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Preliminary QTL detection for *Corynespora* Leaf Fall disease resistance in rubber plant

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Abstract. *Oktavia F, Sudarsono, Dinarti D. 2021. Preliminary QTL detection for Corynespora Leaf Fall disease resistance in rubber plant. Nusantara Bioscience 13: 53-61. Corynespora* Leaf Fall (CLF) disease caused by *Corynespora cassiicola* fungus is one of the most important diseases of rubber trees. Conventional breeding to obtain the resistant rubber clones was constrained by the long time required for selection processes. This study was aimed to identify resistant loci linked with CLF disease on the rubber tree. Analysis was conducted on 104 F1 of BPM 1 x RRIM 600. The resistance evaluation of the population had been done against two *C. cassiicola* isolates and the molecular analysis was generated by using 28 selected SSR markers. There was a phenotypic variation in F1 resistance to both isolates with leaf wilting intensities ranged from 5.2% to 33.4% on CC-06 isolate, and from 3.5% to 36.4% on CC-22 isolate. There was no QTL identified on the genetic linkage map, but a single marker analysis showed that some loci associated with CLF disease. The associated loci can be used as a preliminary information to develop molecular markers linked with resistance to CLF disease to assist the rubber breeding programs.

Keywords: CLF disease, *Hevea brasiliensis*, MAS, progeny, rubber breeding

Abbreviations: CLF: *Corynespora* Leaf Fall; QTL: Quantitative Trait Loci; MAS: Marker Assisted Selection

INTRODUCTION

Rubber tree (*Hevea brasiliensis*) is one of the ten members of the *Hevea* genus which became the main cultivated species in the natural rubber producers. The plants coming from South America are growing well in Southeast Asia and it contributes more than 90% of the natural rubber production in the world. However, this potential was restricted by *Corynespora* leaf fall (CLF) disease caused by the *Corynespora cassiicola* fungus which causes a decrease in latex production, and in severe conditions, the disease can lead to plant death. CLF disease has caused serious damage in almost all of the centers of rubber plantations in Southeast Asia and Africa.

Some efforts have been done to control the CLF disease and chemical control is commonly used to control the disease. Although the chemical method becomes the first choice in the field, it is not the best solution. Chemical control is not only expensive but its application also causes a negative impact on the environment. Continuous use of pesticides can cause the pathogen to develop resistance against the pesticide, thus the pesticide is no longer effective against the pathogen.

Use of high and stable resistant clones is one of the most effective and economical ways to control the CLF disease. Breeding activities to produce new resistant clones have been done by breeders and there are some problems mainly related to the limited area and the lengthy time of selection. One alternative to overcome these problems is the use of *Marker Assisted Selection* (MAS).

MAS can help accelerate the breeding programs mainly on the perennial crops which have a long life cycle. To obtain an accurate MAS, information on and the availability of genetic linkage maps are required. The development of genetic mapping studies on the perennial crops which are cross-pollinated and heterozygous are not as advanced as that in horticultural crops. This is because perennial plants such as Hevea have a long life cycle and low ability to produce seeds due to inbreeding depression, so the preparation of progeny for genetic studies is very difficult (Lespinasse et al. 2000). These limitations also caused only F1 progenies obtained from crossing of the heterozygous parents are possible to be prepared as a mapping population of which these conditions may cause segregation of alleles in one locus. The generated data can be analyzed as a double pseudotestcross and preparation of the genetic linkage map for each parent has to be carried out separately (Grattapaglia and Sederoff 1994). The use of F1 population that was obtained from a cross had been widely used as a mapping population to construct a linkage map as was reported in *Eucalyptus* (Grattapaglia and Sederoff 1994), tea plant (Hackett et al. 2000; Ma et al. 2014), and rubber tree (Le Guen et al. 2003; Le-Guen et al. 2007; Le Guen et al. 2011; Le Guen et al. 2013; Rattanawong et al. 2009; Novalina 2013; Souza et al. 2011; Souza et al. 2013; Conson et al. 2018; Rosa et al. 2018; An et al. 2019).

In this study, we reported the construction of a genetic linkage map by using F1 population that was obtained from crossing of BPM 1 and RRIM 600 clones. The availability of genetic linkage maps and information about the molecular markers related to the loci that control resistance to CLF disease can be used as a marker for early detection of resistant accessions so that it will accelerate the selection process in breeding programs to obtain new superior clones in the future.

