The DUBs Family in Populus: Identification, Characterization, Evolution and Expression Patterns

Wenqing Zheng

Beijing Forestry University

Liang Du (✉ duliang@bjfu.edu.cn)

Beijing Forestry University <https://orcid.org/0000-0003-4903-524X>

Research article

Keywords: Populus, deubiquitinases, evolution, expression

Posted Date: December 4th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-120328/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at BMC Genomics on July 15th, 2021. See the published version at <https://doi.org/10.1186/s12864-021-07844-3>.

The DUBs family in *Populus*: Identification, Characterization, Evolution and Expression Patterns

Wenqing Zheng^{1,2}, Liang Du^{1,2*}

¹Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing Forestry University, Beijing 10083, China

²College of Biological Sciences and Technology, Beijing Forestry University, Beijing 100083, China

* Correspondence: duliang@bjfu.edu.cn

Abstract

Background: The deubiquitinases (DUB) family are a class of enzymes that regulate the stability or reverse the ubiquitination modification of many proteins in the cell, participating in cell cycle regulation, cell division and differentiation, various physiological activities such as DNA damage repair, growth and development, and response to stress. However, little is known about these genes in the woody plants.

Results: In the present study, 88 DUB genes were identified in woody model plant *Populus trichocarpa*, including 44 *PtrUBP*, 3 *PtrUCH*, 23 *PtrOTU*, 4 *PtrMJD*, and 14 *PtrJAMM* with similar domains. According to the phylogenetic analysis, the 44 *PtrUBP* genes were classified into 14 subfamily, three *PtrUCHs* were classified into two groups, 23 *PtrOTUs* had six groups, four *PtrMJDS* had two groups, and 14 *PtrJAMMs* had six groups. The structure and motif analysis indicated that the same subfamily had similar genome structure and motif distribution characteristics. Ks/Ka analysis showed that the segmental duplication events played a major role in the expansion of *Populus* DUB genes. Synteny analysis of *Populus* DUB genes and four other species provided deep perception into the evolutionary traits of DUB genes. Expression profiles derived from transcriptome data exhibited distinct expression patterns of DUB genes in various tissues. Based on the result of promoter *cis*-regulatory elements analysis, we selected 16 representative *PtrUBP* genes to test their response to different hormonal treatments. The results showed that most of *PtrUBPs* were upregulated in the ABA, SA, and MeJA treatments, implying that their potential roles in abiotic stress response in *Populus*.

Conclusion: The results in this study broaden our understanding of the DUB gene family in plants, and the analysis of the structure, conserved elements, and expression patterns of the DUBs provide a solid foundation for exploring their specific functions in *Populus* as well as indicate potential role of PtrUBP gene in abiotic stress.

Keywords: *Populus*, deubiquitinases, evolution, expression

Background

Ubiquitin-mediated post-translational modification of proteins is an important means of protein function regulation [1, 2]. The dynamic balance of ubiquitination and deubiquitination is key in fine regulation of protein level. When the ubiquitination, the ubiquitin molecule (Ub) binds to substrate protein through an isopeptide link between the glycine residue at its very C-terminus and lysine residues in the substrate protein in the form of monomer or polymer chains. There are three main enzymes that mediate this process: ubiquitin-activating enzyme E1 (E1), ubiquitin-conjugating enzyme E2 (E2) and ubiquitin-ligase E3 (E3)[3,4,5,6,7]. On the contrary, the deubiquitination is the removal of ubiquitin from the ubiquitinated substrate protein, which is mediated by the deubiquitination enzymes (DUBs)[8,9]. By removing the ubiquitin carried by the substrate protein, the DUBs modulate the activity or stability of the substrate protein and recycle the ubiquitin molecules into the ubiquitin-pool. [10]. It is obviously that the DUBs provide reversibility to the process of ubiquitination and allow modification, editing or nixing decisions to be made by the ubiquitination machinery[11].

Like other eukaryotes, plant deubiquitination enzymes can be divided into 5 subfamilies according to their catalytic domains[10 ,12]. Four of the subfamilies are cysteine proteases, including UBP/USP (the ubiquitin-specific proteases) family, UCH (the ubiquitin C-terminal hydrolases) family, OTU (the Ovarian Tumor proteases) family, and MJD (the Machado-Joseph domain) family. The rest one JAMM (the JAB1/MPN/MOV34 proteases) subfamily belongs to metalloproteinases, which are deubiquitinases whose activity depends on metal ion zinc[8]. Beyond the five subfamilies, two new putative DUB subfamilies containing monocyte chemotactic protein-induced protein (MCPIP) and the motif interacting with Ub-containing novel DUB family (MINDY) were identified [13,14,15].

So far, total 53 DUBs have been identified in model plant *Arabidopsis thaliana*. Among these

DUBs, there are 27 UBP (UBP1-UBP27) enzymes [16], 3 UCH (UCH1-UCH3) enzymes [17], 12 OTU (OTU1-OTU5, OTLD1, OTU7-OTU12) enzymes [18], 3 MJD (JOSL, At1gAt1g07300, and At3g54130) enzymes [19], and 8 JAMM (AMSH1-AMSH3, RPN11, CSN5A, CSN5B, BRCC36A, BRCC36B) enzymes[19,20,21,]. Previous studies demonstrated that the DUBs perform multiple functions in plants, such as morphogenesis[17,22,23] , programmed cell death [24], signal transduction[25,26,27] , and transcriptional regulation[8].

Poplar is a model plant in woody plant research due to its fast growth and easy genetic transformation. With the completion of *Populus trichocarpa* species genome sequencing, it provides convenience for the identification of genes and the study of gene function. In the present work, we identified 88 PtrDUB genes, including 44 PtrUBPs, 3 PtrUCHs, 23 PtrOTUs, 4 PtrMJDs, and 14 PtrJAMMs by taking *Arabidopsis* DUBs and related conservative domains for reference. The evolutionary tree analysis showed that PtrDUB were classified into different numbers of group, respectively. More detailed analysis were conducted including gene structure, motif component, chromosome distribution, syntenic analysis, expression profile, promoter *cis*-acting elements, and hormone treatments. The analysis of gene structure and conserved motif showed that the same subfamily had similar gene structure and motif distribution. Evolutionary patterns analysis indicated that plant DUBs had undergone a negative selection during the process of evolution. Promoter and hormone treatment analysis showed that PtrUBPs may participate in the stress or hormone response process. Above all, these studies would provide useful clues underlying the functional analysis of PtrDUB gene family in *Populus*.

Results

Identification of *Populus* DUB gene family

To conduct the genome-wide identification of DUB gene family in *Populus trichocarpa*, *Arabidopsis* UBP protein sequences were used as query sequences against the *Populus trichocarpa* databases. A total of 88 DUB members were obtained by comparison with the DUB amino acid sequences of *Arabidopsis thaliana*. And the conserved domain of these sequences were analyzed through the Pfam website. Finally, 88 DUB genes were identified in *Populus trichocarpa*, including 44 PtrUBP genes, 3 PtrUCH genes, 23 PtrOTU genes, 4 PtrMJD genes, and 14 PtrJAMM genes.

