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Pod Characteristics of Cocoa (*Theobroma cacao* L.) Related to Cocoa Pod Borer Resistance

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Abstract

The characteristics of cocoa (*Theobroma cacao* L.) pod related to cocoa pod borer resistance (CPB) had been identified in a series of study. The objective of this research is to evaluate the characteristics of cocoa pod using more diverse of genetic background to obtain selection criteria. Genetic materials for this study were 25 cocoa clones planted in Central Sulawesi for resistance evaluation. Field evaluation of the resistance were assessed by using variable of the percentage of unextractable beans, number of larvae entry and exit holes by which the clones were grouped into 5 groups of resistance. A laboratory works were carried out to assess pod characteristics based on the number of trichomes, granules of tannin and thickness the lignified-tissue of sclerotic layer using micro-technique method at the different level of pod maturity (3.0; 3.5; 4.0 months). Correlation between groups of those variables was analyzed using canonical correlation. The result performed a positive association between the thickness of sclerotic layer at the secondary furrow with the number of entry holes and the number of entry holes through sclerotic layer. The thickness performed a higher value of the coefficient in association with the variables of canonical for pod characteristics 0.59; 0.55; 0.43 and the variables of canonical correlation for CPB resistance 0.54; 0.51; 0.39 that would presenting the characteristics of pod related to CPB resistance in 3.0, 3.5 and 4.0 months of pod maturity. Lignification at sclerotic layer was considered as genotypic expressions due to the thickness at the secondary furrow at 3.0, 3.5 and 4.0 months of pod maturity performed high value of broad-sense heritability i.e. 0.75, 0.89 and 0.92 respectively. A qualitative assessment of the lignification clearly differentiated the resistant clones of ARDACIAR 10 with the susceptible clones of ICCRI 04, KW 516, and KW 564.

Key words *: cocoa pod borer,* Theobroma cacao *L., pod characteristics, resistance*

INTRODUCTION

Cocoa pod borer (CPB) is the main pest on cocoa in the Asia-Pacific region which seriously reduce yield potency due to the infected pods cannot be harvested properly that defecting the bean quality. CPB firstly infected cocoa in North Sulawesi last century ago (1860) then spread to Java in the early 1900's (Wardojo, 1980). Intensity of CPB infestation varies among farms depend on how farmers are able to succeed the application on pest management. For controlling the pest it was recommended an integrated management by combining the component of biological control, technical culture and environmental precondition (Sulistyowaty & Wiryadiputra, 2008). To support the methods,

some resistant clones were released which be developed by exploring and selecting resistant genotypes through cocoa farms in the endemic area during last ten years (McMahon *et al*., 2009; Susilo *et al*., 2012).

Process on selecting CPB-resistant genotypes take time as the resistance has to be confirmed during several periods of harvest time to make sure the resistant expression would not be escaped mechanism. Susilo *et al*. (2004) reported process on selecting CPB-resistant genotypes by exploring the promising genotypes through population of Upper Amazon Hybrid (UAH) in North Sumatera in where resistant genotypes were confirmed during two years of harvest time. Performance of the resistance was evaluated based on an accumulative assessment of the unextractable beans per harvested pod to present intensity of CPB infestation. Effectiveness on selection using these variables was low (Susilo *et al*., 2006) due to expression of the resistance still biased with the unstable mobility of imagoes which possibly were affected by environmental condition. To support method on resistant evaluation the number of larvae entry holes pass through mesocarp and the number of larvae exit holes were also observed (Azhar, 2000) for presenting the activity of larva movement through pod. A positive correlation between the percentages of unextractable beans with the number of larvae entry hole pass through mesocarp was confirmed in the previous study (Susilo *et al*., 2004) from which the intensity of larva activity could be estimated by the number of entry hole. Further study should be focused on identifying criteria for selection related to characteristics of pod which are able to be assessed easily and genetically expressed.