MATERIALS AND METHODS

Evaluation of CLF disease resistance

104 F1 progenies which consist of 30 plants and 74 embryos were obtained from crossings the CLF disease-resistant rubber clone (BPM 1) and the susceptible clone (RRIM 600) (Oktavia et al. 2016). The CLF disease resistance level of 30 F1 plants was tested by their resistance to the toxin filtrate produced by the two isolates of *C. Cassiicola,* namely CC-06 and CC-22 collected from GT 1 and RRIM 600 rubber clones in the Sembawa Research Centre, Palembang. The isolates were classified as highly virulent isolates based on previous study (Oktavia et al. 2017). Procedures of toxin production and resistance evaluation were based on Breton et al. (2000) with some modifications. Resistance evaluation was conducted in the laboratory-based on the activity of the toxin filtrate of isolate using immature leaves (B2C growth stage). The leaves were taken from plants grown in the greenhouse and then soaked in water for one night (16 hours). Subsequently, 125 ml toxin filtrate (concentration of 5 mg L^{-1}) was inserted in the tray covered with perforated styrofoam (3 cm diameter) and the petiole was inserted into the hole until submerged in toxin filtrate. As a control, sterile water was used instead of toxin filtrate, and all treatments were incubated at room temperature for 48 hours. Each treatment was repeated three times.

Assessment of plant resistance level was based on the water loss estimation due to toxin activity that was observed 48 hours after soaking the leaves in the toxin filtrate. Water loss estimation was calculated as the leaf wilting intensity (LWI) which showed the percentage of difference in weight of the leaves before and after the treatment with the toxin minus the control.

Linkage map analysis

Total DNA was extracted from 104 rubber leaf samples according to the procedure described by Orozco-Castillo et al. (1994). The DNA stocks were either dissolved in TE for storage at -20 ^{o}C in the freezer or diluted in ddH_2O as the working solution.

PCR amplification was carried out in a total volume of 12.5 µl by using the selected SSR primers. The PCR reaction mixture consisted of 2 µl of 25 ng μl^{-1} DNA template, 0.75 µl (10 nm) each of the forward and reverse primer, 2.75 µl ddH_2O and 6.25 µl of PCR mix (Kapa Biosystem Inc. USA). The Amplifications were performed in a DNA thermal cycler (Model T-100 Thermal Cycler, Bio-Rad, USA). The amplification program was as follow: one cycle of pre-denaturation at 95 ^{o}C for 3 minutes, followed by 35 cycles of amplification consist of denaturation at 95 ^{o}C for 15 seconds, primer annealing at 53-56 ^{o}C for 15 seconds, and primer extension at 72°C for 30 seconds, and terminated by one cycle of final primer extension at 72 ^{o}C for 3 min. The amplified PCR products were evaluated in 1% agarose in TBE buffer horizontal gel electrophoresis. All of the samples that positively produced PCR product were subsequently separated in a vertical denaturing SDS polyacrylamide gel electrophoresis (SDS PAGE) containing 7 M urea, by using a single gel dedicated manual sequencer (Cole-Parmer®). The observed allelic patterns of the individual accession were visualized by staining the gel in a silver nitrate according to procedure developed by Creste et al. (2001).

The SSR primers used to construct genetic linkage map were obtained from two steps of selection. The first selection was polymorphism of 135 SSR primers (An et al. 2013; Cubry et al. 2014; Le Guen et al. 2011; Li et al. 2012; Mantello et al. 2014; Silva et al. 2014; Triwitayakorn et al. 2011) on both parental clones. Subsequently, the selected primers were used to amplify all individuals in the mapping population. The progenies that carried a specific allele from the resistant parent were indicated as a heterozygous genotype (H) and the ones that did not carry the allele were characterized as a homozygous genotype (A). Furthermore, the loci in the population having segregation of H:A that fitted the ratio of 1:1 based on chi-square test were used to construct the linkage map by converting the frequency of recombination into map distance (centiMorgan = cM). Construction of the linkage map was done using the Kosambi mapping function employing the MapMaker / EXP 3.0 program of the model backcross on the value of LOD minimum 3.0 and 2.0 with fractions of recombinant of 0.5 (Lincoln and Landers 1993). Identification of QTLs linked to CLF disease was done based on the observed resistance level and molecular data by using QGene 4.0 software (Joehanes and Nelson 2008).

Identification of SSRs markers associated with CLF disease resistance

The association between SSR markers with CLF disease resistance was determined by using a single marker analysis (Champoux et al. 1995; Collard et al. 2005). The analysis was performed by combining the disease resistance data and SSR markers data employing SPSS 20 statistical package. This method was done by using analysis of variance, where the plant accessions scoring result was the independent variable (X) and the level of resistance as the dependent variable (Y). Analysis of variance was conducted on each of the SSR markers. Linear model of each variance was $y_{ij} = \mu + X_i + \varepsilon_{ij}$ where: yij: resistance variable on the accession of the i-th and j-th repetition, µ: the average general, Xi: the effect of accession to-i, and εij: environmental effect.

RESULTS AND DISCUSSION

Resistance evaluation of mapping population to CLF disease

One of the problems to prepare a mapping population on the rubber plant is a low success rate of hand pollination to produce F1 progenies. In this study, 6031 crosses were made between BPM 1 and RRIM 600 clones, and resulted in only 30 F1 plants and 74 F1 embryos which were collected from seeds with a crossing success rate of 0.005%. Thus, only 30 F1 plants were evaluated.

