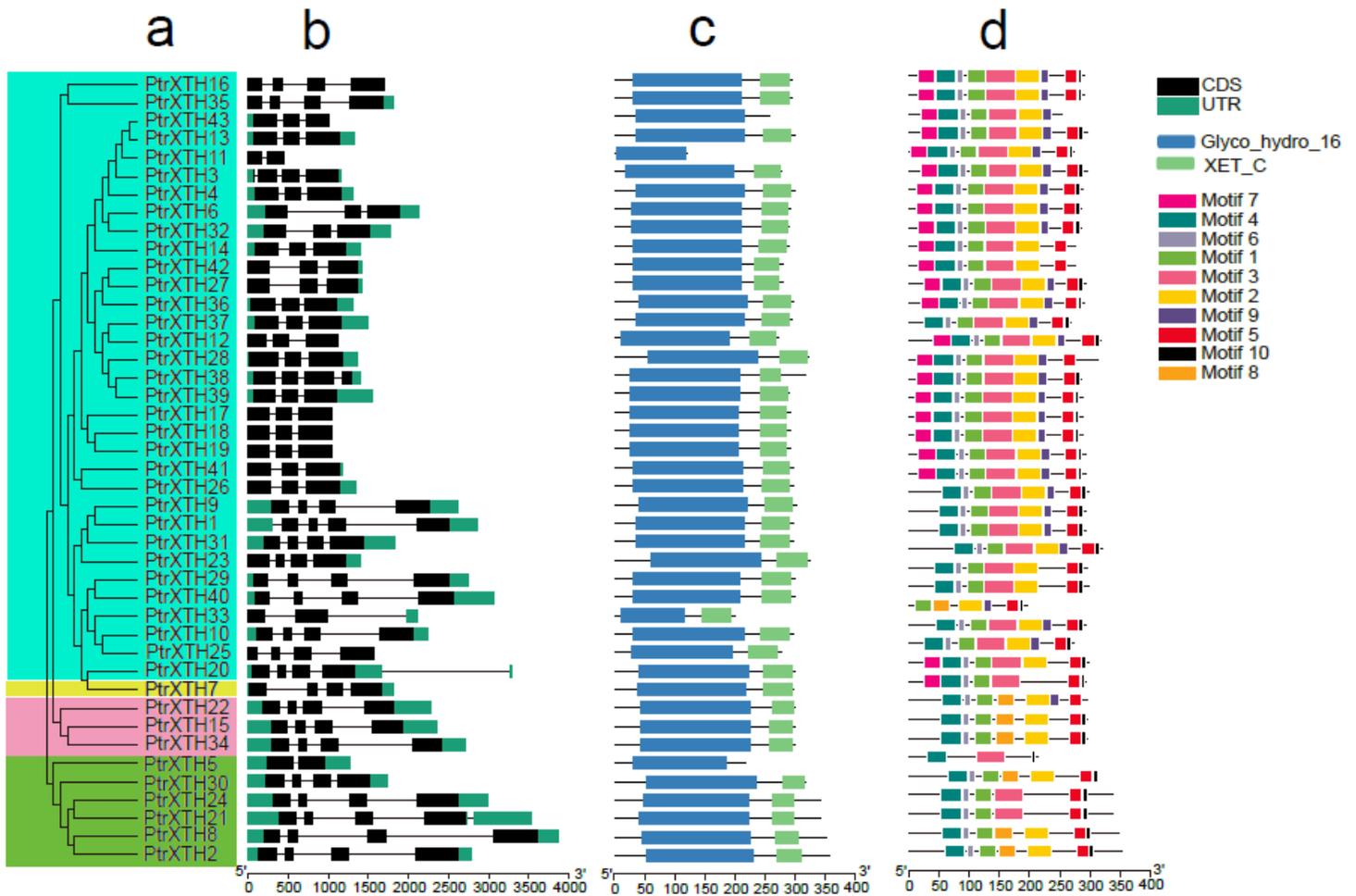


Figure 2

Characterizations of poplar XTH genes structure, conserved protein domain and motif. (a) Phylogenetic tree of poplar XTH genes family. (b) Gene structures. (c) Conserved protein domain. (d) Protein conserved motifs.

