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杨树和葡萄 UBX 蛋白质家族分析*

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摘要: UBX (泛素调控 X 因子) 蛋白质家族在泛素化相关的过程中起着重要的作用, 如细胞周期调控、转录调控、信号转导、发育、胁迫响应、细胞程序性死亡、内吞作用和 DNA 修复。然而, 到目前为止, UBX 家族在杨树和葡萄中还没有被研究过。为了更好的弄清这两个植物的 UBX 家族, 我们对 UBX 的基因结构、染色体位置、基因重复、系统发育关系作了分析。该研究对葡萄和杨树的 UBX 蛋白质家族作了第一个系统的分析。基因的外显子/内含子结构和蛋白质基序组成在同一个组里相对比较保守。基因重复分析表明, 串联重复和片段重复对于杨树和葡萄的 UBX 基因家族的扩张有一定贡献, 基因缺失在 UBX 基因家族的扩张过程中也发生了作用。本研究为 UBX 蛋白质功能的研究奠定了基础。

关键词: 泛素调控 X 因子; 基因结构; 基因重复; 系统发育
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Analyses of the UBX Protein Family in *Populus* and *Vitis*

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Abstract: The UBX (Ubiquitin regulatory X) protein family plays important roles in ubiquitin-related processes including cell-cycle control, transcriptional regulation, signal transduction, development, stress response, programmed cell death, endocytosis and DNA repair. However, this family has not been studied to date in *Populus* and *Vitis*. To better understand the UBX in these two plants, relevant analyses about gene structure, chromosomal location, duplication, phylogenetic relationships were performed. Our study provides the first systematic analysis of the *Vitis* and *Populus* UBX proteins. The exon/intron gene structure and motif composition were relatively conserved in the same group. Duplication analyses suggested that tandem duplication and segmental duplication contribute to the expansion of *Populus* and *Vitis* UBX gene family, while some gene loss has also occurred. The results presented basic information on UBX proteins, which may show a scaffold for future functional analysis of this family.

Key words: UBX; Gene structure; Gene duplication; Phylogeny

As sessile organisms, plant growth, development and distribution are often affected by environmental and endogenous signals, such as plant hormones and stress. On the one hand, environmental stimuli, including cold, heat, drought, high salinity and damage stimulus, can seriously affect plant growth and productivity worldwide (Boyer, 1982). On the other hand, plant hormones, which include auxin, the gibberellins (GAs), abscisic acid (ABA), and so on, also participate in regulating plant growth

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and development (Gray, 2004).

Proteins in cells keep in a continuous metabolism process of degradation and update, which is essential for the normal life function. Ubiquitin is an evolutionarily highly conserved small protein, which plays important roles in plant hormone synthesis and signaling cascades process (Dreher and Callis, 2007). The ubiquitin system controls the selective degradation of many short-lived regulatory proteins in eukaryotic cells, which plays an important role in cell-cycle control, transcriptional regulation, signal transduction, development, vesicular traffic, stress response, DNA repair, programmed cell death *and so on* (Hershko and Ciechanover, 1998). The pivotal role of the selective degradation process controlled by ubiquitin protein has increasingly gained the attention of researchers. The regulation of ubiquitin in such a variety of cellular processes relies on the interaction with its distinct binding specificities and effector functions. Consequently, more and more ubiquitin associated proteins that contain ubiquitin-binding domain have been identified (Buchberger, 2002).

UBX domain (PF00789, pfam) is an 80 amino acid residue module, which is proved to be present typically at the carboxyl terminus of many kinds of eukaryotic proteins (http://pfam.sanger.ac.uk/). The UBX family is more structurally homologous to ubiquitin than other members of the ubiquitin clan (Buchberger *et al.*, 2001). Recent research revealed that the highly conserved AAA ATPase p97 in yeast participates in ubiquitin-dependent proteolysis (Ghislain *et al.*, 1996). P97 was thought to be a molecular chaperone which is a key component in the ubiquitin-proteasome system. This function is dependent on association with cofactors (Schuberth and Buchberger, 2008; Kloppsteck *et al.*, 2012). UBX domain containing proteins are mainly the cofactors of p97 (Schuberth and Buchberger, 2008). Ubx2, a transmembrane protein, participated in Endoplasmic Reticulum-associated degradation (ERAD) process, which selectively transports the misfolded proteins from the endoplasmic reticulum to the cytoplasm for further ubiquitylation and degradation (Römisch, 2006) (Schuberth and Buchberger, 2005). SAKS1, a UBX domain containing adaptor for p97, can negatively modulate ERAD and p97-dependent degradation (LaLonde and Bretscher, 2011). Ubx2/Ubxd8 regulates lipid droplet homeostasis (Wang and Lee, 2012). Another UBX domain-containing protein, TUG can also regulate the p97 ATPase (Orme and Bogan, 2012). All above suggest an important role of UBX involved in processes including protein degradation, endocytosis and so on (Buchberger *et al.*, 2001).

