DENDROBIOLOGY

2018, vol. 79, 111–118

http://dx.doi.org/10.12657/denbio.079.010

Dehuo Hu†, Yan Su†, Shujuan Wu, Jiezhen Wu, Runhui Wang, Shu Yan, Ruping Wei, Huiquan Zheng*

Association of SRAP markers with juvenile wood basic density and growth traits in *Cunninghamia lanceolata* (Lamb.) Hook

Received: 1 October 2017; Accepted: 15 January 2018

Abstract: Application of sequence-related amplified polymorphism (SRAP) markers to unravel variations and relationships with biological and morphological traits has been reported in a variety of plant species, and their potential for breeding has also been highlighted.
(1) Assess the diversity level of a *Cunninghamia lanceolata* (Chinese fir) genetic panel based on phenotypic traits and SRAP markers, (2) identify SRAP loci linked to juvenile wood basic density (JWBD) and growth traits, and (3) address the overlap of the trait-associated SRAP markers during the juvenile and mature stages of this species.
A total of 227 Chinese fir genotypes were subjected to phenotype, SRAP genotyping, and marker-trait association analyses.
A total of 564 unambiguous SRAP bands and 558 polymorphic loci were identified from the genotypes. The overall percentage of polymorphic bands, polymorphism information content, Nei's gene diversity, and Shannon's Information Index were 98.9%, 0.2576, 0.3196 and 0.4838, respectively. An analysis of molecular variance further demonstrated that the genotypes varied significantly at SRAP polymorphisms ($p < 0.01$). A wide genetic distance span from 0.0531 to 0.9097 was also observed; most (94.9%) fell within the range of 0.3000–0.6999. An association analysis based on general linear model (GLM) and mixed linear model (MLM) unraveled 21, 26, 25, and 19 marker-trait associations for JWBD, height (H), diameter at breast height (DBH, 1.3 m) and stem volume (V), respectively. These marker-trait associations corresponded to 64 different SRAP markers; 46 of these were linked to only one trait, while the other 18 markers appeared to be associated with more than one trait but limited to growth traits. Overall, the SRAP markers represented R^2 (percentage of the phenotypic variation explained by marker) values of 1.7–9.2% for the GLM and 1.7–5.6% for the MLM. Strikingly, the significant trait-associated marker list seemed to be rather different from that of the previous study performed on mature traits (WBD, H, DBH and V), except for overlap of two markers. This study demonstrated an association of SRAP markers with JWBD and growth traits in Chinese fir. The results further our understanding of the genetic basis of the Chinese fir WBD and growth traits at the juvenile stage.

Keywords: Chinese fir, SRAP, diversity, wood basic density, association analysis

Addresses: D. Hu, S. Wu, J. Wu, R. Wang, S. Yan, R. Wei, H. Zheng, Guangdong Provincial Key Laboratory of Silviculture, Protection and Utilization; Guangdong Academy of Forestry, 510520 Guangzhou, People's Republic of China, e-mail: zhenghq@sinogaf.cn
Y. Su, College of Forestry and Landscape Architecture, South China Agricultural University, 510642 Guangzhou, People's Republic of China

†These authors contributed equally to this work.
*Corresponding author

Introduction

Cunninghamia lanceolata (Lamb.) Hook, known as Chinese fir, is a fast-growing conifer tree species that widely grows in southern China and northern Vietnam (Zheng et al., 2015a; Zhang et al., 2016). This conifer occupies ~21.4% of man-made plantations and provides up to 30% of the harvested logs for the Chinese timber industry (Zhang et al., 2016; Li et al., 2017). Over the last five decades, breeding success for growth rate and success intensive silvicultural management have significantly reduced the harvest time of this species. However, fast-growing trees harvested at an early age contain a large proportion of juvenile wood commonly characterized as poorer quality (e.g. low wood basic density), which has been a challenge for the current conifer forestry (Li et al., 2012; Boruszewski et al., 2017).

Wood basic density has been demonstrated to be under moderate to strong genetic control, and it can be improved through selection (Li et al., 2012; Zheng et al., 2015b). However, this trait commonly shows a significant negative relationship with growth traits, making the simultaneous improvement of wood density and growth traits challenging in a conventional breeding program. Molecular markers provide a useful tool to track the genetic loci linked to complex traits at the DNA level and may help to accelerate multi-trait selection through a marker-assisted breeding strategy (Agarwal et al., 2008; Aneja et al., 2012; Khadivi-Khub et al., 2014). Of the available marker systems, sequence-related amplified polymorphism (SRAP) is one of the most popular markers based on the polymerase chain reaction (PCR). This marker system specifically amplifies coding regions of the genome and produces a moderate number of genome-wide fragments with high reproducibility and versatility (Li & Quiros, 2001; Robarts & Wolfe, 2014). Moreover, it is simple, efficient, cost-effective, and does not require any prior knowledge of the genome sequence (Aneja et al., 2012; Zheng et al., 2015c). The use of SRAP markers to unravel variations and relationships with biological and morphological traits have been reported in a variety of plant species, and their potential for breeding has also been highlighted (Aneja et al., 2012).