Identification of the criteria for selection should be based on the characteristics of pod due to the larvae activity mainly on the pods. In the previous study it has been identified some characteristics of pod related to CPB resistance such as trichomes at upper layer of mesocarp, granules of tannin distributed through mesocarp and lignification at the sclerotic layer (Susilo, 2005; Susilo *et al*., 2007; Susilo *et al*., 2009). From those studies it can be preliminary concluded the mechanism of CPB resistance may be controlled by both of antixenosis and antibiosis mechanism. The trichomes at upper layer of pod possibly play a significant role on antixenosis by deterring the imagoes hatching on the surface of pod then of the larvae movement through the pod to reach the placental tissue would be suppressed with the tannin compound and lignification at sclerotic layer both act as the chemical or physical barrier. Azhar (2000) and Flood *et al*. (2004) found more number of laid eggs on rough pod than on smooth pod in which of the rough pod may perform higher density of trichomes. It was reported the clones having lower number of larvae entry holes performing higher number of trichomes and granules of tannin (Susilo *et al*., 2007; Susilo *et al*., 2009). It was also identified that the non-lignified tissue (channel) through sclerotic layer more frequent belong to the clones having higher number of larvae exit holes (Susilo, 2005). Further study, Susilo *et al*. (2007) found significant differences of the lignification among clones which perform different background on CPB resistance. Expressions of the characteristics depend on pod development in which the characteristics were performed maximally during 3.0–4.0 months of pod maturity (Susilo *et al*., 2009). To identify criteria for selection these should be confirmed using more diverse of genetic background of CPB resistance that expression of the characteristics can be confirmed as genetic expression.

MATERIALS AND METHODS

Plant materials for this study were 25 cocoa (*Theobroma cacao* L.) clones which mostly be exploratory collected through cocoa farms in Indonesia based on CPB resistance (Table 1). The clones were field-tested in Central Sulawesi for evaluating the response to CPB infestation in a randomized complete block design with 3 blocks as replication; each plot consisted of 4-6 sidegrafted trees. Evaluation for CPB resistance were based on the parameters of the percentage of unextractable bean, the number of larvae entry holes, the number of larvae entry holes pass through sclerotic layer and the number of larvae exit holes. Based on the parameter the tested clones were grouped into five groups of resistance, namely resistant, moderate resistant, moderate susceptible, susceptible and highly susceptible (Susilo *et al*., 2009). All of the tested clones were also assessed for genetic background using simple sequence repeat (SSR) markers that were confirmed all of the tested clones genetically different (Susilo *et al*., 2013).

Pod samples for assessing the characteristics were detached from the selected tree on each plot through four blocks of replication. The pod samples were categorized based on the maturity, namely 3.0; 3.5; and 4.0 months. Tissue of the detached pod (± 1 cm^3) then fixed in 70% alcohol before being shipped for analysis in Genetics Laboratory of Gadjah Mada University Yogyakarta.

A laboratory work was carried out to confirm expressions of the pod characteristics following the method reported in the previous study (Susilo *et al*., 2007). Sample of tissue then treated using microchemical analysis to identify the appearance of trichomes at upper layer cocoa mesocarp, the granules of tannin distributed through mesocarp and lignification at sclerotic layer. Each pod sample was excised at three different positions of pod, namely furrow, primary furrow and secondary furrow then transverse-sectioned for microchemical treatment, namely chemical treatment for microscopic analysis.

Procedure on characterization of the granules of tannin distributed through mesocarp follows the micro chemical treatment in which the tissue was prepared using micro technique approach. The excised tissue of mesocarp were cross-sectioned using a sliding microtome at the thickness of 20-40 µm then dipped in the mix solution of 2 g ferrous sulfate, 10 mL formalin and 90 mL aquadest sterile for 24-48 hours. After the chemical treatment, the sample tissues were placed on deck glass with a drop of glycerin for microscopic visualization in magnification of 250 times. By using a photomicrographs, the granule of tannin can be recorded by counting the number per specified area. It was also altogether enable be evaluated the appearance of trichome at the upper layer of epidermis tissue (Susilo *et al*., 2007).