Details regarding the PtrDUBs, including gene identifier (Gene ID), the number of amino acid

of protein, the length of CDS region, theoretical isoelectric point (pI), molecular weight (MW), predicted subcellular location, and the chromosome mapping were listed in Table 1. Among the 88 DUB proteins, the PtrMJD3 protein was the smallest protein with 112 amino acids (aa), whereas the longest protein identified was the PtrUBP32 protein with 2100 amino acids. The pI of the DUB proteins ranged from 4.45 (PtrMJD4) to 9.77 (PtrMJD3), and the MW ranged from 12.77 (PtrMJD3) to 239.34 kDa (PtrUBP32). The predicted subcellular localization results showed that the majority of proteins were located in the nucleus, while some of the proteins were located in the chloroplast, cytosol, endoplasmic reticulum, mitochondria, or vacuole. Additionally, three poplar DUB proteins (PtrUBP13.2, PtrUBP16, and PtrUBP14.2) were predicted to target to two organelles. The chromosome mapping results showed that the PtrDUBs were distributed on almost every chromosome, except for chromosome 13 and 19.

Table 1. Detailed information about poplar DUB genes

		Gene ID	CDS length(bp)	Amid acid length(aa)	pI	MW(Da)	Predicted subcellular location	Chro
Gene name								moso me No.
UBP	PtrUBP1	Potri.017G055600	2994	997	5.22	109246.8	nucleus	17
UBP	PtrUBP2	Potri.001G315600	3024	1007	5.27	109986.6	nucleus	1
UBP	PtrUBP3	Potri.007G093600	1113	370	5.79	42106.52	nucleus	7
UBP	PtrUBP4	Potri.005G074900	1113	370	5.84	42066.55	nucleus	5
UBP	PtrUBP5.1	Potri.016G031900	2778	925	5.71	104347.9	nucleus	16
UBP	PtrUBP5.2	Potri.006G033800	2748	915	5.90	103662.9	nucleus	6
UBP	PtrUBP6	Potri.003G033700	1449	482	5.79	54210.48	nucleus	3
UBP	PtrUBP7	Potri.001G197400	1440	479	6.34	54282.93	nucleus	1
UBP	PtrUBP8.1	Potri.016G067400	1227	408	4.88	46047.34	nucleus	16
UBP	PtrUBP8.2	Potri.006G201100	2829	942	5.01	107345.3	cytosol	6
UBP	PtrUBP8.3	Potri.001G214800	2655	884	5.40	99506.22	nucleus	1
UBP	PtrUBP9	Potri.001G449000	2817	938	5.19	105047.4	nucleus	1
UBP	PtrUBP10	Potri.011G152500	2829	942	5.44	105347.0	nucleus	11
UBP	PtrUBP12.1	Potri.006G198300	3348	1115	5.58	131373.8	nucleus	6
UBP	PtrUBP12.2	Potri.016G064100	3348	1115	5.54	131067.3	cytosol	16
UBP	PtrUBP13.1	Potri.008G012600	3516	1171	5.72	136580.5	mitochondria	8
UBP	PtrUBP13.2	Potri.010G245100	3513	1116	5.68	130749.7	nucleus, cytoplasm	10
UBP	PtrUBP14.1	Potri.011G125800	2418	805	5.00	89702.12	mitochondria	11
UBP	PtrUBP14.2	Potri.001G408800	2424	807	4.93	89745.45	chloroplast, nucleus	1
UBP	PtrUBP15.1	Potri.001G378900	2814	937	8.32	104330.8	nucleus	1
UBP	PtrUBP15.2	Potri.011G095200	2496	831	7.61	92149.2	nucleus	11
UBP	PtrUBP16	Potri.002G104800	3426	1141	8.14	124114.5	chloroplast, nucleus	2
UBP	PtrUBP17	Potri.005G156900	3378	1125	6.51	121979.7	vacuole	5
UBP	PtrUBP18.1	Potri.018G009500	2181	726	5.33	79907.58	nucleus	18
UBP	PtrUBP18.2	Potri.018G011200	1470	489	6.75	54888.45	endoplasmic reticulum	18

UBP	PtrUBP19	Potri.006G270600	1902	633	5.31	70172.1	chloroplast	6
UBP	PtrUBP20	Potri.003G092400	2430	809	5.31	90719.56	nucleus	3
UBP	PtrUBP21	Potri.001G142000	2508	835	5.27	93581.6	nucleus	1
UBP	PtrUBP22.1	Potri.018G123200	1764	587	8.59	66096.89	nucleus	18
UBP	PtrUBP22.2	Potri.006G266100	1668	555	8.42	63801.75	nucleus	6
UBP	PtrUBP22.3	Potri.018G017000	1662	553	8.4	63397.46	nucleus	18
UBP	PtrUBP23.1	Potri.006G185200	2853	950	8.89	104806.4	nucleus	6
UBP	PtrUBP23.2	Potri.018G107500	2850	949	9.34	104936.0	nucleus	18
UBP	PtrUBP24	Potri.003G202800	1632	543	6.4	59434.23	cytosol	3
UBP	PtrUBP25.1	Potri.011G112800	1974	657	9.03	72492.98	chloroplast	11
UBP	PtrUBP25.2	Potri.001G394600	1926	641	8.88	70967.01	chloroplast	1
UBP	PtrUBP26.1	Potri.005G179500	3255	1084	6.11	121468.9	mitochondria	5
UBP	PtrUBP26.2	Potri.002G081600	3258	1085	6.22	121337.2	nucleus	2
UBP	PtrUBP27	Potri.007G077100	1620	539	5.96	60895.59	extracellular	7
UBP	PtrUBP28	Potri.006G010100	1674	557	5.83	64713.28	cytosol	6
UBP	PtrUBP29	Potri.016G014000	1638	545	5.58	63370.81	cytosol	16
UBP	PtrUBP30	Potri.015G066500	4722	1573	5.46	176909.0	nucleus	15
UBP	PtrUBP31	Potri.012G071900	4635	1544	5.52	174203.1	nucleus	12
UBP	PtrUBP32	Potri.017G089250	6303	2100	5.44	239341.4	nucleus	17
UCH	PtrUCH1	Potri.004G130400	1005	334	5.99	38389.2	cytosol	4
UCH	PtrUCH2	Potri.017G075200	1005	334	5.75	38348.07	cytosol	17
UCH	PtrUCH3	Potri.003G081000	699	232	4.53	25324.61	cytosol	3
OTU	PtrOTU1.1	Potri.003G162600	882	293	4.64	33028.88	nucleus	3
OTU	PtrOTU1.2	Potri.001G067400	858	285	4.74	32270.4	nucleus	1
OTU	PtrOTU2.1	Potri.011G139500	726	241	5.29	26983.53	cytosol	11
OTU	PtrOTU2.2	Potri.001G430200	444	147	4.67	16707.84	cytosol	1
OTU	PtrOTU2.3	Potri.001G435500	627	208	4.9	23513.36	cytosol	1
OTU	PtrOTU3	Potri.016G110400	681	226	8.97	25616.25	nucleus	16
OTU	PtrOTU4.1	Potri.016G050900	1029	342	8.88	37955.08	chloroplast	16
OTU	PtrOTU4.2	Potri.006G057400	1212	303	9.63	34235.05	chloroplast	6
OTU	PtrOTU4.3	Potri.008G177400	492	163	8.85	18732.46	cytosol	8
OTU	PtrOTU4.4	Potri.010G234300	957	318	5.76	35963.03	chloroplast	10
OTU	PtrOTU4.5	Potri.008G026100	981	326	6.05	36874.26	chloroplast	8
OTU	PtrOTU5	Potri.014G134100	981	326	5.59	36659.82	nucleus	14
OTU	PtrOTLD1.1	Potri.009G160100	1605	534	5.05	58738.69	chloroplast	9
OTU	PtrOTLD1.2	Potri.004G196800	1608	535	4.94	58529.95	nucleus	4
OTU	PtrOTU7	Potri.005G140500	1155	384	6.37	42740.81	nucleus	5
OTU	PtrOTU9.1	Potri.008G036900	555	184	5.37	21158.97	nucleus	8
OTU	PtrOTU9.2	Potri.008G036700	1086	361	4.76	41450.21	nucleus	8
OTU	PtrOTU9.3	Potri.010G225400	1083	360	4.76	41021.39	nucleus	10
OTU	PtrOTU10.1	Potri.016G094700	1017	338	4.75	38991.68	nucleus	16
OTU	PtrOTU10.2	Potri.006G125900	1062	353	4.71	40686.63	nucleus	6
OTU	PtrOTU11.1	Potri.016G019700	693	230	7.13	26414.60	nucleus	16
OTU	PtrOTU11.2	Potri.006G021700	681	226	7.13	25798.05	nucleus	6
OTU	PtrOTU12	Potri.014G140200	681	226	9.00	26085.80	nucleus	14
MJD	PtrMJD1	Potri.001G249400	549	182	7.02	20971.02	cytosol	1
MJD	PtrMJD2	Potri.006G095700	696	231	4.51	25897.92	nucleus	6
MJD	PtrMJD3	Potri.009G043400	339	112	9.77	12771.85	cytosol	9
MJD	PtrMJD4	Potri.016G110300	843	280	4.45	31210.47	nucleus	16

