ANALYSIS OF MOLECULAR MARKERS LINKED TO CORYNESPORA LEAF FALL DISEASE RESISTANCE IN RUBBER PLANTS

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Abstract

Rubber cultivation in Indonesia and other major natural rubber producing countries faced the constraint of the Corynespora leaf fall (CLF) disease attack that significantly reduced the production of latex. The use of resistant rubber clones as a planting material is one of the effective and economic solutions in CLF disease control. The rubber breeding program to obtain superior clones resistant to CLF disease is constrained by the length of time required for the selection process. The progress of the molecular biology provides a chance to solve this problem. To identify moleculer markers linked with resistance to CLF disease a series of experiments that consisted of genetic analysis of C. cassiicola isolates, evaluation of the CLF disease resistance level of the rubber germplasm accessions and identification of the QTL linkage to CLF disease on the rubber tree were carried out. The rDNA-ITS sequences and virulence analysis of 23 C. cassiicola *isolates showed that there were 5 haplotypes of isolates and that differences were present in the virulence level of isolates to the rubber clones tested. Three isolates collected from rubber clones PR 303, RRIM 600 and Tjir 1 have a very high virulence level. Resistance test of 56 rubber germplasm accessions with four of* C. cassiicola *isolates showed that 12 accessions could be classified as very resistant, 13 as resistant, 23 as susceptible and 8 as very susceptible. Three accessions of the IRRDB 1981 population namely PN 451, PN 494 and PN 604 have better resistance levels compared to Wickham population. The resistant (BPM 1) and susceptible (RRIM 600) rubber clones were selected as a parent clones to obtain a mapping population for QTL analysis that produced 30 F1 plants and 74 embryonic F1. Linkage analysis by using 30 selected SSR primers showthat there were four linkage groups on the LOD 3 with total 8 loci, while LOD 2 had two linkage groups with total 11 linked loci. QTLs linked to CLF disease have not been identified on the linkage map, but based on the single marker analysis, four loci (EHB70, EHB081, EHBp18 and SSRH548) associated with two isolates used (CC-06 and CC-22) were found. The informations obtained in this study are the first step for the development of Marker Assisted Selection (MAS) of rubber resistant to CLF disease.*

Keywords: Corynespora cassiicola, *genetic linked map, Hevea brasiliensis, QTL, rDNA-ITS*

INTRODUCTION

One of the important problems in the rubber (*Hevea brasiliensis*) cultivation in Indonesia and other natural rubber producing countries in Asia and Africa is the attack of Corynespora leaf fall (CLF) disease caused by *Corynespora cassiicola*. This disease attacks rubber plant at all

leaf and plant growth stages. Under favourable environmental conditions, the disease attack may occur and cause fall of leaves continuously and decrease latex production. Severe disease conditions can lead to loss of trees. In Indonesia, almost all of rubber plantations are affected by CLF disease which caused huge losses in production even up to 60% (Situmorang et al., 2007).

Some of efforts to early control the CLF disease is using resistant clones. The main problem of rubber breeding in obtain a new superior clones is the length of selection period. One alternative to overcome this problem is by using the *Marker Asissted Selection* (MAS) so that the selection cycle can be shorthened due to the selection can be conducted in the early of plant growth.

The development of genetic mapping on the perennial crops which cross-pollinated and heterozygous are not as fast as in horticultural crops. This is because the perennial plants have a long life cycle and low ability to produce seeds due to inbreeding depression. So the development of progeny for the genetic studies is very difficult (Lespinasse et al. (2000). This limitation also cause production of only F1 from crossing of the heterozygous parents and may cause the segregation of four alleles in one locus. The generated data have to be analyzed as a double pseudo test cross and preparation of the genetic linkage map for each parent has to be carried out separately (Grattapaglia and Sederoff 1994). The use of F1 as a mapping population in the rubber plant was reported earlier (Le-Guen et al., 2007; Le Guen et al., 2011; Le Guen et al., 2013; Le Guen et al., 2003; Novalina and Sagala, 2013; Rattanawong et al., 2009; Souza et al., 2013; Souza et al., 2011).

This paper reports the research related to the development of molecular markers linked to CLF disease resistance on rubber plants. Identification of *C. cassiicola* isolates based on pathogenicity, rubber germplasm resistant to CLF disease and QTL (Quantitative Trait Loci) linked to CLF disease resistance in the rubber plants are reported here.

