QTL mapping for growth-related traits by constructing the first genetic linkage map in Simao pine

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Abstract

Background: Simao pine is one of the primary economic tree species for resin and timber production in southwest China. The exploitation and utilization of Simao pine are constrained by the relatively lacking of genetic information. Construction a fine genetic linkage map and detecting quantitative trait locis (QTLs) for growth-related traits is a prerequisite section of Simao Pine's molecular breeding program.

Results: In our study, a high-resolution Simao pine genetic map employed specific locus amplified fragment sequencing (SLAF-seq) technology and based on an F_1 pseudo-testcross population has been constructed. There were 11,544 SNPs assigned to 12 linkage groups (LGs), and the total length of the map was 2,062.85 cM with a mean distance of 0.37 cM between markers. According to the phenotypic variation analysis for three consecutive years, a total of seventeen QTLs for four traits were detected. Among 17 QTLs, there were six for plant height (Dh.16.1, Dh16.2, Dh17.1, Dh18.1-3), five for basal diameter (Dbd.17.1-5), four for needle length (Dnl17.1-3, Dnl18.1) and two for needle diameter (Dnd17.1 and Dnd18.1) respectively. These QTLs individually explained phenotypic variance from 11.0-16.3 %, and the logarithm of odds (LOD) value ranged from 2.52 to 3.87.

Conclusions: In our study, a fine genetic map of Simao pine applied the technology of SLAF-seq has been constructed for the first time. Based on the map, a total of 17 QTLs for four growth-related traits were identified. It provides helpful information for genomic studies and marker-assisted selection (MAS) in Simao pine.

Introduction

Pinus kesiya Royle ex Gordon var. *langbianensis* (A. Chev.) Gaussen (2n = 24) (Wu & Zhao, 1999), also called Simao/Szemao pine in China, is a geographic variant of *Pinus kesiya* (Wang et al., 2012), mainly distributed in the mountainous areas where altitude ranging from 600 to 1800 m of Pu'er, Xishuangbanna and Lincang city in Yunnan Province (Zhu et al., 2017; Li et al., 2018). Compared with other conifer tree species, it has unique rapid growth characteristics that its branches can grow 2 or more rounds a year (Xu et al., 2012; Ou et al., 2016), and the timber is widely used in fiber, furniture, and the building industry (Cai et al., 2017). Simao pine is not only a high commercial value plant for its higher turpentine output (Dong et al., 2009; Wang et al., 2018) but also essential tree species in the forest ecosystem for the function of biological carbon sequestration and water conservation (Wen et al., 2010; Zhu et al., 2016; Cao & Zhang, 2017; Li et al., 2017). However, its exploitation and utilization are constrained by relatively lacking genetic information (Cai et al., 2017).

Highly saturated genetic linkage map construction is a useful tool for QTL mapping and MAS (Zhao et al., 2016; Gao et al., 2018). During the last two decades, a large number of genetic maps for perennial woody plants had constructed (Freeman et al., 2006; Jiang et al., 2011; Wang et al., 2014), various QTLs associated with essential traits had been identified based on these maps (Devey et al., 2004; Rönnberg et al., 2005; Lowry et al., 2015). However, a great majority of them were low saturated frame-work maps that

lowered the degree of accuracy of QTL mapping (Li et al., 2014). Single nucleotide polymorphism (SNP) is a sort of molecular marker technique developed by high-throughput sequencing. It is convenient, abundant, highly polymorphic and commonly used in genetic map construction (Troggio et al., 2007; Liu et al., 2012; Sun et al., 2013). In pace with the rapid development technology of next-generation sequencing (NGS), the technology of SLAF-seq becomes one of the popular methods for SNP markers development and high-resolution genetic map construction (Zhang et al., 2013; Shang et al., 2016; Chang et al., 2018). Until now, various plants genetic linkage maps had established by SLAF-seq, and it greatly heighten the efficiency and the degree of QTL mapping accuracy (Zhang et al., 2015; Luo et al., 2016; Zhang et al., 2016; Conson et al., 2018).

The construction of the first genetic linkage map will provide facilities to understand the genetic information of the genome in Simao pine. Growth-related traits were important economic traits for woody tree breeding, and detecting QTLs for these traits is a crucial section in the molecular breeding program for Simao Pine. Therefore, we employed the technology of SLAF-seq to actualize the fast SNPs development and a high-density linkage map will be constructing. The QTLs linked to growth-related traits will be identified based on the genetic linkage map. It will provide a powerful tool for future detection of other economic characteristics QTLs and MAS in Simao pine breeding.

