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SHORT COMMUNICATION

Transcriptome-wide analysis to dissect the transcription factors orchestrating the phase change from vegetative to reproductive development in *Larix kaempferi*

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Abstract

The timing of phase change from vegetative to reproductive development during aging of forest trees is important for wood and seed production. In a previous study, we investigated the effects of aging on wood formation by measuring the transcriptomic changes in the uppermost main stems of 1-, 2-, 5-, 10-, 25-, and 50-year-old *Larix kaempferi*. Based on the published transcriptome data, here we investigated the transcriptomic differences between the juvenile vegetative (1- and 2-year-old) and adult reproductive (25- and 50-year-old) phases to determine the molecular mechanisms underlying the phase change. In total, 12,789 transcripts were identified as differentially expressed genes, including 573 transcription factors. Further analysis showed that 27 transcription factors belonging to 8 families were common to all four comparisons between old and young life stage categories: (I) 25 vs 1 year old; (II) 50 vs 1 year old; (III) 25 vs 2 years old; and (IV) 50 vs 2 years old. The analysis of their expression patterns in six age categories showed that members of the AP2 and Dof families were expressed highly in 1- and 2-year-old trees, weakly in 25- and 50-year-old trees, while members of the C3H, G2-like, GRAS, MYB-related, and MADS families had the opposite patterns. Notably, one member of the MADS family was only detected in 25- and 50-year-old trees. These results suggest that the phase change might (1) occur in the early stage of the *L. kaempferi* lifetime and (2) be controlled by a complex regulatory network of different transcription factors, some of which are known to play roles in the phase change in model plants. These findings not only provide molecular markers to distinguish different stages of tree growth and development and potential targets for genetic manipulation to improve the reproductive traits of trees, but also improve our understanding of the phase change with aging in trees.

Keywords Larch · Phase change · Transcriptome · Transcription factor · MADS

Wei-Bo Xiang and Wan-Feng Li contributed equally to this work.

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Introduction

Many aspects of tree growth and development change with age, such as wood formation (Xu et al. 2016; Li et al. 2013a, 2014, 2017; Zeng et al. 2017), growth rate (Lai et al. 2014), rooting ability (Peer and Greenwood 2001), and the phase change (Brunner et al. 2017), and these traits are important in forestry. So, revealing the underlying mechanisms is not only helpful to understand the processes of tree growth and development, but also helpful for wood, cutting seedlings, and seed production. Seeds are used to propagate many forest trees, which is still the main means of producing seedlings, at least for larch in China. In addition, the seeds of some trees, such as *Pinus koraiensis*, are edible and sold commercially. So, shortening the time required to enter into the reproductive phase is of great relevance and economic value, but still poses a challenge. Maintaining the juvenile phase or delaying the

onset of the reproductive phase could maintain a high rate of growth and wood production, as well as other traits important for clonal forestry (Wendling et al. 2014b).

Usually, many years are required for forest trees to be able to flower and produce seeds, and so far, the genetic mechanisms underlying this process of sexual reproductive development are still not clear compared with those in herbaceous plants (Bergonzi and Albani 2011; Khan et al. 2014). Mainly due to the prolonged juvenile phase, the efficiency of forest tree breeding is low and needs to be improved. So, investigating the effects of aging on the processes of reproductive development in forest trees is of great theoretical and practical importance.

The phase transition in trees occurs gradually with age and is controlled by genetic and environmental cues as well as silviculture (García-López et al. 2014; Khan et al. 2014; Brunner and Nilsson 2004). During the phase transition, many aspects of growth and development change (Wendling et al. 2014a): the capacity to produce seeds occurs after the transition from the juvenile vegetative to the adult vegetative phase, and reproductive organs appear after the transition from the adult vegetative to the adult reproductive phase. Depicting the molecular features of phase transition with age will help to understand these processes and provide more information about the other physiological changes.