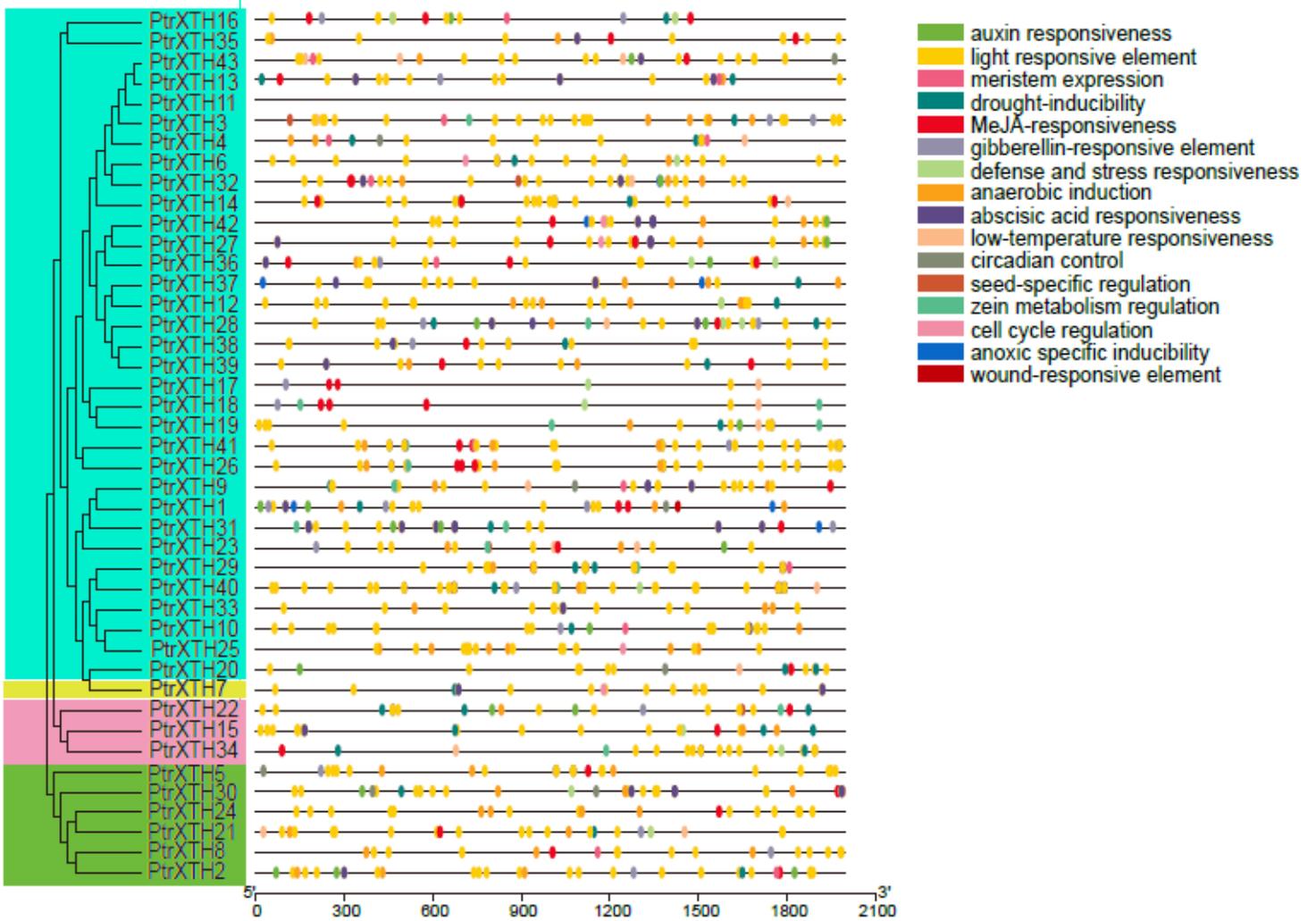
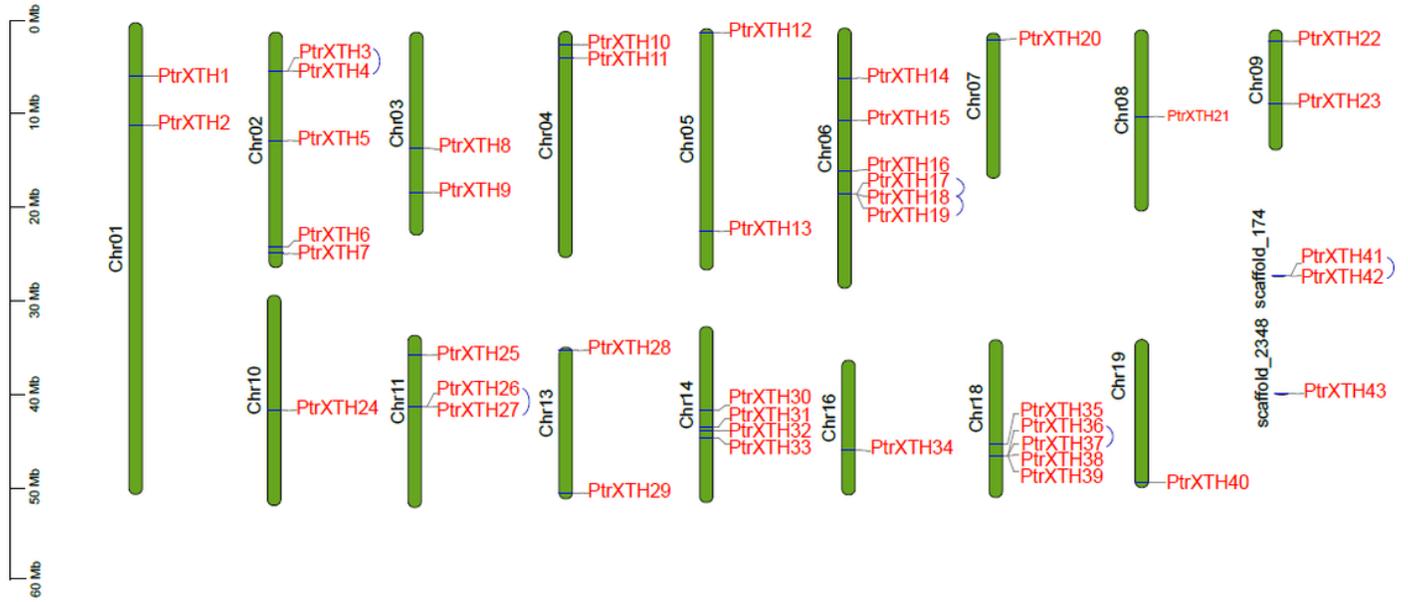