Results

Analysis of sequencing data and SLAF markers

The Simao pine SLAF libraries were constructed successfully. A total of 461.41 M reads (guanine-cytosine of 40.23 % and Q30 of 92.25 %) were obtained. The number of reads for the maternal and paternal parents was 22,947,158 and 18,534,664, the mean for the F_1 individual was 4,659,126 (Table 1). After filtering out the low-quality reads, the number of SLAFs for the two parents and average in F1 progeny was 535,598, 482,851, and 375,315. The average depth of the SLAFs for the maternal and paternal parent was 10.48-fold and 9.77-fold, and the average for each F1 individual was 3.47-fold (Table 1). A total of 633,086 high-quality SLAFs were obtained. Among these markers, 239,790 were polymorphic markers (37.88 %), 385,976 were non-polymorphic markers (60.97 %), and 7,320 were repetitive markers (1.15 %). Finally, 140,485 polymorphic SLAFs of 8 segregation patterns were achieved (Figure 1). After removing the markers showing aa × bb segregation pattern, 97,891 (15.46 %) polymorphic SLAFs will be used to further Simao pine genetic map construction.

Construction and evaluation of genetic linkage map

After discarding the unsuitable markers, 5,643 SLAFs were used successfully for the linkage map construction. Among them, 11,544 SNP markers were detected, and SNP types were summarized (Table 2). Based on these markers, we constructed a high saturated genetic linkage map covering 2062.85 cM, comprising 12 LGs, and a mean distance of 0.37 cM (Figure 2, Table 2). The genetic length of individual LGs varied from 147.38 (LG4) to 194.85 cM (LG9) with a mean of 171.90 cM. Among 12 LGs, LG8 was

the largest linkage group, contained 523 SLAFs, while the smallest group (LG7) included 367 SLAFs. The average number of markers for each LG was 470. For the density, LG5 was the densest linkage group with the minimum marker distance (0.32 cM), whereas LG1, LG3, and LG7 were the lowest density linkage groups (0.40 cM). The max gap in the map was 11.17 cM located in LG2 and LG3.

Three approaches were performed for evaluated the quality of Simao pine genetic map. The result of markers integrity analysis showed that each individual mapped marker's complete degree was 99.99% (Figure 3), and the average depth for parents was more than five times of the offspring (Table 3). It suggested that genotyping was accurate and the mapping population was suitable for further analysis. According to the Haplotype maps (Supplementary Figure 1), most of the recombination blocks were distinctly defined, suggested that the constructed high saturated genetic map was adaptive for subsequent genetic analysis. The map quality was also assessed by Heat maps (Supplementary Figure 2). It indicated that the markers were well ordered in most linkage groups and the constructed Simao pine genetic map with high accuracy.

Phenotypic variation analysis

The 3 years phenotypic data and statistical values for growth-related traits were summarized (Table 4). The results showed that the four traits were normal distribution for three years (Figure 4). And a relatively higher degree of genetic variation was found. The CV of plant height was 21.74 %, 14.80 % and 12.07 % during 2016, 2017 and 2018. The CV of basal diameter was 25.21 % (2016), 23.42 % (2017), and 22.33 % (2018) respectively. The CV of needle length was 21.69 % (2017) and 18.89 % (2018). The CV of needle diameter was 25.96 % (2017) and 21.44% (2018). The Pearson correlations analyses showed the significant correlation among four traits (Table 5).