Cassiicolin toxin is the main effector in the pathogenicity of *C. cassiicola* isolate, so that it can be used as an evaluation method for resistance to CLF disease. Evaluation of parental clones against two *C. cassiicola* isolates showed that the resistant parent clone BPM 1 decreased in water content of 6.9% in CC-06 and 7.8% in CC-22, respectively, while the susceptible parent RRIM 600 had a water content reduction of 33.6% in CC-06 and 41.3% in CC-22 due to the toxin activity, respectively. This indicated that the parental clones used to obtain the mapping population had a contrasting resistance level to CLF disease (Figure 1). These results could support the mapping of quantitative character loci related to resistance of the rubber plant to CLF disease. McCouch and Tanksley (1991) stated that the selection of the parents having a resistant and susceptible extreme response was one of the important factors determining the success of genetic mapping to be able to obtain a segregating progeny in those two parental crossings.

Figure 1 also shows the resistance level of F1 plant population to the two *C. cassiicola* isolates ranged from 5.2% - 33.4% to CC-06 and 3.5% - 36.4% to CC-22 isolates, respectively. The differences in the resistance level to both isolates could be caused by the differences in virulence level of the isolates caused by specific interactions between the plants and the isolates. Lieberei (2007) stated that the disease occurrence can be influenced by many factors such as the level of genetic variability of pathogens and plants, environment and interaction between them. These factors can cause a plant genotype to become resistant to one isolate but susceptible to the other. Three progenies had a better resistance level to both isolates as compared to the resistant parental clone of BPM 1, i.e., F1.1, F1.5, and F1.10. While the F1.27 progeny had a better resistance just to the CC-06 isolate, and F1.11, F1.12, F1.14, F1.23, F1.28, and F1.29 progenies had a better resistance level to CC-22 isolate.

The information about genetic studies of rubber plant resistance to CLF disease was still limitedly reported. Tan and Tan (1996) reported that resistance to CLF disease was complex that is regulated by polygenic, and on the other hand, Hadi and Hartana (2004) reported that the resistance was oligogenic with an epistatic effect. The characters that were regulated by polygenic (quantitative) had a normal distribution, on the contrary, the characters that were regulated by monogenic or oligogenic (qualitative) have a skewed distribution. Figure 2 shows the distribution of leaf wilting intensity of F1 progeny to two *C. cassiicola* isolates. The shape of distribution does not look like a normally distributed trait, and it showed that resistance to CLF disease was regulated by a few major and minor genes (oligogenic).

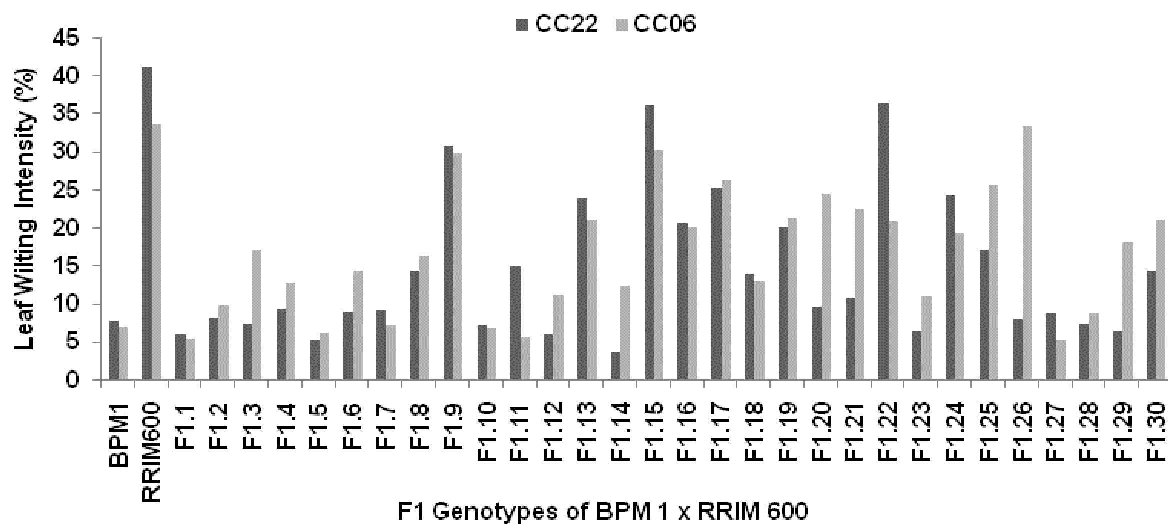

Figure 1. Leaf wilting intensity of 30 F1 progenies of BPM 1 x RRIM 600 to toxin filtrate of *C. cassiicola* isolates