Chemical treatment for lignification, the cross sectioned tissue were dipped in alcohol 70% and then colored using a solution of 2 g phloroglucin and 100 mL alcohol 95% for 15 minutes. After colorization the tissue were rinsed using 25% of HCl for 5 minutes then placed on a deck glass for microscopic analysis. Evaluation of lignification used a photomicrograph in magnification of 400 times. The lignified tissue performed red color which then can be measured for the thickness of the layer and described compactness of the non-lignified tissue. Considering tissue of young pod was not so lignified then measurement of thickness and number of channels were just performed in mature pods.

Table 1. Cocoa clones established in Central Sulawesi used for evaluation the CPB resistance

KW series	Clone	Origin	Response to CPB field infestation*)
KW 264	KPC 1	Local clone explored from java criollo population in East Java	Moderate susceptible
KW 265	KPC 2	Local clone explored from java criollo population in East Java	Susceptible
KW 162	Sul-01	Local clone	Susceptible
KW 163	Sul-02	Local clone	Moderate susceptible
KW 165	Bal 209	Introduced clone	Susceptible
KW 571	ARDACIAR 25	Local clone explored in Central Sulawesi	Moderate susceptible
KW 524	Toli-toli	Local clone explored in Central Sulawesi	Moderate susceptible
KW 525	Nob 1	Local clone explored in East Kalimantan	Moderate susceptible
KW 527	Nob 3	Local clone explored in East Kalimantan	Moderate susceptible
KW 570	ARDACIAR 10	Local clone explored in South Sulawesi	Resistant
KW 516	Paba/VIII/78B/2	Local clone explored in North Sumatra	Highly susceptible
KW 30	ICCRI 03	Recommended clone selected from breeding population	Moderate resistant
KW 216	Pengawu	Local clone explored in Central Sulawesi	Moderate susceptible
KW 396	Na 32	Introduced clone	Moderate susceptible
KW 397	Na 33	Introduced clone	Resistant
KW 529	HF 3	Local clone explored in East Kalimantan	Moderate susceptible
KW 528	HF 2	Local clone explored in East Kalimantan	Moderate susceptible
KW 403	Pound 7	Introduced clone	Susceptible
KW 422	KKM 22	Introduced clone	Moderate resistant
KW 48	ICCRI 04	Recommended clone selected from breeding population	Susceptible
KW 566	Paba/V/81L/1	Local clone explored in North Sumatra	Resistant
KW 564	Paba/IX/90O/2	Local clone explored in North Sumatra	Highly susceptible
KW 572	ARDACIAR 26	Local clone explored in Central Sulawesi	Moderate resistant
KW 215	Sausu Piore	Local clone explored in Central Sulawesi	Moderate susceptible
KW 514	Paba/I/Pbrk	Local clone explored in North Sumatra	Moderate resistant

Note : *) Source of data Susilo *et al.* (2005).

Further analysis was also carried out to identify histological characteristics of the lignified tissue. Laboratory works for this analysis was carried out at the Histology Laboratory, School of Biological Science the University of Reading, United Kingdom follow the method of Steven (1999). For this study, only a few number of tested clones were selected which representing the susceptible and resistance, namely KW 516 (very susceptible), ARDACIAR 10 and KW 566 (resistant). Data analysis based on the qualitative performance of the lignified tissue.

Variance analysis was performed for quantitative data at $\alpha = 5\%$ then follow up by canonical analysis using SAS 9.1 Program. To support correlation between field study and the laboratory study some of data reported by Susilo *et al*. were used (2009). The covariance of genetic and phenotypic were classified as low (0% - ≤ 25%), moderate (25% - ≤ 50%) and high (50% - ≤ 75%) (Moedjiono & Mejaya, 1994).

RESULTS AND DISCUSSION

Analysis of variance shows a significant effect of cocoa clones to the variables of pod characteristics related to CPB resistance at the different level of pod maturity

(Table 2) that could be confirmed that the characteristics of pod are genetic expression of the tested clones. Higher value of coefficient variation was identified for trichome density (>30%) indicating the trichomes might not be distributed evenly through the surface of pod. This result also performed any differences of the characteristics in different pod maturity as was reported in the previous study wherein the characteristics were maximally expressed during 3.0-4.0 months of pod maturity (Susilo *et al.*, 2009).