QTL mapping

Using the constructed map and analyzing the data of phenotypic characteristics in the mapping population, 17 QTLs linked to 4 traits were identified (Table 6, Supplementary Figure 3). The individual QTL explained the phenotypic variation varied from 11.0-16.3 %, and the LOD value ranged from 2.52 to 3.87. There were 6 plant height QTLs detected in the map. In 2016, two plant height QTLs were located on LG3 (Dh16.1) and LG10 (Dh16.2) explained 12.7 % and 12.4 % of the phenotypic variance. In 2017, only one plant height QTL located on LG9 (Dh17.1) explained 15.5 % of the phenotypic variation was detected. In 2018, the other three plant height QTLs were identified which located on LG11 (Dh18.1, Dh18.2), and LG12 (Dh18.3), and explained 16.3 %, 16.1 % and 13.8 % of the phenotypic variation, respectively. A total of 5 QTLs associated with basal diameter were detected on LG3 (Ddb17.1, 11.6 %), LG4 (Ddb17.2, 12.7%) and LG6 (Ddb17.3, 11.6 %; Ddb17.4, 13.1 %; Ddb17.5, 11.4 %), respectively. Four needle length QTLs were identified which located on LG1 (Dnl17.1, 12.1 %; Dnl17.2, 11.0 %; Dnl17.3, 12.1 %) and LG12 (Dnl18.1, 13.5%), respectively. There were two needle diameter QTLs located on LG4 (Dnb17.1 and Dnb18.1) explained 12.9 % and 13.1 % of the phenotypic variation were detected.

Discussion

Construction of the high-resolution map for Simao pine will provide helpful information for genomic studies and facilitate the breeding applications. In our study, a fine genetic map of Simao pine applied the technology of SLAF-seq has been constructed. It contained 12 LGs and 11,544 SNPs spanned 2,062.85 cM with a mean marker distance of 0.37 cM, representing a significant improvement over the previous linkage maps in coniferous plants (Eckert et al., 2009; Chen et al., 2010; Chancerel et al., 2011; Echt et al., 2011; Neves et al., 2014; Westbrook et al., 2015). As we know, this was one of the highest saturated genetic maps to date in coniferous tree species. A total of 17 QTLs for four growth-related traits were identified based on the constructed genetic map, and these QTLs were valuable genetic resources for MAS in Simao pine.

An appropriate mapping population laid a solid foundation for the genetic map construction (Zhu et al., 2015). It's hard for perennial woody trees to get Backcross (BC), Recombination Inbred Lines (RILs) and F_2 populations in the short term because of the long generation constraints. The pseudo-testcross strategy has been put forward that the F_1 population was created to replace the other populations (Grattapaglia & Sederoff 1994). This strategy has been successfully applied to various forestry trees, especially non-model and un-sequenced species (Ukrainetz et al., 2008; Wu et al., 2014; Liu et al., 2016; Xia et al., 2018). In this report, nine F_1 populations were obtained by artificial hybridization, based on the analysis of field phenotypic characteristics variation among populations and genetic similarity coefficient among parents, superior clones SM11 (high resin content) and JG1 (fast growth) were chosen for maternal and paternal parents. The F_1 hybrid population was applied as the mapping population for map construction in our study. The obvious variation will present in the segregation population due to the significant difference in the resin content and the parents' growth speed, which could facilitate QTL mapping for these traits.

Molecular markers were powerful tools for genetic map construction (Alsaleh et al., 2015). The mainstream molecular markers for genetic linkage map construction of heterozygous perennial forest tree species included SNP, simple sequence repeat (SSR), inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) et al. (Casasoli et al., 2001; Gulsen et al., 2010; Li et al., 2014; Wang et al., 2017; Mortaza et al., 2018). Among these markers, SNP was thought of as one of the ideal markers for genetic map construction for the merits of abundance, fast and covering the whole genome (Baird et al., 2008; Elshire et al., 2011). Significant changes have taken place in genetic map construction with the development of low-cost and high-throughput sequencing technology (Eckert et al., 2009). Recently, SLAF-seq technique has become one of the most popular SNP marker development assays (Sun et al., 2013). A high-density genetic linkage map had been successfully constructed by SNP markers using this approach. It indicated that SNP markers could be efficiently applied in constructing a genetic linkage map of Simao pine.

High-quality genetic maps can increase the accuracy of QTL mapping (Li et al., 2014; Liu et al., 2016). The number of markers in the genetic map is one of the essential indicators to evaluate its quality. A

genetic map with a large number of markers has the characteristics of suitable distance and high-resolution (Zhang et al., 2016). This constructed genetic map was the first map that contained over ten thousand SNP markers in coniferous tree species. It supported that we have built a high-quality genetic map for Simao Pine. Moreover, the other three approaches were performed for map quality evaluation. All results indicated that the current high-accuracy map would provide sufficient information for QTL mapping.