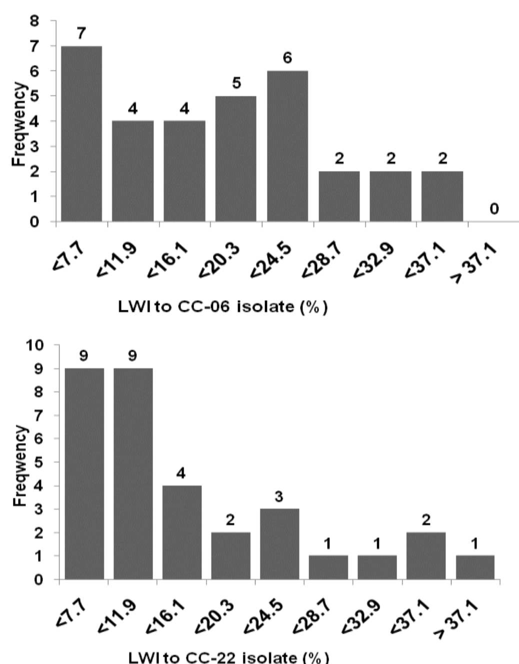

Figure 2. The distribution of leaf wilting intensity (LWI) of mapping population to two of *C. cassiicola* isolates

Selection of SSR markers for construction of genetic linkage map

The first step selection of 135 SSR loci on both parental clones revealed 31 SSR markers that produced polymorphic loci (22.9%). These loci were heterozygous in the resistant parent clone (BPM 1) and homozygous in the susceptible parent clone (RRIM 600), or heterozygous on both clones with different positions of at least one allele. The percentage of polymorphic loci in this study was higher than that of previous studies on the RRIM 600 and RRII 105 clones (18.27%) (Triwitayakorn et al. 2011) but was lower than that produced from primer selection conducted on PB 260 and RO 38 clones (46.15%) (Lespinase et al. 2000) as well as on PB 217 with PR 255 clones (51%) (Souza et al. 2013). These differences are caused by the different types of clones used in the selection, where polymorphic loci in one pair of clones could become monomorphic in other clones due to the differences in the clone's genetic background. In addition, the different type and number of SSR markers selected also caused difference in the percentage of polymorphic loci among studies.

The first step selected markers were used to amplify all population members consisting of 104 F_1 (30 plants and 74 embryos) and their parents. Allele obtained only in the resistant parent clone (BPM 1) was considered as a specific allele that is assumed to be associated with CLF disease resistance in the rubber plant. Individuals carried a specific allele were grouped as individuals with H genotype and those without the specific allele were grouped as individuals with A genotype.

The second step of primer selection was based on the results of PCR amplification of the whole population that was analyzed by using chi-square test. The results showed that 28 of SSR loci had H and A genotypes with segregation ratio of 1:1, while the others did not fit the 1:1 ratio (Table 1). The loci with segregation ratio of 1:1 were used to generate the genetic linkage maps.

Figure 3 shows the results of linkage analysis of 28 SSR loci segregation ratio of 1:1 in the population using the MapMaker / EXP software. In the LOD 3 FR 0.5, 4 linkage groups were obtained, consisted of eight linkage loci, while the remaining 20 loci segregated independently. Two loci distributed on each linkage group with group size range of 30-47.8 cM (Figure 3.A). If the LOD was lowered to 2 with FR 0.5, there was an increasing number of linkage loci to be 11 loci while the remaining 17 loci segregated independently. There was also a decreasing number of linkage groups into two groups, six loci distributed in group 1 and five loci in group 2 with a distance range of linkage groups of 203-228.5 cM (Figure 3.B). This showed that the linkage groups 3 and 4 on LOD 3 were merged into linkage group 1 in LOD 2 with the addition of two bridge loci between EHBla-2 and mHbCIRA-2715. Two loci that were previously independently segregated and then changed to linked were HB-52 and EHB-122. While the linkage groups 1 and 2 on LOD 3 merged to linkage group 2 on LOD 2 with the addition of one linked locus of gSSR-165. These showed that the decrease of LOD limit can increase the number of linked loci, but the chances of the possibility of recombination also increased.

The number of linkage groups obtained in this study was lower compared to the number of rubber plant chromosomes. Similarly, the locus density on each linkage group, which in the closest distance between locus was about 30 cM. It was due to low number of individuals in population and loci used. The same results were also reported in other studies that obtained 2 linkage groups in the construction of genetic linkage map on 22 F1 of PB 260 x PN 711 by using 94 RAPD markers (Novalina and Sagala 2013). Compared to previous studies, the genetic map generated in this study was able to detect a small part of the rubber plant genome. Lespinasse et al. (2000) reported 18 linkage groups with a markers density every 3 cM on the analysis of 106 F1 progenies of PB 260 x RO38 using 717 loci consisting of 301 RFLP, 388 AFLP, 18 SSR, and 10 isozymes. Le-Guen et al. (2007) used 234 SSR markers on the same population and obtained markers density every 10-15 cM. Likewise, the analysis of 81 F1 plants of RRIM 600 x RRII 105 using 97 SSR loci obtained 23 linkage groups with interval between loci 11.9 cM (Triwitayakorn et al. 2011). While Le Guen et al. (2011) on 351 F1 progeny of PB 260 x MDF 180 using 203 SSR, 96 AFLP, and 1 STS markers produced 18 of linkage groups. Using of 1,079 markers on 146 F1 of GT 1 x RRIM 701, Conson et al. (2018) identified 38 QTLs on 18 linkage groups with an average marker density of 3.5 cM. One of the SSR loci in one of the linkage groups was also found in this study. The locus was called mHbCIRA 2715 currently on the linkage group 3 with a distance of 0 cM, and this locus was in linkage group 12 with a distance of 70.8 cM.