In a previous study, we employed SRAP markers to survey the genomic variability of 50 Chinese fir genotypes and preliminarily identified 99 significant marker-trait associations for their mature growth and wood property traits (Zheng et al., 2015c). Herein, we employed a 6-year-old (juvenile state) population (n = 227) to carry out an association analysis as part of the Chinese fir breeding program with the aim to (1) assess the diversity level of the population based on phenotypic traits and SRAP markers, (2) identify SRAP loci linked to juvenile wood basic density (JWBD) and growth traits, and (3) address the overlap of the trait-associated SRAP markers during the juvenile and mature stages of Chinese fir.

Materials and methods

Association population

A total of 227 Chinese fir genotypes grafted for the breeding program were used in this study. These genotypes had divergent geographical origins (covering the main breeding regions of China including Guangdong, Fujian, Hunan, Guangxi, Jiangxi, and Guizhou), family background and morphological characteristics. The plant material was established by synchronously grafting the scions onto 2-year-old rootstock with 4–6 repeats (4–6 ramets per genotype) and a 4 × 4 m spacing since 2010 at the Xiaokeng State Forest Farm (Guangdong, China, 24°70'N, 113°81'E, 328–339 m above sea level). The trees were maintained using standard commercial practices.

Phenotype measurements

Three ramets for each genotype similar in size and vigor were measured for height (H), diameter at breast height (DBH, 1.3 m) and wood basic density (WBD) in 2016 (6-year-old, juveniles). The tree stem volume (V) was evaluated by the formula of $V = 5.8777042 \times 10^{-5} \times DBH^{1.9699831} \times H^{0.89646157}$ (Zheng et al., 2012). An incremental core method was employed for the WBD measurement according to Zheng et al. (2015b)

Genomic SRAP-PCR

Genomic SRAP-PCR was carried out with 35 authentic SRAP primer sets and a standard protocol according to Zheng et al. (2015a). The PCR products were separated on a 2% agarose gel with 0.002% GoldView™ Nucleic Acid Gel Stain (10,000×) (Beijing Dingguo Changsheng Biotechnology Co., LTD, China), and directly recorded with a Universal Hood II imaging system (Bio-Rad, Hercules, CA, USA). Each PCR assay for the same DNA sample was repeated three times in separate experiments. The D2000 DNA ladder (Tiangen, Beijing, China) served as a molecular weight marker.

Data analysis

Variability in each trait among genotypes was assessed by *F* value (F-statistic) and coefficient of variation (*CV*) following the one-way analysis of variance (ANOVA) calculation in Statistical Analysis System (SAS V 8.1) (SAS Institute, Cary, NC,

USA). Repeatability (R) for each trait was evaluated based on the formula of: $R = 1 - 1/F$. The SAS PROC CORR program was employed to calculate the phenotypic correlation coefficient.

The SRAP bands were manually scored as present (1) or absent (0) at a particular location in each lane. Only bright, reliable, and clearly distinguishable bands were recorded. The SRAP-based genetic variability was assessed using binary data of 1 and 0. The polymorphism information content (PIC) value for each primer set was calculated with PowerMarker V 3.25 (Liu & Muse, 2005). The software POPGENE V 1.31 was employed to calculate Nei's gene diversity (h) and estimate the Shannon's Information Index (I) (Yeh et al., 1999). GenAlEx V 6.5 incorporated into Microsoft Excel 2010 was implemented to perform analysis of molecular variance (AMOVA) (Peakall & Smouse, 2012). Numerical Taxonomy Multivariate Analysis System (NTSYSpc V 2.10e) was used to generate the distance matrix and the two-dimensional principal coordinate analysis (PCoA) plot of the genotypes (Rohlf, 2000).

An association analysis was conducted based on general linear model (GLM) and mixed linear model (MLM) with TASSEL V 5.0 software (Bradbury et al., 2007). Markers exhibiting a statistic p-value < 0.05 in both models were considered significantly associated with a particular phenotypic trait.

Results

Variation in juvenile wood basic density and growth traits

Extensive variations were observed for JWBD and growth traits (H, DBH, and V) in the present 6-year-old (juvenile state) Chinese fir population (n = 227) (Table 1). JWBD varied from 0.2176 g/cm^3 to 0.3334 g/cm^3 (mean = 0.2845 g/cm^3) at a significantly different level similar to the growth traits. However, the magnitude of the difference seemed to be consistently lower than that of the growth traits (1.53 vs. 3.15, 4.15, and 37.59). The *CV* for JWBD appeared to be profound (7.4%) but still lower compared to those for the growth traits (15.3–40.3%). Notably, estimates of repeatability (broad-sense heritability) for JWBD, H, DBH and V were 0.50, 0.72, 0.69 and 0.83 respectively, indicating moderate to high genetic control. This finding agreed with previous observations on 12-year-old and 24-year-old Chinese fir populations in terms of their growth and wood property traits (Zheng et al., 2015b, 2015c).