The percentage of unextractable beans was vary among clones in the range of 35.8–91.7% which performing the range of genetic variation of CPB resistance among tested clones. Furthermore it could be expected that the clone which having lower number of that percentage performing more resistance to CPB. ARDACIAR 10 of the clone which have lowest value of the percentage (35.78%) would be the most resistant in contrast to the clone of KW 516 and KW 564 which have highest value of 83.2% and 91.7% respectively would be most susceptible (Table 3). A positive correlation was confirmed between the percentage of unextractable beans with the number of larvae exit holes ($r = 0.62^{*}$) and indirectly correlated with the number of entry holes as the number of exit holes positively correlated with the ratio of the number of exit holes to the number of entry holes ($r = 0.59^{*}$). This result confirms the severity damage due to CPB depend on how many larvae are able to complete its life cycle inside the pod which be performed with the number of larvae exit holes.

Table 2. Mean square of variance analysis the pod characteristics related to CPB resistance

Sources of variation	Degree of freedom	Pod age		
		3.0 months	3.5 months	4.0 months
		Thickness of sclerotic layer at primary furrow (PF), mm		
Clone	24	0.13 ns	0.21 *	0.35 *
Block	3	0.06 ns	0.13 ns	0.03 ns
Error	72	0.05	0.06	0.10
CV (%)		26.55	25.95	30.82
		Thickness of sclerotic layer at the segment between primary furrow (F), mm		
Clone	24	0.02 *	0.03 *	0.04 *
Block	3	0.01 ns	0.002 ns	0.001 ns
Error	72	0.004	0.003	0.003
CV (%)		18.78	15.08	12.72
		Number of tannin granules through mesocarp (Tn) per mm^2 of unit area		
Clone	24	0.38 *	0.26 *	0.30 *
Block	3	0.03 ns	0.07 ns	0.04 ns
Error	72	0.07	0.05	0.04
CV (%)		18.69	20.24	19.93
		Number of trichomes (Tr) per mm		
Clone	24	19.42 *	16.32 *	16.56 *
Block	3	0.29 ns	0.18 ns	0.81 ns
Error	72	2.92	1.56	3.26
CV (%)		37.22	33.21	55.20

Note : CV = Coefficient of variation; * Fisher test was significantly different at $\alpha = 5\%$; ns Fisher test was not significantly different at $\alpha = 5\%$.

Table 3. Mean of the variables correspond to CPB resistance of cocoa clones tested in Central Sulawesi

KW series	Clone	Unextract-able beans, %[1)]	No. of entry holes[2)]	No.of entry holes through sclerotic layer	No. of exit holes	Ratio exit holes to entry holes
KW 264	KPC 01	64.26 MS	30.78 a	22.89 ab	5.36 abcdef	0.21 def
KW 265	KPC 02	76.95 S	17.68 bcde	12.77 cdef	7.04 a	0.51 ab
KW 162	Sulawesi 01	72.97 S	22.52 abcd	16.36 abcd	5.51 abcde	0.26 cdef
KW 163	Sulawesi 02	66.16 MS	14.21 bcde	10.49 cdef	7.00 a	0.49 ab
KW 165	Bal 209	69.45 S	25.13 abc	18.73 abc	2.38 fg	0.09 ef
KW 571	ARDACIAR 25	63.01 MS	12.87 de	8.03 def	3.38 bcdefg	0.26 cdef
KW 524	Toli-toli	60.92 MS	19.90 abcde	14.12 cdef	5.51 abcde	0.30 bcde
KW 525	Nob 1	67.74 MS	18.38 bcde	14.45 cdef	4.41 abcdef	0.26 cdef
KW 527	Nob 3	66.50 MS	31.01 a	24.22 a	2.81 defg	0.09 f
KW 570	ARDACIAR 10	35.78 R	11.44 de	6.69 ef	2.47 efg	0.22 def
KW 516	Paba/VIII/78B/2	83.19 HS	25.90 ab	18.72 abc	6.41 ab	0.25 cdef
KW 30	ICCRI 03	48.37 MR	10.55 de	7.80 def	2.81 defg	0.31 bcd
KW 216	Pengawu	62.95 MS	17.95 bcde	13.55 cdef	3.43 bcdefg	0.19 def
KW 396	Na 32	62.43 MS	14.65 bcde	11.19 cdef	4.71 abcdef	0.32 bcd
KW 397	Na3 3	40.29 R	10.21 e	6.93 ef	1.03 g	0.13 def
KW 529	HF 3	65.20 MS	14.35 bcde	8.56 def	3.26 cdefg	0.22 def
KW 528	HF 2	64.74 MS	15.82 bcde	11.44 cdef	5.01 abcdef	0.33 bcd
KW 403	Pound 7	69.07 S	20.14 abcde	15.18 cdef	2.73 defg	0.25 cdef
KW 422	KKM 22	51.01 MS	13.47 cde	7.82 def	5.65 abcd	0.45 abc
KW 48	ICCRI 04	75.50 S	10.85 de	8.06 def	5.22 abcdef	0.48 ab
KW 566	Paba/V/81L/1	42.12 R	25.80 ab	15.22 bcde	2.55 efg	0.09 f
KW 564	Paba/IX/90O/2	91.72 HS	11.09 de	9.52 def	5.88 abc	0.55 a
KW 572	ARDACIAR 26	44.62 MR	11.71 de	6.41 f	3.33 cdefg	0.33 bcd
KW 215	Sausu Piore	62.02 MS	19.73 abcde	13.03 cdef	3.97 bcdefg	0.20 def
KW 514	Paba/I/Pbrk	53.41 MR	21.50 abcde	13.41 cdef	3.64 bcdefg	0.16 def