Genes functioning to delay reproductive growth or maintain the state of vegetative growth have been identified from herbaceous plants, such as *Arabidopsis*, barley, and rice, including microRNA miR156 (Wang et al. 2009; Wu et al. 2009), miR171 (Curaba et al. 2013; Fan et al. 2015), *DHD1* (*DELAYED HEADING DATE1*) (Zhang et al. 2019), *EMF1* (*Embryonic Flower 1*) and *EMF2* (Sung et al. 2003), *TEM* (*TEMPRANILLO*) (Sgamma et al. 2014), and *TFL1* (*Terminal Flower 1*) (Mohamed et al. 2010; Kotoda and Wada 2005). In gymnosperm trees, genes functioning to change the timing of reproductive growth have also been identified; among them, *DAL1*, a MADS-box transcription factor from *Picea abies*, is expressed increasingly from age 5 years, and its over-expression in *Arabidopsis* results in early flowering (Carlsbecker et al. 2004); in contrast, the over-expression of *LaAP2L1* (a heterosis-associated AP2/EREBP transcription factor from *Larix*) results in late flowering in *Arabidopsis* (Li et al. 2013b) and over-expression of the PEBP genes identified in conifers also represses flowering in *Arabidopsis* (Karlgren et al. 2011; Klintenäs et al. 2012). Furthermore, 38 members of the MADS family in *Larix*, including the putative homologue of *DAL1*, are also expressed increasingly from age 2 or 5 years (see Table S3 in Li et al. 2017), indicating their involvement in the transition from the juvenile to the adult phase. While the capacity to produce seeds occurs at about 20 years in *Picea* and 10 years in *Larix*, the molecular regulatory mechanism of the vegetative-to-reproductive phase transition in trees mediated by these genes is still unclear.

In our previous study of the *Larix* transcriptome, 10,604 transcripts were assigned to the GO term "vegetative to reproductive phase transition," 2619 to "vegetative phase change," 1072 to "regulation of timing of transition from vegetative to reproductive phase," and 94 to "regulation of vegetative phase change" (Li et al. 2017), all showing genetic features of the control of the phase transition in *Larix* aging. In addition, based on GO enrichment analysis, the GO term "reproduction" was enriched in genes with upregulated expression in comparison (I) 2 vs 1 year (Fig. 1), indicating that the molecular aspects of reproductive processes occur very early in the life span of *Larix*, consistent with the findings in *Olea europaea* L. (García-López et al. 2014). Detailed analysis of these genes is needed if they are to be used as targets for the improvement of reproductive traits. Studying the regulation of their expression with age is helpful to understand the relationships between their expression and the timing of the phase change, which are still not clear for most trees.

As for larch, about 10 years is required to achieve the capacity to produce seeds. We hypothesized that the levels of regulators of the phase change increase or decrease gradually with age, and genes, including transcription factors, controlling the phase change have different levels in the juvenile (before 10 years) and mature stages (after 10 years). By comparing the transcriptomes of juvenile vegetative (1- and 2-year-old) and adult reproductive (25- and 50-year-old) phases, which were generated in our previous study (Li et al. 2017), we set out to identify these regulatory genes.

Materials and methods

Sample preparation

The transcriptome data for analysis were generated in our previous study (Li et al. 2017). The uppermost main stems produced in the current year were collected from 1-, 2-, 5-, 10-, 25-, and 50-year-old *L. kaempferi* trees in July 2011 for RNA extraction and transcriptome library construction and sequencing (Li et al. 2014, 2017). The trees were located in Dagujia seed orchard (42° 22′ N, 124° 51′ E), Liaoning province, in northeast China, from young (1-, 2-, 5-, and 10-year-old), middle-aged (25-year-old), and mature (50-year-old) stands. They were grown from seed and include the vegetative and reproductive phases of *L. kaempferi*. After removal of branches and needles, the stems from at least three trees from each age category were pooled, frozen in liquid nitrogen, and stored at − 80 °C until RNA extraction.

Identification of differentially expressed genes

Transcript quantification from RNA-Seq data was performed in our previous study (Li et al. 2017). Six sets of sequencing

Accession	Gene Ontology term	(I)		(II)		(III)		(IV)		(V)	
		Down	Up	Down	Up	Down	Up	Down	Up	Down	Up
GO:0000003	reproduction										
GO:0022414	reproductive process										
GO:0044702	single organism reproductive process										
GO:0003006	developmental process involved in reproduction										
GO:0048506	regulation of timing of meristematic phase transition										
GO:0048510	regulation of timing of transition from vegetative to reproductive phase										
GO:2000241	regulation of reproductive process										
GO:0009553	embryo sac development										
GO:0009909	regulation of flower development										