Table 2. Phenotypic correlations among juvenile wood basic density and growth traits in Chinese fir. H – height; DBH – diameter at breast height; V – stem volume; JWBD – juvenile wood basic density. *$p < 0.05$, **$p < 0.01$

	JWBD	H	DBH	V
JWBD	1.0000			
H	–0.1043	1.0000		
DBH	–0.1778**	0.7785**	1.0000	
V	–0.1789**	0.8411**	0.9551**	1.0000

As expected, the growth traits were strongly correlated with each other ($p < 0.01$), while JWBD appeared to be negatively correlated to all growth traits, particularly DBH and V ($p < 0.01$) (Table 2).

SRAP profile

Thirty-five SRAP primer sets utilized for the present genetic panel (n = 227) yielded 11–21 bands with an average of 16.1 bands per primer set (Table 3). The percentage of polymorphic bands (PPB) spanned from 92.3 to 100.0% with an average of 98.9% per primer set. Collectively, a total of 564 bands were scored, of which 558 (98.9%) were polymorphic. The overall PPB was higher than that of previous studies (89.4% and 93.1 %) (Zheng et al., 2015c; Duan et al., 2016), possibly due to the increased population size and/or diversity level.

According to the population SRAP frequency, we grouped SRAPs into three categories: rare SRAPs (found in < 76 genotypes), medium frequency SRAPs (found in 76–150 genotypes), and common SRAPs (found in > 150 genotypes). Strikingly, most of the SRAPs (274; 48.6%) belong to the rare group, only 124 SRAPs (22.0%) were common (Fig. 1).

SRAP-based diversity assessment

A comprehensive knowledge of the level of intra-population diversity would improve the

Table 1. Descriptive statistics of the juvenile wood basic density and growth traits for 227 6-year-old Chinese fir genotypes. H – height; DBH – diameter at breast height; V – stem volume; JWBD – juvenile wood basic density; SD – standard deviation; *F* value – the ANOVA (analysis of variance) F-statistic (**$p < 0.01$); *CV* – coefficient of variation; *R* – repeatability

Trait	Range	Mean±SD	*F* value	*CV* (%)	R
JWBD (g/cm^3)	0.2176–0.3334	0.2845±0.0205	2.01**	7.4	0.50
H (m)	3.4–10.7	6.8±1.3	3.62**	15.3	0.72
DBH (cm)	6.1–25.3	14.1±3.5	3.19**	19.4	0.69
V (m^3)	0.0071–0.2669	0.0692±0.0445	6.01**	40.3	0.83

Table 3. Genetic diversity analysis for the 227 Chinese fir genotypes as revealed by 35 sequence-related amplified polymorphism (SRAP) primer sets. TNB – total number of bands; NPB – number of polymorphic bands; PPB – percentage of polymorphic bands; PIC – polymorphic information content; *h* – Nei's gene diversity; *I* – Shannon's Information Index; SD – standard deviation

SRAP Primer set*	TNB	NPB	PPB (%)	PIC	h	I
Me1-Em1	19	19	100.0	0.2173	0.2622	0.4158
Me1-Em19	18	18	100.0	0.2347	0.2935	0.4457
Me1-Em20	11	11	100.0	0.2637	0.3242	0.4941
Me2-Em26	13	13	100.0	0.1984	0.2392	0.3808
Me3-Em17	16	15	93.8	0.2695	0.3364	0.5026
Me4-Em5	16	16	100.0	0.2433	0.3000	0.4572
Me4-Em21	17	17	100.0	0.2604	0.3175	0.4896
Me9-Em14	17	16	94.1	0.2153	0.2638	0.4079
Me10-Em20	15	15	100.0	0.2578	0.3203	0.4845
Me10-Em21	17	17	100.0	0.2662	0.3357	0.4999
Me10-Em26	19	19	100.0	0.2839	0.3541	0.5301
Me11-Em5	15	15	100.0	0.2599	0.3205	0.4887
Me11-Em13	16	16	100.0	0.3345	0.4348	0.6196
Me11-Em20	16	16	100.0	0.3005	0.3745	0.5578
Me11-Em21	13	13	100.0	0.2436	0.3075	0.4584
Me12-Em1	14	13	92.9	0.2946	0.3733	0.5457
Me12-Em19	17	17	100.0	0.3179	0.4037	0.5893
Me13-Em5	13	12	92.3	0.2576	0.3214	0.4808
Me13-Em18	15	15	100.0	0.2727	0.3356	0.5108
Me15-Em1	13	13	100.0	0.2325	0.2916	0.4411
Me15-Em26	15	14	93.3	0.2578	0.3218	0.4814
Me16-Em1	17	17	100.0	0.2285	0.2716	0.4336
Me16-Em19	18	18	100.0	0.2276	0.2775	0.4317
Me17-Em19	17	16	94.1	0.2614	0.3269	0.4881
Me17-Em21	21	21	100.0	0.2449	0.3061	0.4620
Me18-Em19	20	20	100.0	0.2214	0.2631	0.4214
Me19-Em1	16	16	100.0	0.2687	0.3349	0.5043
Me19-Em18	15	15	100.0	0.2519	0.3073	0.4755
Me19-Em19	18	18	100.0	0.2696	0.3304	0.5046
Me19-Em22	12	12	100.0	0.2706	0.3374	0.5050
Me20-Em1	19	19	100.0	0.2926	0.3673	0.5458
Me20-Em17	14	14	100.0	0.2280	0.2755	0.4348
Me20-Em19	20	20	100.0	0.2767	0.3468	0.5186
Me20-Em26	17	17	100.0	0.2327	0.2907	0.4424
Me21-Em19	15	15	100.0	0.2577	0.3172	0.4831
Total	564	558	98.9	–	–	–
Mean	16.1	15.9	98.9	0.2576	0.3196	0.4838
SD	2.4	2.5	2.5	0.0293	0.0410	0.0510