Growth-related traits were important economic traits for woody tree breeding, and detecting QTLs for growth-related traits is an introductory section in the molecular breeding program for Simao Pine. In this study, a total of 17 QTLs for four growth-related traits were identified. The individual QTL explained the phenotypic variation varied from 11.0% to16.3 %, and it indicated that several significant useful genes might control the growth-related traits of Simao pine (Rönnberg et al., 2005; Li et al., 2014). In the four traits, only the QTLs for plant height were consistently detected during the three years, but QTLs for the other three traits were not consecutively expressed. It suggested that different genes/QTLs might influence Simao pine's growth-related traits in different seasons/ages or that the QTLs stabilization varied by the effect of environmental change. In agreement with previous studies in the woody tree, growth-related traits were mainly quantitative traits, which dominated by involved genes and easily affected by the environment, probably changes as the tree matures (Yang et al., 2013; Kenis & Keulemans, 2007). In other words, the multi-environment QTL analysis is more accurate than a single-environment experiment for the heterozygous perennial woody tree growth-related traits QTL mapping (El-Soda et al., 2014). Thus, to eliminate interference brought by the environment and improve QTLs accuracy, the multi-environment QTL test in other domains using different backgrounds for Simao pine must be carried out in the future (Li et al., 2014; Adhikari et al., 2018).

Methods

Mapping population and DNA extraction

According to factorial mating design, eleven superior clones with the good characters of rapid growth and high resin content were selected as the hybrid parents from 105 clones. Among them, five clones as the male parents (superior clone JG1, NR7, LC3, ZY1, PW2) and the other six clones for the female parents (PW12, LC9, JG7, JD5, PW3, SM11). In the spring of 2014, a total of 30 hybridized combinations of artificially controlled pollination were conducted. Two years later, a total of 9 full-sib families were obtained and grown at the farm of Pu'er city institute of forestry sciences (N 22◦ 47'/E 100◦ 59') by harvesting, sowing and culturing the seedlings. Proceed to the next step, family 9 was selected as the mapping population for Simao pine genetic linkage map construction by analyzing population phenotypic variation and parent's genetic similarity coefficient (Wang et al., 2018). The parents for the mapping population were superior clone SM11 (maternal, with the characteristic of high resin content) and JG1 (paternal, with the aspect of rapid growth). Fresh and young healthy needles from parents and mapping population (100 hybrid individuals) were collected and frozen in liquid nitrogen at once. The

genomic DNA was isolated using the improved cetyl trimethyl ammonium bromide (CTAB) method (Wang et al., 2019).

SLAF library establishment and sequencing

The similar experiment procedure of high-throughput sequencing and establishment of the SLAF library for the mapping population was performed according to the previous study by Zhang et al. (2015) with minor modified. Briefly, two different steps were applied. First, all of the genomic DNA for SLAF library construction were digested by a single enzyme *Hae* III (New England Biolabs, NEB, USA). Second, only the SLAF fragments in which the length ranging from 414 to 464 bp will be excised and diluted for pair-end sequenced by Illumina HiSeq 2500 platform (Illumina, Inc; San Diego, CA, USA).

Analysing and genotyping for sequence data

The SLAF-seq data grouping and SNP genotyping were the same as Wang et al. (2017). After discarding the low-quality reads, the remaining reads with more than 90% similarity will gather in the same SLAF locus. Simao pine is a diploid plant, so only the SLAF has 2 to 4 alleles that will designate as the potential and polymorphic marker. The aa × bb segregation pattern markers will not be used to construct the genetic map, as the mapping population is obtaining by a cross between two heterozygote parents of Simao pine (Jansen 2005; He et al., 2017).

Linkage map construction and evaluation

The HighMap software (He et al., 2017) with the cross-pollination (CP) option was utilized for Simao pine genetic linkage map construction. The estimation parameters were set for a minimum LOD threshold of 5.0 for linkage groups, and the map distance was estimated with the Kosambi mapping algorithm (Vinod 2011). After linkage grouping, the maximum likelihood method was used to order the SLAFs markers in all LGs (Van Ooijen 2011; Luo et al., 2016). The SMOOTH algorithm was utilized to put correct genotyping errors (Van et al., 2005). To evaluate the quality of the constructed Simao pine genetic map, mapped markers integrity and construction of haplotype maps and heat maps for each LG were carried out (West et al., 2006; Liu et al., 2017; Wu et al., 2018).

