Development and characterization of microsatellite markers for the rubber tree powdery mildew pathogen *Oidium heveae*

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Abstract Powdery mildew, caused by *Oidium heveae*, is a major threat to rubber plantations worldwide. Population studies are scarce for this pathogen due to the lack of polymorphic molecular markers. In this study, sixteen polymorphic microsatellite loci were identified using DNA extracted from single lesions based on the whole-genome sequences of the pathogen. Primers of these loci were applied to 138 *O. heveae* samples from five counties in Hainan, China. The number of alleles per locus ranged from 2 to 11 and the gene diversity varied from 0.014 to 0.728. In total, 119 multilocus genotypes (MLGs) were observed for the 138 samples. However, only one sample was significantly distinct from the other samples base on DAPC analysis. Further study indicated that there were three subclusters for the other 137 samples, but no evidence for regional genotypic subdivision was identified. The microsatellite markers developed in this study are very useful to study the genetic structure and dispersal route of *O. heveae*, especially as it can use DNA extracted directly from infected leaves.

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Keywords Microsatellite markers · *O. heveae* · Rubber tree · Population structure

Introduction

Rubber tree (*Heveae brasiliensis*), a tropical deciduous tree, is economically important for the primary source of natural rubber latex. Rubber trees were successfully planted in South China, mainly in Hainan, Yunnan and Guangdong provinces with plantation area approximately 24.14×10^4 km^2 by the end of 2015 (Liu et al., 2015). However, these regions were considered as marginal areas for rubber plantations and provided favorable conditions for the proliferation and development of rubber tree diseases such as powdery mildew (Liyanage et al., 2016). Powdery mildew is a major threat to rubber plantations worldwide as the disease can result in a reduction of latex yields of up to 45% (Liyanage et al., 2016). In China, the disease was first reported in Hainan in 1951 and now it occurs annually in all rubber plantations (Wu et al., 2019).

Due to lack of a teleomorph, the causal agent of rubber tree powdery mildew is *Oidium heveae*, which was first described by B. A. Steinmann in 1925. However, based on morphological and phylogenetic data, recent studies suggested that *O. heveae* is the asexual morph of *Erysiphe quercicola* using rubber tree powdery mildew sampled from Brazil, Thailand, Malaysia, Vietnam, Sri Lanka and China (Limkaisang et al., 2005; Tam et al. 2016; Limkaisang et al., 2017; Wu et al., 2019). Whether sexual reproduction occurs for the pathogen on rubber tree hosts is still unknown.

As an obligate parasite, the pathogen only infects tender tissues of rubber tree i.e. leaves, buds, and inflorescences, so the disease mainly occurs in spring during

refoliation season after annual wintering. The fungus survives from one season to the next on young leaves that emerge periodically within the canopy or on tender leaves in rubber nurseries and serve as primary inoculum for epidemics in spring.

Population genetics is the study of the distribution of allele frequencies (patterns) in space and time which can be used to infer the origin, migration and evolution of plant pathogens (Grünwald et al., 2017). This information is critical for devising strategies to control the disease. Molecular markers are highly valued in population genetic studies for their capability to distinguish genotypes. Among the numerous marker types available, microsatellite, also known as simple sequence repeats (SSRs), have become most widely used in studies of plant pathogens. Microsatellites are abundant, codominant, universal, easy to automate, reliable and genetic neutrality markers (Grover and Sharma, 2016). Microsatellite markers were developed and used for some powdery mildew pathogens such as *E. necator* (Frenkel et al., 2012), *Blumeria graminis* f.sp. *tritici* (Wang et al., 2014), *B. graminis* f. sp. *hordei* (Tucker et al., 2015), *E. pulchra* (Wyman et al., 2019) and *Podosphaera leucotricha* (Lederson et al., 2021). However, there was no microsatellite markers developed for *O. heveae*.

One practical issue for population genetic analyses arises with obligate pathogens such as rusts or powdery mildews that cannot be cultured on routine media to obtain sufficient biomass for DNA extraction (Grünwald et al., 2017). One of the approaches to cope with this issue was the direct extraction of fungal and plant DNA from infected leaves and developed PCR primers for amplification of species specific loci. DNA was extracted from infected leaves and then used for SSR genotyping had

been reported for some obligate pathogens i.e. *Puccinia striiformis* f.sp. *tritici* from single sporulating lesion (Ali et al., 2011; Ali et al., 2014; Khan et al., 2019), *E. pulchra* from fresh leaves of *C. florida* (Wyman et al., 2019) and *Plasmopara viticola* from single oilspots of grapevine (Maddalena et al., 2020).