However, only very few UBX domain proteins have been studied up to now. A lot of researchers have thoroughly studied other gene families in *Populus* or in *Vitis*. But they have not studied UBX family in *Populus* or in *Vitis*. Few information about UBX family in woody plant species such as *Populus trichocarpa* (poplar) and *Vitis vinifera* (grape) have been commended.

In this study, the UBX proteins were analyzed *in silico* for gene structure, chromosomal location, phylogenetic relationships, protein sequence motifs, gene duplication in *Populus* and *Vitis*. We aimed to better understand the evolution of UBX in these plants. Our results show that the gene structure and motif composition were highly conserved in the same group, and tandem duplication and segmental duplication contribute to the expansion of *Populus* and *Vitis* UBX gene family, while some gene loss has also occurred. These will provide a fundamental basis for further functional investigations on these proteins.

1 Methods

1.1 Sequence retrieval and identification

To identify potential members of the UBX protein family in *Populus* and *Vitis*, we performed multiple database searches. *Arabidopsis* UBX proteins were obtained from the TAIR database (http://www.arabidopsis.org/) The UBX protein number present in the Arabidopsis was reported to be 15

(Bednarek, 2009). Additional searches were also performed based on keyword "UBX", "Ubiquitin regulatory X". *Arabidopsis* UBX protein sequences were retrieved and used as queries in blastp searches against the Poplar Genome database (http://genome.jgj-psf.org) and the Genoscope Grape Genome database (http://www.cns.fr). Blastp searches were also performed against the Poplar and Grape genomes at Phytozome 7.0 (http://www.phytozome.net). To perform a thorough search of UBX proteins, HMMER version 3.0 (http://hmmer.janelia.org/) was used to perform searches against the entire protein set.

SMART (Simple Modular Architecture Research Tool, http://smart.embl-heidelberg.de/) and Pfam (http://pfam.sanger.ac.uk/) were used to ensure the presence of UBX domain (Punta *et al.*, 2012). To estimate the isoelectric point (pI) and grand average hydropathy (GRAVY) values, ProtParam tool from ExPASy (http://us.expasy.org/tools/protparam.html) was used.

1.2 Phylogenetic analyses of the UBX protein family

The software MEGA 5 (Tamura *et al.*, 2011) was used to construct the phylogenetic tree in this study. The amino acid sequences were first aligned using the MUSCLE program with default parameters in this software, followed by manual comparisons and refinement. Gaps and ambiguously aligned regions were removed before phylogenetic analyses. A neighbor-joining phylogenetic tree based on the alignment described above was generated with *p*-distance model and the method of pairwise deletion of gaps. Bootstrap replicates (1000) were used to evaluate the significance of each node of the tree. Other options were default. The phylogenetic tree was displayed using MEGA software.

1.3 Chromosomal location and gene structure of the UBX proteins

The chromosomal locations of the UBX proteins were determined using the *Populus* genome browser http://www.phytozome.net/poplar and *Vitis* genome browser http://www.genoscope.cns.fr/spip/Vitis-vinifera-e.html. Fig. 2 and Fig. 3 were drawn according to the chromosome size and the position of UBX genes. We just used the figures of the references so we can easily see whether UBX is located in segmental blocks (Jaillon *et al.*, 2007; Licausi *et al.*, 2010; Tuskan *et al.*, 2006). Gene intron/extron structure information was collected from the genome annotations of *Populus* and *Vitis* from NCBI and Phytozome databases (7.0, http://www.phytozome.net). Gene structure display server program (GSDS, http://gsds.cbi.pku.edu.cn/index.php) was used to display the UBX gene structures by comparison of the full-length cDNA sequences with the corresponding genomic DNA sequences (Guo *et al.*, 2007). Because cDNA sequences are obtained from the corresponding databases, which are already full-length cDNA.