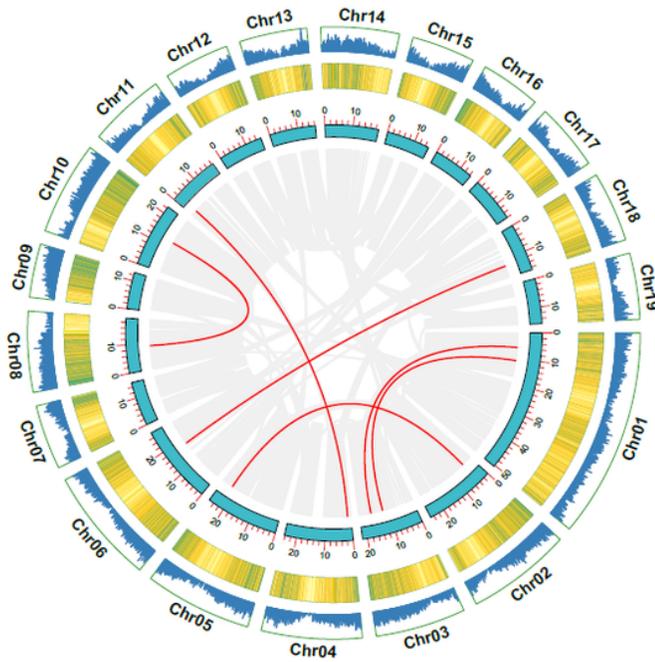