Fig. 1 Distribution of Gene Ontology (*GO*) terms associated with the vegetative-to-reproductive phase change after enrichment analysis of differentially expressed genes (DEGs) during aging of *Larix kaempferi* (Li et al. 2017). The enriched GO terms are highlighted in *yellow* (upregulated) and *purple* (downregulated). Five pairwise comparisons were performed in chronological order to identify DEGs: (I) 2 vs 1 year, (II) 5 vs 2 years, (III) 10 vs 5 years, (IV) 25 vs 10 years, and (V) 50 vs 25 years

reads were mapped to the assembled reference transcripts using Bowtie (Langmead and Salzberg 2012). RNA-Seq by Expectation Maximization was used to estimate the transcript abundance (Li and Dewey 2011). The expression of genes was normalized with edgeR (empirical analysis of digital gene expression data in R) (Nikolayeva and Robinson 2014). Fragments per kilobase of transcript per million fragments was used to measure the normalized expression value. Based on the result of the quantification of transcripts (Li et al. 2017), here we made four pairwise comparisons to identify the differentially expressed genes (DEGs) in the juvenile vegetative (1- and 2-year-old) and adult reproductive (25- and 50-year-old) phases: (I) 25 vs 1 year old; (II) 50 vs 1 year old; (III) 25 vs 2 years old; and (IV) 50 vs 2 years old (Fig. 2). After pairwise comparison, the DEGs were obtained with stringent cutoffs: a false discovery rate (FDR)-corrected *P* value cutoff of 0.001 and a minimum expression fold change of 2.

Transcription factor prediction and their expression patterns

The nucleic acid sequences of 299,637 assembled transcripts (Li et al. 2017) were input into a server to predict the transcription factors (http://planttfdb.cbi.pku.edu.cn/prediction.php) (Jin et al. 2017). When the nucleic acid sequences were input, ESTScan 3.0 (Iseli et al. 1999) was employed to identify the coding regions of the input nucleic acid sequences and translate them to protein sequences, which were used to predict transcription factors based on the family assignment rules (http://planttfdb.cbi.pku.edu.cn/help_famschema.php).

The expression patterns of the predicted transcription factors in the juvenile vegetative and adult reproductive phases were analyzed based on the identification of DEGs (Fig. 2). The Venny 2.1 tool was used to find the transcription factors shared among the four comparisons (http://bioinfogp.cnb.csic.es/tools/venny/index.html) (Fig. 2). Then the expression patterns of the shared transcription factors in six age categories were further analyzed to characterize the changes in their transcript levels during aging throughout an entire rotation period. Quantitative reverse transcription polymerase chain reaction analysis had been performed in our previous study to confirm the results of transcript quantification from RNA-Seq data, so this was not repeated here.

Interaction prediction

The translated protein sequences of the transcription factors shared among the four comparisons were input into the STRING server to analyze their interactions in the context of *Arabidopsis thaliana* (https://string-db.org/) (Szklarczyk et al. 2017).

Result and discussion

Prediction of *Larix* transcription factors

After input of 299,637 assembled transcripts, 142,475 protein sequences were obtained with the length longer than 50 amino acids; among them, 4377 (3.07%) were predicted to be transcription factors and classified into 55 families (Fig. 3). At least three members were identified in each family and the largest number of transcription factors (411) was in the basic helix-loop-helix family (Fig. 3).

Up to 8 July 2019, 320,370 transcription factors classified into 58 families were identified from 165 species in