Growth-related traits Assessment and QTL analysis

Growth-related traits, including plant height, basal diameter, needle length, and needle diameter of the progenies are determined during three consecutive years. The plant height and needle length were measured by a line tape, while the basal diameter and needle diameter were measured with the vernier caliper in December from 2016–2018. The phenotypic variation analysis, including coefficients of variation (CV) and the correlation coefficients between all investigated traits, was performed with software SPSS 20.0. The QTLs underlying the growth-related traits were identified by MapQTL 6.0 software and the interval mapping method (Van Ooijen 2009). The 95% Bayesian credible interval method was used to calculate the confidence intervals for all QTLs (Sen & Churchill, 2001). One thousand permutations decided the threshold value. According to the permutations, the minimum LOD score of 2.5

was conducted in our study. The percentage of phenotypic variance explained of each detected QTL was achieved based on the phenotypic variance in the population (Xia et al., 2018).

Abbreviations

QTL: Quantitative trait loci; LG: Linage group; SLAF-seq: Specific locus amplified fragment sequencing; LOD: Logarithm of odds; MAS: Marker-assisted selection; SNP: Single nucleotide polymorphism; NGS: Next-generation sequencing; CTAB: cetyl trimethyl ammonium bromide; CP: cross-pollination; CV: Coefficients of variation; BC: Backcross; RILs: Recombination Inbred Lines; SSR: Simple sequence repeat; ISSR: Inter-simple sequence repeat; AFLP: Amplified fragment length polymorphism; RAPD: Random amplified polymorphic DNA.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this article (and its supplementary information files).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DW, AD and HG conceived and designed the study. DW, CS, SL and NC performed the experiments. CH and HT analyzed the data, DW and CS wrote the manuscript. HG revised the whole manuscript and helped in preparing the tables and references. All authors have read and approved the manuscript