Figure 3

Cis-elements analysis of poplar XTH genes promoter. The left side represents the phylogenetic tree of the poplar XTH genes, and the rights of different colored ellipses represent different elements.

a**b****Figure 4**

Gene location and repetitive events of poplar XTH family. (a) Poplar XTH family genes location information on the chromosome, and the blue arcs represent different pairs of tandem repeat genes. (b) Fragment duplication analysis of poplar XTH genes, the line and heat map in the outer circle represent poplar genes density on the chromosome, the red line represents the fragment duplicate gene pair, and the gray line represents the syntenic blocks of genes in the poplar genome.

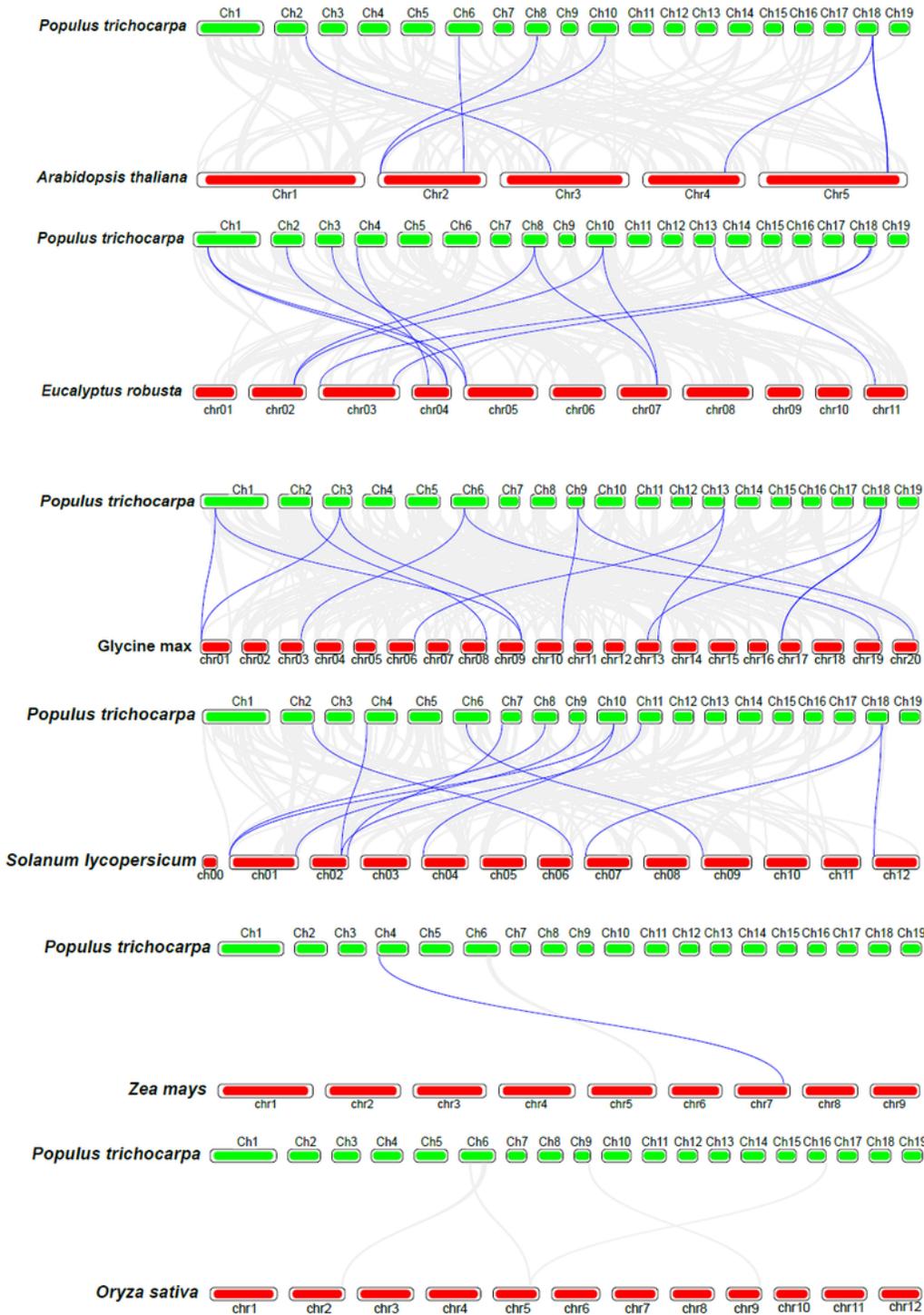


Figure 5

Collinearity analysis of XTH genes in poplar to other six species. The blue lines represent XTH syntenic gene pairs between poplar and other species, and the gray lines represent orthologous of poplar genomes to other six species.