*The primer sequences of present 35 authentic SRAP primer sets referred from Zheng et al. (2015a).

effectiveness of breeding programs (Dadras et al., 2014), while high genetic diversity facilitates a trait association analysis. In the present investigation, diversity parameters (polymorphic information content (PIC), Nei's gene diversity (*h*), Shannon's Information Index (*I*)) varied for each primer set but at a comprehensive level cross genotypes (Table 3). The PIC was 0.1984–0.3345 (mean = 0.2576), while *h* was 0.2392–0.4348 (mean = 0.3196) and *I* was

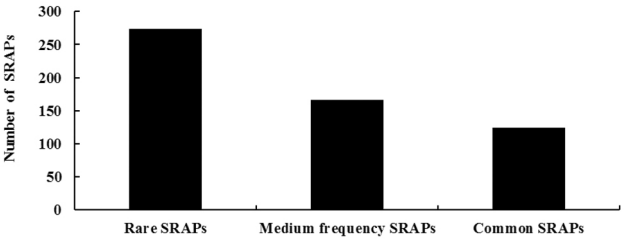

Fig. 1. Distribution of different sequence-related amplified polymorphism (SRAP) types in 227 Chinese fir genotypes. SRAPs were grouped into three categories: rare SRAPs found in < 76 genotypes, medium frequency SRAPs found in 76–150 genotypes, and common SRAPs found in > 150 genotypes

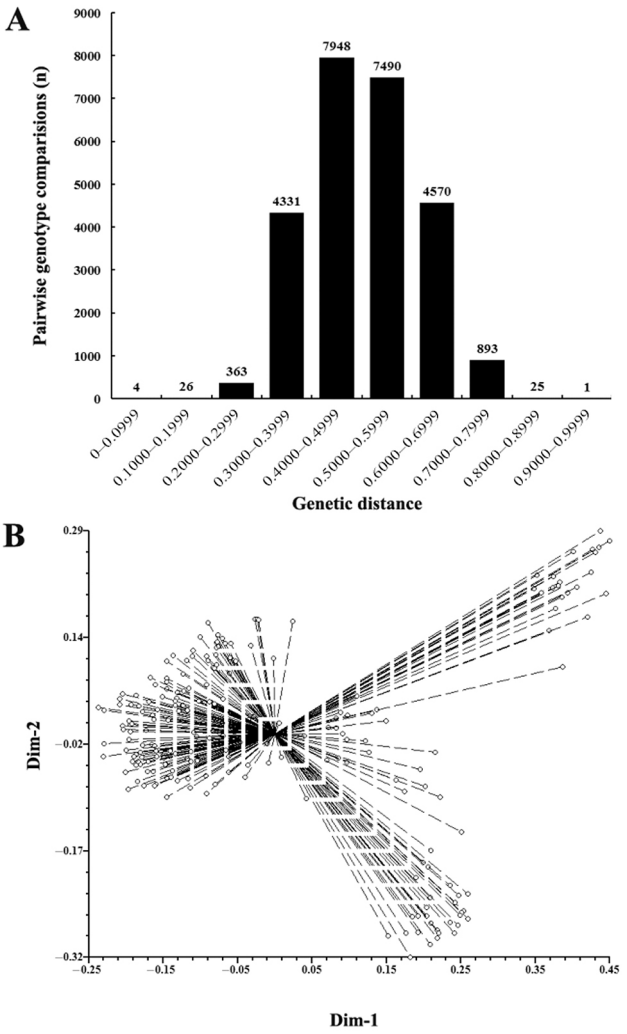

Fig. 2. Divergence of the 227 Chinese fir genotypes revealed by sequence-related amplified polymorphism (SRAP) markers. (A) Distribution of pairwise genetic distance for the genotypes; (B) Genetic similarity matrix-based principal coordinate analysis for the genotypes

0.3808–0.6196 with an average value of 0.4838. The AMOVA further demonstrated that the genotypes varied significantly at SRAP polymorphisms ($p < 0.01$).