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Figures

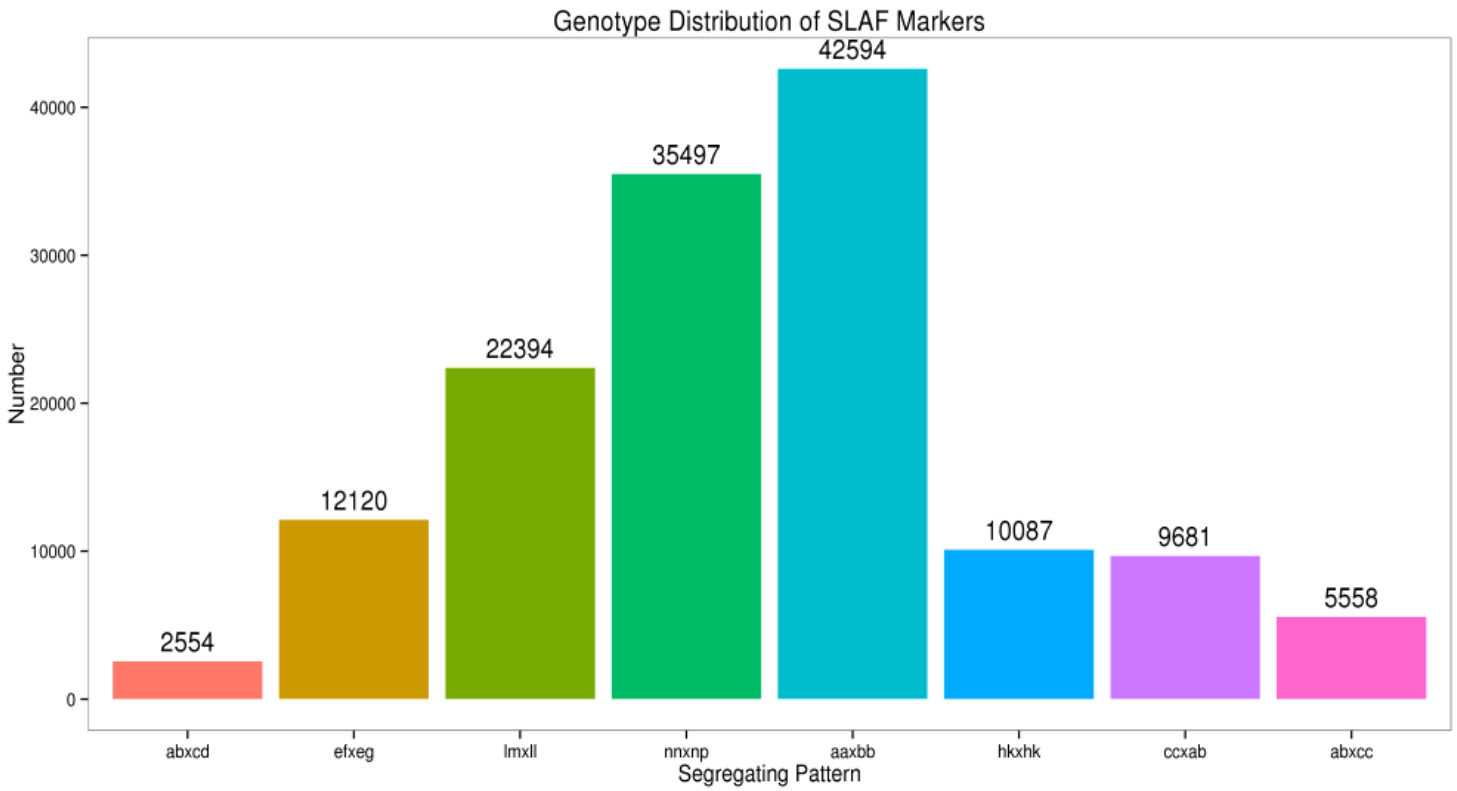

Figure 1

Segregation pattern of polymorphic SLAF markers

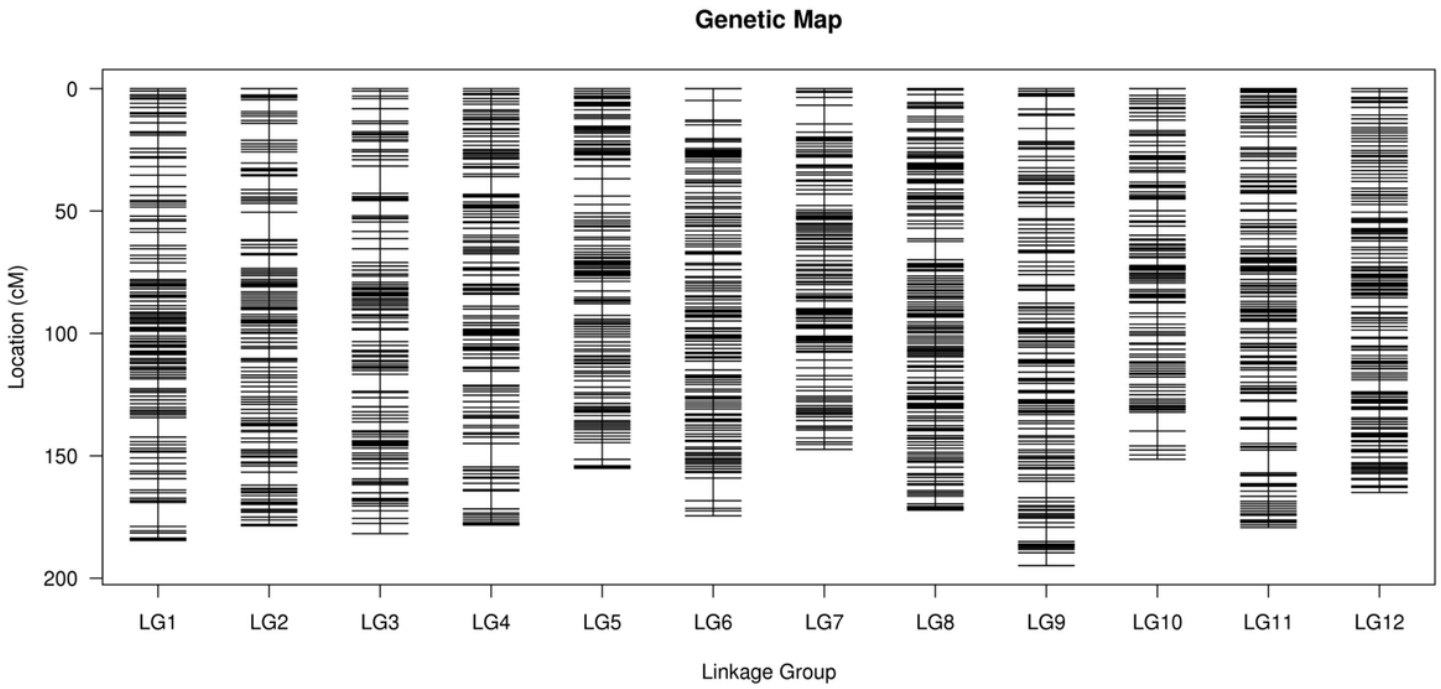

Figure 2

The high-density linkage map of Simao pine A black bar indicates a SLAF marker. The x-axis represents the linkage group number and the y-axis indicates the genetic distance (cM) within each linkage group.