1.4 Estimation of duplication time

Paralogous alignments were performed using ClustalW (codons) in Mega 5. K-Estimator 6.0 was used to estimate the *Ka* and *Ks* values of the paralogous genes. And then the *Ks* was used to estimate the approximate date of the duplication event. That is $T = Ks/2\lambda$, where λ represent clock-like rates of synonymous substitution. For *Populus*, $\lambda = 9.1 \times 10^{-9}$ was used (Lynch and Conery, 2000), and 6.5×10^{-9} for *Vitis* (Gaut *et al.*, 1996).

1.5 Conserved motifs analyses and sequence logo

Multiple Expectation Maximization for Motif Elicitation (MEME, Version 4.9.0, http://meme.sdsc.edu) tool was used to identify motifs constitution in *Populus* and *Vitis* UBX protein sequences (Bailey *et al.*, 2006). MEME was run locally with the following parameters: Distribution of motif occurrences: Any number of repetitions; Number of different motifs: 30; Minimum motif width: 6; Maximum motif width: 200.

2 Results and discussion

2.1 Identification of the UBX protein family in *Populus* and *Vitis*

To identify potential members of the UBX protein family in *Populus* and *Vitis*, *Arabidopsis* UBX

prtoteins were used as queries in blastp searches against the *Populus* Genome database and the *Vitis* Genome database. Sequences returned were identified with SMART and Pfam tools. The UBX protein number present in the *Arabidopsis* was reported to be 15 (Bednarek, 2009). Twenty-three UBX proteins were identified in *Populus* (Table 1) and 10 in *Vitis* (Table 2). The UBX proteins in *Vitis* and *Populus* range from 165 to 738 amino acids in length. The isoelectric point (pI) in *Populus* ranged from 4.58 to 9.78, and 4.81 to 6.22 in *Vitis*. The grand average hydropathy (GRAVY) value is −0.807 to −0.245 in *Populus* and −0.739 to −0.436 in *Vitis* respectively. Lower GRAVY values indicate that they are soluble.

2.2 Phylogenetic analyses of the UBX proteins in *Arabidopsis*, *Populus* and *Vitis*

In order to investigate the evolutionary relationships of the UBX proteins in *Arabidopsis*, *Populus*

Table 1 UBX genes identified in *Populus*

Gene name	Gene ID	Location: chr: start-end (strand)	Locus name	Protein length	PI	GRAVY
popUBX1	7479342	scaffold_1: 6752650-6757124 (+)	PUBXR_0001s08840	592	4.76	−0.753
popUBX2	7467049	scaffold_1: 8688279-8691670 (+)	PUBXR_0001s11200	473	5.04	−0.264
popUBX3	7456202	scaffold_1: 23043626-23044670 (+)	PUBXRDRAFT_844989	165	9.78	−0.245
popUBX4	7480069	scaffold_1: 26097804-26099520 (+)	PUBXR_0001s27170	229	6.46	−0.575
popUBX5	7494211	scaffold_1: 34653537-34654649 (+)	PUBXR_0001s36150	176	9.51	−0.319
popUBX6	7496891	scaffold_2: 18119451-18121869 (−)	PUBXR_0002s21600	429	5.2	−0.694
popUBX7	7489882	scaffold_2: 19060655-19062952 (+)	PUBXRDRAFT_711401	254	5.57	−0.471
popUBX8	7465837	scaffold_3: 12879636-12884009 (+)	PUBXR_0003s12230	589	4.58	−0.779
popUBX9	7497510	scaffold_3: 14581207-14584774 (+)	PUBXR_0003s14510	474	5.06	−0.29
popUBX10	7492568	scaffold_4: 222264-226984 (−)	PUBXR_0004s00590	305	5.52	−0.64
popUBX11	7492568	scaffold_4: 236182-238326 (+)	PUBXR_0004s00620	218	5.24	−0.616
popUBX12	7491781	scaffold_7: 1416610-1421004 (+)	PUBXR_0007s02410	520	5.6	−0.586
popUBX13	7482908	scaffold_8: 8746906-8751006 (+)	PUBXR_0008s13320	480	6.28	−0.57
popUBX14	7457839	scaffold_8: 9414593-9422375 (+)	PUBXR_0008s14230	455	4.89	−0.526
popUBX15	7482291	scaffold_9: 6203578-6205202 (+)	PUBXR_0009s06420	250	6.61	−0.472
popUBX16	7462631	scaffold_10: 11284948-11292772 (−)	PUBXR_0010s10910	451	4.98	−0.525
popUBX17	7484509	scaffold_11: 357597-358981 (+)	PUBXR_0021s00560	253	5.36	−0.406
popUBX18	7484546	scaffold_11: 1135741-1138580 (−)	PUBXR_0011s01620	305	5.32	−0.581
popUBX19	7496924	scaffold_14: 5422940-5428222 (+)	PUBXR_0014s07170	464	5.62	−0.713
popUBX20	7462075	scaffold_14: 11585957-11588052 (−)	PUBXR_0014s15560	408	5.11	−0.659
popUBX21	7453782	scaffold_15: 10460579-10463003 (−)	PUBXR_0015s09180	401	8.07	−0.505
popUBX22	7463293	scaffold_17: 4926068-4932450 (+)	PUBXR_0017s06430	543	5.69	−0.592
popUBX23	7457047	scaffold_Un: 2571-4878 (−)	PUBXRDRAFT_792398	409	5.33	−0.807

