BIODIVERSITAS
Volume 20, Number 9, September 2019
Pages: 2698-2706

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d200935

Fingerprinting sengon (*Falcatria moluccana*) accessions resistant to boktor pest and gall rust disease using microsatellite markers

ULFAH J. SIREGAR[1,2,♥], **DEWI RAHMAWATI**[2], **APRILIYA DAMAYANTI**[1]
[1]Department of Silviculture, Faculty of Forestry, Institut Pertanian Bogor. Jl. Ulin, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia.
Tel/fax.: +62-812-1062154, ♥email: ujsiregar@gmail.com.
[2]Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP). Jl. Raya Tajur Km 6, Bogor 16134, West Java, Indonesia

Manuscript received: 25 July 2019. Revision accepted: 27 August 2019.

Abstract. *Siregar UJ, Rahmawati D, Damayanti A. 2019. Fingerprinting sengon (*Falcatria moluccana*) accessions resistant to boktor pest and gall rust disease using microsatellite markers. Biodiversitas 20: 2698-2706.* Sengon (*Falcataria moluccana* Miq.) is a multipurpose fast-growing tree species and widely planted as community forest in Indonesia. According to Indonesian Light Wood Association (ILWA) *F. moluccana* wood products coming from forest industries in Jawa worth US$ 244.46 million export to China alone. The wood dust also has high potential as source for biomass-based energy in the form of wood-pellet..Monoculture plantation however often suffers from stem borer pest, known as boktor (*Xystrocera festiva*) and a gall rust disease, caused by fungi *Uromycladium falcatarium*. This research was aimed to characterize accessions of resistant and susceptible sengon individuals to both gall rust disease as well as stem borer pest using microsatellite markers. Totally 50 accessions of resistant and of resistant to stem borer pest were collected from Sumedang, West Jawa Province, while 88 accessions of resistant and of resistant to gall rust disease were sampled from Kediri, East Jawa Province and Sukabumi, Indonesia. Eight microsatellite markers could amplify most of the accessions used in this study and produce polymorphic fragments. High genetic diversity was detected in all of *F. moluccana* populations, with He ranged from 0.431 to 0.650. AMOVA showed that most genetic variations come from within populations. A dendrogram based on Nei's genetic distance (1972) clustered some resistant accessions to either stem borer pest or gall rust disease separately from susceptible ones.

Keywords: Gall rust disease, microsatellite, resistant, sengon, stem borer, susceptible

INTRODUCTION

Sengon (*Falcataria moluccana* Miq.) is a fast-growing, leguminous tree species that is widely cultivated in community forests, particularly in Jawa Island. It has short ripe period, easily managed, multipurpose timber, and uncomplicated growing requirements. *F. moluccana* is categorized as pioneer species if occurs in primary forest, but more often found in secondary lowland rainforest, light montane forest, grassy plains and along roadsides near the coast (Krisnawati et al. 2011). Nowadays, this tree species is usually utilized for afforestation in degraded forest and marginal land. *F. moluccana* does not need to be fertilized, especially nitrogen (N), because it has N_2 fixing *bacteria* in its root *nodules* and thus able to increase soil fertility (Iskandar and Ellen 2008). The high demand of *F. mollucana*'s wood in Indonesia, especially Jawa, has caused expansion of its plantation. According to Indonesian Light Wood Association (ILWA) *F. moluccana* wood products, which mostly coming from forest industries in Jawa worth US$ 244.46 million exported to China alone (Anggraini 2019).

Falcataria moluccana is commonly planted with monoculture pattern that has advantages in managing, harvesting, and monitoring, however it is susceptible to biotic invasions, such as stem borer pest commonly known as boktor (*Xystrocera festiva*), and gall rust disease affected by *Uromycladium tepperianum* (Endang and Farikhah 2010; Indresputra et al. 2013; Sunandar et al. 2017). Boktor pest and gall rust disease epidemics reduce the quality of wood and lead to severe damage at all stages of *F. molucana*'s growth. Infestation of *X. festiva* usually begins when the trees are 2-3 years old and the percentage of trees infested increases with age, ranging from 4.2% to 73.5% (Notoatmodjo 1963; Husaeni 1992; Matsumoto 1994). Gall rust causes damage to *F. moluccana* up to 90% (Old and Cristovao 2003; Charomaini and Ismail 2008).

Efforts to control *X. festiva* pest and *U. falcatarium* disease in Indonesia has been carried out, however, there is no effective and efficient method yet. Mechanical method by cutting or removing the affected part of the tree could potentially prevent boktor and gall rust spread, however, this method could only reduce the attack rate by 4-10% (Krisnawati et al. 2011). An effective way for controlling the pest and gall rust disease would be using resistant *F. moluccana* lines. Therefore, research on genetic background of *F. moluccana* is needed, especially to characterize *F. moluccana* resistant and susceptible to boktor pest and gall rust disease. Fortunately, observations in the field showed some individuals seemed more resistant to the pest and disease compared to others. It is interesting to find out whether the resistance is genetically based.

Genetic diversity level could serve as indicator of plant adaptability to its environment. Previous research on genetic diversity of *F. mollucana* evaluated by RAPD marker (Suharyanto et al. 2003; Siregar and Olivia 2013) showed considerable diversity in Jawa populations. RAPD marker however, failed to distinguish resistance accessions from susceptible ones used in the study by Neo *et al*. (2018) which showed that both accessions were actually overlapping populations. Inheritance pattern of resistance and susceptibility to pest and disease is still unknown for tropical forest trees. It's merely speculation whether such inheritance is governed by a simple Mendelian pattern or complex characters with many genes involved, such as QTL. In the case of QTL it is necessary to have saturated maps of many markers associated with the characters under study. Characterization of populations or accessions using more reliable and appropriate markers, such as SSR (*Simple Sequence Repeats) or microsatellite*, which is a repetition of the di-nucleotide to tetra-nucleotide sequences, would be beneficial for later study on QTL.