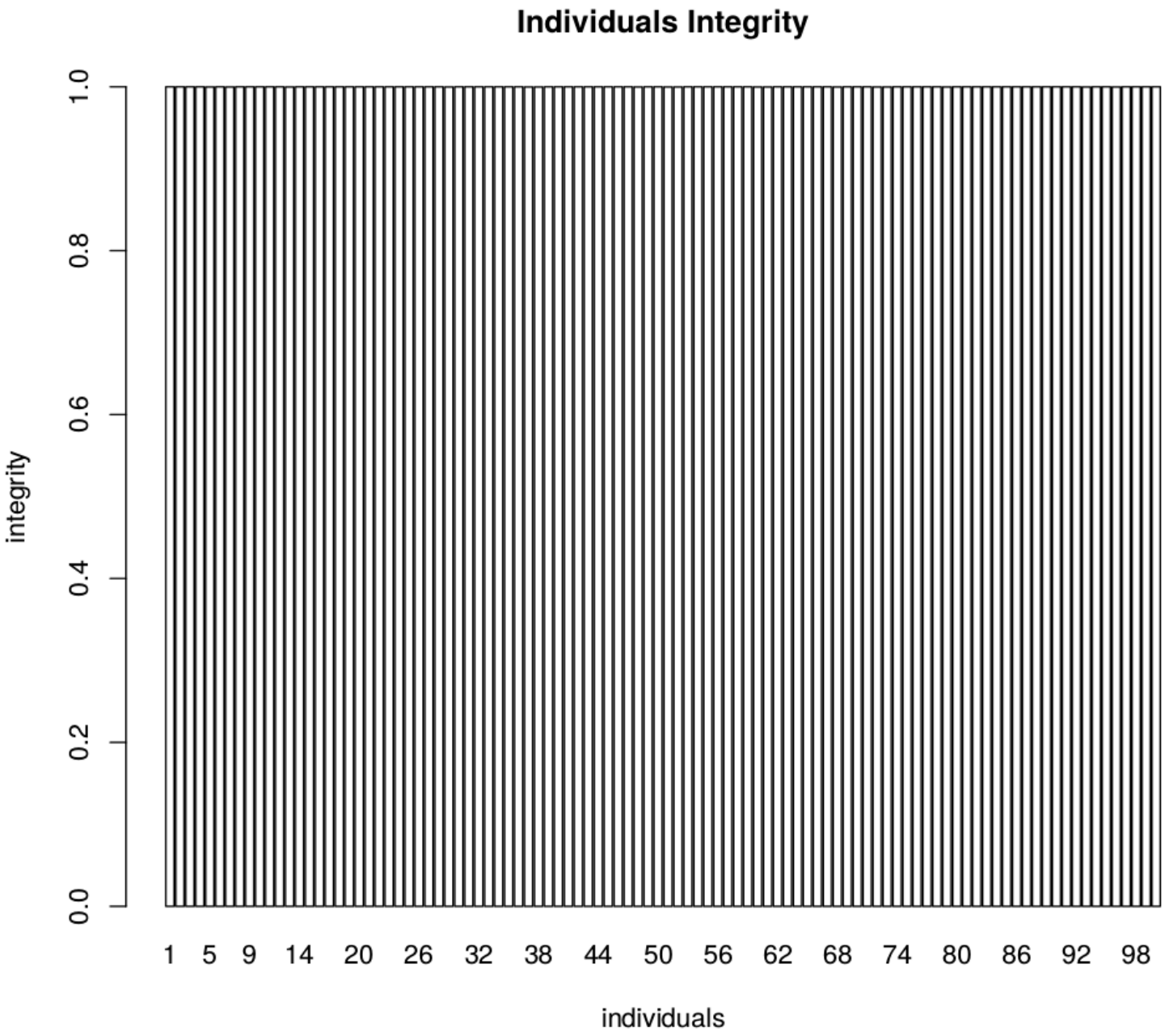

Figure 3

The integrity distribution map of all individuals The x-axis represents the 100 individuals and y-axis represents the complete degree of mapped markers.