JAMM	PtrBRCC36A	Potri.001G172800	1305	434	5.82	47367.14	nucleus	1
JAMM	PtrBRCC36B	Potri.002G233500	1023	340	4.83	38881.34	chloroplast	2
JAMM	PtrBRCC36C	Potri.014G147100	1026	341	4.73	39215.61	chloroplast	14
JAMM	PtrBRCC36D	Potri.010G192000	864	287	4.88	31535.87	cytosol	10
JAMM	PtrBRCC36E	Potri.010G192200	933	310	5.85	34822.22	cytosol	10
JAMM	PtrBRCC36F	Potri.008G065300	933	310	5.96	34836.25	chloroplast	8
JAMM	PtrBRCC36G	Potri.008G065200	933	310	5.76	34990.33	cytosol	8
JAMM	PtrRPN11.1	Potri.002G127900	939	312	6.31	34822.05	cytosol	2
JAMM	PtrRPN11.2	Potri.014G032900	939	312	6.31	34821.07	cytosol	14
JAMM	PtrAMSH1	Potri.015G045800	1545	514	7.21	58188.23	nucleus	15
JAMM	PtrAMSH2	Potri.010G041200	609	202	4.60	22556.11	nucleus	10
JAMM	PtrAMSH3	Potri.010G141100	1512	503	6.00	56958.20	nucleus	10
JAMM	PtrCSN5A	Potri.018G006100	1101	366	5.17	40888.00	nucleus	18
JAMM	PtrCSN5B	Potri.006G275100	1101	366	5.04	40878.89	nucleus	6

The analysis of conserved domain, phylogenetic analysis, and classification of PtrDUB family

The analysis of conserved domain of these five family of DUB enzymes were conducted through the Pfam website. According to the results (Fig. 1), the 44 PtrUBP proteins exhibited conserved a typical UCH domain with cysteine (Cys) and histidine (His) boxes [41]. Also, the PtrUBP proteins contained other conserved non-UBP domains such as zf-UBP, DUSP, ubiquitin, MATH, etc. The PtrUBP proteins were grouped into 16 subfamily according to the AtUBP proteins[42]. The Peptidase_C12 domain was included in all three PtrUCH proteins, and the UCH_C domain only existed in PtrUCH1 and PtrUCH2 proteins. OTU domain was extensively existed in most of the PtrOTU proteins except PtrOTU1.1 and PtrOTU1.2 both of which harbored Peptidase_C65. For PtrMJD and PtrJAMM proteins, the typical features were the Josephin domain and JAB domain, respectively. Additionally, there were also some other domains appeared in the PtrJAMM proteins.

To analyze the phylogenetic relationship of the DUB proteins among different species including *Arabidopsis thaliana*, rice, and *Populus trichocarpa*, five Neighbor-joining phylogenetic trees were constructed based on the alignments of full-length DUB protein sequences from *Arabidopsis thaliana* (27 UBPs, 12 OTUs, 3 MJDs, 3 UCHs, and 8 JAMM), rice (44 UBPs, 22 OTUs, 2 MJDs, 5 UCHs, and 9 JAMM) [33], *Populus trichocarpa* (44 UBPs, 23 OTUs, 4 MJDs, 3 UCHs, and 14 JAMMs) (Fig. 2). The detailed information of Arabidopsis and rice DUB was listed in the supplementary table S1. According to the UBPs phylogenetic tree results, all sequences were classified into 16 groups. The number of different groups was not same. G11 and G14 each had only one, two in G1, G2, G4, G6, G8, G10, G12, G13, and G15, three in G9 and G16, four in G5, seven

in G7 and G3. The all OTUs sequences were divided into the 9 groups. There was one PtrOTU gene in G3, G5 and G7, two in G1 and G6, three in G2, five in G4, eight in G8, but zero in G9. The UCH family mainly was classified into three groups, UCH1, UCH2 and UCH3. PtrMJD proteins were grouped into three groups, G1, G2, and G3. PtrJAMM proteins were grouped into six groups. PtrRPN proteins and PtrCSN proteins were classified as G1 and G2 groups, respectively. PtrBRCC36 and PtrAMSH proteins were classified into G3, G4, G5, and G6 group, respectively.

Gene structure and motif distribution of PtrDUB gene family

To gain more insight into the evolution of the DUB family in poplar, the exon-intron gene structure organizations of all the identified PtrDUB genes were examined. It showed that different sub-families had different gene structures, but there was little difference among members of the same sub-family, especially PtrUBP family (Fig. 3). For example, in UBP sub-family, all G1 members had two exons and one intron, and all G2 contained six exons and five introns. In the G5 group of PtrUBP, the DUB genes possessed the most 32 exons. For 5' and 3'-UTR, most of the PtrDUB genes had both of them except the PtrUBP8.1, PtrUBP18.2, PtrOTU10.2, PtrMJD3, and PtrCSN5A gene all of which had only 5'-UTR region, PtrUBP18.1 and PtrUBP13.1 both of which had neither 5'-UTR nor 3'-UTR region, and PtrOTU2.2 which had only 3'-UTR region.

To analysis the DUB protein motif distribution, the schematic motif characteristic of all PtrDUB proteins was carried out from the MEME motif analysis (Fig. 4). It showed that a total of 34 motifs were identified, and detailed information about the length and sequence of motif were showed in the Supplemental table 2. PtrDUB members within the same subfamily were usually found to share a similar motif composition. For example, motif 11 is unique to OTU subfamily, whereas motif 25 is specific to UCH subfamily. In the MJD subfamily, motif 31 were all found.