Herein, pairwise genetic distances (Nei's 1972) of the genotypes were also calculated based on SRAPs. A broad genetic distance span from 0.0531 to 0.9097 (mean = 0.5053) was observed, and most (94.9%) of these fell in the range of 0.3000–0.6999 (Fig. 2 A). To better represent intra-population diversity, a two-dimensional PCoA plot graph is shown for the genotypes (Fig. 2 B). Apparently, most of the genotypes differed genetically from each other with a diffuse distribution pattern.

Marker-trait association

The SRAP markers were then deployed to detect the association of the putative markers linked to JWBD and growth traits using TASSEL V 5.0 software and the GLM and MLM models. A total of 91 authentic marker-trait associations ($p < 0.05$ in both the GLM and MLM models) corresponding to 64 SRAP markers were identified (Table 4). Overall, the SRAP markers represented a R^2 (percentage of the phenotypic variation explained by marker) values of 1.7–9.2% for GLM and 1.7–5.6% for the MLM. Of the identified SRAP markers, 46 linked to only one trait. The other 18 SRAP markers were associated with more than one trait but were limited to the growth traits (e.g., Me17/Em19$_{200}$ was significantly associated with H, DBH, and V), possibly due to pleiotropism or QTL interactions of the genomic loci relevant to growth (Wang et al., 2013). Such crosslinking also agreed with previous findings that growth traits are positively correlated with each other at significant level (Table 2). According to the previous definition of the SRAPs, 50% (32) of the trait-associated SRAPs were rare SRAPs (found in < 76 genotypes). Ten of the 21 JWBD associated SRAPs (Me1/Em1$_{570}$, Me2/Em26$_{470}$, Me4/Em5$_{900}$, Me4/Em21$_{930}$, Me10/Em26$_{1100}$, Me11/Em21$_{450}$, Me12/Em1$_{480}$, Me15/Em26$_{740}$, Me16/Em1$_{250}$, and Me20/Em1$_{1500}$) were rare and only five SRAPs (Me3/Em17$_{750}$, Me4/Em5$_{830}$, Me10/Em20$_{750}$, Me11/Em20$_{1000}$, and Me20/Em17$_{420}$) belonged to the common group.

Strikingly, the significant trait-associated marker list seemed to be rather different from that of the previous study performed on mature traits (WBD, H, DBH and V), except for overlap of two markers including Me2/Em26$_{470}$ and Me13/Em18$_{150}$ that corresponded to JWBD/WBD and H respectively (Zheng et al., 2015c).

Discussion

Association analysis is a useful tool to decipher the genetic architecture for the observed variation in phenotypes. This study demonstrated an association of SRAP markers with JWBD and growth traits in Chinese fir. The association information could help breeders to purge the trait negative correlation (JWBD vs. growth traits) through marker-assisted repeated selection.

SRAP has proven to be a useful tool for genetic analysis of woody plant species (Duan et al., 2016). This technique has been successfully employed to elucidate genetic variation in a variety of Chinese fir populations (Zheng et al., 2015c; Duan et al., 2016; Li et al., 2017). In this study, we once again used the previous methodology to carry out the SRAP assay

Table 4. Sequence-related amplified polymorphism (SRAP) markers associated with juvenile wood basic density and growth traits in Chinese fir. JWBD – juvenile wood basic density; H – height; DBH – diameter at breast height; V – stem volume; R^2 – the percentage of the phenotypic variation explained by marker. Only the markers with significant marker-trait associations ($p < 0.05$) in both the GLM (general linear model) and MLM (mixed linear model) are shown. The marker is named with its original primer set followed by size (bp). Markers linked to only one trait are underlined.