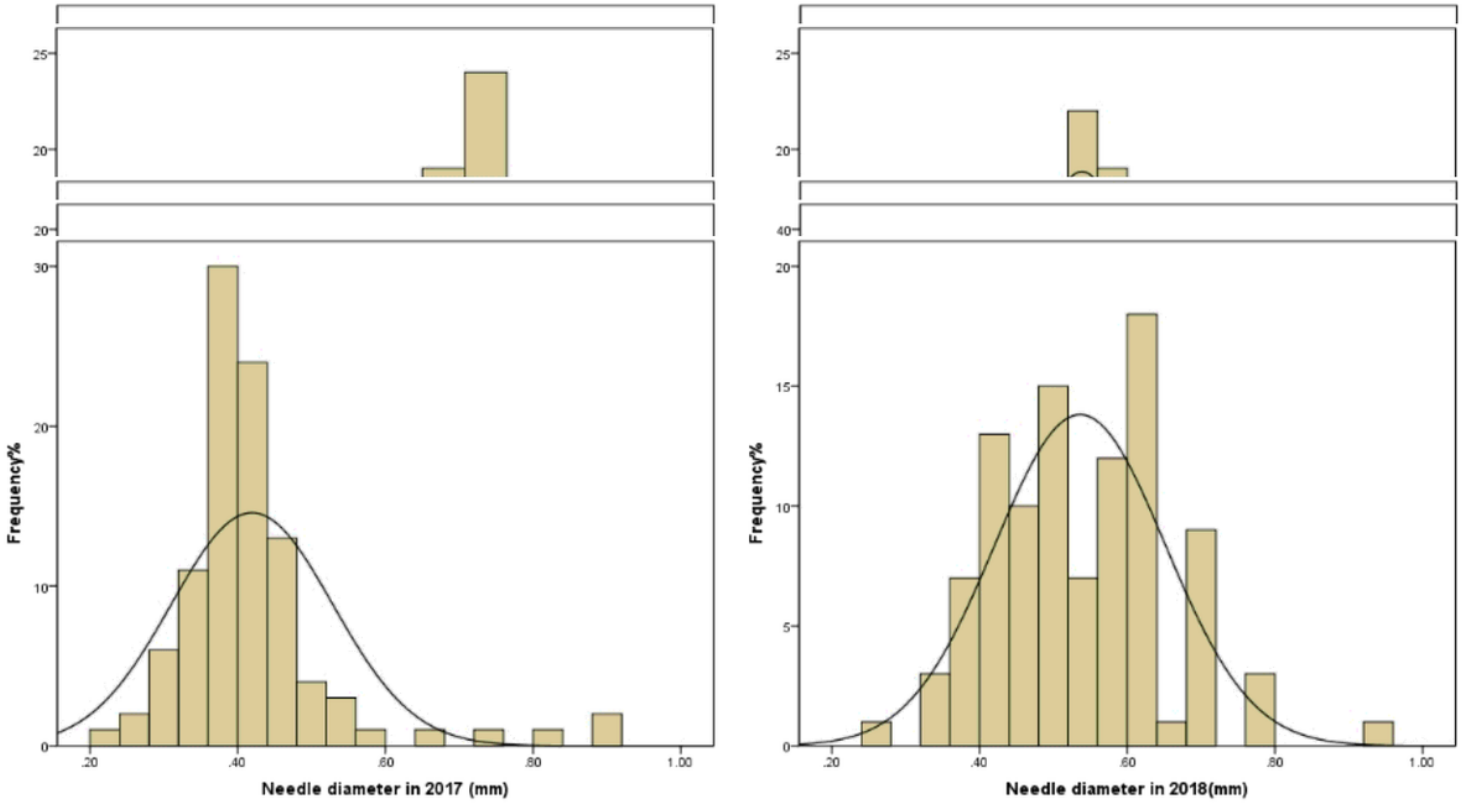

Figure 4

Frequency distributions of growth traits in mapping population during three consecutive years

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