To date, there is currently limited information regarding the genetic diversity of *O. heveae*. Only ITS and 28S sequences were used for the pathogen identification and phylogenetic analyses (Limkaisang et al., 2017; Wu et al., 2019). The purpose of this study was to (i) develop microsatellite markers based on whole-genome sequence data of *O. heveae* using DNA extracted from single lesions, (ii) to use the newly developed method to characterize the population structure of this pathogen in Hainan, China.

Materials and methods

Sample collection and DNA extraction

Leaves showing powdery mildew symptoms were randomly collected between February and April 2021 from 25 rubber tree plantations located in 5 different counties of Hainan, China: Sanya (five plantations), Qiongzhong (five plantations), Danzhou (six plantations), Qionghai (three plantations) and Haikou (six plantations). A single lesion with a 1 cm diameter cork borer per leaf was randomly chosen and considered to be a sample. Four to six samples were used in each plantation and a total of 138 samples were collected in this study (Table S). Furthermore, four samples from Yunan and Guangdong (two from each province) were also included to screen and identify polymorphisms of SSR loci. Genomic DNA for each sample was

extracted using the Tiangen Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions.

Microsatellite development and selection

The genome of *O. heveae* strain HO-73 was downloaded from NCBI database (Accession No: QVIK00000000). Tandem Repeat Finder 4.08 (Benson 1999) was used to identify microsatellite motifs. Only di- to hexanucleotide motifs was included in this study. The copy number criteria were 6 or more repeat units for dinucleotide repeats (DNRs), and 5 or more repeat units for trinucleotiderepeats (TNRs), tetranucleotide repeats (TeNRs), pentanucleotide repeats (PNRs), and hexanucleotide repeats (HNRs).

Primers were designed within flanking regions of microsatellite motifs (≥10 repeat units for DNRs, ≥ 8 repeat units for TNRs, ≥ 6 repeat units for TeNRs, PNRs and HNRs) using PRIMER3 software, with parameters set to primer length from 18 to 23 bp, GC content of 35 to 65%, and primer melting temperatures in the range 52 to 65°C for amplicons of 140–350 bp. A total of 65 primer pairs were designed initially. Six samples (each two from Hainan, Yunnan and Guangdong, respectively) as well as a positive control (DNA of the pathogen) and a negative control (sterile distilled water) were used for all PCR reactions to ensure validity of our data. Furthermore, to eliminate the possibility of amplifying host DNA, all primers were tested against DNA from uninfected leaves from four rubber tree varieties (CATAS7-33-97, Haikeng 1, PR107 and RRIM600).

For each SSR primer pair, PCR was performed with a Veriti™ Dx 384-well

Thermal Cycler (Applied Biosystem, Carlsbad, CA, USA) in a 25 µl reaction volume. The PCR mixtures contained 12.5 µl of Taq-Plus PCR Forest Mix (GeneTech，GuangZhou Jitaike Gene Sci-Tech Co.,Ltd., Guangdong, China), 1 µl of 5 µM of each primer, and 1 µl of DNA template. A touchdown polymerase chain reaction was employed using the following thermal cycling conditions: 95°C/5 min; (95°C/30 s, 62°C/30 s –1°C/ cycle, 72°C/30 s) ×10; (95°C/30 s, 52°C/30 s, 72°C/30 s) ×25, 72°C/20 min. PCR products were visualized on 1% agarose gels.

DNA sequences (four of the six samples) of the primer pairs with expected product sizes that did not amplified rubber tree DNA or the size of the amplicon for rubber tree were different from those for the samples were obtained to confirm amplification of the targeted motif. DNA was sequenced by SinoGenoMax Co., Ltd. (Chinese National Human Genome Center, Beijing, China). Sixteen microsatellite loci showed different repeat units based on sequences analysis and these sequences were submitted to GenBank (see Table 1).

Microsatellite genotyping

For the 16 selected primer pairs, the 5’ end of the forward primer was labeled with one of the three fluorescent dyes (FAM, HEX and TAMRA; Applied Biosystems, Foster City, CA) and they were used to amplify gDNA from the *O. heveae* samples in the study. The PCR mix for each primer contained 1ul of template DNA, 5µL 2×TaqPCR MasterMix (GeneTech，GuangZhou Jitaike Gene Sci-Tech Co.,Ltd.，Guangdong, China), 0.5µl each of forward and reverse primers (10 µM) and 3 µl of double-distilled H_2O. Amplicons were analyzed with an ABI 3730xl DNA Analyzer

(Applied Biosystems, Carlsbad, CA, USA) for capillary electrophoresis. Because *O. heveae* is a haploid, a single peak (amplicon) was expected for each sample near the expected size (Fig. 1). The GeneScan™500 LIZ (Thermo Fisher Scientific) was used for the standard dye size. The amplicon sizes were determined using GeneMarker software version 3.0.0 (Applied Biosystems, Foster City, CA).

