Industrial Crops & Products 120 (2018) 351–356

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

An ultra-high density map allowed for mapping QTL and candidate genes controlling dry latex yield in rubber tree

Zhiqiang Xia[a,b,*], Kede Liu[a], Shengkui Zhang[a,b], Wencai Yu[c], Meiling Zou[a,b,*], Ligang He[c,*], Wenquan Wang[a,b,*]

[a] Huazhong Agricultural University, Wuhan, China
[b] The Institute of Tropical Biosciences and Biotechnology, Chinese Academy of Tropical Agriculture Sciences, Haikou, China
[c] Yunnan Institute of Tropical Crops, Xishuangbanna, China

ARTICLE INFO

Keywords:
Rubber tree
Ultra-high density linkage map
Quantitative trait loci
QTL gene
Dry latex yield

ABSTRACT

Rubber tree (*Hevea brasiliensis*) is the most important commercial producer of high-quality natural rubber in the world. Here, we identified 571,267 SNPs and 134,184 indels, and constructed the first ultra-high density genetic linkage map in rubber tree population. This map consisted of 6940 markers, with average marker density and recombination rate 0.30 cM and 0.97 cM/Mb, respectively. Then the whole genome QTL scanning for dry latex yield (DLY) trait were performed and seventeen repQTLs were obtained, among which *qDFY-10* and *qDFY-18-4* could explain up to 38.3% and 33.3% phenotypic variability, respectively. Numerous highly-promising QTL candidate genes were identified and then verified significant associated with DLY trait, including some first found in rubber tree, such as *thioredoxin h, plastin-like protein, calmodulin binding protein, cytochrome-c oxidase* and *methylglutaconyl-CoA hydratase*. This ultra-high density linkage map supplies a base for important traits QTL mapping and will be useful in the improvement of the assembly of genome sequences in rubber tree. The verification of candidate QTL genes showed great importance for understanding the possible function of mapped QTLs and the comprehensive relations between phenotypes and genotypes. Accurate QTL mapping will be enhance genetic studies that can be applied in rubber tree breeding programs.

1. Introduction

Rubber tree (*Hevea brasiliensis*), belongs to family Euphorbiceae, is a stabilized diploid (2n = 36), with large and complex genome of approximately 2.15 Gbp (Shearman et al., 2015). It is the most important commercial producer of natural rubber, because of its advantages of high yield and good quality rubber production, long economic life and low cost in plant cultivation and latex tapping (Shearman et al., 2015; Pootakham et al., 2015). *H. brasiliensis* produces more than 98% of the total natural rubber in the world (Priyadarshan and Goncalves, 2003). Latex biosynthesis, controlled by a series of metabolic processes (including sucrose loading in the lactiferous vessels and enzyme activity regulation for latex synthesis), was concerned with the biosynthetic energy requirements and the mechanism of senescence phenomena (Tang et al., 2010; Xiao et al., 2014; Li et al., 2016). The latex regeneration usually needs at least 2 days, and the amount of latex production is related with season, tapping hour, tree age, rubber clone, and exploitation system. For its long growing cycles and high heterozygous, the selection of high latex yield cultivars are still very slow by conventional breeding. Using latex yield trait related markers through molecular marker-assisted selection (MAS) system will save the selection time and improve the latex yield in rubber tree breeding.

MAS breeding has been successfully used for plant genetic improvement (Graham et al., 2010). For MAS breeding, the whole genome molecular markers, linkage maps and QTLs are necessary (Balsalobre et al., 2017). In many plants, a large number of markers in genetic linkage maps have been developed, and the QTLs with important traits, located in the linkage map, laid the foundation to accelerate genetic improvement through MAS breeding (Balsalobre et al., 2017; Huang et al., 2016; Triwitayakorn et al., 2011; Delourme et al., 2013). Although there were some markers developed in rubber tree, the number of QTL markers is still limited. The first rubber tree genetic linkage map was developed in 2000, consisted of 301 restriction fragment length polymorphisms (RFLPs), 388 amplified fragment length polymorphisms (AFLPs), 18 simple sequence repeat (SSR) and 10 isozyme markers (Lespinasse et al., 2000). Followed by Feng et al. (2010), Souza et al.

* Corresponding authors.
E-mail addresses: xiazhiqiang@itbb.org.cn (Z. Xia), kdliu@mail.hzau.edu.cn (K. Liu), zsk8920@foxmail.com (S. Zhang), ywckgh@163.com (W. Yu), zoumeiling@itbb.org.cn (M. Zou), ynhlg@163.com (L. He), wangwenquan@itbb.org.cn (W. Wang).

https://doi.org/10.1016/j.indcrop.2018.04.057
Received 28 October 2017; Received in revised form 31 October 2017; Accepted 20 April 2018

(2011), Triwitayakorn et al. (2011), Le Guen et al. (2011), Souza et al. (2013), and Novalina and Sagala, (2013) using SSR, AFLP or RAPD markers to construct genetic maps, (Triwitayakorn et al., 2011; Feng et al., 2010; Souza et al., 2011; Le Guen et al., 2011; Souza et al., 2013; Novalina and Sagala, 2013). These genetic linkage maps were used in QTL mapping for resistance, growth, and yield related traits (Novalina and Sagala, 2013; Le Guen et al., 2003; Le Guen et al., 2007; Rattanawong et al., 2011). Recently, Pootakham et al. (2015) constructed genetic linkage maps for P and C Populations (Pootakham et al., 2015), comprised 1704 and 1719 markers and covered 2041 cM and 1874 cM, respectively, with the average marker densities 1.23–1.25 cM. However, the average distances between these adjacent markers were still large, requiring a higher density genetic linkage map. Besides, so far there is no report about QTL detection based on high-density linkage map in rubber tree. In this study, we generated an ultra-high density linkage map, performed QTL mapping of dry latex yield trait and detected the candidate genes in rubber tree.