Table 1. Chi-square analysis of 30 SSR loci segregated into 1:1 ratio on the mapping population

Locus	Accession no.		Segregation	X^2	P
	H	A			
EHB-069	49	55	1:1	0.35	Ns
EHB-070	43	55	1:1	1.47	Ns
EHB-079	58	46	1:1	1.38	Ns
EHB-081	67	32	1:1	12.37	S
EHB-087	62	42	1:1	3.85	Ns
EHB-088	45	57	1:1	1.41	Ns
EHB-113	53	51	1:1	0.04	Ns
EHB-122	58	46	1:1	1.38	Ns
EHB-133	53	50	1:1	0.09	Ns
EHB-151	62	42	1:1	3.85	Ns
HB-52	53	47	1:1	0.36	Ns
HB-68	37	58	1:1	4.64	S
HB-78	46	57	1:1	1.17	Ns
HESR-032	44	57	1:1	1.67	Ns
EHBp-15	44	56	1:1	1.44	Ns
EHBp-18	54	49	1:1	0.24	Ns
EHBla-2	60	46	1:1	2.96	Ns
EHBc-34	55	48	1:1	0.48	Ns
SSRH-103	52	37	1:1	2.53	Ns
SSRH-358	58	43	1:1	2.23	Ns
SSRH-548	56	43	1:1	1.71	Ns
gSSR-165	60	43	1:1	2.81	Ns
gSSR-268	44	60	1:1	2.46	Ns
HBE-329	49	50	1:1	0.01	Ns
P070	55	46	1:1	0.80	Ns
mHbCIRA-2715	58	46	1:1	1.38	Ns
mHbCIRA-2425	52	52	1:1	0.00	Ns
gSSR-194	54	50	1:1	0.15	Ns
gSSR-212	52	45	1:1	0.51	Ns
HESR-029	51	50	1:1	0.01	Ns

Note: P (5%) on the df 1: 3.84. Ns: nonsignificant, number of the H and A genotypes are not significantly different (segregation of alleles 1:1). S: significant, number of the H genotype is significantly different from A genotype (segregation of alleles deviated from 1:1 ratio)

Based on the information of genetic linkage map generated, the identification of QTLs position on the linkage map can be conducted by using information of the resistance phenotype and SSR data. Analysis using QGene program showed that with a total distance of 387.4 cM and markers density of 35.3 cM, this analysis could not identify the presence of QTLs linked with CLF disease resistance on the linkage map. A few of the linkage group numbers and a low locus density were major limited factors on the QTLs identification process. Other studies reported that the presence of QTLs associated with SALB (South American Leaf Blight) disease was identified on 18 linkage groups with a markers density of 10-15 cM (Le Guen et al. 2007) and 8-10.6 cM (Le Guen et al. 2011). Likewise, the QTLs associated with the growth of rubber plants were identified on 23 linkage groups with a markers density of 10 cM (Souza et al. 2013), 21 linkage groups with 5 cM (Rosa et al. 2018), 18 linkage groups with 3.5 cM (Conson et al. 2018) and 15 linkage groups with 3.0 cM (An et al. 2019).

Association of molecular markers with phenotypic characters could be identified by using a single marker analysis without the information of genetic linkage map (Champoux et al. 1995; Collard et al. 2005). However, the information obtained was limited to the association between the markers with the phenotypic character, while their position and distance of the QTLs from the marker were unknown. This might be because markers and the resistance character were not in the same chromosome, or they may be in the same chromosome but they had a long-distance so that it would not be segregated together (Collard et al. 2005).