Trait	The significantly associated SRAP markers ($p < 0.05$)	Range of GLM R^2 (%)	Range of MLM R^2 (%)
JWBD	Me1/Em1$_{570}$, Me1/Em19$_{440}$, Me2/Em26$_{470}$, Me2/Em26$_{630}$, Me3/Em17$_{750}$, Me4/Em5$_{620}$, Me4/Em5$_{830}$, Me4/Em5$_{900}$, Me4/Em21$_{930}$, Me10/Em20$_{750}$, Me10/Em26$_{1100}$, Me11/Em20$_{1000}$, Me11/Em21$_{450}$, Me12/Em1$_{480}$, Me13/Em5$_{250}$, Me15/Em26$_{740}$, Me16/Em1$_{250}$, Me20/Em1$_{430}$, Me20/Em1$_{1500}$, Me20/Em17$_{420}$, Me20/Em19$_{1000}$	1.9–5.6	1.8–4.6
H	Me1/Em20$_{380}$, Me2/Em26$_{1100}$, Me3/Em17$_{560}$, Me10/Em20$_{650}$, Me10/Em26$_{180}$, Me10/Em26$_{1700}$, Me11/Em20$_{1900}$, Me11/Em20$_{2000}$, Me12/Em19$_{220}$, Me13/Em5$_{150}$, Me13/Em5$_{700}$, Me13/Em18$_{150}$, Me15/Em26$_{550}$, Me16/Em19$_{1600}$, Me17/Em19$_{200}$, Me17/Em19$_{1450}$, Me17/Em19$_{1600}$, Me17/Em21$_{180}$, Me18/Em19$_{260}$, Me19/Em19$_{270}$, Me19/Em19$_{1400}$, Me19/Em22$_{350}$, Me20/Em1$_{110}$, Me20/Em19$_{750}$, Me20/Em26$_{350}$, Me20/Em26$_{620}$	1.7–9.2	1.8–4.3
DBH	Me1/Em1$_{700}$, Me1/Em1$_{2000}$, Me1/Em19$_{250}$, Me1/Em20$_{380}$, Me2/Em26$_{510}$, Me2/Em26$_{750}$, Me2/Em26$_{1200}$, Me3/Em17$_{560}$, Me4/Em5$_{1400}$, Me4/Em21$_{750}$, Me10/Em26$_{1500}$, Me11/Em20$_{150}$, Me11/Em20$_{1900}$, Me12/Em1$_{230}$, Me12/Em19$_{500}$, Me13/Em5$_{150}$, Me15/Em1$_{160}$, Me15/Em26$_{550}$, Me15/Em26$_{600}$, Me16/Em1$_{340}$, Me17/Em19$_{200}$, Me17/Em19$_{1450}$, Me20/Em26$_{350}$, Me20/Em26$_{620}$, Me21/Em19$_{800}$	1.8–7.9	1.7–5.6
V	Me1/Em1$_{700}$, Me1/Em1$_{2000}$, Me1/Em19$_{250}$, Me1/Em20$_{380}$, Me2/Em26$_{510}$, Me2/Em26$_{1100}$, Me2/Em26$_{1200}$, Me3/Em17$_{560}$, Me4/Em5$_{1400}$, Me11/Em20$_{1900}$, Me13/Em5$_{150}$, Me13/Em5$_{400}$, Me15/Em26$_{550}$, Me15/Em26$_{600}$, Me17/Em19$_{200}$, Me17/Em19$_{1450}$, Me20/Em26$_{350}$, Me20/Em26$_{620}$, Me21/Em19$_{800}$	1.7–8.3	1.7–5.0

(Zheng et al., 2015a), and found that most of the obtained SRAPs (48.6%) belong to the rare group, only 124 SRAPs (22.0%) were common. Next-generation sequencing-based SNP markers have also been used in a Chinese fir genetic analysis, but most of the available SNPs appeared to be common (Su et al., 2016). This divergence indicates that the medium-throughput SRAP technique is also valuable when paired with the next-generation approach (Robarts & Wolfe, 2014). Lees et al. (2016) demonstrated that SRAP has similar efficacy to next-generation sequencing technology for a cluster analysis of *Brassica napus* genotypes.

Several studies have applied SNP markers to elucidate the causative genetic differences linked to observed phenotypic variation in conifers (González-Martínez et al., 2007; Eckert et al., 2009; Beaulieu et al., 2011; Eckert et al., 2012; Parchman et al., 2012; Prunier et al., 2013; Uchiyama et al., 2013; Lamara et al., 2016; Ganthaler et al., 2017). SNPs are concentrated on transition or transversion variation regardless of the presence/absence information, whereas the PCR-based marker technique facilitates breeders to capture presence/absence information of genomic fragments that give rise to phenotypic variation. Such a demonstration has been represented by some ISSR or SSR association studies on quince (*Cydonia oblonga*), white savory (*Satureja mutica*), *Coffea canephora* and *Calophyllum inophyllum*, respectively (Ganopoulos et al., 2011; Pawar et al., 2011; Khadivi-Khub et al., 2014; Achar et al., 2015). Based on amplified fragment length polymorphism (AFLP)-PCR, Dadras et al. (2014) successfully identified a set of AFLP markers that closely linked to seven growth traits in tobacco. Pereira da Costa et al. (2014) reported applying SRAP to identify the DNA loci linked to tomato fruit quality traits. In a previous study, we utilized SRAP technique to detect the trait-associated markers in Chinese fir, and 77 different SRAPs related to 24-year-old growth and wood property traits were identified (Zheng et al., 2015c). Herein, we searched for trait-associated markers specifically linked to Chinese fir JWBD and growth traits (6-year-old). A set of significant trait-associated SRAPs were found, but the marker list seemed to be different from that of a previous study performed on mature traits (WBD, H, DBH, and V; 24-year-old), suggesting a divergent genetic architecture for JWBD and growth traits in Chinese fir when compared to the mature stage. Nevertheless, an overlap was still observed in the studies, as evidenced by $Me2/Em26_{470}$ and $Me13/Em18_{150}$ that corresponded to JWBD/WBD and H respectively. $Me2/Em26_{470}$ has been sequenced (469-bp in length) and deposited in NCBI with Accession No. KP314214.