Notes: [1)]Clone's response to CPB were grouped as resistant (R), moderate resistant (MR), moderate susceptible (MS), susceptible (S) and highly susceptible (HS) using fustclass analysis; [2)]number in the column with same letter indicate not significant different by Duncan Multiple Range Test at $\alpha = 5\%$.

Relationship Between Resistant Characteristics

Analysis of canonical correlation showed a positive association between the number of entry holes and number of entry holes through sclerotic layer with the thickness of sclerotic layer at the secondary furrow at the different level of pod maturity. In contrast, the ratio of the number of exit holes to the number of entry holes was negatively associated with the thickness (Table 4). Further analysis support the result that the thickness of sclerotic layer at the secondary furrow performed a higher value of the coefficient in association with the variables of canonical for pod characteristics (0.59; 0.55; 0.43) and the variables of canonical for CPB resistance (0.54; 0.51; 0.39) (Table 5). Therefore it could be concluded that the thickness of sclerotic layer at the secondary furrow as the representative characteristics related to CPB resistance. However, association between the thicknes with the variable of CPB

resistance would not be interpreted as linier combination but just performing a higher contribution of the characteristics on CPB resistance. The sclerotic layer would play role on buffering larvae movement into the placental area for feeding but it is still unclear mechanism of the layer as physical or chemical barrier. Alonzo-Díaz *et al*. (2008) reported an extracted-tannin of some plants which was able to suppress larvae movement of *Trycho-strongylus colubiformis*. Lignin is the second compound of plant tissue after cellulose contributing on plant structure and resistance to pest and disease (Weiting Ni & Jung, 1998).

Mean differences of those characteristic were different among tested clones in which the more resistant clones performed higher value of the thickness of sclerotic layer at secondary furrow but not for others characteristics (Figure 1). Measurement of the thickness is an approach how to quantify the lignification at the sclerotic layer. However, the most resistant clone of ARDACIAR 10 performed similar of the thickness with the susceptible clones. Qualitative assessment of the lignification at sclerotic layer showed a significant differences of the lignification performance between the resistant clones of ARDACIAR 10 and KW 397 and the susceptible clones of ICCRI 04, KW 516 and KW 564 in which the resistant clones performed more intensive and more compact of the lignified-tissues compared to the susceptible clones (Figure 2). Xi Liu *et al*. (2011) also reported the lignin content of the resistant cotton to wilt fungus of *Verticillium dahlia* higher than of the susceptible plant that lignin was the important compound on cotton resistance. Histological analysis of the lignified tissue detected more cells on the resistant clones (ARDACIAR 10 and KW 397) differentiated to woody tissue indicating a physical barrier of the layer to larvae movement (Figure 3).