2. Materials and methods

2.1. Plant materials

In this study, rubber tree population derived from a cross between a female parent Yunyan277-5 and amale parent IAN873 (population YI), consisted of 187 F1 progeny. The female parent, Yunyan277-5, a widely cultivated accession for its high dry latex yield and fast growth, was obtained from across between PB5/63 and Tjir1 (Anon., 1996). The male parent IAN873 was a descendent of a PB86 × FA1717 cross, with fast growth and medium dry latex production (Liu et al., 2010).

2.2. AFSM library construction, sequencing and data analysis

Young leaves were immediately frozen in liquid nitrogen, after collected at Yunnan Institute of Tropical Crops in China, and then preserved at −80 °C. Using Plant DNeasy Maxi Kit (QIAGEN, Valencia, CA) to isolated DNA. DNA quality and quantity was checked on agarose gel and NanoDrop Spectrophotometer. AFSM libraries were constructed using AFSM (Xia et al., 2014) method for 189 rubber tree accessions. Then using Illumina HiSeq2500 with pair-end 150 bp lengths to sequence the AFSM libraries. The Illumina AFSM sequencing raw data was processed using custom Perl scripts (Xia et al., 2014) and then aligned to the recently publicly available rubber tree genome (Tang et al., 2016) using Bowtie2 (Langmead and Salzberg, 2012), allowing one mismatch. To identify the SNPs and indels, SAMtools and VCFtools_v0.1.9 (http://vcftools.sourceforge./net/) were used.

2.3. Rubber tree genetic linkage map construction

The rubber tree genetic linkage map was constructed using JoinMap4.1 (Van Ooijen, 2011), with LOD value > 5. Bi-allelic markers were identified by querying the filtered tags for pairs of sequences with the following characteristics: 1) all pairs of tags were identical in at least two reads; 2) passed a Fisher's exact test for independence; 3)fit to the expected Mendelian segregation ratio as demonstrated by a chi-squared test at a $P < 0.01$; 4) Remove those markers present less than 30% in individuals; 5) possessed a recombination frequency of < 0.4; and 6) had AFSM markers specific to the female or male parent that fit to a 1:1 segregation ratio in addition to shared AFSM markers that fit to a 3:1 segregation ratio in the YI population. If a SNP or indel site call was heterozygous, presumably due to sequencing errors, then the call was classified as missing data. According to JoinMap 4.1, the YI population could be considered as a CP population according to the genetic background if the two parents were heterozygous. The parent-specific AFSM markers, which segregated at a 1:1 ratio in the population, were recorded as lm × ll (marker in the female parent) and nn × np (marker in the male parent). The AFSM markers that were present in both parents and segregated at a 3:1 ratio in the population were recorded as hk × hk (marker present in both parents).

2.4. Dried latex yield (DLY) data collection

We performed two Dried Latex Yield (DLY, g) measurements. DLY1 and DLY2 measurements came from Yunnan field experiments in two key months, May and October 2013, respectively. In each month, the YI lines were tapped 10 times, using S/2, d/2 tapping method (S: spiral; d: day). YI lines were tapped 2 times at first, and then collected the followed 8 times tapping latex. After dried, each collected DLY was recorded. The average DLYs in May and October 2013 were recorded as DLY1 and DLY2, respectively. DLY1, DLY2 and combined DLY were used for further QTL analysis.

2.5. QTL analysis

After constructing the rubber tree genetic linkage map, QTL analysis was performed using Restricted MQM Mapping (rMQM) model in MapQTL5 (Van Ooijen, 2004), with the threshold LOD > 3.8. The maximum LOD value was considered as the QTL position. The confidence interval was 2 LOD. The percent contribution of each identified QTL interpretation variance (PVE) was assessed by variance component analysis. The QTL name started with "*q*".

2.6. Association analyses

Association analyses was performed using EMMAX algorithm (Kang et al., 2010). Only SNPs with a minor allele frequency > 5% were used.

2.7. Availability of data and materials

Raw sequence data that support the findings of this study have been submitted to the NCBI Sequence Read Archive (SRA) with accession number SRR5477593-SRR5477594.