Table 2 UBX genes identified in *Vitis*

Gene name	Gene ID	Location: chr: start-end (strand)	Locus name	Protein length	PI	GRAVY
vitUBX1	100247740	chr1: 2244794-2294793 (−)	GSVIVG01010276001	456	4.81	−0.525
vitUBX2	100257839	chr1: 5295143-5301096 (−)	GSVIVG01011678001	474	6.22	−0.438
vitUBX3	100263375	chr2: 2079818-2091015 (+)	GSVIVG01019629001	354	5.24	−0.482
vitUBX4	100251323	chr2: 3558065-3566452 (−)	GSVIVG01019815001	738	4.84	−0.739
vitUBX5	100244691	chr5: 21017108-21042799 (−)	GSVIVG01013567001	542	5.25	−0.599
vitUBX6	100262315	chr10: 111796-117184 (+)	GSVIVG01004812001	299	5.47	−0.636
vitUBX7	100261938	chr10: 120527-137361 (−)	GSVIVG01004815001	428	5.79	−0.559
vitUBX8	100243031	chr12: 6128660-6135333 (−)	GSVIVG01030489001	366	4.97	−0.477
vitUBX9	100249155	chr14: 26293468-26297939 (−)	GSVIVG01001850001	231	5.77	−0.533
vitUBX10	100241512	chr17: 10309381-10311030 (−)	GSVIVG01007694001	335	5.86	−0.436

and *Vitis*, we constructed a neighbor-joining phylogenetic tree from a multiple sequence alignment of full length UBX proteins using Mega 5. Based on the bootstrap values, gene structure and conserved motif, we further divided them into seven subgroups, namely Groups 1−7 (Fig. 1).

Each group contains all the three species, which suggested that some ancestor UBX genes had branched before species evolved into woody plant and herbarium lineages. In a single homologs, which can be called as homologous, most *Populus* UBXs clustered with their homologous in *Populus* (*Populus*−*Populus*), sometimes clustered together with a grape UBX (*Populus*−*Vitis*), but never clustered together