Table 4. Canonical correlation among the characteristics of CPB resistance and the variables of plant's resistance to CPB

Pod characteristics*)	Pod maturity, month	No. of entry holes	No. of entry holes through sclerotic layer	No. of exit holes	Ratio between exit holes to entry holes
PF	3.0	0.20	0.21	-0.11	-0.27
PF	3.5	0.17	0.17	-0.15	-0.31
PF	4.0	0.18	0.14	-0.21	-0.36
F	3.0	0.62	0.64	-0.01	-0.39
F	3.5	0.56	0.55	-0.01	-0.35
F	4.0	0.45	0.48	-0.02	-0.29
Tn	3.0	-0.09	-0.05	-0.07	0.07
Tn	3.5	0.02	0.06	-0.05	0.01
Tn	4.0	-0.08	-0.02	0.08	0.17
Tr	3.0	0.29	0.45	0.35	0.15
Tr	3.5	0.17	0.33	0.14	0.08
Tr	4.0	0.15	0.32	0.25	0.13

Notes: PF = thickness of sclerotic layer at primary furrow (mm); F = thickness of sclerotic layer at the secondary furrow (mm); Tn = number of tannin granules per mm^2; Tr = number of trichomes per mm, numbers inside the ellips-circles indicate higher value of canonical coefficient.

Genetic Resistant Characteristics

Thickness at furrow on 3.0, 3.5 and 4.0 months of pod maturity was also analyzed performing high value of broad-sense heritability i.e. 0.75; 0.89 and 0.92 respectively (Table 6) that confirmed the thickness as genetic expression of the tested clones. This was also supported by small difference in genetic coefficient of variation to phenotypic coefficient of variation for the thickness at the secondary furrow. A phenomenon of genotype by environment interaction was reported on yam resistance to anthracnose however genetic factor was more influenced on the resistance (Egesi *et al.*, 2009). Further analysis show a low classification of genetic coefficient of variation (≤ 25%) of the thickness at the secondary furrow indicating a low variability of CPB resistance from this study. Study on the analysis of the quantitative trait

Table 5. Correlation among the characteristics of CPB resistance with their canonical variables [A1] and the canonical variables of plant's resistance to CPB infestation [B1]

Variables*)	[A1]	[B1]
PF-3.0	0.12	0.11
PF-3.5	0.07	0.07
PF-4.0	0.09	0.09
F-3.0	0.59	0.54
F-3.5	0.55	0.51
F-4.0	0.43	0.39
Tn-3.0	-0.01	-0.01
Tn-3.5	0.09	0.09
Tn-4.0	-0.04	-0.04
Tr-3.0	0.29	0.26
Tr-3.5	0.21	0.19
Tr-4.0	0.13	0.12

Note: *) Notation of variables refer to Table 4, numbers in the ellips circle indicate a higher value of canocial correlation coefficient.

Table 6. Genetic parameters of pod characteristics related CPB resistance among the tested clones

Pod characteristics*)	Pod maturity, month	h^2_{bs}	PCV (%)	GCV (%)	Mean
PF	3.0	0.38	16.43	10.06	0.86
PF	3.5	0.60	20.84	16.14	0.93
PF	4.0	0.60	23.81	18.44	1.05
F	3.0	0.75	17.60	15.24	0.36
F	3.5	0.89	21.19	19.98	0.39
F	4.0	0.92	22.72	21.78	0.42
Tn	3.0	0.77	20.09	17.68	1.39
Tn	3.5	0.76	20.47	17.87	1.12
Tn	4.0	0.85	25.34	23.31	1.01
Tr	3.0	0.82	44.25	40.14	4.59
Tr	3.5	0.89	51.15	48.38	3.76
Tr	4.0	0.75	55.79	48.47	3.27

Note: *) code of pod characteristics refer to note in Table 4, h^2_{bs} = broad sense heritability, PCV = phenotypic coeficient of variation, GCV = genotypic coeficient of variation.