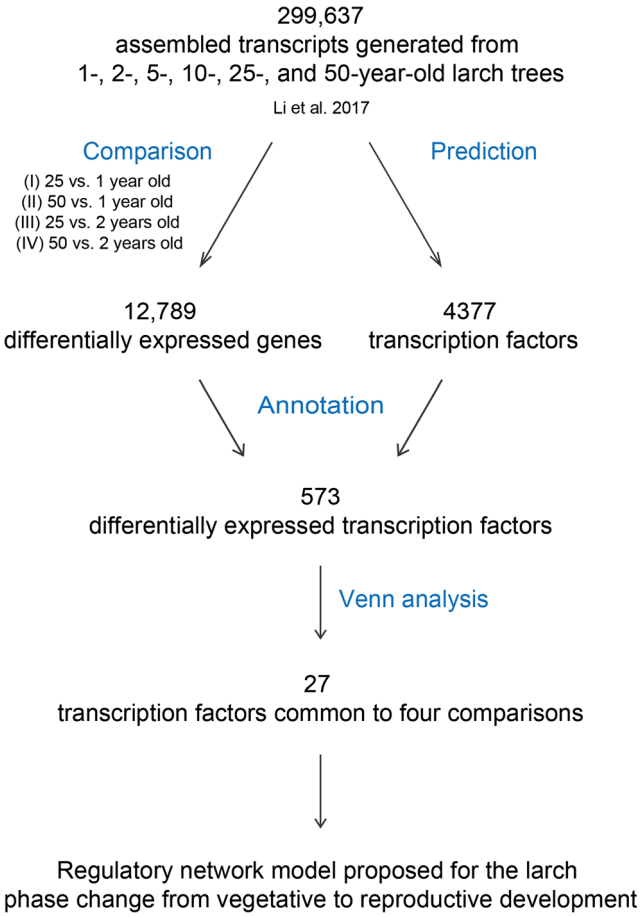

Fig. 2 Analytical flowchart of RNA-Seq analysis. 299,637 assembled transcripts generated from the uppermost main stems of 1-, 2-, 5-, 10-, 25-, and 50-year-old larch trees in a previous study (Li et al. 2017) were used to predict the transcription factors, and their expression levels in the juvenile vegetative (1- and 2-year-old) and adult reproductive (25- and 50-year-old) phases were compared. In total, 27 transcription factors shared in four comparisons: (I) 25 vs 1 year, (II) 50 vs 1 year, (III) 25 vs 2 years, and (*IV*) 50 vs 2 years, were identified

PlantTFDB 4.0 (http://planttfdb.cbi.pku.edu.cn/), which covers the main lineages of green plants (Jin et al. 2017), providing a powerful platform for predicting the transcription factors across green plants and studying the transcriptional control of plant growth and development. Genome-wide identification of transcription factors from *P. abies* 28,354 genes (Nystedt et al. 2013) and *Pseudotsuga menziesii* 47,874 genes (Neale et al. 2017) shows that there are 1107 (3.9%, classified into 54 families) and 1915 (4%, classified into 54 families) transcription factors, respectively (http://planttfdb.cbi.pku.edu.cn/index.php).

Here transcriptome-wide identification of transcription factors from *L. kaempferi* assembled transcripts showed that the ratio of transcription factors to genes from *L. kaempferi* was similar to that from *P. abies* and *P. menziesii*, and that almost the same number of transcription factor families existed in these three conifers. These results suggested the reliability and accuracy of the prediction of transcription factor with PlantTFDB 4.0 and conserved features of transcriptional control of conifers' growth and development. The prediction of transcription factors from trees' genomes and transcriptomes not only helps to study the transcriptional control of many aspects of tree growth and development, such as wood formation, phase change, and seasonal activity of the meristem, but also helps to identify the targets of larch microRNA to construct regulatory networks at the post-transcriptional level.

Transcriptional regulation of gene expression is a relatively stable mechanism in a time window of 50 years

To identify the transcription factors involved in the control of the phase change, we first made four pairwise comparisons: (I) 25 vs 1 year old; (II) 50 vs 1 year old; (III) 25 vs 2 years old; and (IV) 50 vs 2 years old (Fig. 2, Table 1). Based on this comparison, 12,789 transcripts were identified as DEGs (Table S1), among which 573 were transcription factors based on the prediction results (Table S2). Second, Venn analysis was used to identify the transcription factors shared in the four comparisons (Fig. 2). The results showed that 27 transcription factors from eight families were common, including 18 members of the MADS family (Fig. 4, Table 2). Third, we detected the expression patterns of 27 transcription factors in six age categories, finding that members of the AP2 and Dof families were expressed highly in 1- and 2-year-old trees, but weakly in 25- and 50-year-old trees, while members of another five families (C3H, G2-like, GRAS, MYB-related, and MADS) had the opposite patterns, and one member of the MADS family was only detected in 25- and 50-year-old trees (Table 2). Notably, the number of transcription factors highly expressed in 25- and 50-year-old trees was larger than that of highly expressed 1- and 2-year-old trees (Table 2) and the ratio of differentially expressed transcription factors to DEGs was higher in the upregulated genes (Table 1), suggesting more transcription factors take part in the positive regulation of the phase change.

In our previous study, 12,927 transcripts were identified as DEGs during larch tree aging (Li et al. 2017). To obtain more information about the transcriptional regulation of tree aging, here we used the 4377 predicted transcription factors to annotate the 12,927 DEGs again, and found that 562 (4.35%) were transcription factors (Table S3). In our present study, 573 transcription factors were differentially expressed, having 421 in common with the 562 transcription factors identified previously, and the ratio of differentially expressed transcription factors to DEGs was 4.48% (573/12,789), almost the same as in our previous study. These findings indicated that changes in the expression of transcription factors might be part of, or responsible for, the extensive and complex transcriptome reprogramming during tree aging. They also suggested that