Data analysis

To evaluate the 16 selected microsatellite markers, the number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I) and Nei's unbiased gene diversity (h) was computed for each marker based on the data of 138 samples in GenAlEx v. 6.5.2 (Peakall and Smouse, 2012). A genotype accumulation curve was created to determine the threshold required to discriminate among unique MLGs given a random sample of *n* loci with 1,000 bootstrap replicates using the package *poppr*.

To determine the genetic diversity of different populations defined on the basis of counties, the following statistics were computed with the *poppr* package (Kamvar et al. 2014) in R 4.1.0, including number of multilocus genotypes (MLGs), the number of expected MLG based on rarefaction (eMLG) to eliminate potential effects different sample sizes, Simpson's index of genotypic diversity (λ), genotypic evenness (E.5) and Nei's gene diversity. The allelic richness (R_a) and private allele richness (R_p) of populations was calculated with the rarefaction approach implemented in the ADZE programme to account for differences in the sampling effort (Szpiech et al. 2008).

The population genetic structure of the 138 *O. heveae* samples was studied using discriminant analysis of principal components (DAPC) in the *adegenet* package (Jombart et al., 2010). The number of clusters that best fitted the original data was determined using a K-means clustering based on Bayesian information criteria (BIC).

Results

Characterization and distribution of microsatellites

In total, 2720 di- to hexanucleotide motifs were identified (Table 2). Among them, the most common repeat motifs were dinucleotide (1619, 59.52%), followed by trinucleotide (682, 25.07%). The observed frequency for tetranucleotide repeats was 12.13% (330), while the proportion for the penta- and hexanucleotide repeats each were below 2%. The number of microsatellite repeat units per locus ranged from 5 to 43. Microsatellites with six tandem repeats (27.39%) were the most common types, followed by five tandem repeats (18.60%), seven tandem repeats (13.71%), and eight tandem repeats (9.49%).

Evaluation of microsatellite markers

Among the 65 microsatellite primer pairs designed, 9 primer pairs did not did not produce an amplification product and the amplicon sizes of another 11 primer pairs were not the same as expected or could not be distinguished from those with rubber tree DNA only. For the other 45 primer pairs with expected product sizes that did not amplify rubber tree DNA, or where the sizes of the amplicon were different from those with rubber tree DNA, 16 microsatellite markers successfully amplified

polymorphic PCR products based on the sequences analysis. Based on the data from 138 samples from Hainan, the number of alleles per primer pairs ranged from 2 (OH-SSR38) to 11 (OH-SSR51). Gene diversity of the 16 loci ranged from 0.014 (OH-SSR38) to 0.728 (OH-SSR28) (Table 3).

Population structure

According to the genotype accumulation curve (Fig. S1), a plateau was reached in the number of each MLG for the number of loci used, which indicated that the 16 loci used were sufficient to discriminate individuals among the tested samples. In total, 119MLGs were detected among the 138 samples, among which only 7 MLGs were detected in different samples, whereas the remaining 112 MLGs were observed only once. MLG 87 was detected in 11 samples from 10 plantations in four counties (except Qionghai) while the other 6 shared MLGs were only identified in two or three samples from one or two counties (Tables S1).

The number of eMLG was similar for populations from different counties. A high genotypic diversity was observed in all populations as revealed by Simpson's index and genotypic evenness (> 0.91) (Table 4). The Nei's gene diversity ranged from 0.272 to 0.353. Allelic richness (R_a) averaged across all loci ranged from 2.813 to 3.813 and the private allele richness (R_p) ranged from 0.248 to 0.685 (Table 4).

Based on the lowest BIC value, the *k*-means method estimated eleven clusters present in the 138 samples; however, only one sample (from Qiongzhong) was significantly separated for the other samples (Fig. 2). Further analysis of for the other samples indicated that nine subclusters were the optimal number to describe the data

according to the *k*-means method. However, the DAPC analysis of the samples indicated that these nine subclusters reduced to three subclusters as the increase of BIC value from K = 9 to K = 3 was small (Fig. S2). Therefore, three subclusters were used in the DAPC analysis for the other 137 samples (Fig. 3). Each subcluster contained 40, 10 and 87 samples, respectively. Both subcluster 1 and subcluster 3 had samples from all five counties while subcluster 2 had samples from four counties (all counties except Qionghai) (Fig. 4).

Discussion

The whole-genome sequences of fungi allow the identification of microsatellites at genome-wide level and this approach has been applied successfully in other powdery mildew pathogens including *B. graminis* f. sp. *hordei* (Tucker et al., 2015), *E. pulchra* (Wyman et al., 2019) and *P. leucotricha* (Lederson et al., 2021). In the present study, the genome sequence of a strain of *O. heveae* was used to develop 16 polymorphic microsatellite markers. Their utility for population studies was demonstrated by assessing samples from Hainan, China.