Expression profiling of poplar DUB genes with RNA-seq

In order to study the expression patterns of all 88 PtrDUB genes, we downloaded and analyzed the transcriptome data of different vegetative tissues and stages of reproductive development derived from public database according to the previous published article [43]. Hierarchical clustering of the heatmap revealed clear differential expression of PtrDUB genes in different poplar tissues and development stages (Fig. 5). RNA-seq data of 88 PtrDUB genes were listed in Table S3. These tissues or stages were separated into three clusters: The DUB expression patterns in FM (female catkin prior to seed release), F (female catkin of post-fertilization), M (male catkin), ML (mature

leaf), and PC (phloem, cortex, and epidermis), the second cluster composed of G43h (germinated seedling sampled 43hr post imbibition), YFB (newly initiated female floral buds), ApB (actively growing shoot apex), AxB (axillary bud), REF (roots from field-grown trees), RTC (roots from plants in tissue culture), and YMB (newly initiated male floral buds), Phloem3 (developing phloem) and Xylem1 (developing xylem) formed another cluster. Based on the expression profiling in the 14 tissues performed, the PtrDUB genes were grouped into nine clusters (Fig. 5). All 3 genes in C1 were expressed highly in xylem1 and phloem3. Genes in C6 were expressed highly in the YFB and ApB. As shown in figure 5, most of these genes (except PtrOTU9.3, PtrUBP14.2, PtrUBP17, PtrAMSH2, PtrMJD4, PtrMJD2, PtrUBP30, PtrUBP23.2, and PtrUBP29) showed lower expression levels in FM, F, and M. And a large number of genes (except PtrUBP32, PtrUBP8.1, PtrOTU9.3, and PtrUBP18.1) showed higher expression profiling in xylem1 tissues.

Chromosomal distribution and syntenic analysis of PtrDUB genes

Figure 6 showed that the PtrDUB genes were unevenly distributed across the 19 poplar linkage groups (LG), except LG13 and LG19. LG1 contained the largest number of DUB genes (14). Some linkage groups (e.g. LG16 and LG6) had more genes, whereas others had few, such as LG4, 7, 9, and 15. LG12 had only one gene. From the analysis, it showed that there was no absolute connection between the LG length and the number of PtrDUB genes.

According to the description of Holub [44], a chromosomal region within 200 kb containing two or more genes is defined as a tandem duplication event, otherwise called segmental duplication. 44 segmental duplication events with 88 DUB genes were also identified (Table S4). These results indicated that PtrDUB genes were possibly generated by gene duplication events and the segmental duplication events played a major part for PtrDUB duplication events.

To further deduce the phylogenetic mechanisms of poplar DUB family, we constructed five comparative syntenic maps of *Populus trichocarpa* associated with five representative species, including two dicots (*Arabidopsis* and *Vitis vinifera*) and three monocots (*Oryza sativa*, *Zea mays*, and *Sorghum bicolor*) (Fig. 7). A number of PtrDUB genes showed syntenic relationship with those genes from *Arabidopsis*, rice, *Zea mays*, *Sorghum bicolor*, and *Vitis vinifera* (Table S4). The numbers of orthologous pairs between the other five species (*Arabidopsis*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor*, and *Vitis vinifera*) were 62, 27, 9, 24, and 55. Some PtrDUB genes were found to be associated with three or four syntenic gene pairs. For example, PtrUBP12.2 was found to be

associated with four gene pairs in *Populus* and *Arabidopsis*.

To better understand evolutionary and divergence patterns of the DUB genes, the Ka/Ks ratios of the DUB gene pairs were calculated. In principle, the value of Ka/Ks ratio less than 1, equal to 1 and greater than 1 represents negative selection, neutral selection and positive selection, respectively [45,46]. The results showed that all segmental duplicated PtrDUB gene pairs, and orthologous DUB gene pairs had $Ka/Ks < 1$, suggesting that the PtrDUB gene might have experienced strong purifying selective pressure during evolution.

Analysis of the putative promoter regions of the PtrDUB gene family

To examine the regulatory elements in promoter regions of PtrDUB gene, 2000bp of genomic sequences upstream of ATG were selected as putative promoter region and analyzed by using the Plant CARE website. The results showed that many *cis*-acting elements associated with stress or hormonal response were present in the promoters of the PtrDUB genes (Fig. 8). For example, promoters of PtrDUB genes contained abundant *cis*-acting elements, such as the SA-responsive elements (TCA-elements), MeJA responsive elements (CGTCA-motif), Box-S and WUN motifs involved in wounding stress, LTR participated in cold stress, ABRE and MBS motifs related to salt/drought/ABA response, W-box(WRKY binding site), etc. More detailed *cis*-acting elements including specific ones contained in each gene were listed in the Table S5. TCA-elements and CGTCA-motif were found in the promoters of 40 and 28 PtrDUB members, respectively. 5 and 43 promoters contained Box-S and WUN motifs related to wounding stress. The LTR (low-temperature-responsive) element was found in 27 promoters of PtrDUB genes. MBS and ABREs were rich in PtrDUB gene promoters. In addition, W-box, which is the WRKY binding site was existed in the promoters of 46 PtrDUBs.

qRT-PCR analysis of PtrDUB genes under some treatments

Among the DUB enzymes, UBP family had been reported to participate in the regulation of ABA [47] and MeJA [27,48,49]. To further confirm whether the expression of PtrUBP genes was influenced by different hormone treatments, the expression of 16 PtrUBP (PtrUBP1, PtrUBP3, PtrUBP6, PtrUBP9, PtrUBP12.1, PtrUBP14.1, PtrUBP15.1, PtrUBP21, PtrUBP22.1, PtrUBP23.1, PtrUBP24, PtrUBP25.1, PtrUBP26.1, PtrUBP27, PtrUBP28, and PtrUBP30) genes from 16 different groups were detected through ABA, SA, and MeJA treatment (Fig. 9). Overall, some PtrUBP genes were induced or suppressed by different treatments. And Each gene had a different

expression trend in different treatments. For example, PtrUBP1 gene was suppressed in the SA treatment but induced in the MeJA treatment. The expression level of most genes was peaked at 6h. In particular, the expression levels of some genes reach their peak in 1h, 3h, 12h, and 24h. For instance, the expression of PtrUBP3, PtrUBP6, PtrUBP14.1, and PtrUBP27 genes under SA treatment reached a peak in 1h.

Figure 1. The analysis of conserved domains from UB, UCH, OTU, MJD, and JAMM five subfamilies. The different colors represent different conservative domains. The PtrUBPs had conserved UCH and some other domains, such as zf-UBP, DUSP, ubiquitin, MATH, DUF629, etc.

The PtrUCHs all had Peptidase_C12 domain. The PtrOTUs had conserved Peptidase_C65 or OTU domain. The PtrMJDs and PtrJAMMs had conserved Josephin and JAB domains.

Figure 2. The phylogenetic trees of DUBs proteins from species of *Arabidopsis thaliana*, rice and *Populus trichocarpa*. The phylogenetic trees were conducted using the Neighbor-Joining (N-J) method through MEGA5.0. A, UBP family; B, OTU family; C, MJD family; D, UCH family; E, JAMM family. The different colors represent different groups.

Figure 3. The exon-intron distribution of PtrDUB genes. The PtrDUBs exon-intron distributions were sorted according to UBP, UCH, OTU, MJD, and JAMM subfamilies. CDS, intron, and upstream/downstream were exhibited in yellow rectangular box, black line, and blue rectangular box, respectively.

Figure 4. The motif distribution of PtrDUB proteins. The same subfamily has similar motif distribution characteristics. Different motifs are displayed in boxes with different colors. The length of the black line represents the size of the protein. The PtrUBP family has motif 1-10. The other families have motif 11-34.