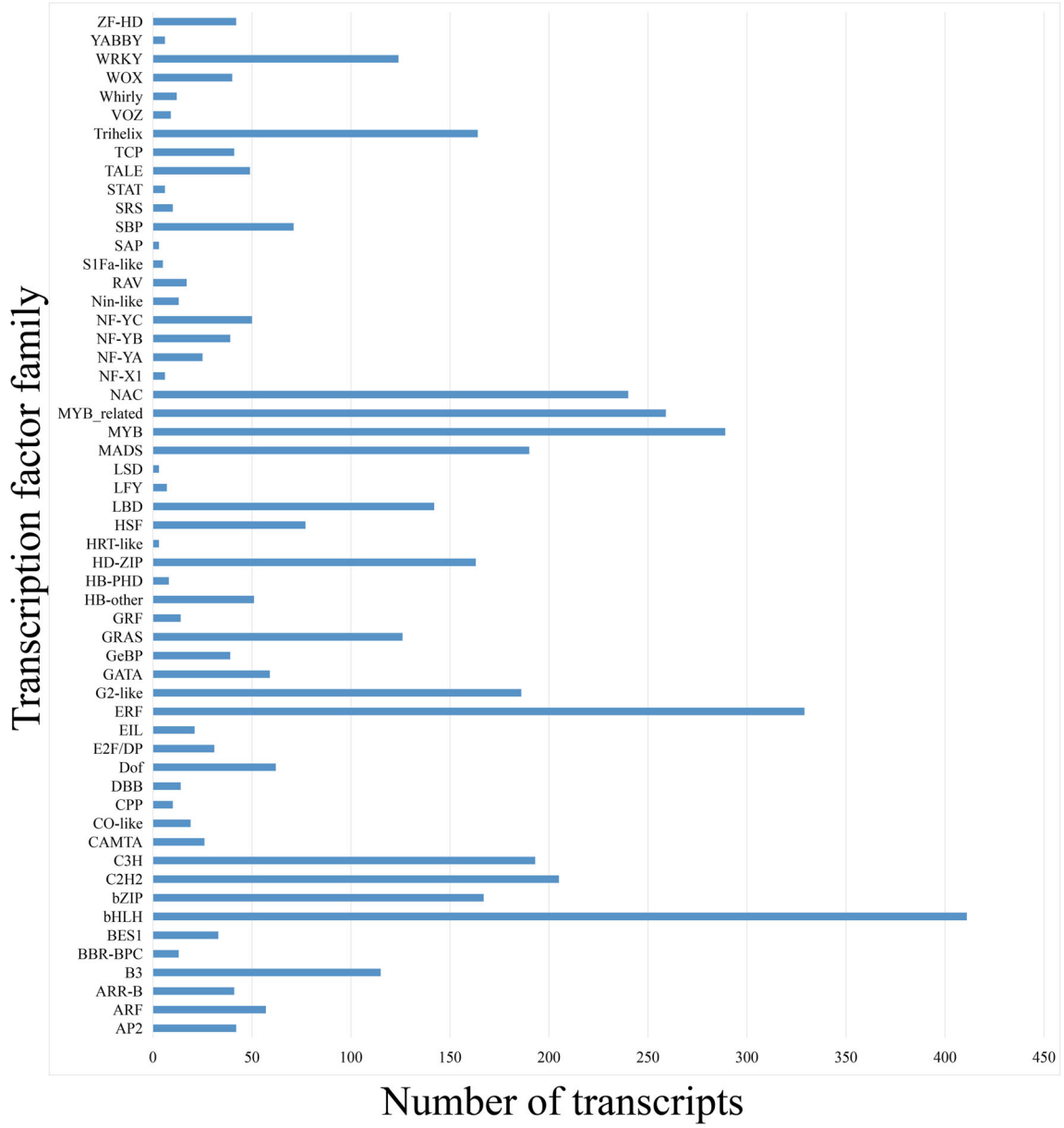

Fig. 3 Transcription factors predicted from the transcriptome generated from the uppermost main stems produced in the current year collected from 1-, 2-, 5-, 10-, 25-, and 50-year-old *L. kaempferi* trees (Li et al. 2014, 2017). In total, 4377 transcripts were predicted to be transcription factors and belonged to 55 families

the transcriptional regulation of gene expression is a relatively stable mechanism underlying tree growth and development at different stages, and is involved in the phase change at an early stage in the life span.