Among the 16 markers, only 8 of them were with the Nei's unbiased gene diversity above 0.25 (Table 3). The main reason may be that the samples were all from a single province of China and the Nei's unbiased gene diversity may be higher if samples had been taken from different provinces. However, the microsatellite markers developed in this study were still useful for studying the genetic diversity and population structure of *O. heveae*.

More importantly, the microsatellite markers developed in this study can be used

for DNA extracted from a single lesion and only a single peak (amplicon) near the expected size was observed for each sample used in this study (Fig. 1). This was very important for the genetic structure research of *O. heveae* because of the difficulty in the preserving and culturing of the pathogen. The pathogen only infects young tissues of rubber tree i.e. leaf, bud and inflorescence and no protective green agent has been reported on rubber tree leaf made it was impossible to culture the pathogen on detached leaves (Tu et al., 2012). As a woody plant, although rubber tree tissue culture of seedlings has been successfully demonstrated (Qin et al. 2017), usually only three small tender leaves are produced per seedling so a lot of seedlings and time would be needed to obtain sufficient powdery mildew biomass for DNA extraction. The technique developed in this study could save time and resources in producing seedlings for population structure research of *O. heveae*.

The 16 markers developed in this study were evaluated based on 138 samples from five counties in Hainan, China. In DAPC analysis of the entire data set, eleven clusters best explained the data. However, only one sample was assigned to a distinct cluster and the other 137 samples were further divided into distinct genetic groups (Fig. 2). Whether the individual represented in the sample that clustered distinctly had originated from another province or region requires further research but dispersal of airborne conidia of powdery mildew is possible over long distances (Glawe 2008). Further analysis indicated that the 137 samples that were clustered together could be grouped into three subclusters but these could not be attributed to sampling counties (Fig. 4). This indicated that the pathogens were dispersed between different counties.

Similar results were reported about the population structure of apple powdery mildew *P. leucotricha* in Washington State, USA (Lederson et al., 2021). Also no regional genotypic subdivision was observed in mildew pathogens such as barley powdery mildew *B. graminis* f. sp. *hordei* from Australia (Tucker et al., 2015) and the dogwood powdery mildew *E. pulchra* in the eastern United States (Wyman et al., 2019). Furthermore, MLG 87 was detected in four counties, which also indicated the dispersal of the pathogen within different counties. This is consistent with that powdery mildew fungi can produce huge numbers of spores, which are wind dispersed (Nicolaisen et al., 2017). In spring, the time for rubber trees to produce young leaves varied in different counties where the time for Sanya was usually earlier, followed by Qiongzhong, Qionghai and Danzhou, while Haikou was the latest in the five counties. The time for rubber trees to produce young leaves also varied among plantations in the same county because of the varieties and cultivation measures. This variation in susceptible periods is thought to make it more likely that a new cycle of infection will occur successfully each year in different regions and may influence the dispersal of the pathogen.

In conclusion, this study developed 16 microsatellite markers to characterize genetic variation of *O. heveae* using DNA extracted from single lesions. This can save a lot of time for the biomass collection for DNA extraction. Furthermore, these markers can be used to study the population structure of the pathogen at the regional level for samples from different regions i.e. Guangdong and Yunnan in China or other countries and may imply the dispersal route of the pathogen. Additionally, whether

there could be a sexual stage present from the diversity of the population can be studied using these markers in future research.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Table 1 Characteristics of 16 polymorphic microsatellite markers developed in this study for *Oidium heveae*