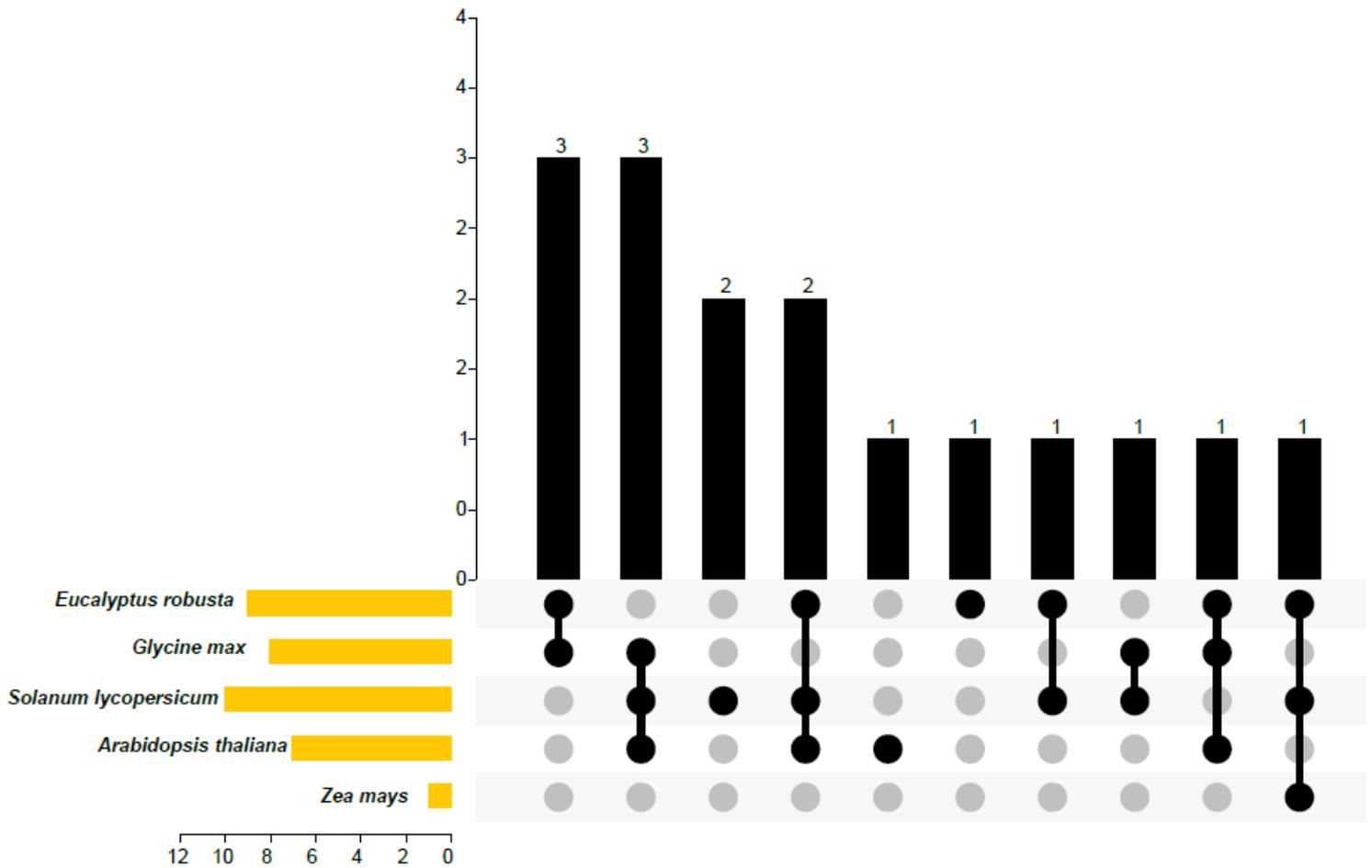


Figure 6

Upset plot diagram of poplar XTH genes throughout diverse species. The yellow color represents the number of genes that have collinearity between the poplar XTH gene and other species, the black circles connected by line segments represent genes that are shared by different species, and the black column represents the number of shared genes.