Transcriptional regulation of the phase change at an early stage in the life span

Modulation of gene expression with genetic transformation methods has been used to shorten the juvenile phase in woody plants (Brunner et al. 2017); for example, downregulation of *TFL1* homologues in *Populus* (Mohamed et al. 2010), *Malus* (Kotoda et al. 2006; Flachowsky et al. 2012), and *Pyrus* (Yamagishi et al. 2016; Freiman et al. 2012) results in precocious flowering. Some homologues of members of the MADS family have been overexpressed to shorten the juvenile phase in *Betula* (Elo et al. 2007; Huang et al. 2014) and *Malus* (Flachowsky et al. 2007). These reports inspired us to further explore the genetic regulation of the timing of the transition from the vegetative to the reproductive phase in forest trees, and here identification of the transcription factors involved in the phase transition in larch offers new potential targets for genetic manipulation to change the length of the juvenile phase (Table 2).

Table 1 Statistics of differentially expressed transcription factors and transcripts in juvenile vegetative (1- and 2-year-old) and adult reproductive (25- and 50-year-old) phases in *Larix kaempferi*

Comparison	Number of differentially expressed transcription factors/transcripts		
	Up	Down	Total
(I) 25 vs 1 year old	149/2712 (5.49%)	80/2539 (3.15%)	229/5251 (4.36%)
(II) 50 vs 1 year old	109/2119 (5.14%)	117/3219 (3.63%)	226/5338 (4.23%)
(III) 25 vs 2 years old	209/2958 (7.07%)	82/2408 (3.41%)	291/5366 (5.42%)
(IV) 50 vs 2 years old	111/2054 (5.4%)	113/3272 (3.45%)	224/5326 (4.21%)

Entry into the reproductive phase occurs earlier in larch than in spruce; however, an increase in the expression of some members of the MADS family, such as the putative homologue of *DAL1* in both larch (Table 2) and spruce (Carlsbecker et al. 2004), is found at the same age, indicating that at that age (5 years), some conserved and unknown biological processes occur in both species. Among the 562 transcription factors identified as DEGs in our previous study, 38 were MADS family members (see Table S3 in Li et al. 2017), also showing the importance of the MADS family in the phase change. So, identification of the targets of MADS family members and their partners will help to reveal these processes, and meanwhile, studying the regulation of the expression of MADS family members with age will also help to understand the age control of tree growth and development.

Based on the expression patterns of the transcription factors identified here (Table 2), some could be used as molecular markers to distinguish the special stages of tree growth and development and determine the state of plant materials after rejuvenation. For example, members of the AP2 (comp128327_c0_seq16) and ERF (comp129386_c0_seq9) families were only detected in 1- and 2-year-old trees, while several members of the MADS family were undetectable in these trees (Table 2). Notably, one member of the MADS family (comp126977_c0_seq2) was only detected in 25- and 50-year-old trees (Table 2). Nevertheless, to dissect changes in their expression with age, examples from more ages or developmental stages are still needed.

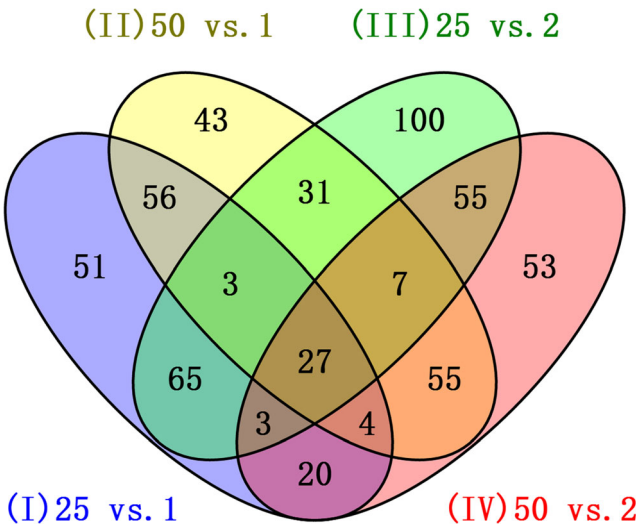

Fig. 4 Venn diagram of differentially expressed transcription factors in four comparisons: (I) 25 vs 1 year, (II) 50 vs 1 year, (III) 25 vs 2 years, and (IV) 50 vs 2 years in *Larix kaempferi*

Regulatory network of the phase change

Environmental control of tree growth and development occurs throughout the lifetime of a tree, and its effects might be evident in the tree at any time—once a certain level of a tree's responses is reached, changes in anatomy and/or physiology appear. Several genes have been identified as both regulators of the reproductive phase change and integrators of environmental signaling in annual plants (Blümel et al. 2015; Song et al. 2013), such as *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *MIR172*. Here, the expression of several putative homologues of *SOC1* in larch were found to increase with age (Table 2); the expression of two putative homologues of *AP2* family members, of which some are targets of miR172 (Zhu and Helliwell 2011), decreased with age (Table 2). These results indicated that photoperiod, ambient temperature, and age pathways take part in the phase change of larch, and conserved mechanisms governing growth and development in response to environmental cues occur in gymnosperms and angiosperms (Blümel et al. 2015; Song et al. 2013). Low levels of key regulators of flowering time at an early stage in the lifetime of larch might be the reason for no "flowering."