MATERALS AND METHODS

Identification and Selection of *C. cassiicola* Isolate

The virulence analysis was done based on filtrate toxin activity on 23 *C. cassiicola* isolates which consisted of 6 isolates from GT 1 on different plantation and 17 isolates from other clones that were isolated from same location. Analysis was performed according to the method described by (Breton et al., 2000) with modifications. Virulence of isolates was quantified based on the leaf water loss caused by toxin activity where observation was made after 48 hour immersion in toxin filtrate. The isolates virulence were grouped into weak, medium, high and extremely virulent based on the Standard Deviation (SD) value i.e. those which have a value under -1 SD were grouped as weak virulence between -1 SD to mean as medium virulence, mean to +1 SD as high virulence, and more than +1 SD as a extremely virulent isolate.

DNA was extracted from fungal mycelia based on (Nghia et al., 2008) method. The PCR was done using universal primers ITS1F (5'CTTGGTCATTTAGAGGAAGTAA3') and ITS4 (5' TCCTCCGCTTATTGATATGC3') (White, 1990). The PCR products were sequenced by a commercial sequencing service (First BASE Laboratories Sdn. Bhd. Malaysia) by using the ITS1F and ITS4 primers. Analysis of isolates clustering based on ITS-rDNA sequences was performed by the Neighbour-Joining method with number of bootstrap of 1000 using Mega6 program (Tamura, 2013).

Identification of Resistance Level to CLF disease of Rubber Germplasm

Analysis was done on 56 rubber tree accessions consisted of six Wickam clones and 50 IRRDB 1981 accessions. Resistant assay was performed according to the method described by (Breton et al., 2000) with modifications. Resistance of accession was quantified based on the leaf water loss caused by toxin activity of *C. cassiicola* isolate where observation was made after 48 hour immersion in toxin filtrate. The plants resistance were grouped into resistant, moderate resistant, moderate susceptible and susceptible based on the Standard Deviation (SD) value i.e. those which have a value under -1 SD were grouped as resistant, between -1 SD to mean as moderate resistant, mean to +1 SD as moderate susceptible, and more than +1 SD as a susceptible plant.

Identification of QTL (Quantitative Trait Loci) Linked to CLF Disease Resistance on the Rubber Plants

The population mapping was obtained from cross of resistant to CLF disease rubber clone (BPM 1) and susceptible to CLF disease clone (RRIM 600). The phenotyping data were generated by using toxin assay of two selected *C. cassiicola* isolate (CC-06 and CC-22) to population. The genotyping data were generated by using SSR marker. The SSR primers that used to construct genetic linkage map were obtained from two steps of selection. The first selection was polymorphisme of 135 SSR primers on both of parents clone. Subsequently the selected primers were used to amplification all of individu in the mapping population. The progeny carried a specific allele to resistant parent were indicated as a heterozygous genotype (H) and which does not carry it were characterized as a homozygous genotypes (A). Furthermore the loci having a segregation ratio of 1: 1 by chi square test in the population were used to construct the linkage map. The association between SSR markers with the resistance to CLF disease was done by using a single marker analysis (Champoux et al., 1995; Collard et al., 2005). The analysis was performed by combining the resistance and markers data which was analyzed by using SPSS 20.

RESULTS AND DISCUSSION

Identification and Selection of *C. cassiicola* Isolate

The results of virulence analysis showed that there was an interaction between isolates and clones which resulted in differences of virulence levels of isolate to six rubber clones (BPM 1, RRIC 100, BPM 24, PB 260, GT 1 and RRIM 600 To evaluate the potency of resistance gene to CLF disease on the rubber germplasm, the resistance of leaf to toxin filtrate of 4 isolates of *C. cassiicola* which was considered very virulent in the previous research was evaluated. Three of 23 isolates namely CC-20, CC-22 and CC-23 from PR 303, RRIM 600 and Tjir 1 rubber clones were found to have extreme virulence levels.