Locus	Repeat unit	GenBank accession no.	Primer sequence (5'-3')	GC%	Tm
OH-SSR10	$(TC)_{22}$	OK041123-OK041126	F: TGCCATGCCATCAATACGGT	42.8	52.2
			R: TCTCTGTTCCCATCATTGCTT	50	60
OH-SSR11	$(GA)_{15}$	OK065947-OK065950	F: GGTGCAGATATGTTCTGGCT	50	60
			R: GTTTAGGGAAGCTCACGGTC	55	62
OH-SSR15	$(CT)_{27}$	OK065951-OK065954	F: GGTCGACAGTTTGCCCATCT	55	62
			R: ACATTGGCGTCCTCGTGATT	50	60
OH-SSR17	$(TTTGAA)_{8}$	OK065955-OK065958	F: CGAGTTCCTGTGATAAGCGT	50	60
			R: GGCCACAGTAGTGCAAAAGG	55	62
OH-SSR26	$(AGA)_{15}$	OK065959-OK065962	F: GGGTATCCCATTGACCTTGCT	52.3	56.1
			R: ATCTGGTCCCTTCAAAACACCG	50	56.4
OH-SSR28	$(GAAAAT)_{15}$	OK065963-OK065966	F: TACCCTACTCCTGAGCGCAT	55	62
			R: CTTCCGTTCAACAGCGCATC	55	62
OH-SSR34	$(AC)_{18}$	OK065967-OK065970	F: AAGCTGTTCATTTGCCTCTGC	47.6	54.2
			R: TAGCCTCAAGAACGTCGGAAA	47.6	54.2
OH-SSR36	$(GA)_{21}$	OK065971-OK065974	F: TCACAATGGGCCAGCTAACTT	47.6	54.2
			R: GTGCAGTGAAAGTCGTCAATGT	45.4	54.5
OH-SSR38	$(TTG)_{9}$	OK065975-OK065978	F: TTTCGTACTCGCTTGGGCTT	50	60
			R: GATCCTACTGCACCTTCGCA	55	62
OH-SSR46	$(CT)_{36}$	OK065979-OK065982	F: CTGTCCCTCATTGCCATATTCT	45.4	54.5
			R: GAATTCCAAAACCCCTTTTTGC	40.9	52.7
OH-SSR47	$(GAA)_{18}$	OK065983-OK065986	F: ACTCCAGCGCCTCAAGATAA	50	60
			R: TCCAGCATCTGATTTTGTGATG	40.9	52.7
OH-SSR48	$(TTC)_{20}$	OK065987-OK065990	F: ACCCACGATGCAGATAGACAA	47.6	54.2
			R: CAAATTAAGAGGAATGAGGGCA	39.1	53.1
OH-SSR49	$(GA)_{20}$	OK065991-OK065994	F: CCAATCACTCGAAGGCCAGAT	52.3	56.1
			R: GGATCAACCGTTTTTGCCCT	50	60
OH-SSR50	$(TTTGA)_{18}$	OK065995-OK065998	F: CGGGAGTCAGCAAATGAGGT	55	62
			R: CAAAGGGTTTCCCCCGTGTA	55	62
OH-SSR51	$(TC)_{28}$	OK065999-OK066002	F: CTACCGGCAGTGGCAGTTTA	55	62
			R: ATTCACGCTCGCTCGCTTAT	50	60
OH-SSR63	$(CAAT)_{11}$	OK066003-OK066006	F: GCGCCATATCAGGCAGAGTT	55	62
			R: CTGGTCTGGTATGTGGTCCG	60	64

Table 2 Relative frequency, proportion (%), and number of selected repeat-motif types in *Oidium heveae*

No. of repeat unit	Motif length					Total	proportion %
	Di-	Tri-	Tetra-	Penta-	Hexa		
5	-	284	186	20	16	506	18.60
6	451	177	95	12	10	745	27.39
7	243	96	24	6	4	373	13.71
8	188	51	13	2	4	258	9.49
9	153	28	4	2	1	188	6.91
10	127	18	2	1	0	148	5.44
11	123	9	3	0	2	137	5.04
12	89	6	0	0	1	96	3.53
13	68	2	0	0	0	70	2.57
14	44	2	0	0	0	46	1.69
≥15	133	9	3	2	6	153	5.63
Total	1619	682	330	45	44	2720	
Proportion %	59.52	25.07	12.13	1.65	1.62		

Table 3 Genetic variation of 16 microsatellite loci used to characterize *Oidium heveae* from Hainan, China[a]

Locus	Product size	Na	1-D	Evenness	He
OH-SSR10	253-259	4	0.111	0.412	0.111
OH-SSR11	202-206	3	0.029	0.329	0.029
OH-SSR15	240-260	7	0.492	0.589	0.496
OH-SSR17	264-276	3	0.070	0.384	0.071
OH-SSR26	250-280	7	0.227	0.387	0.229
OH-SSR28	293-341	8	0.722	0.700	0.728
OH-SSR34	239-249	6	0.512	0.608	0.516
OH-SSR36	251-257	4	0.164	0.415	0.165
OH-SSR38	163-166	2	0.014	0.333	0.014
OH-SSR46	310-320	6	0.461	0.611	0.464
OH-SSR47	264-304	10	0.441	0.418	0.444
OH-SSR48	255-300	9	0.264	0.384	0.266
OH-SSR49	196-202	4	0.233	0.518	0.235
OH-SSR50	232-302	9	0.573	0.530	0.577
OH-SSR51	197-241	11	0.573	0.686	0.577
OH-SSR63	180-212	3	0.029	0.329	0.029

Na = Number of alleles; 1-D = Simpson’s diversity index; He = Nei's gene diversity.