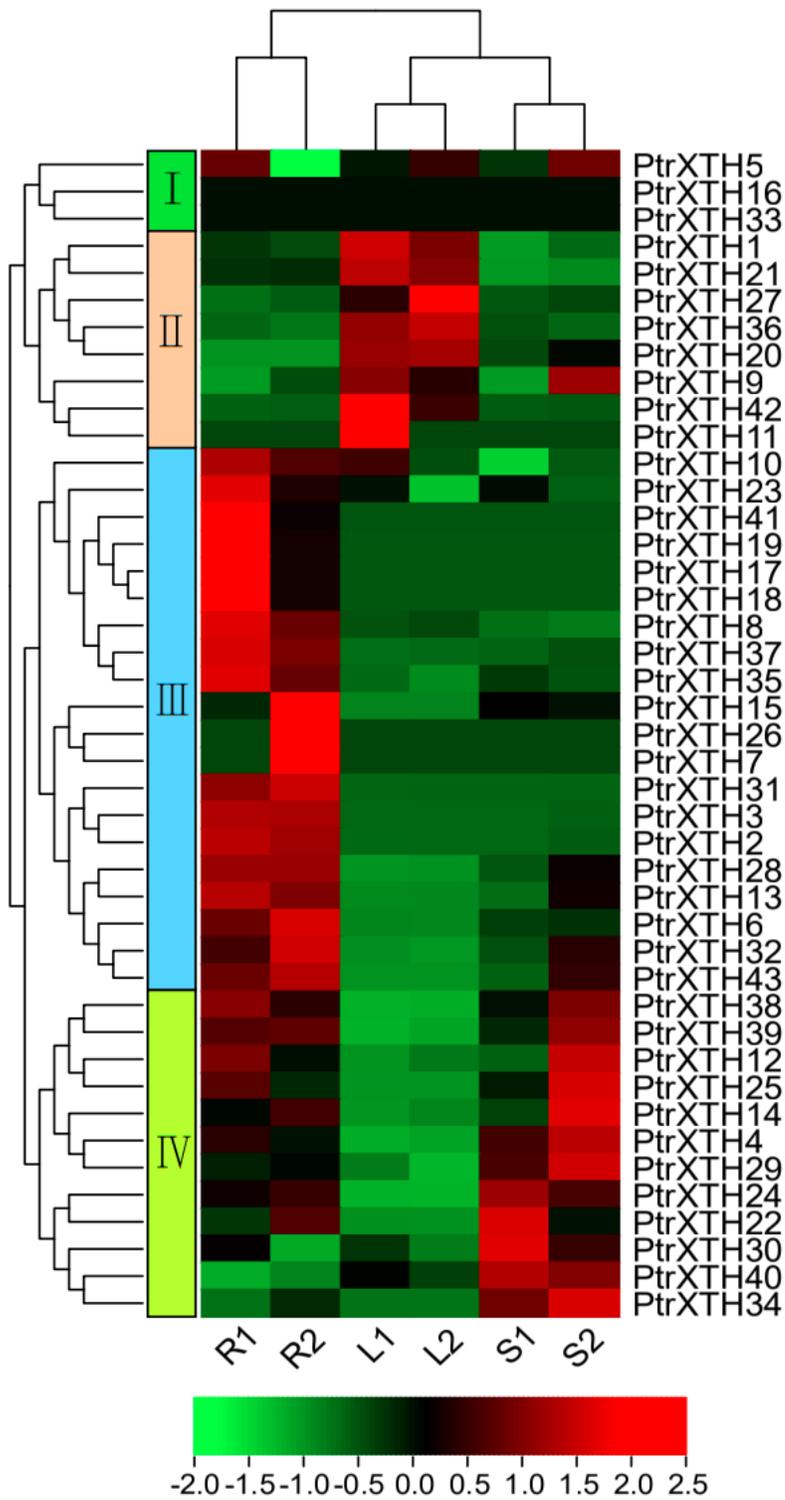


Figure 7

Expression pattern of poplar XTH genes in different tissues. Red represents high expression, green represents low expression. The left side represents different four gene clusters, R, L, and S represents roots, leaves and stems without salt stress, respectively.