In addition, we concluded that other transcription factors identified here also play roles in the phase change, because the functions of their putative homologues in other plants have been studied. For example, plants with elevated levels of a *Dof* transcription factor, *CYCLING DOF FACTOR 1*, flower late (Imaizumi et al. 2005); a member of the MYB family, *ASYMMETRIC LEAVES 1*, accelerates flowering by increasing the amount of gibberellin-4 and facilitating CONSTANS to induce the expression of *FLOWERING LOCUS T* (Song et al. 2012); in barley and rice, miR171 regulation of some members of the GRAS (GAI-RGA-SCR) family mediate the phase change from vegetative to reproductive development (Curaba et al. 2013; Fan et al. 2015), and in rice, another member of the GRAS family, *DHD1*, also controls the flowering time (Zhang et al. 2019).

Table 2 Expression patterns of 27 transcription factors in six age categories of *Larix kaempferi* shared in four comparisons: (I) 25 vs 1 year, (II) 50 vs 1 year, (III) 25 vs 2 years, and (IV) 50 vs 2 years

Transcript ID	1-year-old	2-year-old	5-year-old	10-year-old	25-year-old	50-year-old	Family	Name	*Arabidopsis*	*E* value
comp122930_c0_seq2	5.52	3.11	0.39	0.15	0	0	AP2	AP2	AT4G36920.1	1.00E-101
comp128327_c0_seq16	6.37	1	0	0	0	0		AP2	AT4G36920.1	1.00E-98
comp111742_c0_seq2	0	0	2.06	1	3.41	4.02	C3H	OZF2	AT4G29190.1	1.00E-52
comp120092_c0_seq3	1.26	1.03	0.03	0.88	0	0	Dof	TMO6	AT5G60200.1	1.00E-35
comp124322_c0_seq3	3.16	2.96	5.68	0	48.06	0	ERF	ERF017	AT1G19210.1	7.00E-37
comp129386_c0_seq9	2.55	3.76	0	0	0	0		ERF082	AT1G50640.1	3.00E-31
comp130729_c0_seq1	0	0	0.39	0	0.73	1.25	G2-1ike	PHL	AT5G29000.1	6.00E-62
comp114072_c0_seq3	0	0	0.2	0	2.31	1.99	GRAS	SCL29	AT3G13840.1	3.00E-97
comp81209_c0_seq1	0	0	0.17	1	2.95	7.65	MADS-box	CAL	AT1G26310.1	3.00E-50
comp125095_c0_seq9	0	0	0.26	1.93	8.96	9.68		AGL6	AT2G45650.1	2.00E-71
comp126977_c0_seq16	0	0	0.73	1.62	3.61	3.52		AGL6	AT2G45650.1	6.00E-70
comp126977_c0_seq2	0	0	0	0	3.62	3.74		AGL6	AT2G45650.1	4.00E-69
comp126977_c0_seq5	0	0	0.34	1.81	5.64	10.32		AGL6	AT2G45650.1	4.00E-69
comp128412_c0_seq11	0.24	0.99	42.31	33.82	25.68	40.85		SOC1	AT2G45660.1	3.00E-56
comp128412_c0_seq23	0	0	27.91	17.82	34.37	55.12		SOC1	AT2G45660.1	4.00E-53
comp128412_c0_seq8	0	0	102.39	87.67	84.99	109.11		SOC1	AT2G45660.1	6.00E-56
comp128471_c0_seq24	0	0	1.24	1.8	4.52	5.34		SOC1	AT2G45660.1	1.00E-54
comp128471_c0_seq5	0	0	8.88	6.72	10.1	20.61		SOC1	AT2G45660.1	1.00E-54
comp128471_c0_seq9	0	0	91.69	76.32	62.42	98.54		SOC1	AT2G45660.1	7.00E-56
comp129709_c0_seq16	0	0	8.41	4.58	6.73	9.88		SOC1	AT2G45660.1	6.00E-57
comp129709_c0_seq2	0	0.11	4.35	5.63	3	8.01		SOC1	AT2G45660.1	4.00E-60
comp125095_c0_seq5	0.08	0	16.35	11.49	30.28	51.89		DAL1	AT3G02310.1	4.00E-843
comp126977_c0_seq13	0	0	16.16	13.3	31.39	49.34		DAL1	AT3G02310.1	4.00E-83
comp129017_c0_seq15	0	0.43	9.61	7.73	7.46	17.01		AGL19	AT4G22950.1	8.00E-22
comp128471_c0_seq14	0	0	5.85	6.91	6.18	7.77		SOC1	AT2G45660.1	4.00E-36
comp128471_c0_seq18	0	0	0	2.53	3.71	5.5		AGL42	AT5G62165.4	9.00E-38
comp128112_c0_seq2	0	0	1.36	1.64	1.67	2.37	MYB_related	TRFL6	AT1G72650.2	2.00E-56