A Microsatellite is widely and evenly spread throughout the genome, easy to assay using PCR technique, and has high repeatability. Its codominance nature with high allelic variation makes microsatellite preferable as genetic markers. Microsatellite consistently displays the same fragment if using the same primer and relatively stable (Hartwell et al. 2004). Verhaegen et al. (2010) used microsatellite markers to determine genetic diversity of teak, Lee et al. (2004) on Shorea, Singh et al. (2014) on agarwood, Budiani et al. (2014) on rubber, Tasma and Arumsari (2013) on oil palm, and Dinarti et al. (2015) on cacao. With these various advantages, microsatellite marker is potential to be developed as molecular marker, particularly for identification, study of genetic diversity, and characterization of the accessions. The purpose of this study was to characterize accessions of *F. moluccana* resistant and susceptible to stem borer pest (*X. festiva*) and gall rust disease (*U. falcatarium*) using microsatellite marker.

MATERIALS AND METHODS

Plant materials

Plant materials used in this research were young leaves of *F. mollucana* individuals which are identified in the field as either resistant or susceptible to boktor pest and gall rust disease. The resistant and susceptible individual pairs were trees which are grown in the same plot, close to each other, in order to eliminate any possible environmental factors affecting the resistance and susceptibility. Susceptible individuals referred to those trees severely attacked by either boktor pest or gall rust (Figure 1) while resistant individuals were the trees showing no signs of the pest and disease attacks. Leaves samples of 25 resistant and 25 susceptible *F. mollucana* trees to boktor pest were taken from community forest in Sumedang, West Jawa, Indonesia. The susceptible tree can be easily identified by holes or wood powders and blackish stains on the stem due to presence of boktor larvae. Meanwhile resistant and susceptible accessions of *F. mollucana* towards gall rust disease were taken from 4 selected populations in PT Bodjongasih, Sukabumi, West Jawa, and Perum Perhutani KPH Kediri, East Jawa, Indonesia. Sukabumi accessions had 31 susceptible and 24 resistant samples, while Kediri accessions had 24 susceptible and 9 resistant samples.

Procedures

DNA extraction

DNA was extracted from 0.1 g fresh leaves with Qiagen DNeasy Plant Mini kit. Extracted DNA quality was analyzed by electrophoresis of 1% agarose gel, at 75 volts for 30 minutes. The electrophoresis result was visualized with Kodak Gel Logic 200.

Polymerase Chain Reaction

The specific SSR primers of *F. moluccana* used in PCR process is the commercial primer published by Saito et al. (2014), and presented in Table 1. PCR reactions were carried out with 25µL of reaction mixture using KOD One^{TM} PCR Master Mix, of which consisted of 3 µL template DNA, 0.5 µL KOD FX Neo, 12.5 µL KOD FX Neo Buffer, 5 µL dNTPs, 2 µL forward primer and 2 µL reverse primer. PCR products that had been visualized with 2% agarose electrophoresis and had good quality, were then electrophoresed on 3% SFR agarose gel. The electrophoresis was at 115 volts for 75 minutes, and then the result was visualized in Kodak Gel Logic 200.

Data analysis

Polymorphic microsatellite loci on the gel was then scored and put in a datasheet, and the data were analyzed using GenAlex 6.501 (Peakall and Smouse 2012) software to estimate the genetic parameters, including percentage of polymorphic loci (PLP), numbers of observed alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho) and expected heterozygosity/Nei's gene diversity (He). DARwin 6.0.14 software was used to calculate the genetic distance and create a dendrogram based on weighted neighbor-joining method. Data on resistant and susceptible accessions to stem borer pest were analyzed separately from those of resistant and susceptible accessions to gall rust disease.

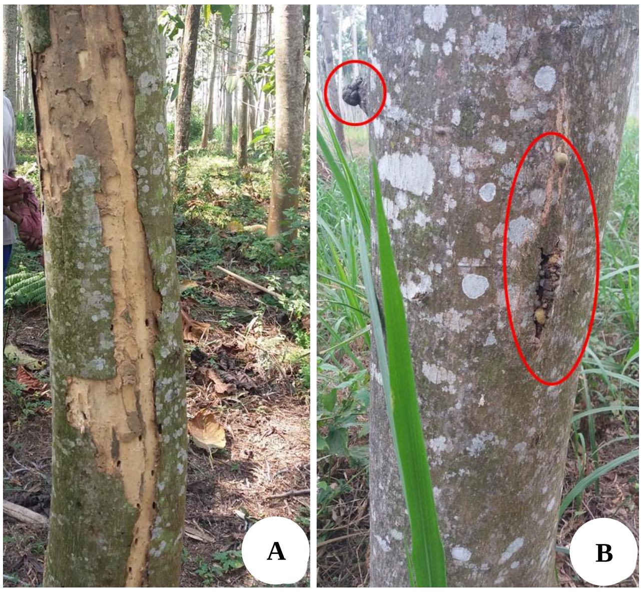

Figure 1. *Falcatria moluccana* tree attacked by (A) stem borer and (B) gall rust

Table 1. Primer pairs of microsatellite used in this research

Loci	Primer sequence (5’-3’)	Product size (bp)	Ta (ºC)
Pafa02	F : (NED) CTCTTGTACGTTTCTGAATGC R : AGGCGTATACATTTACAAATC	149-163	54
Pafa03	F : (VIC) AACTTTGATTTGGGAGCTTG R : ACCACTTCCTTAATTACCGTG	149-165	54
Pafa04	F : (VIC) CTACACAAATTACCACATGC R : GGGATTATAAAGGAGACCATTGTGGTG	191-193	58
Pafa05	F : (FAM) TACCGAATTACTTAGTGCAG R : ATTAATAGCTTCTGACCGAAG	172-176	52
Pafa06	F : (FAM) TGCGAATTCCTTATCTGAAC R : GAGTATTATTTCCCAAAGTGGTGAGGG	170-208	54
Pafa07	F : (FAM) TAACCAAATGGTATTGCATG R : TCAGTACTCAGTACCTGGTAC	122-134	54
Pafa08	F : (PET) AAGTCAGCTGTAAGTCATTG R : GAGCAGAGAAAAAAGCTGATG	204-234	54
Pafa10	F : (NED) ACGAGTACCCCGTTATTTTG R : TGTGGTAATTGCAGTGTTAG	171-185	54