3. Results

3.1. SNPs and indels markers discovery in rubber tree

To determine the SNPs and indels in rubber tree population YI (derived from a cross between a female parent Yunyan277-5 and a male parent IAN873), 187 F1 offsprings and two parents were sequenced and obtained 606,479,584 raw reads. Cleaned reads with quality scores of ≥20 were mapped against the publicly available rubber tree genome (Tang et al., 2016) (4,212,231, 6,401,246 and 95,884–6,600,365 clean reads for IAN873, Yunyan 277-5 and F1 lines, respectively). Relative to the reference sequences, 571,267 SNPs and 134,184 indels were identified. The frequency of SNPs discovered was higher than indels, with an average ratio of 4:1.

3.2. Rubber tree genetic linkage map construction

Out of 705,451 markers, 20,066 SNPs and 2672 indels were used for linkage analysis. A total of 6940 markers could be assigned to 18 linkage groups (LGs), covering 2094.10 cM (Table 1, Fig. 1). The genome-wide recombination rate (GWRR) estimated at 0.97 cM/Mb. The markers density were ranged from 0.09 cM (LG4) to 0.59 cM (LG11), with an average of 0.30 cM. The average length of linkage groups was 116.34 cM.

3.3. Anchoring sequence of scaffolds to the genetic linkage map

The rubber tree genome, recently assembly, consisted of 7453 scaffolds, covering 1.37 Gb of sequences (Tang et al., 2016). We were able to anchor a total of 1086 scaffolds onto our genetic linkage map

Table 1
Summary statistics of linkage map constructed from a rubber tree population of a cross between IAN873 and Yunyan277-5.

Linkage group	Number of markers	Length (cM)	Average marker density (cM)	Number of anchored scaffolds (The total length of anchored scaffolds, Mb)
LG 1	428	80.84	0.19	94 (89.58)
LG 2	243	85.93	0.35	48 (48.28)
LG 3	289	61.53	0.21	65 (45.28)
LG 4	131	11.77	0.09	28 (26.17)
LG 5	102	9.43	0.09	2 (1.04)
LG 6	432	146.08	0.34	86 (105.16)
LG 7	114	12.87	0.11	14 (10.13)
LG 8	703	241.46	0.34	112 (91.78)
LG 9	537	218.79	0.41	70 (61.11)
LG 10	329	117.45	0.36	87 (86.11)
LG 11	186	110.01	0.59	26 (15.03)
LG 12	140	28.77	0.21	23 (15.02)
LG 13	408	107.84	0.26	54 (62.69)
LG 14	367	64.31	0.18	74 (66.67)
LG 15	156	66.97	0.43	24 (14.21)
LG 16	601	153.82	0.26	72 (61.80)
LG 17	787	235.13	0.30	90 (76.34)
LG 18	987	341.09	0.35	117 (85.54)
Average	385.56	116.34	0.30	–
Total	6940	2094.10	0.30	1086 (961.97)

(Table 1). The combined length of these scaffolds was 961.97 Mb, which was equivalent to 70.04% of the sequenced genome.

3.4. QTL mapping

QTL mapping was performed for DLY trait by applying an rMQM model in rubber population. The value of LOD score threshold for DLY trait was 3.8. The results of the QTL mapping were summarized in Table 2. On the basis of the high-density genetic linkage map, a total of 17 repQTLs were detected on LG 1, LG 6, LG 8, LG 10, LG 13, LG 14, LG 16, LG 17 and LG 18. Most of these QTLs were distributed in the upper parts of LG8, 14 and the lower parts of LG13, 18 (Fig. 1). Phenotypic variability explained by each QTL ranged from 11.1% to 38.3% (Table 2). The confidence intervals were ranged from 0.101 cM to 2.59 cM. Particularly, two repQTL, *qDFY-10* (LVXX01000148.1_1091479) and *qDFY-18-4* (LVXX01000430.1_338729) were found at 101.042 cM on LG10 and 304.929 cM on LG18, and the confidence intervals were at 100.837-101.363 cM and 304.88-304.981 cM, with a percentage of phenotypic variance explained (PVE) of 38.3% and 33.3%, respectively.

3.5. Identification of candidate QTL gene

Due to the availability of the whole genome sequence of rubber tree, it became possible to identify candidate QTL genes underlying QTLs mapped in this study. Several defense mechanisms-related candidate QTL genes were detected, such as *heat shock cognate 70 kDa protein* (LVXX01003015.1_9361), *thioredoxin h* (LVXX01000085.1_2317233), *APO protein* (LVXX01000457.1_744390), *plastin-like protein* (LVXX01000194.1_899614), *calmodulin binding protein* (LVXX01001048.1_251238), *cytochrome-c oxidase* (LVXX01000171.1_1778368), *chromatin remodeling complex subunit* (LVXX01001358.1_21130), *cytochrome P450 mono-oxygenase (CYP81D4)* (LVXX01000269.1_225265), *DEAD-box ATP-dependent RNA helicase* (LVXX01000724.1_229497), *chromatin remodeling factor17 (CHR17)* (LVXX01000309.1_533645), *transcription factor PIF3* (LVXX01001148.1_234332), *AP2/ERF domain-containing transcription factor* (LVXX01000105.1_1351840), and *ethylene-responsive transcription factor RAP2* (LVXX01000494.1_230329). Three biosynthesis pathway-related candidate QTL genes (*acetyl-CoA carboxylase biotin carboxyl carrier protein* (LVXX01000157.1_1787078), *HbGGPS mRNA for geranylgeranyl-diphosphate synthase* (LVXX01000434.1_537739), and *methylglutaconyl-CoA hydratase* (LVXX01000076.1_48625) were also be found. In addition, two energy metabolism-related candidate genes were identified: *Mitochondrial uncoupling protein (UCP2)* (LVXX01000941.1_74888), which located on the mitochondrial inner membrane, and related with ATP synthesis and ROS production, and *ATP binding protein* (LVXX01001571.1_19153).