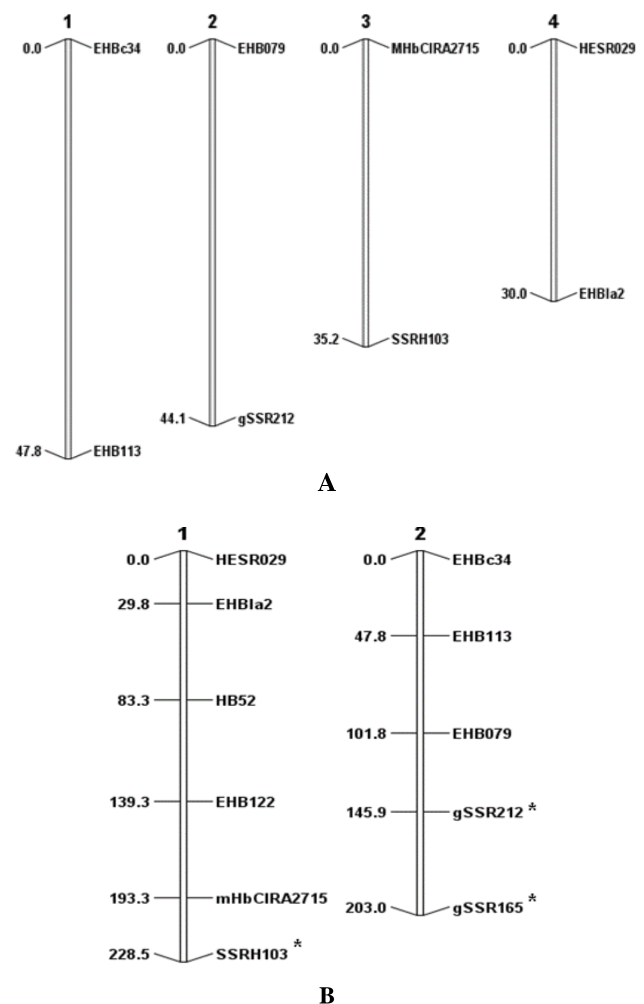

Figure 3. Genetic linkage map of F1 population from BPM 1 x RRIM 600 clones resulted from Qgene analysis. A. Analysis on the LOD (Log of Odd) 3 with RF (Recombinant Fraction) 0.5 and B. Analysis on the LOD 2 RF 0.5. Distance between locus in centi Morgan (cM). * is associated locus with CLF disease based on single-marker analysis.

Identification of SSR marker associated with CLF disease resistance

The single-marker analysis by using SPSS program successfully identified the presence of SSR loci associated with the resistance of the rubber plant to *C. cassiicola* isolates (Table 2). The result showed that there were four loci associated with resistance to both isolates, one locus only associated with CC-06 isolate and five loci associated only with CC-22 isolate ($P < 0.05$). Loci associated with resistance to the CC-06 isolate were EHB-70, EHB-081, EHBp 18 SSRH-548, and HB-68, while loci associated with CC-22 isolate were gSSR-165, HBE-329, EHB-070, SSRH-103, HB-78, EHB-081, EHBp-18, SSRH-548, and gSSR-212. Among these, EHB-70, EHB-081, EHBp-18, and SSRH-548 loci are associated with resistance to the two isolates used.

The number loci associated with resistance to CC-22 isolate were higher than that of CC-06 isolate. This may be correlated with the host origin of the isolates; the CC-22 isolate was obtained from RRIM 600 clone. This clone was the susceptible parent clone, so it was expected that there was a specific relationship between the population resistance with isolate tested. The locus association with resistance to CC-22 isolate was also highly significant ($P < 0.01$).

Furthermore, four of the ten loci associated with resistance to CLF disease were an EST-SSR (Expressed Sequence Taq-Simple Sequence Repeat) which was designed based on the cDNA. These loci were HBE-329 from latex cDNA (Feng et al. 2009), EHB-070 and EHB-081 from apical meristem cDNA (Triwitayakorn et al. 2011), and EHBp-18 from bark cDNA (Silva et al. 2014). The HBE-329, EHB-070, and EHB-081 were designed by using cDNA of RRIM 600 clone, while EHBp-18 was from cDNA of PB 217 clone.

Figure 4 shows the combinations of allele from five loci associated with CC-06 isolate. Each locus has a variation of allele combinations with a different average leaf wilting percentage, where the lower the percentage would cause the higher the plant resistance level. Based on the average resistance value of each allele combination, the selected associated allele with the resistance to CLF disease was estimated. Estimated loci carried the selected allele were red-circled. Resistance to CLF disease at the EHB-070 and HB-68 was estimated to be controlled by the A1 allele, whereas in the EHB-081, EHBp-18 and SSRH-548 were controlled by the A2 allele.

Likewise, ten of loci associated with the CC-22 isolate have allele variations with a different average leaf wilting percentage. A1 allele at the SSRH 548, SSRH 103, HB 78, and gSSR 212 was predicted to be associated with the resistance to CC-22 isolate, while EHB 70, EHB 081, EHBp 18, and HBE 329 were estimated to be associated with the A2 allele and A3 allele at the gSSR 165 locus (Figure 5).