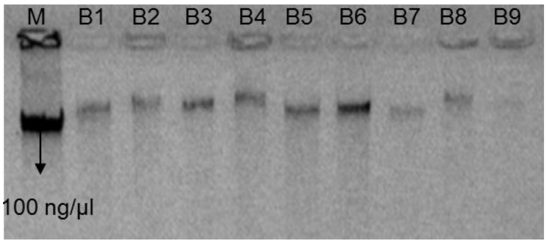

Figure 1. Electropherograms of extracted total DNA from *Falcatria moluccana* leaf samples in this study. S: DNA Standard; B1-B9: Susceptible accessions from boktor pest

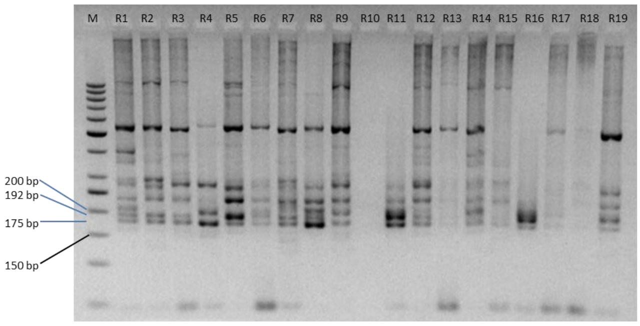

Figure 2. Electropherograms of PCR products. M: GeneRuler 50 bp DNA Ladder; R1-R19: Resistant accessions of *Falcatria moluccana* from boktor pest

RESULTS AND DISCUSSION

DNA extraction and Polymerase Chain Reaction

Clear and strong DNA bands (Figure 1) showed that extracted total DNA samples had good quality and enough quantity for subsequent PCR process. It can be seen by the absence of smear or contamination, even though the bands were relatively thin and less bright. The thin band indicated the concentration of extracted DNA was less than 100 ng/µL. Such concentration is still enough for PCR that DNA template of 10-50 ng/µL was able to produce relatively stable amplification results (Guo and Gao 2017; Li and Geng 2015). In addition, SSR or microsatellite marker does not require extracted DNA with high-concentration, on the other hand, DNA polymerases that have high amplification efficiency are required for DNA with low concentration.

PCR process relies on thermal cycling and it is highly dependent on the primer used. PCR products of *F. moluccana* (Figure 2) clearly showed codominant microsatellite loci in homozygote and heterozygote states. Electropherogram of PCR products also showed some un-amplified samples, that was marked by the absence of DNA bands or null alleles. The accessions that could not be amplified at some loci are probably caused by PCR process error. Another probability is most-likely due to nucleotide-sequence alterations within the priming recognition sites. This alteration is due to point mutations, insertions, deletions or inversions, which lead to no primer matching, and the absence of the corresponding PCR band (Liu et al. 2001). The resolution of each amplified DNA band was not always clear. It was affected by the number of DNA fragments amplified in the plant genome on the PCR process, plentiful amplify produced clearer band. Theoretically, the number of templates should double after each cycle. The competition of primer attachment in genomic DNA leads some fragments able to amplify in large quantities and other fragments in small quantities. Thus impact to the band resolution.

Genetic diversity of *Falcatria moluccana* accessions

Genetic diversity of *F. moluccana* accessions that are resistant and susceptible to stem borer and gall rust from Sumedang, Sukabumi and Kediri populations were considerably high (Table 2). Polymorphic loci percentage obtained in the *F. moluccana* populations was high ranging from 87.50% to 100%. It explains the high ability of the markers to amplify the target sequences and detect polymorphisms between the populations tested. Accessions from Sumedang, which were related to boktor pest had higher Na and Ne than accessions from Sukabumi and Kediri which were related to gall rust disease. Na per population ranged from 2.375 to 3.500, and as expected they are higher than Ne, which varied from 2.015 to 2.909. Observed heterozygosity in each population ranged from 0.219 to 0.630, while expected heterozygosity varied from 0.431 to 0.650. Shannon information index ranged from 0.719 to 1.134. Genetic diversity within the population could be estimated by Shannon Index and Nei's gene diversity (Zhang et al. 2007). Overall Ho, He and I value of Sumedang accessions were higher than Sukabumi and Kediri accessions, indicating that Sumedang community forest of *F. moluccana* had a relatively higher level of genetic diversity, and thus adaptability and vitality compared to populations in Sukabumi and Kediri. Indeed, Kediri population has been known as endemics of the pest and disease. Population characterized by high genetic diversity has strong positive correlation between heterozygosity and population fitness, which is important for the long-term adaptation of populations to novel environmental conditions (Stojnić et al. 2019). The Ho, He and I value of resistant accession infested by gall rust disease were lower than the susceptible. The values were similar to those studied by Lelana et al. (2018). A population is more susceptible to unfavorable environmental conditions if there is a lack of heterozygosity (Basyuni et al. 2012) and genetic diversity for disease resistance in host populations is generally thought to reduce the risk of spread for infectious diseases (Lively 2010).

The genetic diversity value also indicated that microsatellite primers used has a high ability to distinguish accessions. Expected heterozygosity values that varied from 0.431 to 0.623 in susceptible populations and 0.496 to 0.650 in resistant populations indicated polymorphism obtained with microsatellite marker was greater than those obtained in a previous study using RAPD by Siregar and Olivia (2013) ranged from 0.1328 to 0.2946 and Lelana et al. (2018) ranged from 0.018 to 0.299. It implied that the usage of microsatellite marker is effective to gain higher polymorphism due to its co-dominant trait.