QTL validation was performed by looking for significant QTL candidate genes in YI population. The results revealed that *HSP, thioredoxin h, plastin-like protein, calmodulin binding protein, cytochrome-c oxidase* and *methylglutaconyl-CoA hydratase* showed highly significant associated with dry latex yield trait, with the $-\log_{10}$ *p*-value range from 4.0447 to 4.6220 (Fig. 2).

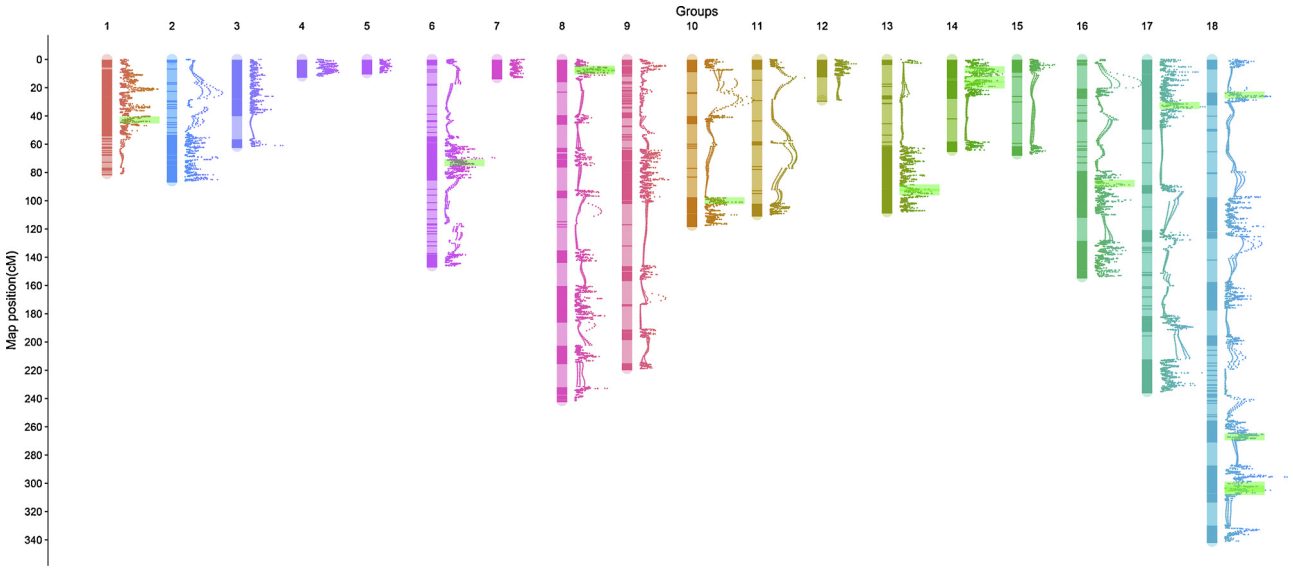

Fig. 1. Map of QTL detected in mapping population IAN873 × Yunyan277-5.
The left cylinder indicate the rubber tree linkage group1-18. Dark lines on linkage groups indicate the markers. On the right, light green indicate the dried latex yield trait QTL position for each linkage group in rubber tree. Positions of markers are shown in cM.

Table 2
Summary of repQTL observed for dried latex yield trait in rubber tree.

repQTL	LG	Position (cM)	Flanking marker	LOD	PVE[a]	Confidence interval[b] (cM)
qDFY-1	1	43.864	LVXX01000817.1_23826	4.53	13.9	43.631–43.922
qDFY-6	6	74.116	LVXX01000197.1_1480327	4.43	17	73.221–74.284
qDFY-8-1	8	7.833	LVXX01000085.1_2317233	6.02	18.2	7.124–7.989
qDFY-8-2	8	9.189	LVXX01002194.1_30899	4.53	11.1	9.011–9.307
qDFY-10	10	101.042	LVXX01000148.1_1091479	5.26	38.3	100.837–101.363
qDFY-13-1	13	91.907	LVXX01001898.1_22013	4.44	15.8	91.285–92.423
qDFY-13-2	13	94.814	LVXX01000277.1_1369636	4.93	12.5	94.176–95.114
qDFY-14-1	14	8.331	LVXX01001505.1_72329	5.08	15.3	6.360–8.482
qDFY-14-2	14	15.019	LVXX01000744.1_242310	4.9	18.7	14.438–15.151
qDFY-14-3	14	19.08	LVXX01001048.1_251238	4.58	12.3	18.991–19.204
qDFY-16	16	88.791	LVXX01000309.1_533645	5.27	12.9	88.135–89.043
qDFY-17	17	33.576	LVXX01000208.1_119381	6.91	19.4	33.480–34.749
qDFY-18-1	18	26.205	LVXX01000808.1_42396	6.98	16.7	25.599–27.434
qDFY-18-2	18	268.134	LVXX01000430.1_172697	4.68	12	267.500–268.382
qDFY-18-3	18	302.241	LVXX01001138.1_167648	4.75	12.2	302.038–302.843
qDFY-18-4	18	304.929	LVXX01000430.1_338729	6.21	33.3	304.880–304.981
qDFY-18-5	18	306.812	LVXX01000494.1_230329	4.6	13.9	305.215–307.805