Figure 5. Expression profiles of DUB family in poplar across different vegetative tissues and stages of reproductive development. FM, female catkin prior to seed release; F, female catkin of post-fertilization; M, male catkin; ML, mature leaf; PC, phloem, cortex, and epidermis; G43h, germinated seedling sampled 43hr post imbibition; YFB, newly initiated female floral buds; ApB, actively growing shoot apex; AxB, axillary bud; REF, roots from field-grown trees; RTC, roots from plants in tissue culture; YMB, newly initiated male floral buds; Phloem3, developing phloem; Xylem1, developing xylem.

Figure 6. Diagrammatic sketch for the chromosomal distribution and synteny relationships of poplar DUB genes. Gray lines indicate all synteny blocks in the poplar genome, the red lines indicate PtrDUB gene pairs. The yellow numbers represent the number of chromosomes.

Figure 7. Synteny analysis of DUB genes between poplar and five representative plant species. Gray lines indicate the collinear blocks within pineapple and other five plant species, while the red lines highlight the syntenic DUB gene pairs. The other five species from top to bottom are *Arabidopsis*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor*, and *Vitis vinifera*, respectively.

Figure 8. The gene number containing various *cis*-elements in the promoter region. TCA-element, SA-responsive element; CGTCA-motif, MeJA-responsive element; Box-S and WUN-motif, wounding responsive element; LTR, low-temperature-responsive element; ABRE, ABA-responsive element; MBS, MYB binding site; W-box, WRKY binding site.

Figure 9. Expression profiles of 16 selected PtrUBP genes from different 16 groups in response to treatments of various hormones. The hormones used here were ABA, SA, and MeJA. Ptr18S gene was selected as a reference gene.

Discussion

Deubiquitinases are important proteolytic enzymes in ubiquitin system, involving in the plant growth and development[50,51,52], immune stress response[26], and signal transduction pathways[53]. According to previous research, 53 deubiquitinases were identified from *Arabidopsis thaliana* [19]. However, the deubiquitinases of *Populus trichocarpa* have not been described until now. Here, 88 poplar deubiquitinases were identified, including 44 UBPs, 3 UCHs, 23 OTUs, 4 MJDs, and 14 JAMMs. The number of UCH subfamily members is the same as that of *Arabidopsis thaliana*, however the other four subfamilies have far more genes than those in *Arabidopsis* plant. For the genome size of the *Arabidopsis* is much smaller than that of poplar tree [54,55], the same

number of UCH in the two species indicates that the UCH subfamily might be more conservative than other four DUB subfamilies.

The PtrUBPs contain more members demonstrating family function diversification

According to the phylogenetic analysis, PtrUBPs were classified into 16 groups, with two more groups than AtUBPs. PtrUBP28 and PtrUBP29 belong to the new group 15 (G15), and PtrUBP30, PtrUBP31, and PtrUBP32 belong to another new group 16 (G16). Each of these 16 groups had similar protein domains. Compared with *Arabidopsis*, PtrUBPs in G16 contain two special conserved domains DUF627 and DUF629, indicating the complexity of PtrUBP family.

The Expression pattern of *Populus* DUBs indicates their potential roles in plant development

The analysis of DUB gene expression patterns from different tissues and development stages was conducive to the understanding of the DUB gene function in the growth and development of *Populus*. We profiled the DUB gene expression according to the published transcriptome data. The result showed that a great amount of DUB genes were highly expressed in the xylem, indicating that they maybe involve in the development of xylem. Previous study showed that UBP family was involved in the plant development, including the embryo development [16,22], seed development [48,51,56], seed size [23], leaf cell size [57], pollen development or transmission [58]. However, the role about xylem or cambium of UBP family was little reported, inferring that the functions of genes in the same family are different in different species.

***Populus* DUBs play an important role in hormone-related signaling response**

Plants have evolved various mechanisms to resist different stresses in response to diverse environmental conditions[59]. The DUB family has been reported to be involved in hormone and stress response, especially the UBP family [16,47,60]. Plant requires a variety of signaling molecules in response to stress such as abscisic acid (ABA), salicylic acid (SA), Methyl jasmonate (MeJA), and ethylene (ET), etc. Additionally, elements of gene promoter play important roles in the growth and development process of plants, especially in the determination of the tissue-specific expression or stress-responsive regulation of genes [61,62]. Based on previous research, we conducted an analysis of *cis*-acting elements. PtrDUB genes contained abundant *cis*-acting elements, including TCA-elements (SA-responsive elements), CGTCA-motif (MeJA responsive elements), Box-S, WUN motif (wounding stress), LTR (cold stress), ABRE, MBS motif (salt/drought/ABA response), and W-box(WRKY binding site)[61,62,63,64,65,66]. Indeed, the plant UBPs show

potential in regulation of stress responses [47]. In view of the important role of UBP family in abiotic stress resistance, the expression level of PtrUBP was conducted based on the qRT-PCR analysis in response to ABA, SA, and MeJA treatments. PtrUBP1 and PtrUBP22.1 were significantly induced in response to MeJA treatment, implying that they may play an important role in jasmonic acid signaling pathway. The AtUBP1(homologous gene of PtrUBP1) was reported to involve in the canavanine (CAN) resistance, indicating that PtrUBP1 maybe play different function in the *Populus* [42]. As for further functional exploration, more experimental verification may be needed.

Conclusion

The DUB family has been reported to involve in various growth and development process. In this study, a total of 88 PtrDUBs containing 44 *PtrUBP*, 3 *PtrUCH*, 23 *PtrOTU*, 4 *PtrMJD*, and 14 *PtrJAMM* were identified. The analysis of conserved domain, exon-intron structure and motif distribution showed that the same subfamily had similar characteristics. Synteny analysis of DUB genes from different plant species provided valuable clues about the evolutionary characteristics of poplar DUB genes. The expression pattern of PtrDUB from different poplar tissues and development stages indicated that PtrDUB genes played important roles in poplar growth and development in different tissues and development stages. The PtrUBPs gene expression analysis response to different hormone treatments offers a new perspective on the stress or hormone response of PtrUBP. These results provide a valuable resource for better exploring the biological function of DUB genes in poplar.

Materials

Identification of *Populus* DUB family

The sequences of DUB family members of *Arabidopsis thaliana* were downloaded from the TAIR website (<https://www.arabidopsis.org/>) according to the accession number (Table S1). In order to identify the *Populus* DUB family members, we used the amino acid sequence of *Arabidopsis* DUB family as the query sequences with the Blast algorithm in the *Populus* genome database (<https://genome.jgi.doe.gov/portal/Poptr1/Poptr1.home.html>). Then the family members were further confirmed by the analysis of conserved domains using the EMBL-EBI Pfam database (<http://pfam.xfam.org/>). The number of amino acids, length of the coding sequence (CDS), the theoretical isoelectric point (pI), and molecular weight of amino acids were obtained using the

ExPASy-ProtParam online tool (<https://web.expasy.org/protparam/>)[28]. The subcellular location of *Populus* DUB proteins was predicted through the WoLF PSORT: Advanced Protein Subcellular Localization Prediction Tool (<https://www.genscript.com/wolf-psort.html?src=leftbar>) [29].