Analysis of molecular variance AMOVA was computed using GenAlEx based on co-dominant microsatellite loci to assess the distribution of gene diversity and estimate the components of variances of the populations. To suppress within-population variance and simply calculate population differentiation based on the genotypic variance, data was inputted as co-dominant allelic distance matrix for Fst calculation (within individual analysis suppressed). Results of AMOVA (Table 3) showed that the highest percentage of variation in each region was coming from within populations. In Sumedang region, AMOVA indicated that 94.088% of the genetic variation occurred among individuals within population while only 5.912% of the genetic variation occurred among populations. Similarly, accessions in Sukabumi (76.004%) and Kediri (80.589%) had more variation inside the population, either for resistant or susceptible accessions. As such gene diversity observed among *F. moluccana* populations was low. The low genetic diversity may be due to environmental conditions, geography, and differences in alternative host species diversity that may have a role in generating variability within populations, as well as recent introduction of the pest and disease in those regions (Moges et al. 2016). As mentioned before all sample pairs of resistant and susceptible accessions in this study were taken from nearby grown individuals in the same plot, to minimize environmental effects.

Population and individual genetic distance of *Falcatria moluccana*

Genetic differences between populations and individuals are quantified by the size of genetic differentiation or genetic distance, and further demonstrated by the clustering method. Genetic distances can be used to measure differences in genetic structure between two individuals or populations at a particular gene locus. Genetic distance is the degree of gene difference (genomic difference) between species or populations that is measured by some numerical method (Dogan and Dogan 2016). Genetic distance for the six *F. moluccana* populations is summarized in Table 4.

Table 2. Genetic diversity of *Falcatria moluccana* accessions in this study

Population	Pest/disease	N	PLP (%)	Na	Ne	Ho	He	I
Susceptible (Sm)	Stem borer	25	100.00	3.250	2.714	0.580	0.623	1.060
Resistant (Sm)	Stem borer	25	100.00	3.500	2.909	0.630	0.650	1.134
Susceptible (Sk)	Gall rust	31	100.00	3.000	2.173	0.315	0.496	0.830
Resistant (Sk)	Gall rust	24	87.50	2.625	2.031	0.219	0.431	0.719
Susceptible (Kd)	Gall rust	24	100.00	3.000	2.300	0.276	0.518	0.866
Resistant (Kd)	Gall rust	9	100.00	2.375	2.015	0.500	0.469	0.729

Note: Sm: Sumedang; Sk: Sukabumi; Kd: Kediri; N: Number of individuals; PLP: Polymorphic Loci Percentage; Na: No of observed alleles; Ne: No of effective alleles; Ho: Observed heterozygosity; He: Expected heterozygosity/Nei's gene diversity; I: Shannon Index

Table 3. Summary AMOVA table

Region/source	**df**	**SS**	**MS**	**Est. Var.**	**%**
Sumedang					
Among Pops	1	5.380	5.380	0.082	5.912
Within Pops	98	127.300	1.299	1.299	94.088
Total	99	132.680		1.381	100.000
Sukabumi					
Among Pops	1	34.322	34.322	0.599	23.996
Within Pops	108	204.987	1.898	1.898	76.004
Total	109	239.309		2.497	100.000
Kediri					
Among Pops	1	15.213	15.213	0.502	19.411
Within Pops	64	133.257	2.082	2.082	80.589
Total	65	148.470		2.584	100.000

Note: df: degree of freedom; ss: sum of squares; ms: mean square; est. var.: estimated variance; %: percent of variance

Table 4. Genetic distance of *Falcatria moluccana*'s populations

Population	**Sumedang**		**Sukabumi**		**Kediri**	
	Susceptible	**Resistant**	**Susceptible**	**Resistant**	**Susceptible**	**Resistant**
Sumedang						
Susceptible	0.000					
Resistant	0.160	0.000				
Sukabumi						
Susceptible			0.000			
Resistant			0.346	0.000		
Kediri						
Susceptible			0.311	0.495	0.000	
Resistant			0.397	0.267	0.337	0.000