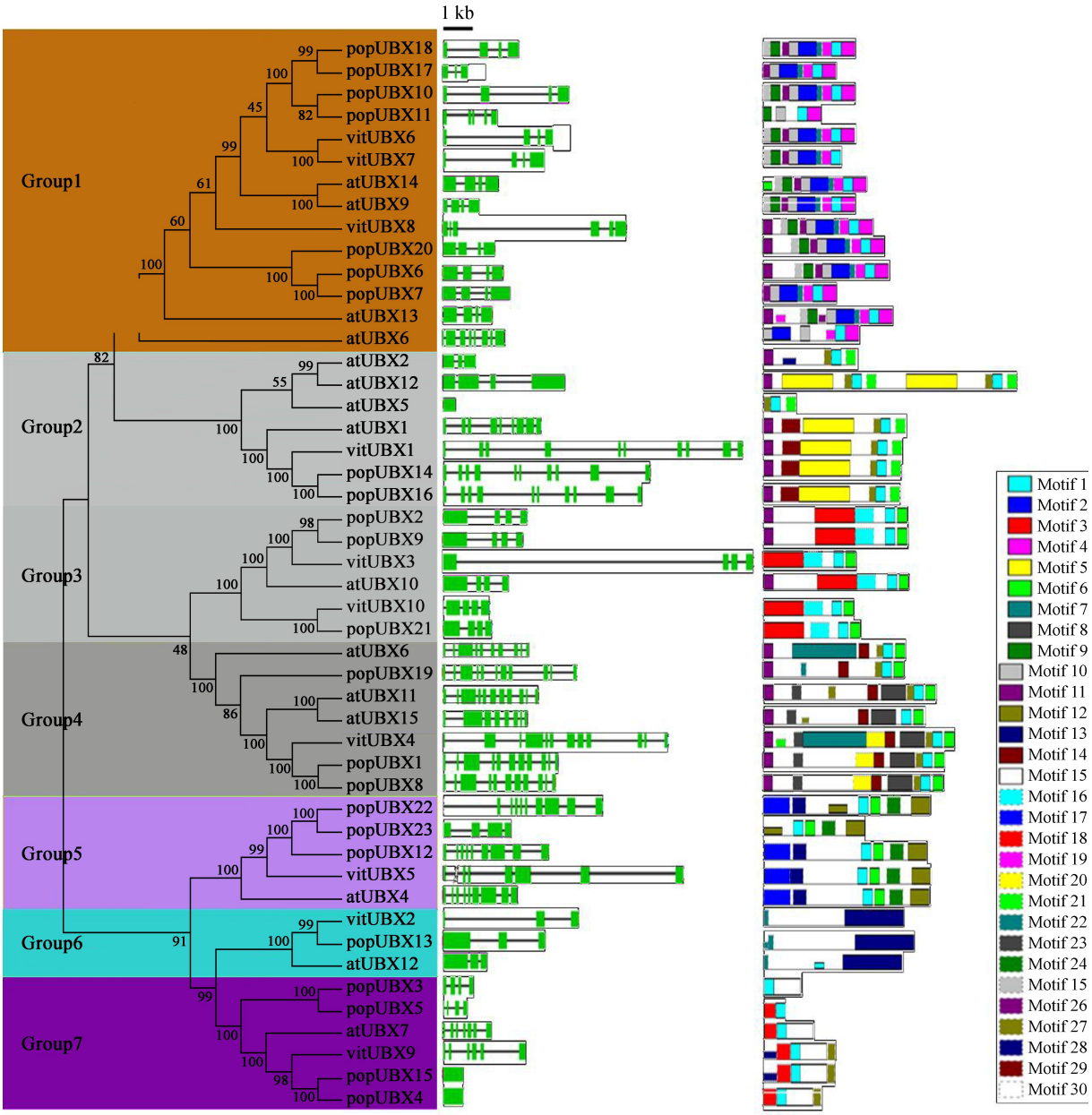

Fig. 1　Phylogenetic relationships, gene structure and conserved motif of UBX proteins in *Arabidopsis* (at), *Populus* (pop) and *Vitis* (vit). The molecular phylogeny (left panel) was constructed using full length UBX protein sequences from the three species. Numbers associated with branches show bootstrap support values for neighbor-joining (NJ) analyses. The 7 major groups designated from 1 to 7 are marked with different color backgrounds. Exon/intron structures of the UBX genes are shown in the middle panel. The long intron was denoted by "//". Green boxes represent exons and black lines represent introns. A schematic representation of conserved motifs (obtained using MEME) in UBX proteins is displayed on the right panel. Different boxes represent different motifs

with any corresponding protein in *Arabidopsis* (*Populus*≠*Arabidopsis*). It's the same to grape, in a single homologs, grape UBXs clustered together with grape UBXs (*Vitis*−*Vitis*), sometimes clustered together with a single *Populus* protein (*Populus*−*Vitis*), but never clustered together with any protein in *Arabidopsis* (*Populus* ≠ *Arabidopsis*). It indicated that some UBX proteins may have evolved after the woody plant and herbs differentiation.