The determination of conserved domain and phylogenetic analysis

The conserved domains of all identified PtrDUB genes were determined through the Pfam32.0 batch sequence search tool of EMBL-EBI (<http://pfam.xfam.org/search#tabview=tab1>) and confirmed by search in Conserved Domain Database (CDD) in NCBI (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>)[30]. The protein sequences of AtDUBs were obtained through the TAIR database based on gene ID [19,31,32]. The protein sequences of OsDUBs were downloaded from the Rice Genome Annotation Project (<http://rice.plantbiology.msu.edu/>)[33]. The protein sequences of PtrDUBs were collected from the Phytozome website (<https://phytozome.jgi.doe.gov>). And the protein sequences firstly aligned through Muscle. Then phylogenetic trees were constructed by using Neighbor-Joining (NJ) method of 1000 bootstrap replications.

Gene structure and motif analysis

Firstly, the CDS and genomic sequences of PtrDUB genes were downloaded from the website (<https://phytozome.jgi.doe.gov>). The gene structure of PtrDUB was determined using these sequences in the online program Gene Structure Display Server (GSDS) (<http://gsds.cbi.pku.edu.cn>)[34]. The conserved motifs in PtrDUB proteins were identified by motif-based sequences analysis tool MEME Suite (<http://meme-suite.org>) setting the maximum number of motifs as 34[35].

Chromosomal distribution and syntenic analysis

The PtrDUB genes were mapped to the corresponding position of *Populus* chromosomes based on the genome database according to the Circos [36]. Gene duplication events were analyzed using Multiple Collinearity Scan toolkit (MCScanX)[37]. The syntenic analysis maps were drawn using the Dual Synteny Plotter software (<https://github.com/CJ-Chen/TBtools>)[38]. The value of synonymous substitution rate (Ks) and nonsynonymous substitution rate (Ka) were computed using KaKs_Calculator 2.0 [39].

Putative promoter region analysis

The 2000bp upstream sequences of the genomic sequences of PtrDUB genes were taken as putative

promoter regions, and the inclusive *cis*-regulatory elements were identified and analyzed through the Plant CARE website (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) [40].

Plant materials treatment, RNA extraction and gene expression analysis

84K poplar (*Populus alba* × *Populus glandulosa*), an aspen hybrid poplar from Korea, was used in the study. Poplar trees were grown in a greenhouse at a temperature of 25 °C for 16 / 8h (light / dark). Poplar seedlings grown in greenhouses for about three months were treated with hormones (ABA, SA, and MeJA). The seedlings were sprayed with MS liquid medium supplied with 100 μM ABA, SA, and MeJA for 1, 3, 6, 12, and 24 h, respectively. Seedlings only sprayed with MS medium solution were treated as control for 0 h. All treated tissue samples were collected and immediately frozen in liquid nitrogen and stored at –80 °C for subsequent analysis.

Total RNA of treated samples was extracted using the RNAPrep Pure Plant Kit (TIANGEN - DP419, Beijing, China). Integrity and concentration of RNA were detected through agarose gel electrophoresis and Nanodrop micro-spectrophotometer respectively. The cDNA was produced by using the TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix kit (TransGen - AT311, Beijing, China) according to the operating instruction. The quantitative RT-PCR was carried out with the Bio-Rad Real-time instrument using TransStart® Top Green qPCR SuperMix (TransGen-AQ132, Beijing, China). The 18S rRNA gene was used as an internal control. The primers was designed with the primer-blast tool of NCBI (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>) according to the CDS sequences. The reaction was conducted as follows: denaturation for 30 s at 94 °C, 40 cycles of 94 °C/5s for denaturation, 60 °C/30 s for annealing, and 72 °C/30 s for extension. The data from qPCR amplification was analyzed using $2^{-\Delta\Delta CT}$ method. The gene expression heatmaps of various tissues were created by the TBtools software [38].

Supplementary Information

Supplementary information accompanies this paper at...

Additional file 1: Table S1. The detailed information of Arabidopsis and rice DUB family.

Additional file 2: Table S2. The detailed information of MEME motif sequences about DUB proteins in *Populus trichocarpa*.

Additional file 3: Table S3. RNA-seq data of 88 PtrDUB genes that were used in this study.

Additional file 4: Table S4. The value of Ka, Ks, and Ka/Ks of syntenic gene pairs from different

species.

Additional file 5: Table S5. The detailed information of cis-acting elements of 88 PtrDUB genes.

Acknowledgements

We are grateful to all the colleagues in our laboratory.

Authors' contribution

L.D. designed the research program. W. Z. analyzed the data and performed the experiment. W.Z. and L.D. wrote the manuscript.

Funding

This project is based on research that was supported by National Natural Science Foundation of China (No. 32070548).

Availability of data and materials

All data supporting the conclusions of this article are included in the article and its additional files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing Forestry University, Beijing 10083, China

²College of Biological Sciences and Technology, Beijing Forestry University, Beijing 100083, China.

References

1. Vierstra RD. The ubiquitin-26S proteasome system at the nexus of plant biology. *Nat Rev Mol Cell Biol.* 2009;385(6):397-10.
2. Vierstra RD. The expanding universe of ubiquitin and ubiquitin-like modifiers. *Plant Physiol.* 2012;2(1):14-160.
3. Herskho A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem.* 1998;425:479-67.

4. Kerscher O, Felberbaum R, Hochstrasser M. Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annu Rev Cell Dev Biol.* 2006;159:180-22.
5. Pickart CM. Mechanisms underlying ubiquitination. *Annu Rev Biochem.* 2001;503:533-70.
6. Neutznier M, Neutznier A. Enzymes of ubiquitination and deubiquitination. *Essays Biochem.* 2012;37:50-52.
7. Ye Y, Rape M. Building ubiquitin chains: E2 enzymes at work. *Nat Rev Mol Cell Biol.* 2009;755(11):764-10.
8. Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, Sixma TK. A genomic and functional inventory of deubiquitinating enzymes. *Cell.* 2005;773(5):786-123.
9. Wilkinson KD. Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. *FASEB J.* 1997;1245(14):1256-11.
10. Komander D, Clague MJ, Urbe S. Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol.* 2009;550(8):563-10.
11. Sowa ME, Bennett EJ, Gygi SP, Harper JW. Defining the human deubiquitinating enzyme interaction landscape. *Cell.* 2009;389(2):403-138.
12. Reyes-Turcu, FE, Ventii, KH, Wilkinson KD. Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annu Rev Biochem.* 2009;363:397-78.
13. Liang J, Saad Y, Lei T, Wang J, Qi D, Yang Q, Kolattukudy P, Fu M. MCP-induced protein 1 deubiquitinates TRAF proteins and negatively regulates JNK and NF-kappaB signaling. *J Exp Med.* 2010;2959(13):2973-207.
14. Fraile JM, Quesada V, Rodriguez D, Freije JM, Lopez-Otin C. Deubiquitinases in cancer: new functions and therapeutic options. *Oncogene.* 2012;2373(19):2388-31.
15. Nishi R, Wijnhoven P, le Sage C, Tjeertes J, Galanty Y, Forment JV, Clague MJ, Urbe S, Jackson SP. Systematic characterization of deubiquitylating enzymes for roles in maintaining genome integrity. *Nat Cell Biol.* 2014;1016(10):1026-16.
16. Liu Y, Wang F, Zhang H, He H, Ma L, Deng XW. Functional characterization of the Arabidopsis ubiquitin-specific protease gene family reveals specific role and redundancy of individual members in development. *Plant J.* 2008;844(5):856-55.
17. Yang P, Smalle J, Lee S, Yan N, Emborg TJ, Vierstra RD. Ubiquitin C-terminal hydrolases 1 and 2 affect shoot architecture in *Arabidopsis*. *Plant J.* 2007;441(3):457-51.
18. Radjacommaré R, Usharani R, Kuo CH, Fu H. Distinct phylogenetic relationships and biochemical properties of Arabidopsis ovarian tumor-related deubiquitinases support their functional differentiation. *Front Plant Sci.* 2014;84-5.
19. Isono E, Nagel MK. Deubiquitylating enzymes and their emerging role in plant biology. *Front Plant Sci.* 2014;56-5.
20. Block-Schmidt AS, Dukowicz-Schulze S, Wanieck K, Reidt W, Puchta H. BRCC36A is epistatic to BRCA1 in DNA crosslink repair and homologous recombination in *Arabidopsis thaliana*. *Nucleic Acids Res.* 2011;146(1):154-39.
21. Isono E, Katsiarimpa A, Müller IK, Anzenberger F, Stierhof YD, Geldner N, Chory J, Schwechheimer C. The deubiquitinating enzyme AMSH3 is required for intracellular trafficking and vacuole biogenesis in *Arabidopsis thaliana*. *Plant Cell.* 2010;1826(6):1837-22.
22. Doelling JH, Yan N, Kurepa J, Walker J, Vierstra RD. The ubiquitin-specific protease UBP14 is essential for early embryo development in *Arabidopsis thaliana*. *Plant J.* 2001;393(5):405-27.