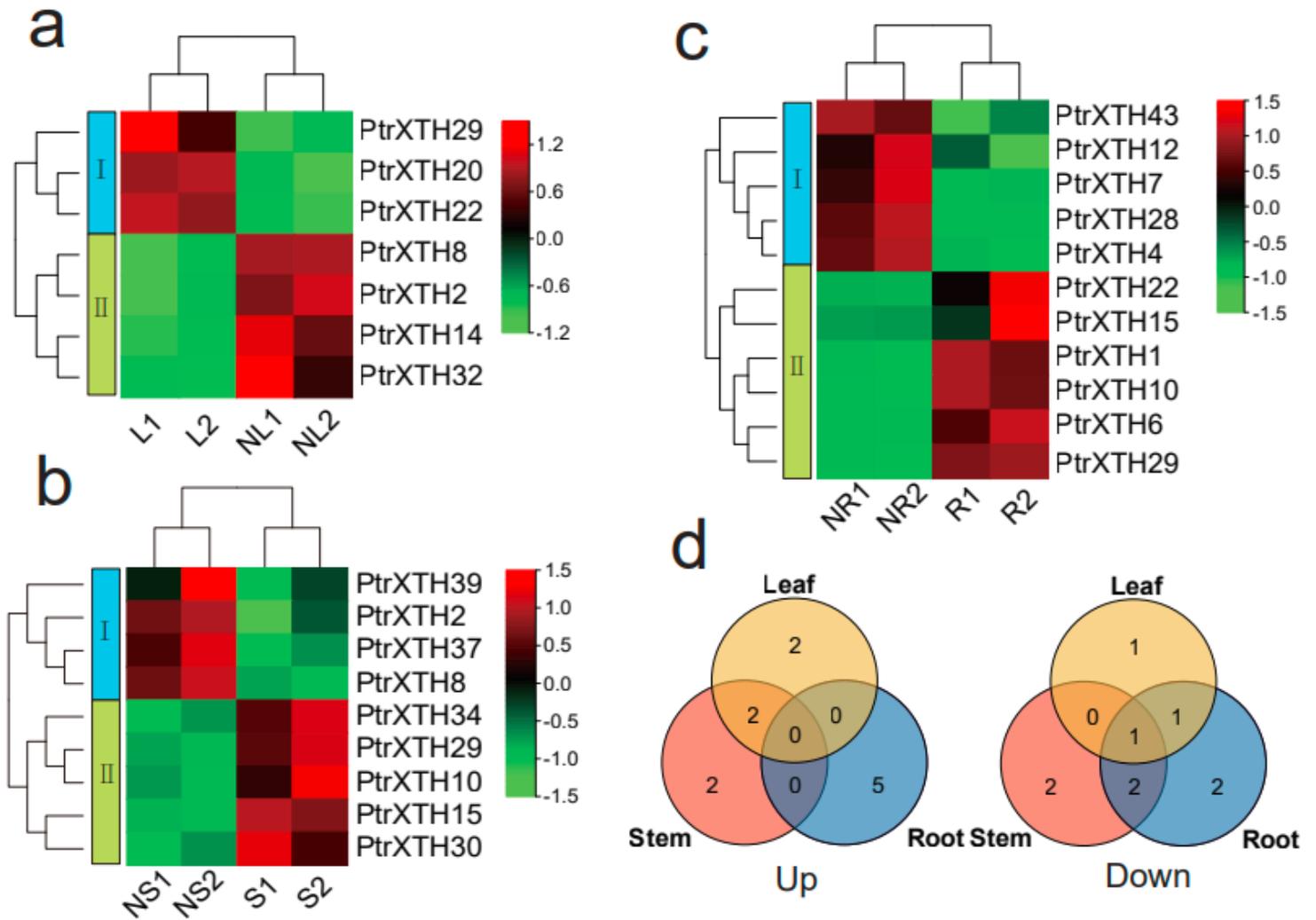


Figure 8

Expression pattern of PtrXTH genes in salt stress. (a-c) Heatmaps of DEGs in leaves, stems and roots. (d) Venn diagrams of DEGs in three tissues.