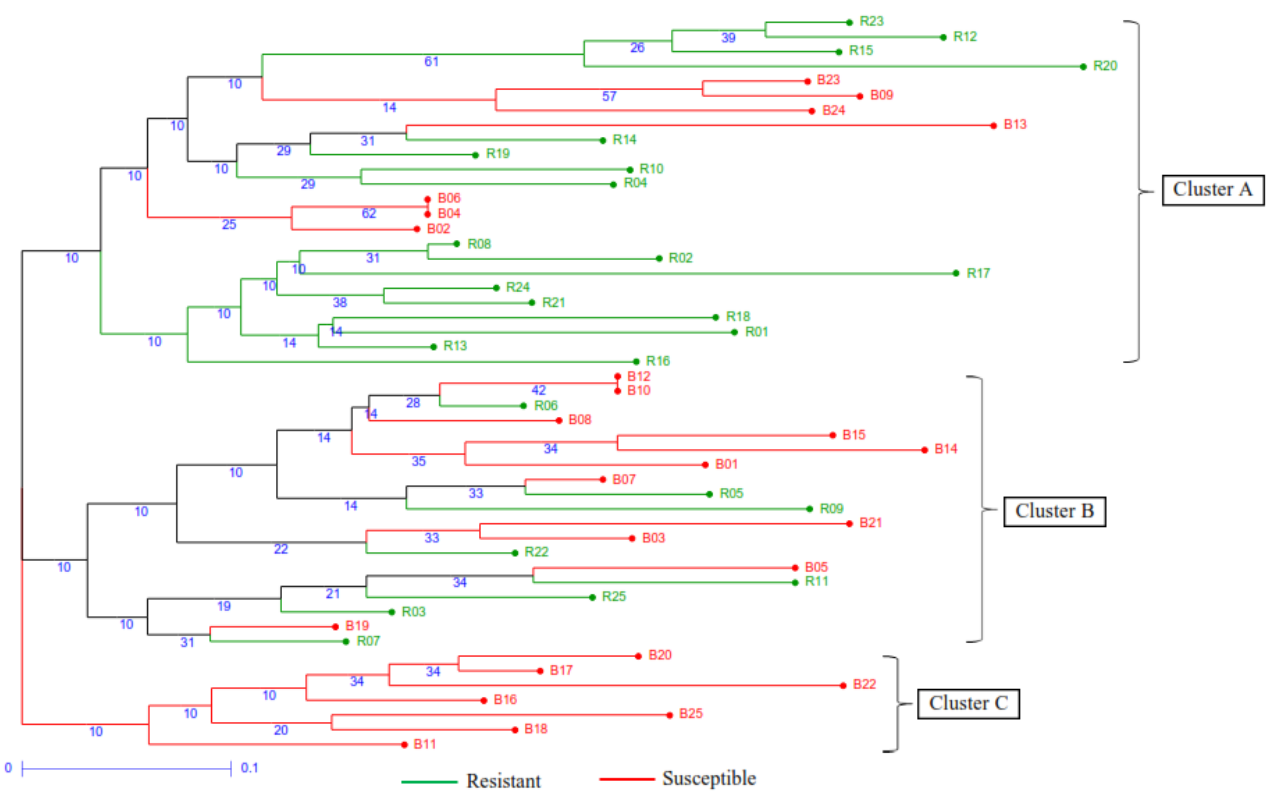

Figure 3. Weighted neighbor-joining dendrogram of 50 sengon accessions that resistant and susceptible to boktor pest. The blue numbers in the branch indicate bootstrap values with option of 10,000, it is shown all (without number category). Names of the individuals are in the right side of line; R indicates 25 accessions of resistant sengon and B indicates 25 accessions of susceptible ones.

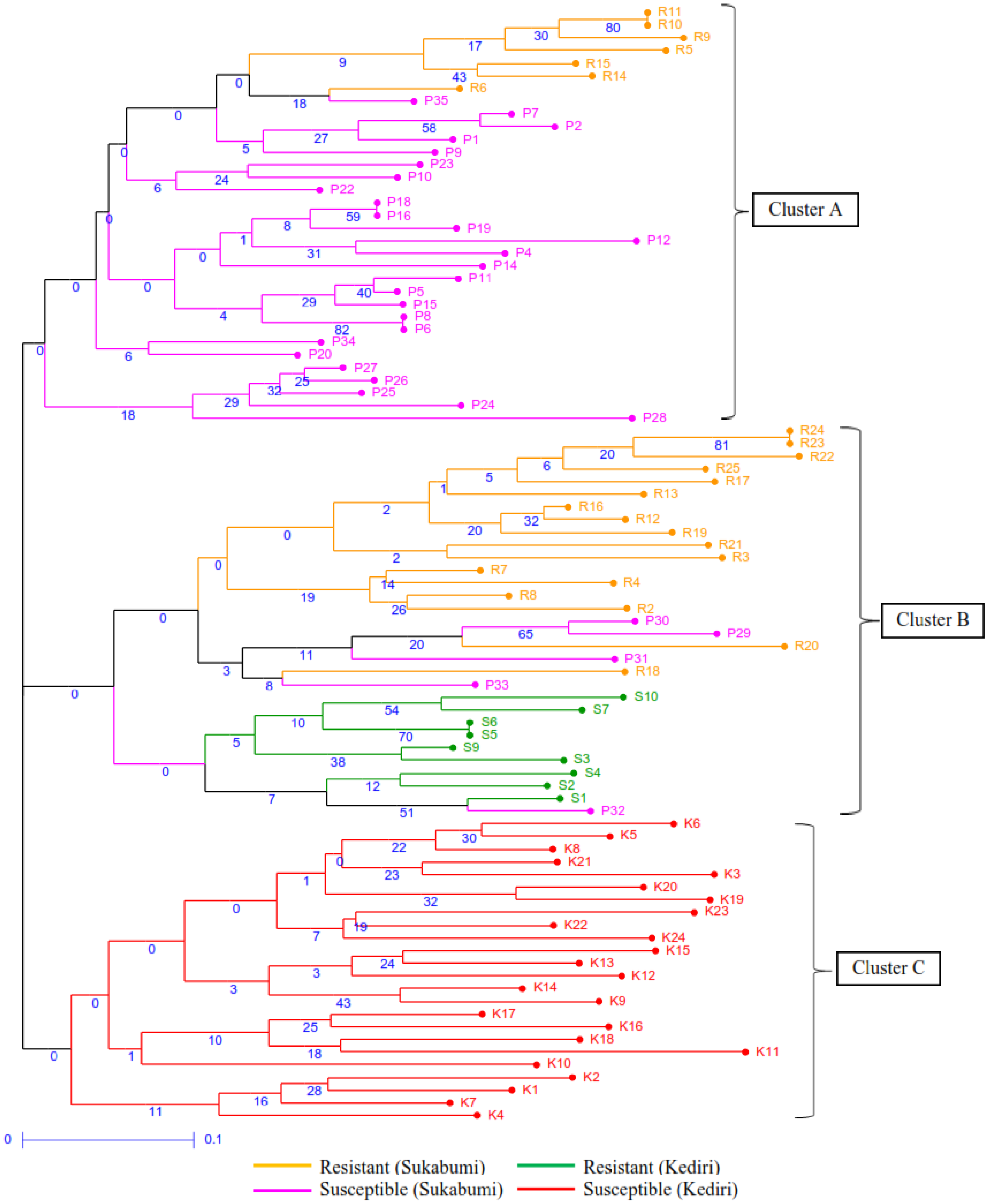

Figure 4. Weighted neighbor-joining dendrogram of 88 sengon accessions that resistant and susceptible to gall rust disease. The blue numbers in the branch indicate bootstrap values with option of 10,000, it is shown all (without number category). Names of the individuals are in the right side of line; R indicates 24 accessions of resistant sengon (Sukabumi) and P indicates 31 accessions of susceptible sengon (Sukabumi), as well as S indicates 9 accessions of resistant sengon (Kediri) and K indicates 24 accessions of susceptible sengon (Kediri).