23. Du L, Li N, Chen L, Xu Y, Li Y, Zhang Y, Li C, Li Y. The ubiquitin receptor DA1 regulates seed and organ size by modulating the stability of the ubiquitin-specific protease UBP15/SOD2 in *Arabidopsis*. *Plant Cell*. 2014;665(2):665-26.
24. Ramakrishna S, Suresh B, Baek KH. The role of deubiquitinating enzymes in apoptosis. *Cell Mol Life Sci*. 2011;15(1):26-68.
25. Moon BC, Choi MS, Kang YH, Kim MC, Cheong MS, Park CY, Yoo JH, Koo SC, Lee SM, Lim CO, Cho MJ, Chung WS. Arabidopsis ubiquitin-specific protease 6 (AtUBP6) interacts with calmodulin. *FEBS Lett*. 2005;3885(18):3890-579.
26. Ewan R, Pangestuti R, Thornber S, Craig A, Carr C, O'Donnell L. Deubiquitinating enzymes AtUBP12 and AtUBP13 and their tobacco homologue NtUBP12 are negative regulators of plant immunity. *New Phytol*. 2011;92(1):106-191.
27. Cui X, Lu F, Li Y, Xue Y, Kang Y, Zhang S, Qiu Q, Cui X, Zheng S, Liu B, Xu X, Cao X. Ubiquitin-specific proteases UBP12 and UBP13 act in circadian clock and photoperiodic flowering regulation in *Arabidopsis*. *Plant Physiol*. 2013;897(2):906-162.
28. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. ExPASy: The proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res*. 2003;3784(13):3788-31.
29. Horton P, Park K, Obayashi T. WoLF PSORT: protein localization predictor. *Nucleic Acids Res*. 2007;suppl_2.
30. El-Gebali S, Mistry J, Bateman A, Eddy SR, Luciani A, Potter SC, Qureshi M, Richardson LJ, Salazar GA, Smart A, Sonnhammer ELL, Hirsh L, Paladin D, Tosatto-Piovesan SCE, Finn RD. The Pfam protein families database in 2019. *Nucleic Acids Res*. 2019;427(1):432-8.
31. March E, Farrona S. Plant Deubiquitinases and Their Role in the Control of Gene Expression Through Modification of Histones. *Front Plant Sci*. 2018;2274-17.
32. Parinita M, Utpal N. De-ubiquitinases on the move: an emerging field in plant biology. *Plant Biol*. 2020;563(4):572-22.
33. Wang DH, Song W, Wei SW, Zheng YF, Chen ZS, Han JD, Zhang HT, Luo JC, Qin YM, Xu ZH, Bai SN. Characterization of the Ubiquitin C-Terminal Hydrolase and Ubiquitin-Specific Protease Families in Rice (*Oryza sativa*). *Front Plant Sci*. 2018;9.
34. Hu B, Jin J, Guo A, Zhang H, Luo J, Gao G. GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics*. 2015;1296(8):1297-31.
35. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS. MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res*. 2009;202:8-37.
36. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. Circos: an information aesthetic for comparative genomics. *Genome Res*. 2009;1639(9):45-19.
37. Wang Y, Tang H, DeBarry JD, Tan X, Li J, Wang X, Lee T, Jin H, Marler B, Guo H. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res*. 2012;e49(7):e49-40.
38. Chen C, Chen H, Zhang Y, Thomas HR, Xia R. Tbtools an integrative toolkit developed for interactive analyses of big biological data. *Mol Plant*. 2020;1194(8):1202-13.
39. Wang D, Zhang Y, Zhang Z, Zhu J, Yu J. KaKs_Calculator 2.0: a toolkit incorporating gamma-series methods and sliding window strategies. *Genom Proteom Bioinformatics*. 2010;77(1):80-8.
40. Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van D, Rouze P, Rombauts S. PlantCARE,

- a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* 2002;325(1):327-30.
41. Amerik AY, Hochstrasser M. Mechanism and function of deubiquitinating enzymes. *Biochim Biophys Acta.* 2004;189(1-3):207-1695.
 42. Yan N, Doelling JH, Falbel TG, Durski AM, Vierstra RD. The ubiquitin-specific protease family from *Arabidopsis*. AtUBP1 and 2 are required for the resistance to the amino acid analog canavanine. *Plant Physiol.* 2000;1828(4):1843-124.
 43. Eli R, Shrinivasrao PM, Palitha D, Gancho TS, Oswald RC, Steven HS, Amy MB, Stephen PD. Contrasting patterns of evolution following whole genome versus tandem duplication events in *Populus*. *Genome Res.* 2012;95(1):105-22.
 44. Holub EB. The arms race is ancient history in *Arabidopsis*, the wildflower. *Nat Rev Genet.* 2001;516(7):527-2.
 45. Cannon SB, Mitra A, Baumgarten A, Young ND, May G. The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. *BMC Plant Biol.* 2004;1-4.
 46. Shiu SH, Karlowski WM, Pan R, Tzeng YH, Mayer KF, Li WH. Comparative analysis of the receptor-like kinase family in *Arabidopsis* and rice. *Plant Cell.* 2004;1220(5):34-16.
 47. Zhao J, Zhou H, Zhang M, Gao Y, Li L, Gao Y, Li M, Yang Y, Guo Y, Li X. Ubiquitin-specific protease 24 negatively regulates abscisic acid signaling in *Arabidopsis thaliana*. *Plant Cell Environ.* 2016;427(2):440-39.
 48. Derkacheva M, Liu S, Figueiredo D, Gentry M, Mozgova I, Nanni P, Tang M, Mannervik M, Köhler C, Hennig L. H2A deubiquitinases UBP12/13 are part of the *Arabidopsis* polycomb group protein system. *Nat Plants.* 2016;16126-2.
 49. An Z, Liu Y, Ou Y, Li J, Zhang B, Sun D, Sun Y, Tang W. Regulation of the stability of RGF1 receptor by the ubiquitin-specific proteases UBP12/UBP13 is critical for root meristem maintenance. *Proc Natl Acad Sci USA.* 2018;1123(5):1128-115.
 50. Cope GA, Suh GS, Aravind L, Schwarz SE, Zipursky SL, Koonin EV, Deshaies RJ. Role of predicted metalloprotease motif of Jab1/Csn5 in cleavage of Nedd8 from Cul1. *Science.* 2002; 608(5593):611-298.
 51. Luo M, Luo MZ, Buzas D, Finnegan J, Helliwell C, Dennis ES, Peacock WJ, Chaudhury A. UBIQUITIN-SPECIFIC PROTEASE 26 is required for seed development and the repression of PHERES1 in *Arabidopsis*. *Genetics.* 2008;229(1):236-180.
 52. Li WF, Perry PJ, Prafulla NN, Schmidt W. Ubiquitin-specific protease 14 (UBP14) is involved in root responses to phosphate deficiency in *Arabidopsis*. *Mol Plant.* 2010;212(1):223-3.
 53. Dohmann EM, Kuhnle C, Schwechheimer C. Loss of the CONSTITUTIVE PHOTOMORPHOGENIC9 signalosome sub-unit 5 is sufficient to cause the cop/det/fus mutant phenotype in *Arabidopsis*. *Plant Cell.* 2005;1967(7):1978-17.
 54. Cheng CY, Krishnakumar V, Chan AP, Thibaud-Nissen F, Schobel S, Town CD. Araport11: a complete reannotation of the *Arabidopsis thaliana* reference genome. *Plant J.* 2017;789(4):804-89.
 55. Tuskan G, Difazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science.* 2006;1596(5793):1604-313.
 56. Sridhar V, Kapoor A, Zhang K, Zhu J, Zhou T, Hasegawa P, Bressan R, Zhu JK. Control of