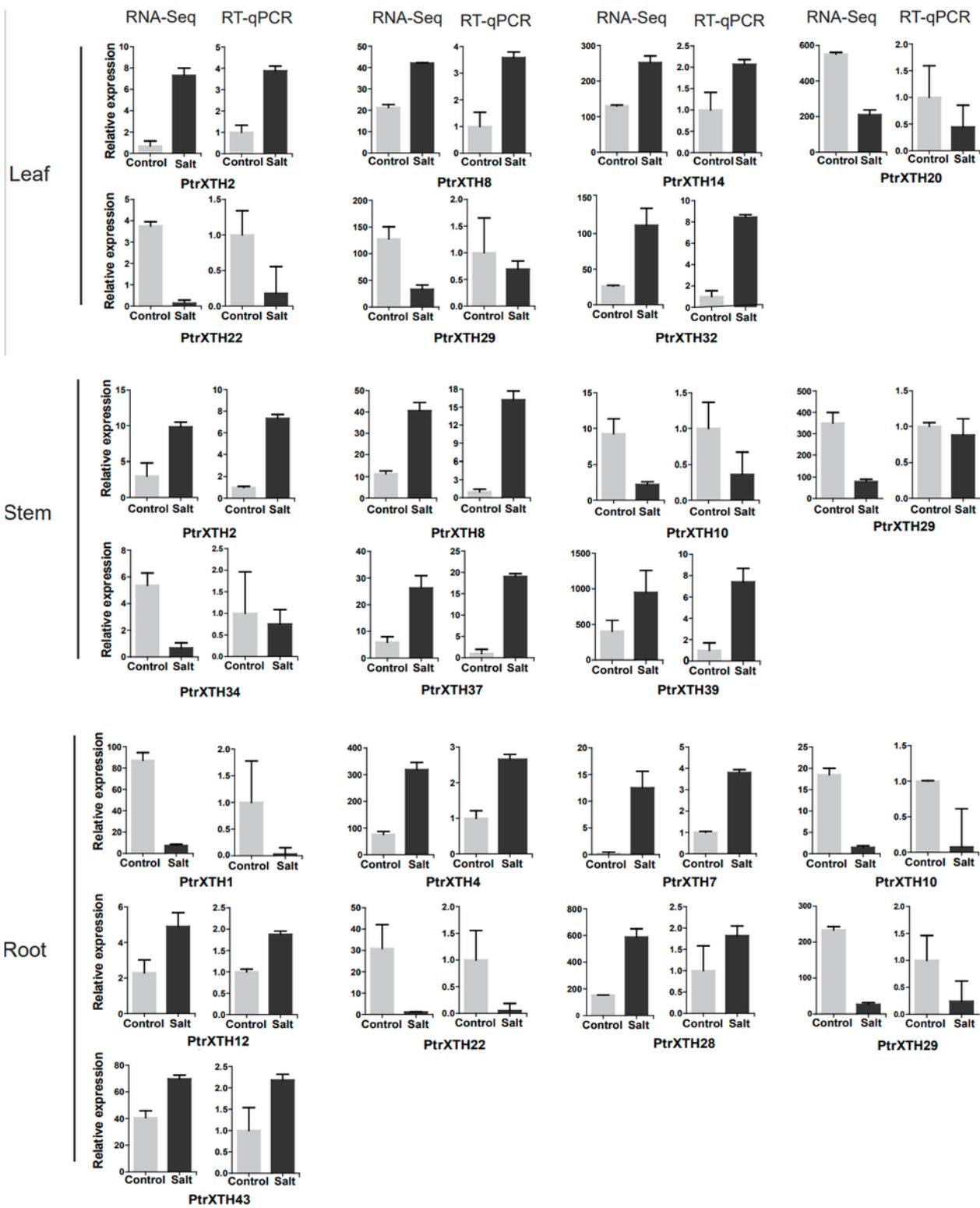


Figure 9

Gene expression levels in three tissues from both RNA-Seq and RT-qPCR platforms. Salt and control represent with and without salt stress.

Supplementary Files

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