[a] Proportion of phenotypic variation explained by each QTL.
[b] 99% Confidence interval for QTL length (cM).

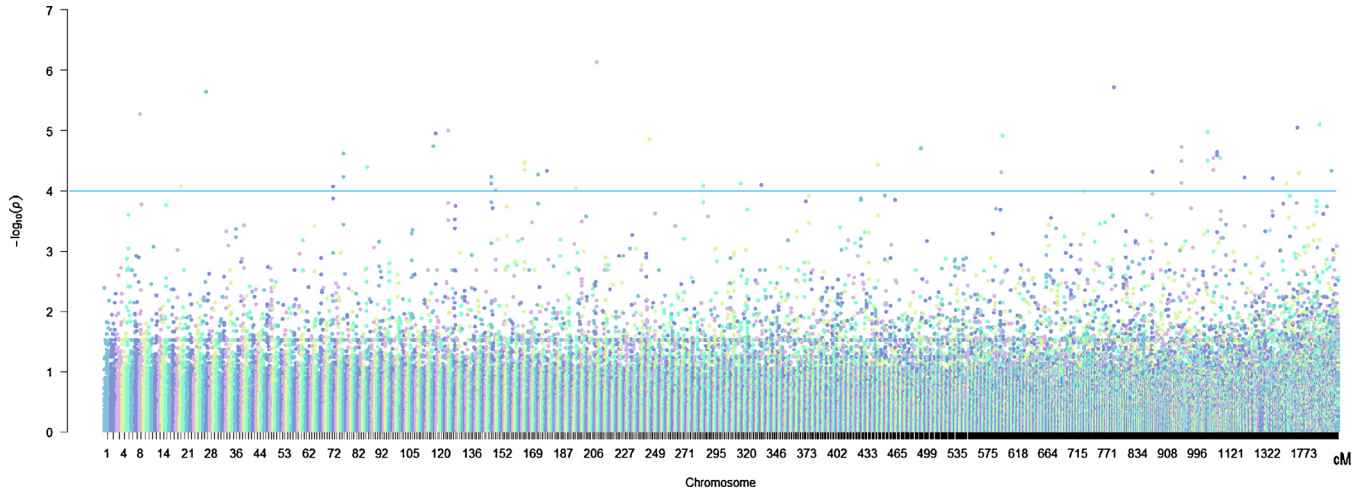

Fig. 2. Manhattan plots of Association analyses results in mapping population IAN873 × Yunyan277-5.
Blue horizontal line indicate threshold $-\log_{10}$ *p*-value = 4.

4. Discussion

Here, we discovered a total of 571,267 SNPs and 134,184 indels from YI population, which was 33 times more than the previously reported maximum marker number in rubber tree (21,353 SNPs) (Pootakham et al., 2015). For SNPs, the majority of nucleotide variation detected were transitions (transitions were 1.61 times more than transversion events), which consistent with the previous study (Pootakham et al., 2015). A high-density fine mapping genomic map is urgent needed, to increase the understanding of the genetic structure of rubber tree. We constructed an ultra-high density linkage map in the YI population, distributed in 18 linkage groups. The number of LGs was consisted with the cytology (Ong, 1975). The average distance between adjacent markers was 0.30, with the highest in LG11 (0.59 cM) and lowest in LG4 (0.09 cM). The marker density in our linkage map (0.30 cM) showed significantly higher, compared to previously reported rubber tree linkage maps [21.29 cM in Feng et al. (2010), 11 cM in Souza et al. (2011), 11.9 cM in Triwitayakorn et al. (2011), 6.37 cM in Le Guen et al. (2011), 9.53 cM in Souza et al. (2013), 1.23–1.25 cM in Pootakham et al. (2015) and 3 cM in Lespinasse et al. (2000) (Pootakham et al., 2015; Triwitayakorn et al., 2011; Lespinasse et al., 2000; Feng et al., 2010; Souza et al., 2011; Le Guen et al., 2011; Souza et al., 2013). The length of linkage map (2094.10 cM) was longer than the previously published linkage maps constructed by Triwitayakorn et al. (2011) (842.9 cM) and Feng et al. (2010) (1937.06 cM), but shorter than that in Le Guen et al. (2011) (2441 cM), Souza et al. (2013) (2688.8 cM) and Souza et al. (2011) (2471.2 cM) and comparable to that in Pootakham et al. (2015) for P populatin (2041 cM) and in Lespinasse et al. (2000) (2144cM) (Pootakham et al., 2015; Triwitayakorn et al., 2011; Lespinasse et al., 2000; Feng et al., 2010; Souza et al., 2011; Le Guen et al., 2011; Souza et al., 2013).