There were differences of allele associated with resistance to CC-06 and CC-22 isolates on the EHB 070 and SSRH 548 loci. However, based on the average value of resistance to both isolates, it was expected that A1 and A2 were the associate alleles on the EHB 070 and SSRH 548 loci, respectively (Figure 6). Although they can not be used as direct markers for resistance selection tools, the presence of these alleles could be used as early information to develop the molecular markers linkage with CLF disease resistance. To improve the accuracy of obtained data to allow the loci could be used in marker-assisted selection, it is necessary to make further analysis by increasing the number of mapping population and loci analyzed.

Table 2. Results of single marker analysis of SSR locus with resistance of mapping population to two of *C. cassiicola* isolates

Locus	Response to isolate		
	F R-CC-06	F R-CC-22	F R-Average
gSSR-268	1.381 ns	0.563 ns	0.976 ns
gSSR-194	1.974 ns	2.360 ns	2.536 ns
EHB-87	0.229 ns	1.505 ns	0.898 ns
EHB-113	0.717 ns	0.038 ns	0.308 ns
SSRH-358	0.254 ns	0.064 ns	0.109 ns
HB-52	0.156 ns	0.906 ns	0.311 ns
HESR-032	0.790 ns	0.519 ns	0.657 ns
EHBc-34	1.048 ns	2.069 ns	1.814 ns
EHB-069	0.267 ns	1.426 ns	0.716 ns
EHB-122	0.166 ns	0.441 ns	0.175 ns
EHB-079	1.028 ns	0.279 ns	0.64 ns
EHB-088	0.774 ns	1.011 ns	0.996 ns
P-070	1.704 ns	1.054 ns	1.216 ns
gSSR-165	1.992 ns	7.677 **	5.461 *
EHB-151	0.542 ns	0.563 ns	0.618 ns
mHbCIRA-2425	0.613 ns	1.283 ns	1.144 ns
HESR-029	0.662 ns	0.318 ns	0.563 ns
EHBla-2	0.119 ns	0.301 ns	0.190 ns
HBE-329	1.890 ns	4.878 **	3.908 *
EHB-133	0.949 ns	2.349 ns	1.585 ns
EHB-070	3.407 *	7.847 **	6.701 **
mHbCIRA-2715	0.664 ns	0.634 ns	0.652 ns
SSRH-103	2.442 ns	8.044 **	6.026 **
HB-78	2.864 ns	8.962 **	6.529 **
EHB-081	3.866 **	8.092 **	8.494 **
EHBp-15	2.079 ns	1.348 ns	1.536 ns
EHBp-18	3.469 *	7.755 **	6.73 **
gSSR-212	1.892 ns	5.044 **	3.723 *
SSRH-548	2.812 *	13.31 **	7.886 **
HB-68	5.516 **	2.250 ns	3.596 *

Note: F R-CC-06 and F R-CC-22: F value of resistance to *C. casiicola* isolate of CC-06 and CC-22. ns: non significant, **: significant in α 0.01, *: significant at α 0.05

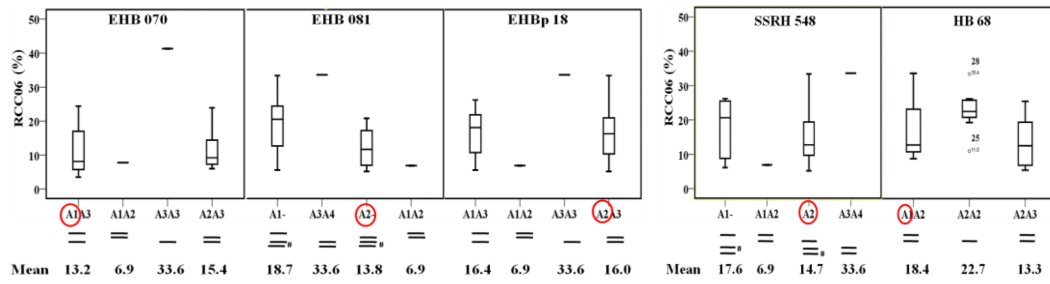

Figure 4. Allele variations and mean resistance level of mapping population to *C. cassiicola* isolate CC-06 on the 5 SSR locus associated with CLF disease. The lower proportion of allele variation is the parents alleles. The circled alleles are the alleles predicted to be associated with CLF disease. # shows the position of one of the allele pairs