The uppermost main stems produced in the current year were collected from 1-, 2-, 5-, 10-, 25-, and 50-year-old *L. kaempferi* trees in July 2011 for RNA extraction and transcriptome library construction and sequencing (Li et al. 2014, 2017). Here by comparing the transcriptomes of juvenile vegetative (1- and 2-year-old) and adult reproductive (25- and 50-year-old) phases, 27 transcription factors were identified, which were shared in four comparisons: (I) 25 vs 1 year, (II) 50 vs 1 year, (III) 25 vs 2 years, and (IV) 50 vs 2 years. Fragments per kilobase of transcript per million fragments was used to indicate the expression value of each transcript

Based on the expression patterns, annotations and predicted interactions of transcription factors identified here (Table 2), and the flowering time gene network in *Arabidopsis thaliana* (Blümel et al. 2015; Song et al. 2013), we propose a regulatory network model for the phase change from vegetative to reproductive development in larch (Fig. 5), which is helpful to understand the relationships of these transcription factors in the context of the current flowering pathways. However more work is still required to verify their functions and interactions with other molecular biology techniques.

Taken together, we conclude that the phase change from vegetative to reproductive development during aging of larch is orchestrated by putative homologues of major developmental regulators in angiosperms, and they constitute a complex regulatory network (Fig. 5). Although the positions of some of these

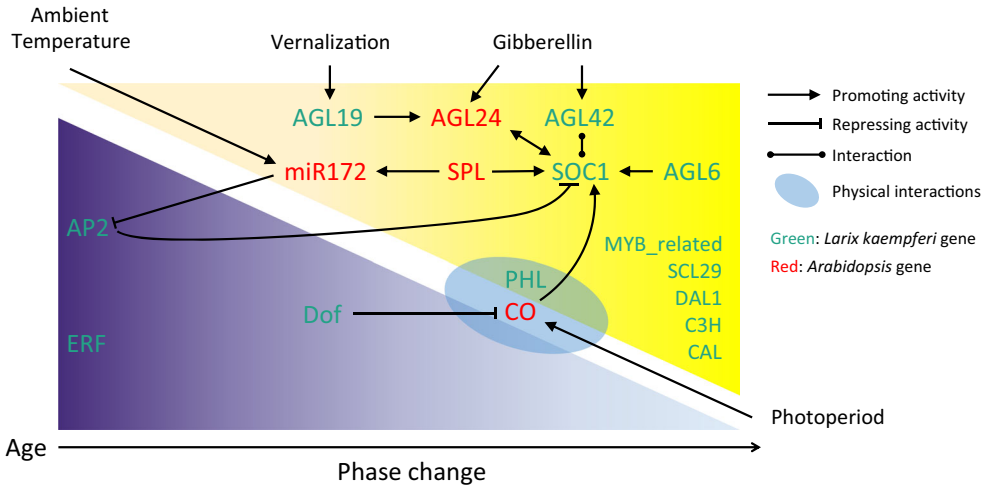

Fig. 5 Regulatory network model proposed for the *Larix kaempferi* phase change from vegetative to reproductive development based on the expression patterns, annotations, and predicted interactions of 27 transcription factors shared in four comparisons: (I) 25 vs 1 year, (II) 50 vs 1 year, (III) 25 vs 2 years, and (IV) 50 vs 2 years, and the flowering time gene network in *Arabidopsis thaliana* (Blümel et al. 2015; Song et al. 2013)

homologues in the network cannot be confirmed, their expression levels increase or decrease gradually year by year over the course of aging, characteristic of regulators of the phase change.

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Author contributions Wan-Feng Li conceived, designed, and carried out the study. Wei-Bo Xiang analyzed the data and wrote the manuscript with Wan-Feng Li. Shou-Gong Zhang and Li-Wang Qi provided suggestions on the experimental design and analyses. All authors read and approved the manuscript.

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