The GWRR, observed in this study (0.97 cM/Mb for rubber tree), was much lower than those angiosperm species with small genomes size (5.52 cM/Mb for *Brachypodium distachyon*, 4.83 cM/Mb GWRR for *Brassica rapa* and 4.73 cM/Mb GWRR for *Oryza sativa*) (Tiley and Burleigh, 2015). This finding was consisted with that GWRR was negatively correlated with genome size in angiosperms (Tiley and Burleigh, 2015; Jaramillo-Correa et al., 2010). However, GWRR detected here was a little higher than in *Zea mays* (0.73 cM/Mb) (Bauer et al., 2013), which may indicated that tree have higher GWRR than short-lived herb. This linkage map allowed us to anchor scaffolds of 961.97 Mb in total length or 70.04% of the sequenced rubber tree genome (Tang et al., 2016). The length of anchored scaffolds was much longer than the previously study (135 Mb, equivalent to 12% of the

sequenced genome) (Pootakham et al., 2015; Rahman et al., 2013). These scaffolds anchored to the genetic linkage map will facilitate genomic sequence assembly.

High polymorphism between parents, a large population, an ultra-high density genetic map, and replicated experiments are necessary factors for precise QTL detection, beside the genetic map construction and experimental design. The dried latex yield (DLY) trait had been observed great difference between IAN873 and Yunyan277-5 in the previous studies. The average annual yield of dried latex in IAN873 was 1310 kg/hm^2/y, lower than in RRIM600 (1969 kg/hm^2/y) (Liu et al., 2010), but that in Yunyan277-5 (2035.5 kg/hm^2) was higher than in RRIM600 (1662.0 kg/hm^2) (Anon., 1996), according to sixteen and eleven years studies, respectively. The population size in this linkage map was larger than the previous studies (Triwitayakorn et al., 2011; Lespinasse et al., 2000; Feng et al., 2010). These seventeen repQTLs could be considered reliable, because each repQTL interprets phenotypic variation > 11%, and their confidence intervals were small (0.101–2.59 cM). Moreover, numerous highly-promising candidate QTL genes, associated with defense mechanisms, energy metabolism and rubber biosynthesis pathways, were identified and then verified significant associated with DLY trait in this study. These candidate QTL genes included some first found in rubber tree population, such as *Methylglutaconyl-CoA hydratase, thioredoxin h, plastin-like protein, calmodulin binding protein, cytochrome-c oxidase, methylglutaconyl-CoA hydratase, ATP binding protein* and *UCP2*. Dried latex yield trait, one of the most important biological feature of rubber tree, has tremendous economic importance. Nature rubber from rubber tree, *Cis*-1, 4-polyisoprene, is made up of *Cis*-isoprene units derived from isopentenyl diphosophate (IPP). The biosynthesis of nature rubber roughly through three processes: IPP biosynthesis, *Trans*-IPP condensation and *Cis*-IPP condensation. IPP is formed from the condensation of three acetyl-CoA moieties in the cytoplasmic mevalonate pathway, while from the condensation of pyruvate and D-glyceraldehyde 3-phosphate in the plastidic 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (http://www.genome.jp/kegg/). In the mevalonate pathway, *hydroxymethylglutaryl-CoA (HMG-CoA)* can be used for synthesize the Mevalonate mediated by *HMG-CoA reductase*. While another enzyme, encoded by *methylglutaconyl-CoA hydratase*, leads to the formation of *trans-3*-methylglutaconyl-CoA from *HMG-CoA*, in a competitive reaction. *Methylglutaconyl-CoA hydratase* was detected in our study (Fig. 3). We also found *HbGGPS mRNA for geranylgeranyl-diphosphate synthase*. Natural rubber is synthesized by the successive addition of IPP molecules to a priming allylic diphosphate: farnesyl diphosphate (FPP) and/or geranylgeranyl diphosphate (GGPP), so the IPP condensation process via two distinct routes: FPP and/or GGPP pathways (Fig. 3). The synthase of GGPP, formed from dimethylallyl diphosphate (DMAPP), is mediated by *geranylgeranyl-diphosphate synthase* (*GGPS*). Genes involved ethylene signalling pathways, play an important role in stress regulation and were associated with rubber tree latex. It has been reported that the yield of rubber tree latex can be significantly increased by the ethylene-releasing agent ethephon (Tang et al., 2016; Han et al., 2000; Duan et al., 2010; Piyatrakul et al., 2014). *Heat shock cognate 70 kDa protein, cytochrome P450 mono-oxygenase, AP2/ERF domain-containing transcription factors* and *ethylene-responsive transcription factor RAP2*, found in this study, were essential in rubber tree ethylene signal transduction and response. *AP2/ERF domain-containing transcription factor* in rubber tree latex, played an extremely important role in ROS-scavenging, ethylene signal response, and programmed cell death (PCD) (Putranto et al., 2015), and *ethylene-responsive transcription factor RAP2* was associated with ethylene stress-induced response (Han et al., 2000; Sivasubramaniam et al., 1995). These findings reveal the genetic control underlying dry latex yield trait and will contribute to the genetic breeding improvement in rubber tree.