2.3 Exon-intron evolution of the UBX family genes in *Arabidopsis*, *Populus* and *Vitis*

The exon/intron structure can provide new clues for our further understanding the evolutionary mechanisms underlying the origin of family genes (Chen et al., 2012). So a comparison between the cDNA and genomic sequences were conducted in *Arabidopsis*, *Populus* and *Vitis*. Fig. 1 illustrated the distribution and position of exons/introns within each of the UBX genes. Generally, intron loss/gain events are important mechanism generating structural diversity and complexity, while the structural diversity may be a mechanism for the gene family expansion (Cao *et al.*, 2011). In this study, we analyzed the structural diversity of UBX proteins and found that exon/intron loss or gain events occurred during the expansion and structural evolution of UBX paralogous. The family proteins in the same group have similar exon/intron structures (include intron number or exon length, Fig. 1); which better support their close phylogeny relationship. For instance, the UBX genes in each group contained at least two introns with exception of atUBX5 in group 2 and popUBX4, popUBX15 in group 7, which had no introns. In contrast to other members in group 7, a gene having simple gene structure and the same function may be an advanced kind of evolved form. Different gene structures in the different phylogenetic subgroups can be a clue of gene family expansion from ancient paralogous or a clue of multiple origins of gene ancestry.

2.4 Chromosomal location and duplication of the UBX proteins

To further study the gene duplication mechanism in the *Populus* and *Vitis* genomes, we mapped the UBX gene onto chromosome. The chromosomal location of UBX illustrated as Fig. 2 (*Populus*) and Fig. 3 (*Vitis*). As presented in Fig. 2, we found these UBX genes are distributed unevenly among twelve chromosomes of the *Populus* genome. Chromosomes 5, 6, 12, 13, 16, 18 and 19 had no UBX, while relatively high densities of UBXs located on chromosome I. PopUBX23 localized to unknown genomic sequence scaffolds and thus could not be mapped to any particular chromosome.

Chromosomal segments duplication, tandem duplication and transposition events are three factors result in family gene expansion (Cannon *et al.*, 2004; Kong *et al.*, 2007). Study of Tuskan *et al.* (2006) has identified the segmental duplication event in the Salicaceae (salicoid duplication) caused the duplicated paralogous and significantly contributed to the expansion of plenty of gene families. Illustrated as Fig. 2, it was found that 95% (21/22) UBX genes are located in duplicated blocks except for popUBX5. Two duplicated paralogous pairs (popUBX-1/8 and popUBX-14/16) are located in paralogous segment blocks, so they can be the direct results of the segmental duplication event. Two duplicated paralogous pair (popUBX 2-9, 4-15, 17-18) located on the blocks, but the position of corresponding block shift, which suggested that dynamic transposition events may have occurred after the segmental duplication. Five duplicated UBX (popUBX-12, 13, 19, 21, 20) also located on the blocks, but lacked duplicated parologous on the corresponding block, suggesting that either the genes losses or transposition may have occurred. According to phylogenetic result, several pairs of UBX proteins can be considered as putative paralogous (Fig. 1). So we computed the duplication time of these paralogous according to *Ks*. These putative paralogous UBX proteins account for 17.4% of all the UBX family in *Populus*. As table 3 illustrated, the duplication events of these UBX in *Populus* occurred from 10.2Ma to 14.59Ma. This period is consistent with the time about