The diversity analysis of the ITS-rDNA sequence used ITS1F / ITS4 primer showed that there were three of SNP points. The SNP positions are an informative character in the genetic variation analysis, where they can be used as a tool to determine the haplotypes (Dixon et al., 2009) or race clustering of *C. cassiicola* isolate (Nghia et al., 2010). Except the third SNP position, the other SNP positions were also found on the some of *C. cassiicola* isolates from other countries (Dixon et al., 2009); (Hieu et

al.,2014; Nghia et al., 2010). Acording to the position of SNPs, the *C. cassiicola* isolates from Indonesia were divided into 5 haplotypes, and most of the isolates belong to haplotype 1. These results indicate that there were at least five different races in *C. cassiicola* isolate from rubber plantation in Indonesia and most of the isolates are classified as race 1.

Identification of Resistance Level to CLF Disease of Rubber Germplasm

Various studies of genetic variability of rubber plants using several of molecular markers have been reported (Besse et al., 1994; Gouvêa, et al., 2010); Lam, Thanh, Chi, & Tuy, 2009; Lekawipat et al. 2003; Oktavia et al., 2011; Saha et al., 2005). These studies show that the cultivated clones that come from Wickham population exhibited a very narrow genetic base. The availability of the new 1981 IRRDB germplasm population is an opportunity to widen the genetic diversity. They are also genetic source of important agronomic characters such as resistance to drought-induced stress (Mercy, 2001) resistance to South American Leaf Blight (Le Guen et al 2002) and Colletotrichum (Le Guen et al. 2009), the source of high quality wood characters (Mydin, 2012; Reghu, 2011) and source of resistance to CLF disease.

To evaluate the potency of resistance gene to CLF disease on the rubber germplasm, so it was evaluated the resistance of leaf to toxin filtrate of 4 isolates of *C. cassiicola* which was considered very virulent in the previous research. The results showed that 19 accessions tested had a high resistance to PGDC and three of them belonged to the 1981 IRRDB germplasm namely PN 451, PN 494 and PN 604, which had a better resistance compared to the BPM 1 as a resistant clone fro Wickham population (Figure 1).

Identification of QTL (Quantitative Trait Loci) Linked to CLF Disease Resistance on the Rubber Plants

The crossing success rate to produce the mapping population was very low. Out of 6031 crossing between BPM 1 and RRIM 600 only30 F1 plants (< 1%) were obtained. The low of success of these crosses may be influenced by, the genetic factors of both the parent clones and environmental factors. To increase the number of mapping population on the DNA isolated from the F1 embryo can be used. But the disadvantage is that data for phenotyping can only be obtained from F1 plants.

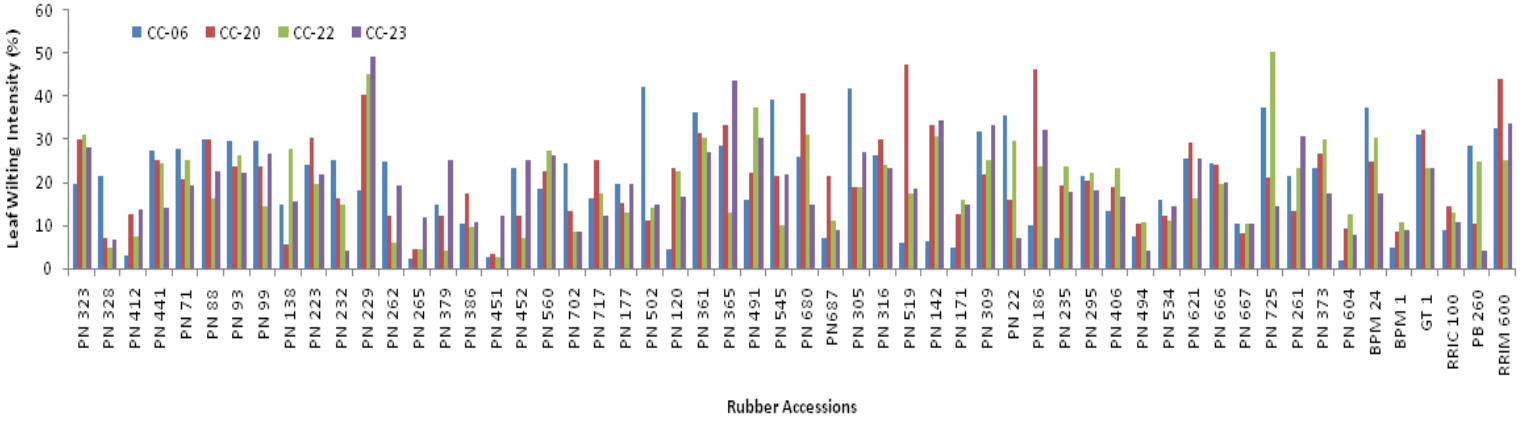

Figure 1. Leaf wilting intensity of 56 rubber accessions to 4 of *C. cassiicola* isolates. CC-01, CC-20, CC-22 and CC-23 were *C. cassiicola* isolates from rubber clones GT 1, RRIM 600, Tjir 1 and PR 303 (Oktavia et al., 2016)