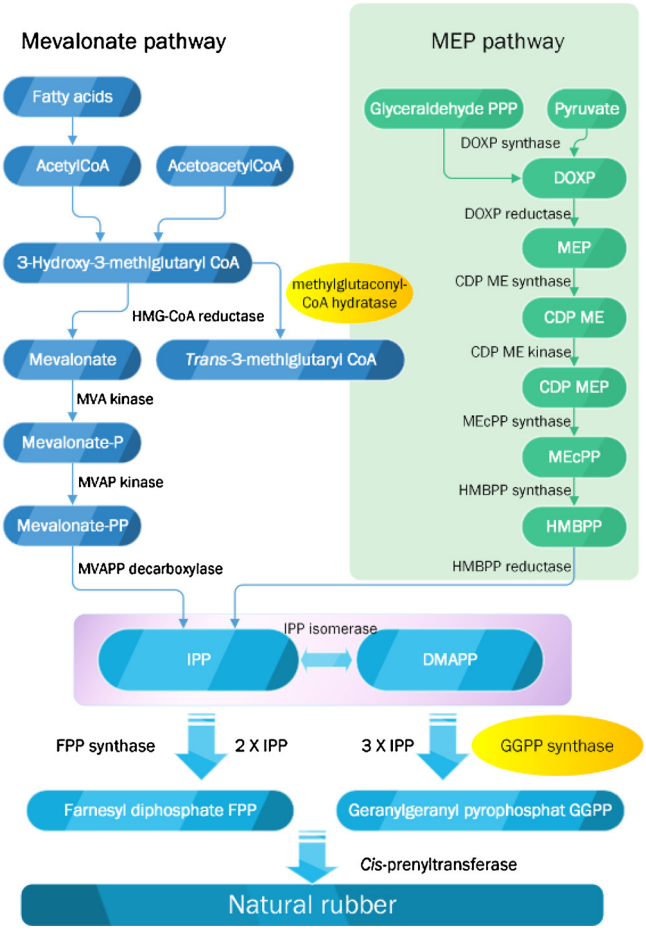

Fig. 3. The candidate QTL genes in rubber biosynthesis pathway.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

This work was supported by National Natural Science Foundation of China (31261140363, 31301102), Natural Science Foundation of Hainan Province (20163118) and Chinese Agriculture Research System Foundation (CARS-31-GW3).

Authors' contributions

WW and ZX conceived and designed the project. ZX and MZ analyzed the data as a whole. MZ prepared the manuscript. LH developed the rubber tree YI mapping population. MZ and SZ performed DNA preparation and prepared libraries for Illumina sequencing. ZX developed the original protocol for AFSM library preparation, created bioinformatics scripts and conducted sequence analysis. MZ and ZX constructed the linkage map and QTL map. ZX performed association analysis. WY and LH planted and collected the field trait. KL gave the revision of manuscript and provided constructive suggestions. All authors read and approved the final manuscript.

Consent for publication

All authors consent for publication.

References

Anon, 1996. Breeding laboratory in yunnan institute of tropical crops. the research of yunyan 277-5 breeding. Yunnan Trop. Crop Sci. Technol. 8–12.

Balsalobre, T.W.A., et al., 2017. GBS-based single dosage markers for linkage and QTL mapping allow gene mining for yield-related traits in sugarcane. BMC Genomics 18, 72.

Bauer, E., et al., 2013. Intraspecific variation of recombination rate in maize. Genome Biol. 14, 103.