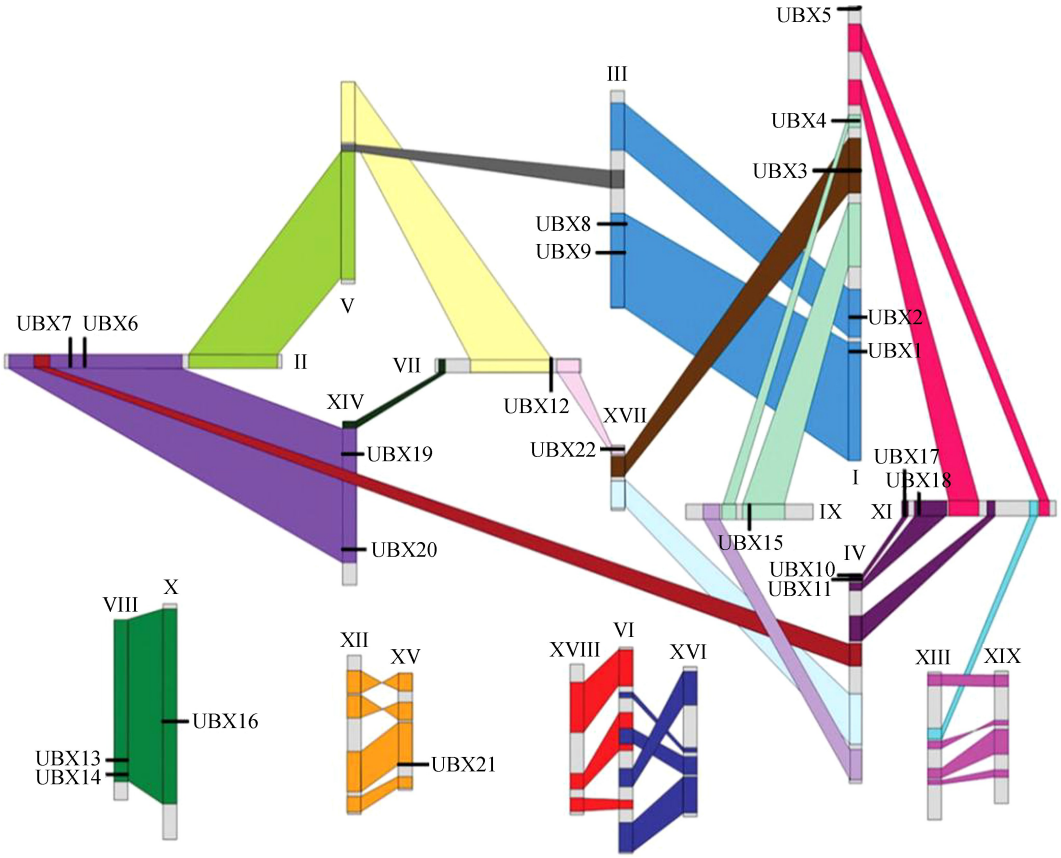

Fig. 2　Chromosomal locations of the *Populus* UBX protein family. The schematic diagram shows the 22 UBX proteins mapped to 12 chromosomes. One remaining genes (popUBX23) is located on unassembled scaffolds. Homologous blocks derived from segmental duplication are indicated using the same colors according to Tuskan *et al.* (2006)

13Ma ago when large-scale genome duplication event has occurred in *Populus*, which suggest that large scale gene loss has occurred after the large scale genome duplication event in *Populus*. For *Vitis*, the segmental duplication event of vitUBX6/7 was estimated to occur in 11.78Ma.

Furthermore, the tandem duplications also contribute to the expansion of UBX gene family (popUBX-10/11). And popUBX22 was located on segmental duplicate blocks with its counterpart popUBX23 not mapped to any chromosome yet. Altogether, the segment duplication, tandem duplication and maybe transposition events, contributed to the expansion of UBX gene family in the *Populus* UBX genome.

And as illustrated in Fig. 3, the result showed that the UBX genes are dispersed randomly throughout the seven chromosomes of *Vitis*. Chromosomes 3, 4, 6, 7, 8, 9, 11, 13, 15, 16, 18 and 19 had no