Analysis of the resistance of 30 F1 plants to the two highly virulent isolates of *C. cassiicola* from GT 1 (CC-06) and RRIM 600 (CC-22) showed that each plant had different resistance levels to both the isolates. These differences can occur due to the specific interactions between plants and isolates. Lieberei (2007) stated that the diversity of disease rates can be affected by various factors such as the genetic variability of pathogens and plants, enviroment and interaction between them. These factors can render a genotype of the plant resistant to one isolate but susceptible to the others. Three progenies namely F1.1, F1.5 and F1.10 had better resistance to both the isolates compared to resistant parent clone of BPM 1 The F1.27 progeny had a better resistance to CC-06 isolate, and F1.11, F1.12, F1.14, F1.23, F1.28 and F1.29 progeny to CC-22 isolate (Table 1).

Table 1. Resistance level of 30 F1 progenies of the cross BPM 1 x RRIM 600 to toxin filtrate of two *C.cassiicola* isolates

Genotype	LWI to isolate (%)		Genotype	LWI to isolate (%)	
	CC-06	CC-22		CC-06	CC-22
BPM1	6.9	7.8	F1.15	19.4	14.1
RRIM600	33.6	41.3	F1.16	20.0	20.8
F1.1	5.5	6.0	F1.17	26.2	25.4
F1.2	9.7	8.1	F1.18	12.8	13.9
F1.3	17.2	7.3	F1.19	21.3	20.1
F1.4	12.7	9.4	F1.20	24.4	9.6
F1.5	6.1	5.1	F1.21	22.5	10.7
F1.6	14.4	9.0	F1.22	20.8	6.8
F1.7	7.2	9.2	F1.23	11.0	6.4
F1.8	16.3	14.4	F1.24	19.3	24.4
F1.9	25.5	16.4	F1.25	25.8	17.1
F1.10	6.8	7.3	F1.26	33.4	7.9
F1.11	5.6	14.9	F1.27	5.2	8.8
F1.12	11.2	6.0	F1.28	8.8	7.4
F1.13	21.1	23.9	F1.29	18.1	6.3
F1.14	12.4	3.5	F1.30	21.2	14.3

LWI : Leaf Wilting Intensity

Another limitation in the preparation of genetic mapping populations on the rubber and other perennial crops is the difficulty of preparing homozygous parents F1 progenies that produced from crosses of heterozygous parents when used as mapping population results in the segregation of four alleles on one locus. The use of F1 population requires the segregation pattern as in the backcross population. In this study out of the 135 of SSR loci selected only 30 (22.2%) polymorphic loci followed the backcross segregation pattern. Selected primers were used for

amplification all the mapping population. Figure 2 show the example of PCR amplification product of EHB 069 locus on part of population and genotyping of them. The alleles found only on the resistant parent clone (BPM 1) was estimated as a specific allele related to CLF disease resistance in the rubber plant. The plants with the specific allele were marked as H genotype and others as A genotype. The last step of primer selection was selection of 30 segregation loci on 104 F1 plants of mapping population based on chi square analysis. The analysis showed that only 28 of SSRs loci had H and A genotypes following the Mendel's law with a 1: 1 segregation (Table 2). The 1: 1 segregated loci was used in the preparation of genetic linkage map.