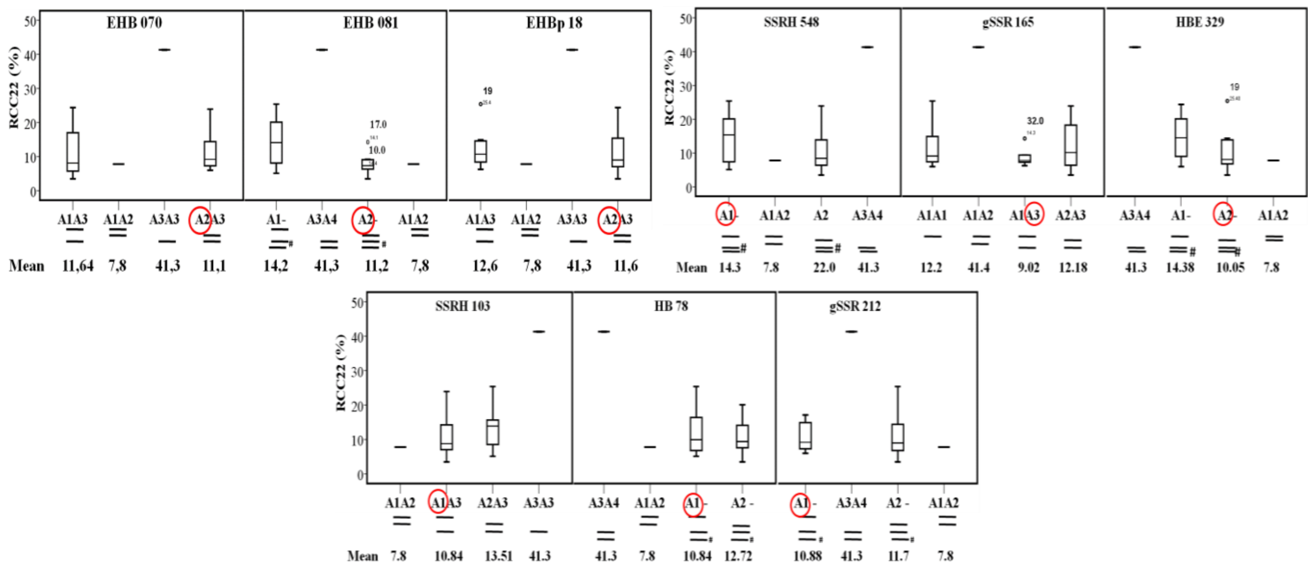

Figure 5. Allele variations and mean resistance level of mapping population to CC-22 isolate on 9 of SSR locus associated with CLF disease. The lower proportion of allele variation is the parents alleles. The red circled alleles are the alleles predicted to be associated with CLF disease. # shows the position of one of the allele pair

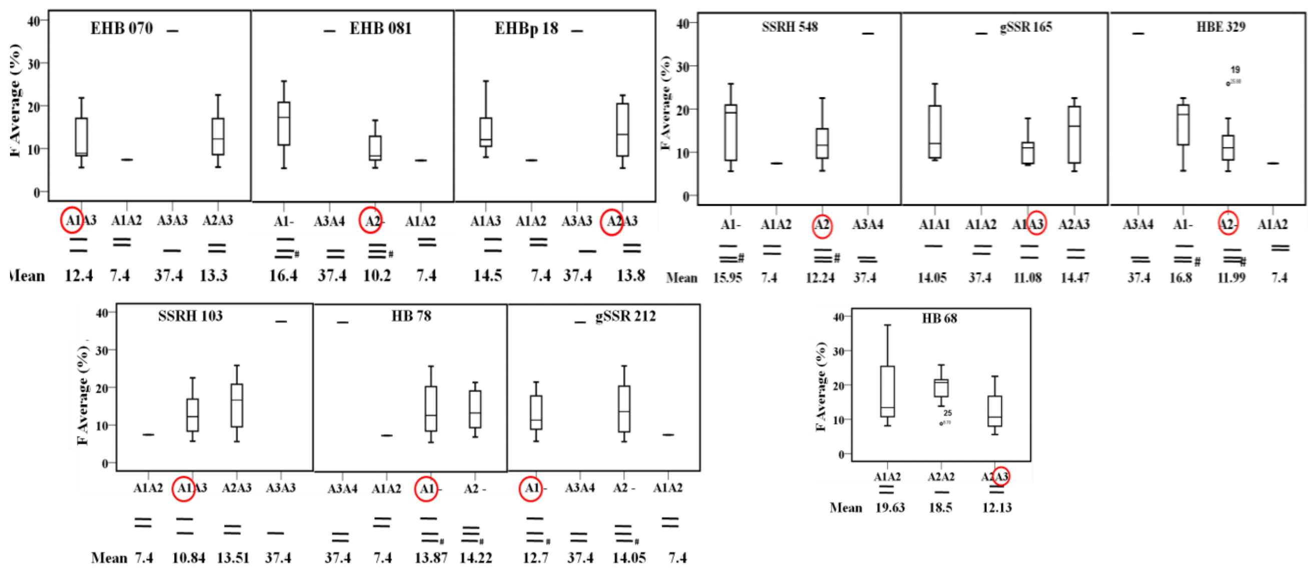

Figure 6. Allele variations and mean resistance level of mapping population of rubber plant to *C. cassiicola* isolates CC-06 and CC-2 on the 10 of SSR locus associated with CLF disease. The lower proportion of allele variation is the parents alleles. The circled alleles are the alleles predicted to be associated with CLF disease. # shows the position of one of the allele pairs.

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