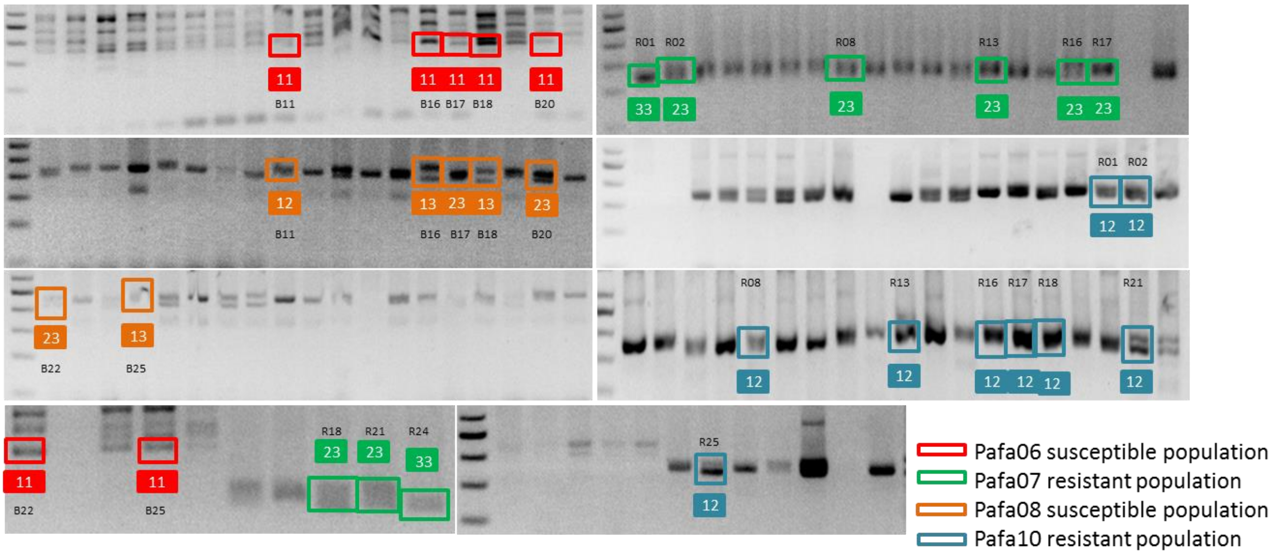

Figure 5. Allele pattern of resistant and susceptible *Falcatria moluccana* attacked by boktor pest

In this study, genetic diversity of *F. moluccana* accessions attacked by boktor pest was 0.160. This low genetic distance between resistant and susceptible populations to boktor pest, which was collected from the same population in Sumedang indicated the close kinship of the two accessions. However, genetic diversity of *F. moluccana* accessions infested by gall rust disease varied from 0.267 to 0.495, indicating the farther genetic relationship between populations or accessions. Highest genetic distance was found between susceptible population from Kediri and resistant population from Sukabumi, indicating the dissimilarity between those populations was quite far. However, resistant population from Kediri and resistant population from Sukabumi has the lowest genetic distance value.

Genetic distance measures have already been established as one of the major tools for analyzing data on gene differentiation between populations. Therefore, genetic diversity between individual accessions was used to construct the dendrogram of individuals in clustering trees based on dissimilarity matrix using weighted neighbor-joining method. In phylogenetic analyses, genetic diversity is considered important as it influences branch lengths and may consequently affect the tree topology. Dendrogram of *F. moluccana* attacked by boktor pest from Sumedang (Figure 4) shows some individuals have already clustered according to the resistant or susceptible characters to boktor pest. However, there is still cluster consisting of mixed characters. Similar dendrogram of *F. moluccana* infested by gall rust disease was also observed (Figure 3). Resistant *F. moluccana* trees that significantly separated from susceptible trees indicate that genetically resistant accession is different from susceptible accession. Those the microsatellite markers tested have been able to produce fingerprints that differentiate the two types of accessions.

Figure 4 contains three clusters, of which cluster 3 consists of fully susceptible accessions. Cluster 1 and 2 consists of resistant and susceptible accessions to boktor pest. However, in Cluster 1 some resistant accessions grouped together and separate from the other mixed sub-cluster, while Cluster 2 contains randomly mix characters. Also, Figure 3 consists of three clusters. Cluster 1 embraces only susceptible accessions from Kediri, which are separate from other accessions cluster. In Cluster 2, resistant accessions from Sukabumi with resistant accessions from Kediri formed separate groups, as well as there is mixed accessions cluster. Cluster 3 is mostly occupied by susceptible accessions from Sukabumi and some resistant accessions from Sukabumi, which could be categorized as intermediate groups. The resistance of a plant can be categorized as high, intermediate, or low. Figure 3 and 4 showed that to some extent *F. moluccana* accessions susceptible to boktor pest and gall rust disease, with main susceptible accession from Kediri, is different from resistant accessions even though those two populations collected from the same plot and location.

The random cluster in Figure 3 and 4 probably caused by random mating and outcrossing tendency of the plant, of which each individual in the population has equal opportunity to mate with another individual in its population, so the chances of a genotype to mate with another genotype are equal depending on relative frequencies of the genotype in those individuals. Outcrossing rates in *F. molucanna* may be strongly influenced by flowering time, tree density, as well as the types and behaviors of pollinator governing the pollen movement (Obayashi et al. 2002). The plant resistance to pest and disease is a trait controlled by supposedly many genes. Horizontal resistance or polygenic is controlled by several genes resulted from random matting (Baskorowati dan Nurrohman 2011). Tolerant plant from pest attack and disease infestation do not show significant loss of yield. Previous study by Siregar et al. (2011) revealed that resistance trait to stem borer insect was also effected by activity of trypsin and α-amylase inhibitor as the defense mechanism of *F. moluccana*, of which resistant tree showed higher inhibitory activity than susceptible ones.

Another factor which can affect genetic diversity is allele pattern. The unique alleles play an important role in genotypes identification. The unique alleles were used as a diagnostic marker for specific varietal identification, and they could distinguish varieties from the rest of the genotypes (Kumbhar et al. 2015). As a sample, Figure 5 presented allele pattern of resistant and susceptible *F. moluccana* attacked by boktor pest to find the unique alleles that might be a diagnostic marker. The observed accessions were sub-cluster 1 consisted of resistant samples; R01, R02, R08, R13, R16, R17, R18, R21 and R24, and cluster 3 consisted of susceptible samples; B11, B16, B17, B18, B20, B22, and B25. The results indicated that Pafa10 primer might identify resistant tree with band pattern of 12 (heterozygous), while Pafa06 primer might identify susceptible tree with band pattern of 11 (homozygous). Pafa07 primer showed that in the second band always appeared allele number 3 in the resistant cluster. Similarly, Pafa08 primer might able to mark the susceptible accession with the appearance of allele number 3 in the second band except B12.