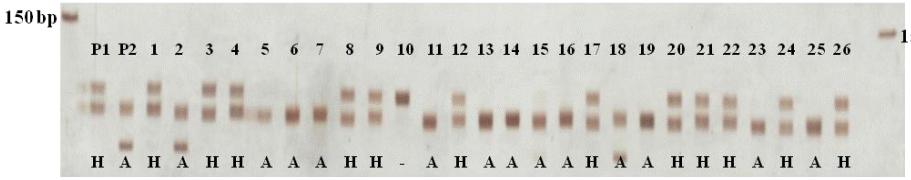

Figure 2. PCR amplification product and genotyping of EHB 69 locus part of mapping population. P1= parent clone of BPM 1, P2= parent clone of RRIM 600, 1-26= F1 progenies no 1-26, 150 bp= position of 150 bp on DNA marker, H= heterozygot genotype dan A= homozigot genotype

Linkage analysis of 28 SSR loci which had 1:1 segregation using the MapMaker / EXP software showed that only 8 loci are linked on LOD 3 FR 0.5 and 11 loci on LOD 2 FR 0.5 while the other segregated independently. As the number of rubber chromosome is 18, it can be inferred that only a small part of the rubber genome was identified. Hence it is not a ideal genetic linked map. QTLs linked to CLF disease have not been identified on the linked map. But the single marker analysis showed that 3 four loci (EHB 70, EHB 081, EHBp 18 and SSRH 548) have association with both the isolates, one locus (HB 68) only with the CC-06 isolate and five loci (gSSR 165, HBE 329, SSRH 103, HB 78 and gSSR 212) only with the CC-22 isolate. All these ten loci associated with CLF disease is a preliminary information for development of MAS for resistance to CLF disease in rubber plants. By increasing the number of mapping populations and the molecular markers used, more CLF-linked markers can be identified and can be used for accurate MAS.

Table 2. Chi square analysis of 30 SSR loci that segregate 1:1 on F1 population from the cross of BPM 1 x RRIM 600

No	Locus	Accession Number		X^2	No	Locus	Accession Number		X^2
		H	A				H	A	
1	EHB 069	49	55	0.35	16	EHBp 18	54	49	0.24
2	EHB 070	43	55	1.47	17	EHBla 2	60	46	2.96
3	EHB 079	58	46	1.38	18	EHBc 34	55	48	0.48
4	EHB 081	67	32	12.37*	19	SSRH 103	52	37	2.53
5	EHB 087	62	42	3.85	20	SSRH 358	58	43	2.23
6	EHB 088	45	57	1.41	21	SSRH 548	56	43	1.71
7	EHB 113	53	51	0.04	22	gSSR 165	60	43	2.81
8	EHB 122	58	46	1.38	23	gSSR 268	44	60	2.46
9	EHB 133	53	50	0.09	24	HBE 329	49	50	0.01
10	EHB 151	62	42	3.85	25	P070	55	46	0.80
11	HB 52	53	47	0.36	26	mHbCIRA 2715	58	46	1.38
12	HB 68	37	58	4.64*	27	mHbCIRA 2425	52	52	0.00
13	HB 78	46	57	1.17	28	gSSR 194	54	50	0.15
14	HESR 032	44	57	1.67	29	gSSR 212	52	45	0.51
15	EHBp 15	44	56	1.44	30	HESR 029	51	50	0.01

P 5% on the df 1 = 3.84. *= significant, number of the H different with A genotypes (segregation of alelles not 1:1).

Each of genetic linkage map is specific to the population based on the parent clone pair used to produce the population. Any tightly linked locus with resistance to CLF disease identified on the one of population can be used as marker on other populations that have a different genetic backgrounds. Such loci found on several different populations are called universal markers. The universal markers can be used to construct consensus maps composed of combining or merging together of several different maps produced from different populations. The consensus map is very useful for preparation of new genetic maps in other populations. Thus a strongly linked universal markers can also be used as a tool to identify resistance of rubber plants in the field, whether a plant has a genetic resistance or only an escape. Information related to this type of resistance is particularly useful in selecting and developing clones with resistance to CLF disease.

CONCLUSION

Based on ITS-rDNA sequence, *C. cassiicola* isolates from Indonesia were divided into two large groups with 5 haplotypes. Each isolate had different virulence levels to six rubber clones. Three isolates from clone PR 303, RRIM 600 and Tjir 1 were highly virulent. The evaluation of rubber germplasm indicated that there is a potentially large number of

resistance genes in the IRRDB 1981 germplasm, where in three accessions of PN 451, PN 494 and PN 604 have higher resistance than BPM 1 (resistant clone of the Wickham population). Preparation of genetic linked map obtained 4 linked groups with a total of 8 linked loci on LOD 3, and 2 linked groups with a total of 11 of linked loci in LOD 2. QTLs linked to CLF disease have not been identified on the genetic linked map, but some loci were found to be associated with CLF. The EHB 70, EHB 081, EHBp 18 and SSRH 548 loci were found to be associated with both isolates, whereas the HB 68 locus was only associated with CC-06 isolate and gSSR 165, HBE 329, SSRH 103, HB 78 and gSSR 212 loci with isolates CC-22. The identification of the associated loci are a preliminary information to develop MAS for CLF disease resistance and accelerate the rubber breeding programme.