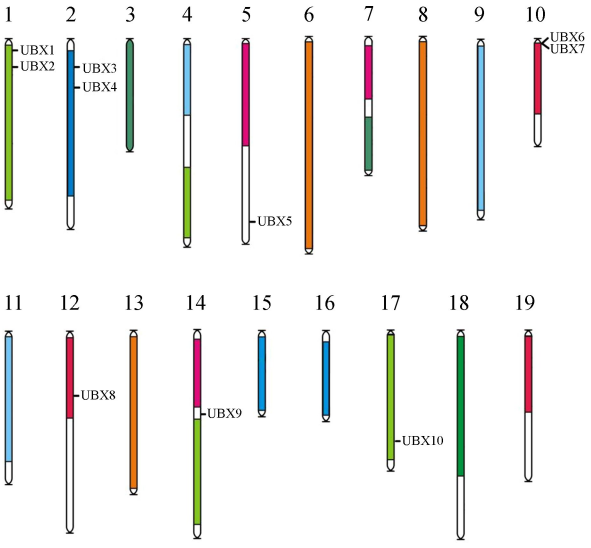

Fig. 3　Chromosomal locations of the *Vitis* UBX family. The 10 UBX proteins mapped to the 8 of the 19 grape chromosomes are shown. Paralogous regions in the putative ancestral constituents of the *Vitis* genome are depicted using the colors according to Jaillon *et al.* (2007) and Licausi *et al.* (2010)

Table 3 Estimation of duplication time

	Ka	*Ks*	time (Ma)
popUBX14/16	0.0522	0.26546	14.59
popUBX1/8	0.08218	0.18562	10.2
vitUBX6/7	0.06831	0.15309	11.78

UBX. We found that some UBX proteins are located in tandem clusters on the chromosomes; examples are vitUBX6-7 (Fig. 3). Tandem duplications may also be a factor contributing to the genesis of family genes in *Vitis*.

2.5 Conserved domains and motifs in UBX proteins

The major domains of the UBX proteins in *Populus*, *Vitis* and *Arabidopsis* were verified using Pfam and SMART (Punta *et al.*, 2012; Letunic *et al.*, 2012). While these tools are unable to recognize smaller or more divergent motifs, we used MEME to discern for more detailed motif information, and thirty distinct motifs were identified in these proteins (right panel in Fig. 1). Our result shows that seven groups have different types of motif composition; these motifs are highly conserved in the same group, suggesting closely evolutionary relationships and similar function among group members.

Most UBX members in all the seven subgroup shared motif 1 (Fig. 1), suggesting its conservative and functional importance. So we illustrated the top one conserved motif (motif 1) with sequence logo (Fig. 4). From the sequence logo, we can determine the consensus sequence and the relative frequency of bases and the information content (measured in bits) at every position in a site (Schneider and Stephens, 1990). The logo displays both significant residues and subtle sequence patterns.

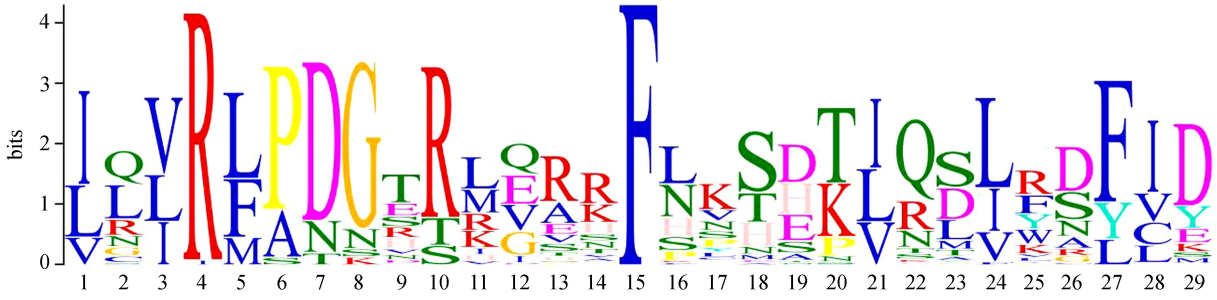

Fig. 4 Sequence logo plot of the top one conserved motif (motif 1) identified by MEME program for all the predicted full-length UBX proteins. The height of a letter indicates its relative frequency at the given position (x-axis) in the motif. Numbers on the x-axis represent the sequence positions in zinc finger motifs. The sequence logos were derived using WebLogo (Crooks *et al.*, 2004)

3 Conclusion

This study provides a comparative genome analysis including phylogeny, chromosomal location, gene structure of the UBX family in *Populus* and *Vitis*. The exon/intron gene structure and motif composition were relatively conserved in the same group. Duplication analyses suggested tandem duplication and segmental duplication contribute to the expansion of *Populus* and *Vitis* UBX gene family, while some gene loss has also occurred. The results presented in this study provide basic information on UBX proteins, which may provide valuable information for future functional investigations of this family.

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