Further observation on the resistant and susceptible accessions which were clustered together in the dendrogram also identified some consistent pattern on certain accession (not shown here). Based on Pafa03 primer, susceptible accessions to gall rust from Kediri and Sukabumi were dominated by presence of allele 2. Meanwhile from primer Pafa05 allele 2 always present in the resistant accessions, while allele 1 present in all susceptible accessions, except K14 and K15. Primer Pafa10 produced monomorphic pattern of homozygous 11 on resistant accessions from Sukabumi, while others showed polymorphic pattern. Resistant lines to pest and diseases are essential part of sustainable and long-term pest and disease management strategies. Microsatellite markers were useful to comprehend the genetic diversity and population structure of F. moluccana attacked by boktor pest and infected by gall rust disease. The result showed that eight microsatellite markers (Pafa02, Pafa03, Pafa04, Pafa05, Pafa06, Pafa07, Pafa08 and Pafa10) could amplify most of the accessions used in this study and to some extent could differentiate resistant accessions to susceptible ones. Sumedang accession of F. moluccana resistant to stem borer has the highest He (0.650) than susceptible population (He = 0.623). The population of F. moluccana susceptible and resistant to gall rust disease from Sukabumi and Kediri has genetic diversity (He) value ranging from 0.431 to 0.518. Most genetic variations (65- 100%) were coming from within population, reflecting the origin of the accessions. Dendrogram based on Nei's genetic distance could clustered some resistant and susceptible accessions together, albeit some others were still mixed, indicating ability of microsatellite marker to differentiate some accessions. Certain allelic pattern could be useful as diagnostic bands for either resistant or susceptible accessions.

ACKNOWLEDGEMENTS

This research was funded by SEAMEO BIOTROP trough DIPA Project and partially by USAID through the SHERA program, Centre for Development of Sustainable Region (CDSR). In year 2017-2021 CDSR is led by Center for Energy Studies, Gadjah Mada University (UGM) Yogyakarta, Indonesia.

REFERENCES

Anggraeni RG, Fitra S (ed.). 2019. Ministry of Foreign Trade will Enlarge Export of Sengon Wood Products to Vietnam [Indonesian]. https://katadata.co.id/berita/2019/03/13/kemendag-bakal-perluas-ekspor-olahan-kayu-sengon-ke-vietnam

Baskorowati L, Nurrohman SH. 2011. Variation of resistance to gall rust of *Falcataria moluccana* seedlings. J Pemuliaan Tanaman Hutan 5 (3): 129-138. [Indonesian]

Basyuni M, Hamzah, Rahayu S, Siregar UJ. 2012. Effect of anthropogenic activities on genetic variation of *Rhizophora mucronata* Lamk. in Secanggang Mangrove Forest, North Sumatra. Foresta 1 (2): 41-48. [Indonesian]

Budiani A, Woelan S, Minarsih H, Haris N, Putranto RA. 2014. Evaluation of 18 SSR primers to develop DNA fingerprint of rubber tree (*Hevea brasiliensis* Muell. Arg.). Menara Perkebunan 82 (2): 81-94. [Indonesian]

Charomaini MZ, Ismail B. 2008. Preliminary indication of resistant on sengon (*Falcataria moluccana*) Irian Jaya provenances to gall rust disease. J Pemuliaan Tanaman Hut*an* 2 (2): 1-9. [Indonesian]

Dinarti D, Susilo AW, Meinhardt LW, Ji K, Motilal LA, Mischke S, Zhang D. 2015. Genetic diversity and parentage in farmer selections of cacao from Southern Sulawesi, Indonesia revealed by microsatellite markers. Breed Sci 65: 438-446. DOI: 10.1270/jsbbs.65.438.

Dogan I, Dogan N. 2016. Genetic distance measures: Review. Turkiye Klinikleri J Biostat 8 (1): 87-93. DOI: 10.5336/biostatic.2015-49517.

Endang AH, Haneda NF. 2010. Infestation of *Xystrocera festiva* in *Paraserianthes falcataria* plantation in East Java, Indonesia. J Trop For Sci 22 (4): 397-402.

Guo L, Gao X. 2017. Genetic diversity and population structure of *Indigofera szechuensis* complex *(Fabaceae)* based on EST-microsatellite markers. Gene 624: 26-33. DOI: 10.1016/j.gene.2017.04.047.

Hartwell LH, Hood L, Goldberg ML, Reynolds AE, Silver LM, Veres RC. 2004. Ed ke-2. Genetics: from genes to genomes. New York.

Husaeni EA. 1992. Financial losses due to attack by sengon stem borer (*Xystrocera festiva* Pascoe) in sengon stands (*Paraserianthes falcataria* (L) Nielsen). In: Mas'ud F et al. (eds.) Proceedings of the Seminar and Field Meetings on the Establishment of Industrial Forest Plantation in Sumatra Region. Palembang, 29-31 October 1992. [Indonesian]

Indresputra F, Rahayu S, Widiyatno. 2013. Effect of pyroclastic cloud from Merapi Volcano to the survival of *Uromycladium tepperianum* on *Falcataria moluccana* in Yogyakarta, Indonesia. Procedia Environ Sci 17: 70-78. DOI: 10.1016/j.proenv.2013.02.013.

Iskandar J, Ellen FR. 2008. The contribution of *Paraserianthes falcataria* (Albizia) to sustainable swidden management practices among the Baduy of West Java. Hum Ecol 28:1-17.