Table 4 Genetic diversity based on 16 microsatellite markers in *Oidium heveae* populations from Hainan, China[a]

Populations	N	MLG	eMLG	λ	E.5	He	R_a	R_P
Sanya	26	24	15.3	0.956	0.963	0.296	3.063	0.445
Qiongzhong	28	25	14.9	0.954	0.916	0.353	3.813	0.685
Qionghai	16	15	15.0	0.930	0.967	0.327	2.813	0.534
Haikou	34	31	15.2	0.964	0.927	0.272	2.938	0.248
Danzhou	34	30	15.0	0.962	0.921	0.311	3.375	0.257
Total	138	119	15.3	0.986	0.677	0.309		

[a] N = number of isolates, MLG = number of multilocus genotypes, eMLG = number of expected MLG based on rarefaction, λ = Simpson's index of genotypic diversity, E.5 = genotypic evenness, which is the distribution of genotypes within a sample, He = Nei's gene diversity, R_a = allelic richness, R_P = private allelic richness.

Legend of figures

Fig. 1 The PCR amplification peaks of some primers determined using GeneMarker software

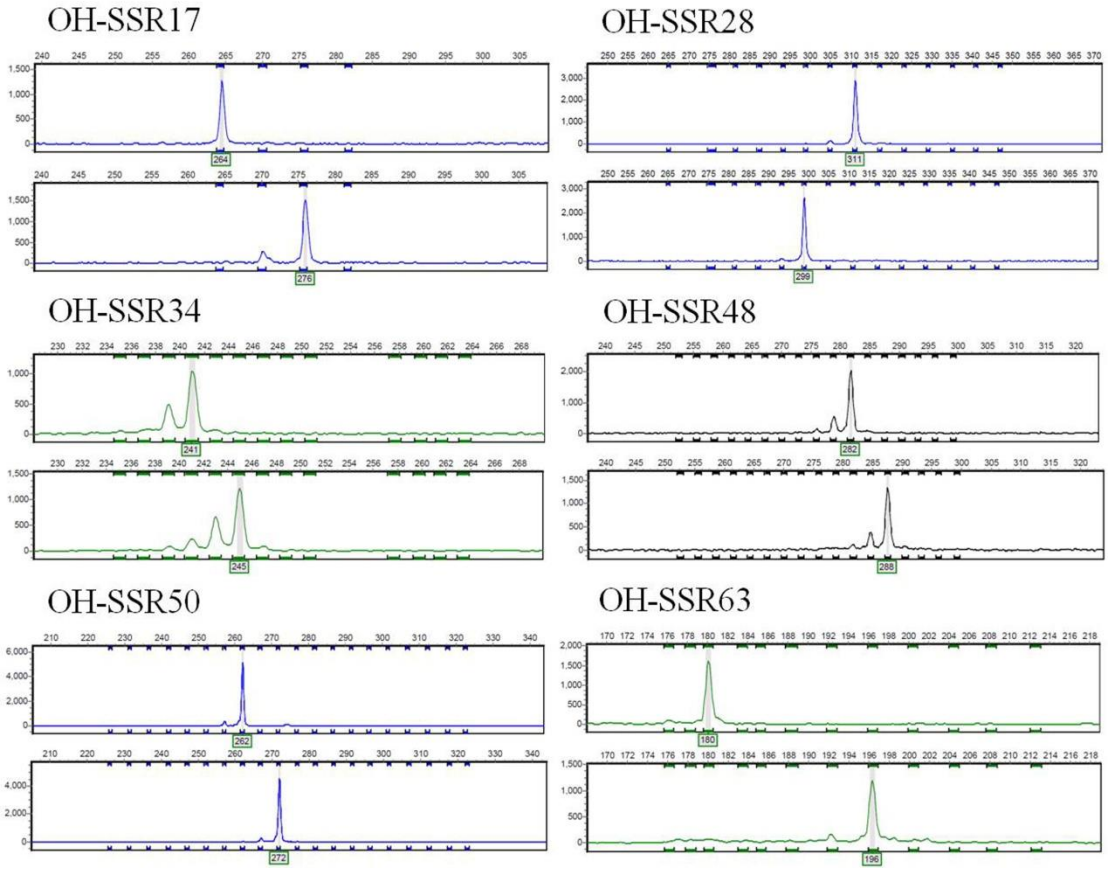
Fig. 2 Discriminant analysis of principal components (DAPC) of the 138 rubber tree powdery mildew samples from Hainan, China. A, Value of Bayesian information criteria (BIC); B, Scatterplot of DAPC.

Fig. 3 Scatterplot of three clusters of 137 rubber tree powdery mildew samples from Hainan based on discriminant analysis of principal components (DAPC).

Fig. 4 Subclusters of the other 137 rubber tree powdery mildew samples from Hainan, China using discriminant analysis of principal components (DAPC)

Fig. S1 The genotype accumulation curve for 138 rubber tree powdery mildew samples from Hainan, China. The boxplots represent the range of MLG numbers observed for each random sample of loci.