REFERENCES

Besse, P., Seguin, M., Lebrun, P., Chevallier, M., Nicolas, D., & Lanaud, C. (1994). Genetic diversity among wild and cultivated populations of *Hevea brasiliensis* assessed by nuclear RFLP analysis. *Theoretical and Applied Genetics, 88*, 199-207.

Breton, F., Sanier, C. and D'Auzac, J. (2000). Role of *cassiicolline*. a host-selective toxin. in pathogenicity of *Corynespora cassiicola*. causal agent of a leaf fall disease of Hevea. *Journal of Rubber Research*, 3, 115-128.

Champoux, M. C., Wang, G., Sarkarung, S., Mackill, D. J., O'Toole, J. C., Huang, N., & McCouch, S. R. (1995). Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. *Theoretical and Applied Genetics, 90*(7-8), 969-981.

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica, 142*, 169-196.

Dixon, L. J., Schlub, R. L., Pernezny, K., & Datnoff, L. E. (2009). Host specialization and phylogenetic diversity of *Corynespora cassiicola*. *Phytopathology, 99*(9), 1015-1027. doi: 10.1094/PHYTO-99-9-1015

Gouvêa, L. R. L., Rubiano, L. B., Chioratto, A. F., Zucchi, M. I., & Gonçalves, P. d. S. (2010). Genetic divergence of rubber tree estimated by multivariate techniques and microsatellite markers. *Genetics and Molecular Biology, 33*, 308-318.

Grattapaglia, D., & Sederoff, R. (1994). Genetic linkage maps of *Eucaliptus grandis* and *Eucaliptus urophylla* using pseudo-test-cross-mapping strategy and RAPD markers. *Genetics, 137*, 1121-1137.

Hieu, N., Nghia, N., Chi, V., & Dung, P. (2014). Genetic diversity and pathogenicity of *Corynespora cassiicola* isolates from rubber trees and other hosts in Vietnam. *Journal of Rubber Research, 17*(3), 187-202.

Lam, L., Thanh, T., Chi, V., & Tuy, L. (2009). Genetic Diversity of Hevea IRRDB'81 Collection Assessed by RAPD Markers. *Molecular Biotechnology, 42*(3), 292-298. doi: 10.1007/s12033-009-9159-7

Le-Guen, V., Garcia, D., Mattos, C., Doare, F., Lespinasse, D., & Seguin, M. (2007). Bypassing of a phyllogenic Microcyclus ulei resistance in rubber tree, analyzed by QTL detection. *New Phytologist, 173*, 335-345.

Le Guen, V., Garcia, D., Doaré, F., Mattos, C., Condina, V., Couturier, C., . . . Seguin, M. (2011). A rubber tree's durable resistance to Microcyclus ulei is conferred by a qualitative gene and a major quantitative resistance factor. *Tree Genetics & Genomes, 7*(877–889).

Le Guen, V., Garcia, D., Mattos, C., Fouet, O., Doaré, F., Condina, V., & Seguin, M. (2013). A newly identified locus controls complete resistance to Microcyclus ulei in the Fx2784 rubber clone. *Tree Genetics & Genomes, 9*(3), 805-812. doi: 10.1007/s11295-013-0599-7

Le Guen, V., Lespinasse, D., Oliver, G., Rodier-Goud, M., Pinard, F., & Seguin, M. (2003). Molecular mapping of genes conferring field resistance to South American Leaf Blight (Microcyclus ulei) in rubber tree. *Theoretical and Applied Genetics, 108*(1), 160-167. doi: 10.1007/s00122-003-1407-9

Lekawipat, N., Teerawatanasuk, K., Rodier-Goud, M., Seguin, M., Vanavichit, A., Toojinda, T., & Tragoonrung, S. (2003). Genetic diversity analysis of wild germplasm and cultivated clones of *Hevea brasiliensis* Muell. Arg. by using microsatellite markers. *Journal of Rubber Research, 6*(1), 36-47.