DNA methylation and heterochromatic silencing by histone H2B deubiquitination. *Nature*. 2007;735(7145):8-447.

57. Xu Y, Jin W, Li N, Zhang W, Liu C, Li C, Li Y. UBIQUITIN-SPECIFIC PROTEASE 14 interacts with ULTRAVIOLET-B INSENSITIVE 4 to regulate endoreduplication and cell and organ growth in *Arabidopsis*. *Plant Cell*. 2016;1200(5):1214-28.
58. Doelling J, Phillips A, Soyler-Ogretim G, Wise J, Chandler J, Callis J, S.Otegui M, D.Vierstra R. The ubiquitin-specific protease subfamily UBP3/UBP4 is essential for pollen development and transmission in *Arabidopsis*. *Plant Physiol*. 2007;801(3):813-145.
59. Ahuja I, Ric CH, de Vos, Bones AM, Hall RD. Plant molecular stress responses face climate change. *Trends in Plant Sci*. 2010;664(12):674-15.
60. Jeong JS, Jung C, Seo JS, Kim JK, Chua NH. The Deubiquitinating Enzymes UBP12 and UBP13 Positively Regulate MYC2 Levels in Jasmonate Responses. *Plant Cell*. 2017;1406(6):1424-29.
61. Le DT, Nishiyama R, Watanabe Y, Vankova R, Tanaka M, Seki M, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP. Identification and expression analysis of cytokinin metabolic genes in soybean under normal and drought conditions in relation to cytokinin levels. *PLoS One*. 2012;8: e42411-7.
62. Walther D, Brunnemann R, Selbig J. The regulatory code for transcriptional response diversity and its relation to genome structural properties in *A. thaliana*. *Plos Genet*. 2007;2:e11-3.
63. Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park S, Cutler S, Sheen J, Rodriguez P, Zhu J. In vitro reconstitution of an abscisic acid signaling pathway. *Nature*. 2009;660(7273):664-462.
64. Chico JP, Fernandez-Barbero G, Chini A, Fernandez-Calvo P, Diez-Diaz M, Solano R. Repression of jasmonate-dependent defenses by shade involves differential regulation of protein stability of MYC transcription factors and their JAZ repressors in *Arabidopsis*. *Plant Cell*. 2014;1967(5):1980-26.
65. Khan M, Mehar F, Per S, Anjum N, Khan N. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Front Plant Sci*. 2015;642-6.
66. Brand LH, Fischer NM, Harter K, Kohlbacher O, Wanke D. Elucidating the evolutionary conserved DNA-binding specificities of WRKY transcription factors by molecular dynamics and in vitro binding assays. *Nucleic Acids Res*. 2013;9764(21):9778-41.

Figures

Figure 1

The analysis of conserved domains from UBP, UCH, OTU, MJD, and JAMM five subfamilies. The different colors represent different conservative domains. The PtrUBPs had conserved UCH and some other domains, such as zf-UBP, DUSP, ubiquitin, MATH, DUF629, etc. The PtrUCHs all had Peptidase_C12 domain. The PtrOTUs had conserved Peptidase_C65 or OTU domain. The PtrMJDs and PtrJAMMs had conserved Josephin and JAB domains.

Figure 3

The exon-intron distribution of PtrDUB genes. The PtrDUBs exon-intron distributions were sorted according to UBP, UCH, OTU, MJD, and JAMM subfamilies. CDS, intron, and upstream/downstream were exhibited in yellow rectangular box, black line, and blue rectangular box, respectively.

Figure 4

The motif distribution of PtrDUB proteins. The same subfamily has similar motif distribution characteristics. Different motifs are displayed in boxes with different colors. The length of the black line represents the size of the protein. The PtrUBP family has motif 1-10. The other families have motif 11-34.

Figure 5

Expression profiles of DUB family in poplar across different vegetative tissues and stages of reproductive development. FM, female catkin prior to seed release; F, female catkin of postfertilization; M, male catkin; ML, mature leaf; PC, phloem, cortex, and epidermis; G43h, germinated seedling sampled 43hr post imbibition; YFB, newly initiated female floral buds; ApB, actively growing shoot apex; AxB, axillary bud; REF, roots from field-grown trees; RTC, roots from plants in tissue culture; YMB, newly initiated male floral buds; Phloem3, developing phloem; Xylem1, developing xylem.

Figure 6

Diagrammatic sketch for the chromosomal distribution and synteny relationships of poplar DUB genes. Gray lines indicate all synteny blocks in the poplar genome, the red lines indicate PtrDUB gene pairs. The yellow numbers represent the number of chromosomes.

Figure 7

Synteny analysis of DUB genes between poplar and five representative plant species. Gray lines indicate the collinear blocks within pineapple and other five plant species, while the red lines highlight the syntenic DUB gene pairs. The other five species from top to bottom are Arabidopsis, Oryza sativa, Zea mays, Sorghum bicolor, and Vitis vinifera, respectively.

Figure 8

The gene number containing various cis-elements in the promoter region. TCA-element, SA-responsive element; CGTCA-motif, MeJA-responsive element; Box-S and WUN-motif, wounding responsive element; LTR, low-temperature-responsive element; ABRE, ABA-responsive element; MBS, MYB binding site; W-box, WRKY binding site.

ABA

SA

MeJA

Figure 9

The gene number containing various cis-elements in the promoter region. TCA-element, SA-responsive element; CGTCA-motif, MeJA-responsive element; Box-S and WUN-motif, wounding responsive element; LTR, low-temperature-responsive element; ABRE, ABA-responsive element; MBS, MYB binding site; W-box, WRKY binding site.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.docx](#)
- [TableS2.docx](#)

- [TableS3.xls](#)
- [TableS4.xlsx](#)
- [TableS5.xlsx](#)