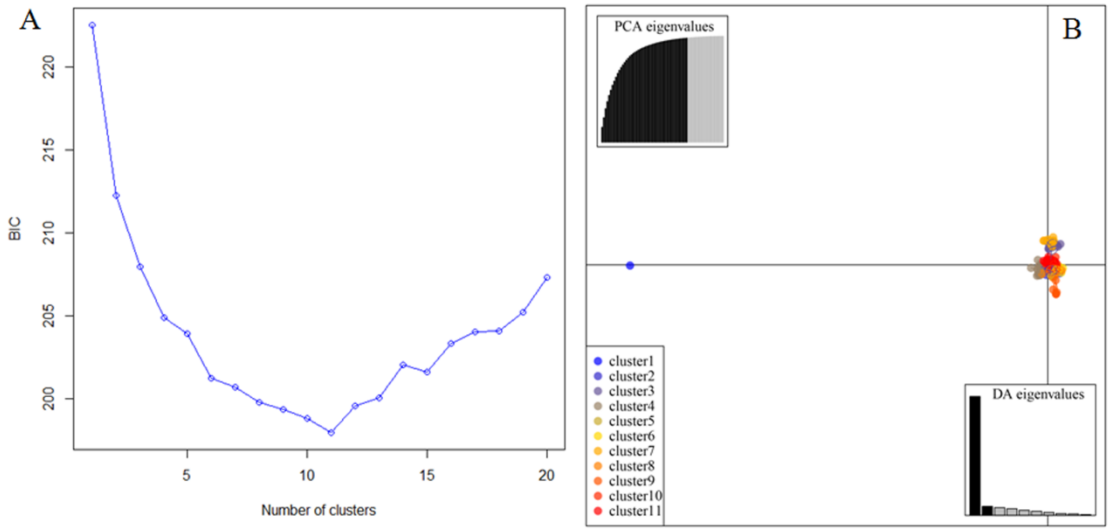
Fig. S2 Discriminant analysis of principal components (DAPC) of the 137 rubber tree powdery mildew samples from Hainan, China. A, Value of Bayesian information criteria (BIC); B, Scatterplot of DAPC.



1

2

3


A
BIC
200
205
210
215
220
5
10
15
20
Number of clusters
B
PCA eigenvalues
DA eigenvalues
cluster1
cluster2
cluster3
cluster4
cluster5
cluster6
cluster7
cluster8
cluster9
cluster10
cluster11