Lespinasse, D., Rodier-Goud, M., Grivet, L., Leconte, A., Legnate, H., & Seguin, M. (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.

Lieberei, R. (2007). South American Leaf Blight of the Rubber Tree (Hevea spp.): New Steps in Plant Domestication using Physiological Features and Molecular Markers. *Annals of Botany, 100*(6), 1125-1142. doi: 10.1093/aob/mcm133.

Mercy, M.A. (2001). Genotypic evaluation and screening for drought tolerance in wild *Hevea* germplasm. Doctor of Philosphy. Kerala Agricultural University, India.

Mydin, K., Narayanan, C. and Abraham, T. (2012). Incorporation of the 1981 IRRDB wild amazonian germplasm in Hevea breeding in India. IRRI-IRRDB Rubber Plant Breeding Seminar. Medan, Indonesia,

Nghia, N., Kadir, J., Sunderasan, E., Abdullah, M., Malik, A. and Napis, S. (2008). Morphological and inter Simple Sequence Repeat (ISSR) Markers Analyses of *Corynespora cassiicola* Isolates from Rubber Plantations in Malaysia. Mycopathologia, 166, 189-201.

Nghia, N., Kadir, J., Sunderasan, E., Abdullah, M., Malik, A., & Napis, S. (2010). Intraspecific variability of *Corynespora cassiicola* inferred from single nucletide polymorphisms in ITS region of ribosomal DNA. *Journal of Rubber Research, 13*(4), 257-264.

Novalina, & Sagala, A. D. (2013). Construction of *Hevea brasiliensis* genetic linkage map and Identification of quantitative trait loci using RAPD markers. *International Journal on Advanced Science Engineering Information Technology, 3*(1), 71-75.

Oktavia, F., Lasminingsih, M., & Kuswanhadi. (2011). Genetic Relationship of Wickham and IRRDB 1981 Rubber Population Based on RAPD Markers Analysis. *HAYATI Journal of Biosciences, 18*(1), 27-32. doi: DOI: 10.4308/hjb.18.1.27

Oktavia, F., Sudarsono, Kuswanhadi, Dinarty, D., Widodo. (2016). Identification of IRRDB 1981 rubber germplasm resistance to Corynespora leaf fall disease based on toxin activity. *Indonesian Journal Rubber Research*, 34(1) : 35-48.

Rattanawong, R., Prapan, K., Lekawipat, N., Seguin, M., & Clement-Demange, A. (2009). *Detection of QTLs assotiation with growth, latex production and rubber quality for the development of Marker Asissted Selection (Hevea brasiliensis)*. Paper presented at the

IRRDB workshop on genome and transcriptome, Montpellier, 3-5 June 2009. France.

Reghu, P., Mercy, M. A. and Lakshmanan, R. (2012). Further evaluation and selection of 1981 IRRDB wild Hevea germplasm collection in India. *Nattural Rubber Research*. 25(1), 31-38.

Saha, T., Roy, C. B., & Nazeer, M. A. (2005). Microsatellite variability and its use in the characterization of cultivated clones of *Hevea brasiliensis*. *Plant Breeding, 124*(1), 86-92. doi: 10.1111/j.1439-0523.2004.01053.x

Situmorang, A., Sinaga, M., Suseno, R., Hidayat, S., Siswanto, & Darussamin, A. (2007). Spreading of Corynespora leaf fall disease on the rubber plantation in Indonesia. *Indonesian Journal of Rubber Research, 25*(1), 76-82.

Souza, L., Gazaffi, R., Mantello, C., Silva, C., Garcia, D., Le Guen, V., . . . AP, S. (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*(4), 1 - 10.

Souza, L., Mantello, C., Suzuki, F., Rodrigo, G., & Le-Guen, V. (2011). *Development of a genetic linkage map of rubber (Hevea brasiliensis) based on microsatellite markers*. Paper presented at the IUFRO tree biotechnology confrence. 26 Jun - 2 Jul 2011, Arraial D'Ajuda, Bahia, Brazil.

White, T. J., Bruns, S., Lee and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelland, D. Sninsky, J. J. and White, T. J., editors. PCR protocols: A guide to methods and applications, (pp. 315-322). Academic Pres**,** New York.