The present study provides new insights for understanding the genetic basis of Chinese fir WBD and growth traits at the juvenile stage. However, further investigations to track the marker-trait association trend on transition of the juvenile stage to mature stage on the same association population are needed.

Acknowledgements

This research was supported by the Special Fund for Forest Scientific Research in the Public Welfare (No. 201404127), the Specific Programs in Forestry Science and Technology Innovation of Guangdong (No. 2017KJCX026) and the Special Plan for the Cultivation of High-level Talents of Guangdong (No. 2014TQ01N140).

References

Achar D, Awati MG, Udayakumar M & Prasad TG (2015) Identification of putative molecular markers associated with root traits in *Coffea canephora* Pierre ex Froehner. Molecular Biology International 2015: 532386. doi:10.1155/2015/532386.

Agarwal M, Shrivastava N & Padh H (2008) Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Reports 27: 617–631. doi:10.1007/s00299-008-0507-z.

Aneja B, Yadav NR, Chawla V & Yadav RC (2012) Sequence-related amplified polymorphism (SRAP) molecular marker system and its applications in crop improvement. Molecular Breeding 30: 1635–1648. doi:10.1007/s11032-012-9747-2.

Beaulieu J, Doerksen T, Boyle B, Clément S, Deslauriers M, Beauseigle S, Blais S, Poulin PL, Lenz P, Caron S, Rigault P, Bicho P, Bousquet J & Mackay J (2011) Association genetics of wood physical traits in the conifer white spruce and relationships with gene expression. Genetics 188: 197–214. doi:10.1534/genetics.110.125781.

Boruszewski P, Jankowska A & Kurowska A (2017) Comparison of the structure of juvenile and mature wood of *Larix decidua* Mill. from fast-growing plantations in Poland. Bioresources 12 : 1813–1825.

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y & Buckler ES (2007) TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics 23: 2633–2635. doi:10.1093/bioinformatics/btm308.

Dadras AR, Sabouri H, Nejad GM, Sabouri A & Shoai-Deylami M (2014) Association analysis, genetic diversity and structure analysis of tobacco based on AFLP markers. Molecular Biology Reports 41: 3317–3329. doi:10.1007/s11033-014-3194-6.

Duan HJ, Hu DH, Li Y & Zheng HQ (2016) Characterization of a collection of Chinese fir elite gen-

otypes using sequence-related amplified polymorphism markers. Journal of Forestry Research 27: 1105–1110. doi:10.1007/s11676-016-0233-2.

Eckert AJ, Bower AD, Wegrzyn JL, Pande B, Jermstad KD, Krutovsky KV, St Clair JB & Neale DB (2009) Association genetics of coastal Douglas fir (*Pseudotsuga menziesii* var. *menziesii*, Pinaceae). I. Cold-hardiness related traits. Genetics 182: 1289–302. doi:10.1534/genetics.109.102350.

Eckert AJ, Wegrzyn JL, Cumbie WP, Goldfarb B, Huber DA, Tolstikov V, Fiehn O & Neale DB (2012) Association genetics of the loblolly pine (*Pinus taeda*, Pinaceae) metabolome. New Phytologist 193: 890–902. doi:10.1111/j.1469-8137.2011.03976.x.

Ganopoulos I, Merkouropoulos G, Pantazis S, Tsipouridis C & Tsaftaris A (2011) Assessing molecular and morpho-agronomical diversity and identification of ISSR markers associated with fruit traits in quince (*Cydonia oblonga*). Genetics and Molecular Research 10: 2729–2746. doi:10.4238/2011.November.4.7.

Ganthaler A, Stöggl W, Mayr S, Kranner I, Schüler S, Wischnitzki E, Sehr EM, Fluch S & Trujillo-Moya C (2017) Association genetics of phenolic needle compounds in Norway spruce with variable susceptibility to needle bladder rust. Plant Molecular Biology 94: 229–251. doi:10.1007/s11103-017-0589-5.

González-Martínez SC, Wheeler NC, Ersoz E, Nelson CD & Neale DB (2007) Association genetics in *Pinus taeda* L. I. Wood property traits. Genetics 175: 399–409. doi:10.1534/genetics.106.061127.

Khadivi-Khub A, Karimi E & Hadian J (2014) Population genetic structure and trait associations in forest savory using molecular, morphological and phytochemical markers. Gene 546: 297–308. doi:10.1016/j.gene.2014.05.062.

Lamara M, Raherison E, Lenz P, Beaulieu J, Bousquet J & MacKay J (2016) Genetic architecture of wood properties based on association analysis and co-expression networks in white spruce. New Phytologist 210: 240–255. doi:10.1111/nph.13762.

Lees CJ, Li G & Duncan RW (2016) Characterization of *Brassica napus* L. genotypes utilizing sequence-related amplified polymorphism and genotyping by sequencing in association with cluster analysis. Molecular Breeding 36: 155. doi:10.1007/s11 032-016-0576-6.