Delourme, R., et al., 2013. High-density SNP-based genetic map development and linkage disequilibrium assessment in *Brassica napus* L. BMC Genomics 14, 120.
Duan, C., et al., 2010. Gene expression pattern in response to wounding, methyl jasmonate and ethylene in the bark of Hevea brasiliensis. Tree Physiol. 30, 1349–1359.
Feng, S.P., Li, W.G., Yu, F., Wang, J.Y., Wu, Y.T., 2010. Construction of genetic linkage map for rubber tree (*Hevea brasiliensis*) based on SSR markers. Hereditas 32, 857–863.
Graham, I.A., et al., 2010. The genetic map of *Artemisia annua* L. identifies loci affecting yield of the antimalarial drug artemisinin. Science 327, 328–331.
Han, K.H., et al., 2000. Genes expressed in the latex of *Hevea brasiliensis*. Tree Physiol. 20, 503–510.
Huang, L., et al., 2016. Development and deployment of a high-density linkage map identified quantitative trait loci for plant height in peanut (*Arachis hypogaea* L.). Sci. Rep. 6, 39478.
Jaramillo-Correa, J.P., Verdú, M., González-Martínez, S.C., 2010. The contribution of recombination to heterozygosity differs among plant evolutionary lineages and life-forms. BMC Evol. Biol. 10, 22.
Kang, H.M., et al., 2010. Variance component model to account for sample structure in genome-wide association studies. Nat. Genet. 42, 348–354.
Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with bowtie 2. Nat. Methods 9, 357–359.
Le Guen, V., et al., 2003. Molecular mapping of genes conferring field resistance to South American Leaf Blight (*Microcyclus ulei*) in rubber tree. Theor. Appl Genet. 108, 160–167.
Le Guen, V., et al., 2007. Bypassing of a polygenic Microcyclus ulei resistance in rubber tree, analyzed by QTL detection. New Phytol. 173, 335–345.
Le Guen, V., et al., 2011. A rubber tree's durable resistance to Microcyclus ulei is conferred by a qualitative gene and a major quantitative resistance factor. Tree Genet. Genomes 7, 877–889.
Lespinasse, D., et al., 2000. A saturated genetic linkage map of rubber tree (*Hevea spp.*) based on RFLP, AFLP, microsatellite, and isozyme markers. Theor. Appl. Genet. 100, 127–138.
Li, D., et al., 2016. Transcriptome analyses reveal molecular mechanism underlying tapping panel dryness of rubber tree (*Hevea brasiliensis*). Sci. Rep. 6, 23540.
Liu, Z., Ning, L., He, L., 2010. Adaptability study on hevea clone IAN873 in yunnan. Trop. Agri. Sci. Technol 33 (13–15), 30.
Novalina, N., Sagala, A.D., 2013. Construction of *Hevea brasiliensis* genetic linkage map and identification of quantitative trait loci using RAPD markers. Int. J. Adv. Sci., Eng. Inf. Technol 3, 71–75.
Ong, S.H., 1975. Chromosome morphology at the pachytene stage in Hevea brasiliensis-a preliminary report. In: Kuala Lumpur, Malaysia. Int. Rubber Conf. 2. pp. 3–12.
Piyatrakul, P., et al., 2014. Sequence and expression analyses of ethylene response factors highly expressed in latex cells from *Hevea brasiliensis*. PLoS One 9, e99367.
Pootakham, W., et al., 2015. Construction of a high-density integrated genetic linkage map of rubber tree (*Hevea brasiliensis*) using genotyping-by-sequencing (GBS). Front. Plant Sci. 6, 367.
Priyadarshan, P.M., Goncalves, P. de S., 2003. Hevea gene pool for breeding. Genet. Resour. Crop Evol 50, 101–114.
Putranto, R.A., et al., 2015. Involvement of ethylene in the latex metabolism and tapping panel dryness of Hevea brasiliensis. Int. J. Mol. Sci. 16, 17885–17908.
Rahman, A.Y.A., et al., 2013. Draft genome sequence of the rubber tree *Hevea brasiliensis*. BMC Genomics 14, 75.
Rattanawong, R., et al., 2011. Application of QTL mapping for early selection on growth and latex yield traits in rubber breeding. RRDB Int. Rubber Conf in Chiang Mai Thailand 1–10.
Shearman, J.R., et al., 2015. SNP identification from RNA sequencing and linkage map construction of rubber tree for anchoring the draft genome. PLoS One 10, e0121961.
Sivasubramaniam, S., Vanniasingham, V.M., Tan, C.T., Chua, N.H., 1995. Characterisation of HEVER, a novel stress-induced gene from Hevea brasiliensis. Plant Mol. Biol 29, 173–178.
Souza, L.M., et al., 2011. Development of a genetic linkage map of rubber tree (*Hevea braziliensis*) based on microsatellite markers. BMC Proceedings 5 (39 Suppl. 7).
Souza, L.M., et al., 2013. QTL mapping of growth-related traits in a full-sib family of rubber tree (*Hevea brasiliensis*) evaluated in a sub-tropical climate. PLoS One 8, e61238.
Tang, C., et al., 2010. The sucrose transporter HbSUT3 plays an active role in sucrose loading to laticifer and rubber productivity in exploited trees of Hevea brasiliensis (para rubber tree). Plant Cell Environ. 33, 1708–1720.
Tang, C., et al., 2016. The rubber tree genome reveals new insights into rubber production and species adaptation. Nat. Plants 2, 16073.
Tiley, G.P., Burleigh, J.G., 2015. The relationship of recombination rate, genome structure, and patterns of molecular evolution across angiosperms. BMC Evol. Biol 15, 194.
Triwitayakorn, K., et al., 2011. Transcriptome sequencing of *Hevea brasiliensis* for development of microsatellite markers and construction of a genetic linkage map. DNA Res. 18, 471–482.
Van Ooijen, J.W., 2004. MapQTL® 5. Software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Wageningen, pp. s1.
Van Ooijen, J.W., 2011. Multipoint maximum likelihood mapping in a full-sib family of an outbreeding species. Genet. Res. 93, 343–349.
Xia, Z., Zou, M., Zhang, S., Feng, B., Wang, W., 2014. AFSM sequencing approach: a simple and rapid method for genome-wide SNP and methylation site discovery and genetic mapping. Sci. Rep. 4, 7300.
Xiao, X., et al., 2014. Structure and expression profile of the sucrose synthase gene family in the rubber tree: indicative of roles in stress response and sucrose utilization in the laticifers. Febs. J 281, 291–305.