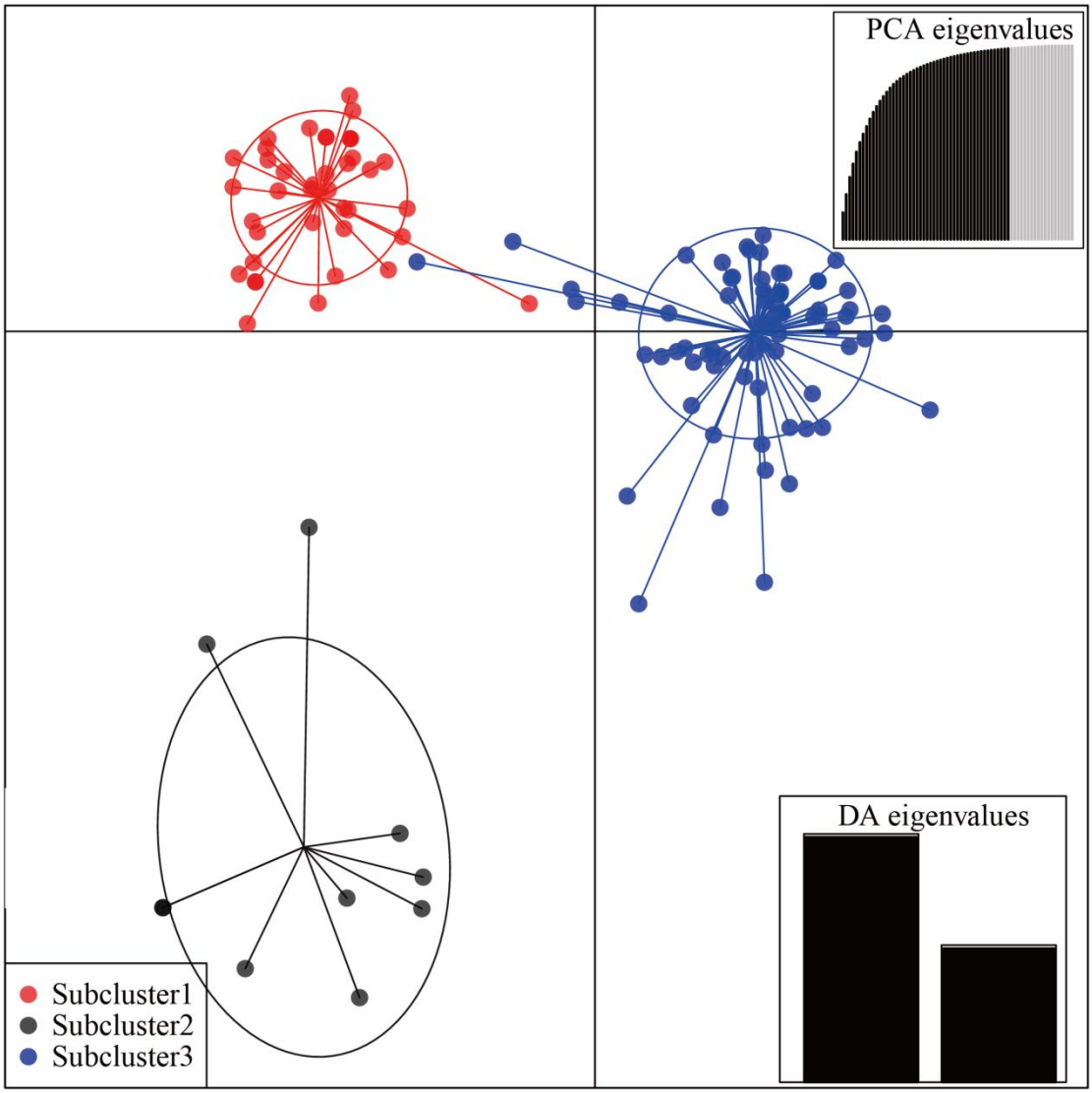
1

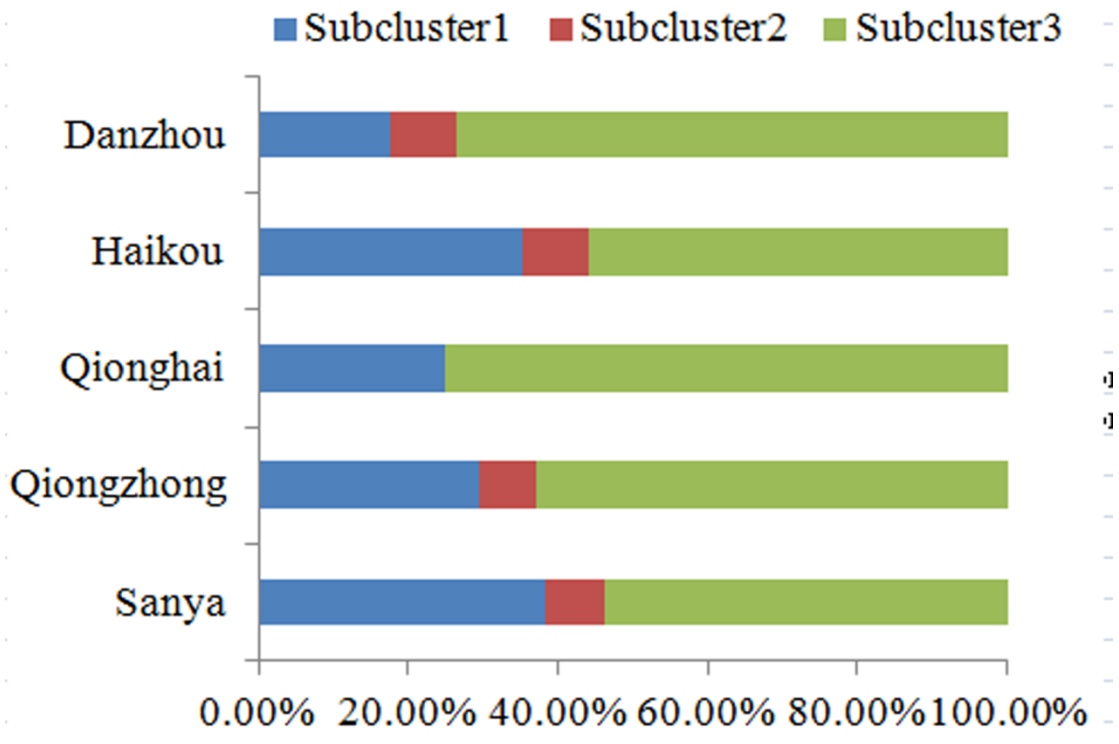
2

3

4

5


PCA eigenvalues
DA eigenvalues
Subcluster1
Subcluster2
Subcluster3


Subcluster1
Subcluster2
Subcluster3
Danzhou
Haikou
Qionghai
Qiongzhong
Sanya
0.00%
20.00%
40.00%
60.00%
80.00%
100.00%

1