Li G & Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theoretical and Applied Genetics 103: 455–461.

Li M, Chen XZ, Huang MS, Wu PF & Ma XQ (2017) Genetic diversity and relationships of ancient Chinese fir (*Cunninghamia lanceolata*) genotypes revealed by sequence-related amplified polymorphism markers. Genetic Resources and Crop Evolution 64: 1087–1099. doi:10.1007/s10722-016-0428-6.

Li X, Wu HX & Southerton SG (2012) Identification of putative candidate genes for juvenile wood density in *Pinus radiata*. Tree Physiology 32: 1046–1057. doi:10.1093/treephys/tps060.

Liu K & Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21: 2128–2129. doi:10.1093/bioinformatics/bti282.

Parchman TL, Gompert Z, Mudge J, Schilkey FD, Benkman CW & Buerkle CA (2012) Genome-wide association genetics of an adaptive trait in lodgepole pine. Molecular Ecology 21: 2991–3005. doi:10.1111/j.1365-294X.2012.05513.x.

Pawar KD, Joshi SP & Thengane SR (2011) Association between chemical and genetic variation in *Calophyllum inophyllum*, a medicinally important tree of the Western Ghats of India. Plant Systematics and Evolution 292: 257–265. doi:10.1007/s00606-010-0409-8.

Peakall R & Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28: 2537–2539. doi:10.1093/bioinformatics/bts460.

Pereira da Costa JH, Rodríguez GR, Pratta GR, Picardi LA & Zorzoli R (2014) Pericarp polypeptides and SRAP markers associated with fruit quality traits in an interspecific tomato backcross. Genetics and Molecular Research 13: 2539–2547. doi:10.4238/2014.January.24.10.

Prunier J, Pelgas B, Gagnon F, Desponts M, Isabel N, Beaulieu J & Bousquet J (2013) The genomic architecture and association genetics of adaptive characters using a candidate SNP approach in boreal black spruce. BMC Genomics 14: 368. doi:10.1186/1471-2164-14-368.

Robarts DWH & Wolfe AD (2014) Sequence-related amplified polymorphism (SRAP) markers: A potential resource for studies in plant molecular biology. Applications in Plant Sciences 2: 1400017. doi:10.3732/apps.1400017.

Rohlf FJ (2000) NTSYSpc, numerical taxonomy and multivariate analysis system version 2.10e. Exeter Software, Setauket.

Su Y, Hu DH & Zheng HQ (2016) Detection of SNPs based on DNA specific-locus amplified fragment sequencing in Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook). Dendrobiology 76: 73–79. doi:10.12657/denbio.076.007.

Uchiyama K, Iwata H, Moriguchi Y, Ujino-Ihara T, Ueno S, Taguchi Y, Tsubomura M, Mishima K, Iki T, Watanabe A, Futamura N, Shinohara K & Tsu-

mura Y (2013) Demonstration of genome-wide association studies for identifying markers for wood property and male strobili traits in *Cryptomeria japonica*. PLoS One 8: e79866. doi:10.1371/journal.pone.0079866.

Wang XQ, Yu Y, Li W, Guo HL, Lin ZX & Zhang XL (2013) Association analysis of yield and fiber quality traits in *Gossypium barbadense* with SSRs and SRAPs. Genetics and Molecular Research 12: 3353–3362. doi:10.4238/2013.September.4.1.

Yeh FC, Yang RC & Boyle T (1999) PopGene: Microsoft Window-based freeware for population genetic analysis, version 1.31. University of Alberta and Center for International Forestry Research, Edmonton.

Zhang YX, Han XJ, Sang J, He XL, Liu MY, Qiao GR, Zhuo RY, He GP & Hu JJ (2016) Transcriptome analysis of immature xylem in the Chinese fir at different developmental phases. Peer Journal 4: e2097. doi:10.7717/peerj.2097.

Zheng HQ, Hu DH, Wei RP, Wang RH & Cai WJ (2012) Fast-growing clone selection and wood quality analysis in Chinese fir. Chinese Agricultural Science Bulletin 28: 27–31.

Zheng HQ, Duan HJ, Hu DH, Wei RP & Li Y (2015a) Sequence-related amplified polymorphism primer screening on Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook). Journal of Forestry Research 26: 101–106. doi:10.1007/s11676-015-0025-0.

Zheng HQ, Hu DH, Wang RH, Wei RP & Yan S (2015b) Assessing 62 Chinese fir (*Cunninghamia lanceolata*) breeding parents in a 12-year grafted clone test. Forests 6: 3799–3808. doi:10.3390/f6103799.

Zheng HQ, Duan HJ, Hu DH, Li Y & Hao YB (2015c) Genotypic variation of *Cunninghamia lanceolata* revealed by phenotypic traits and SRAP markers. Dendrobiology 74: 85–94. doi:10.12657/denbio.074.009.