Krisnawati H, Varis E, Kallio M, Kanninen M. 2011. *Paraserianthes falcataria* (L.) Nielsen: Ecology, silviculture, and productivity. IPB, Bogor. [Indonesian]

Kumbhar SD, Kulwal PL, Patil JV, Sarawate CD, Gaikwad AP, Jadhav AS. 2015. Genetic diversity and population structure in landraces and improved rice varieties from India. Rice Sci 22 (3): 99-107. DOI: 10.1016/j.rsci.2015.05.013.

Lee SL, Tani N, Ng KKS, Tsumura Y. 2004. Isolation and characterization of 20 microsatellite loci for an important tropical tree *Shorea leprosula* (Dipterocarpaceae) and their applicability to *S. parvifolia.* Mol Ecol Notes 4 (2): 222-225. DOI: 10.1111/j.1471-8286.2004.00623.x.

Lelana NE, Wiyonon S, Giyanto, Siregar IZ. 2018. Genetic diversity of *Falcataria moluccana* and its relationship to the resistance of gall rust disease. Biodiversitas 19 (1): 12-17.

Li H, Geng S. 2015. Assessment of population genetic diversity of *Derris elliptica* (Fabaceae) in China using microsatellite markers. Ind Crops Prod 73: 9-15. DOI: 10.1016/j.indcrop.2015.04.023.

Liu XM, Smith CM, Gill BS, Tolmay V. 2001. Microsatellite markers linked to six Russian wheat aphid resistance genes in wheat. Theor Appl Genet 102 (4): 504-510. DOI: 10.1007/s001220051674.

Lively CM. 2010. The effect of host genetic diversity on disease spread. Am Nat 175 (6): 149-152. DOI: 10.1086/652430.

Matsumoto K. 1994. Studies on the ecological characteristics and methods of control of insect pests of trees in forested area in Indonesia. Final report submitted to Forestry Research and Development Agency, Ministry of Forestry, Jakarta, Indonesia.

Moges AD, Admassu B, Belew D, Yesuf M, Njuguna J, Kyalo M, et al. 2016. Development of microsatellite markers and analysis of genetic diversity and population structure of *Colletotrichum gloeosporioides* from Ethiopia. PLoS One 11 (3): 1-18. DOI: 10.1371/journal.pone.0151257.

Notoatmodjo SS. 1963. Preventing mass attack by sengon stem borer *Xystrocera festiva* Pascoe on the stands of *Albizia falcataria*. Board Report for the Institute of Forestry Research, Bogor, Indonesia. [Indonesian]

Obayashi K, Tsumura Y, Ihara-Ujino T, Niiyama K, Tanoushi H, Suyama Y, Washitani I, Lee CT, Lee SN, Muhammad N. 2002. Genetic diversity and outcrossing rate between undisturbed and selectively logged forests of *Shorea curtisii* (Dipterocarpaceae) using microsatellite DNA analysis. Intl J Plant Sci 163 (1): 151-158. DOI: 10.1086/324549.

Old KM, Cristovao CS. 2003. A rust epidemic of the coffee shade tree (*Paraserianthes falcataria*) in East Timor. ACIAR Proceeding No. 113, Canberra.

Peakall ROD, Smouse PE. 2012. GenAlex 6.5: Genetic analysis in excel, population genetic software for teaching and research. Bioinformatics Appl Note 28 (19): 2537-2539. DOI: 0.1093/bioinformatics/bts460.

Saito Y, Lian C, Ishio S, Ide Y. 2014. Isolation and characterization of eight microsatellite markers in *Paraserianthes falcataria*, a fast-growing tropical leguminous tree species. J Trop For Sci 26 (2): 295-297.

Singh P, Sharma H, Nag A, Bhau BS, Sharma RK. 2014. Development and characterization of polymorphic microsatellites markers in endangered *Aquilaria malaccensis*. Conserv Genet Resour 7 (1): 61-63. DOI: 10.1007/s12686-014-0287-4.

Siregar UJ, Haneda NF, Flowrensia L. 2011. Correlation of enzyme inhibitor activity in sengon with boktor larval growth on artificial diet. J Silv Trop 03 (01): 101-108. [Indonesian]

Siregar UJ, Olivia RD. 2013. Genetic diversity of sengon population *(Paraserianthes falcataria* (L)*)* in Java Community Forest based on RAPD marker. J Silv Trop 3 (2): 130-136. [Indonesian]

Stojnić S, Avramidou EA, Fussi B, Westergren M, Orlović S, Matović B, Trudi´ B, Kraigher H, Aravanopoulos FA, Konnert M. 2019. Assessment of genetic diversity and population genetic structure of Norway spruce *(Picea abies* (L.) Karsten*)* at its Southern Lineage in Europe. Implications for conservation of forest genetic resources. Forests 10 (3): 258. DOI: 10.3390/f10030258.

Suharyanto, Rimbawanto A, Isoda K. 2003. Genetic diversity and relationship analysis on *Paraserianthes falcataria* revealed by RAPD marker. Pp 81-85 in Advances in Genetic Improvement of Tropical Tree Species. Proceedings of the International Conference. Yogyakarta, 1-3 October 2002. [Indonesian]

Sunandar A, Dorly, Supena EDJ. 2017. Induction of somatic embryogenesis in sengon *(Falcataria moluccana)* with thidiazuron and light treatments. Hayati J Biosci 24 (2): 105-108. DOI: 10.1016/j.hjb.2017.08.002.

Tasma IM, Arumsari S. 2013. Genetic diversity analysis of the Cameroon-originated oil palm accessions assessed with SSR markers. Littri 19 (4): 194-202. [Indonesian]

Verhaegen D, Fofana IJ, Logossa ZA, Ofori D. 2010. What is the genetic origin of teak *(Tectona grandis* L.*)* introduced in Africa and in Indonesia*?* Tree Genet Genomes 6 (5): 717-733. DOI: 10.1007/s11295-010-0286-x.

Zhang C, Chen X, He T, Liu X, Feng T, Yuan Z. 2007. Genetic structure of *Malus sieversii* population from Xinjiang, China, revealed by SSR markers. J Genet Genomics 34 (10): 947-955. DOI: 10.1016/s1673-8527 (07)60106-4.