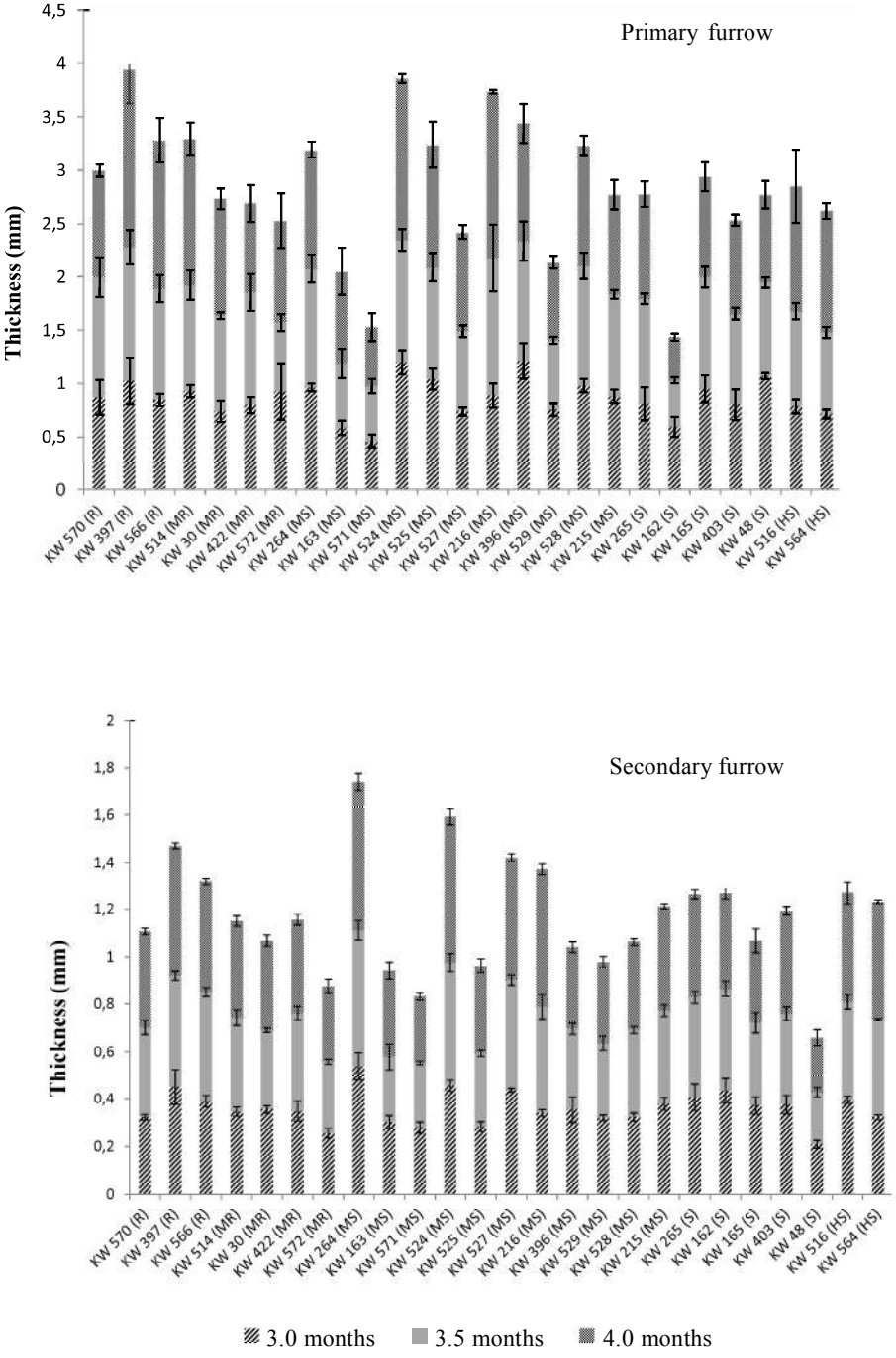

Figure 1. Sclerotic layer thickness at primary furrow (top) and secondary furrow (below) of the tested clones which perform different response to CPB resistance (Bars indicate standard deviation of mean)

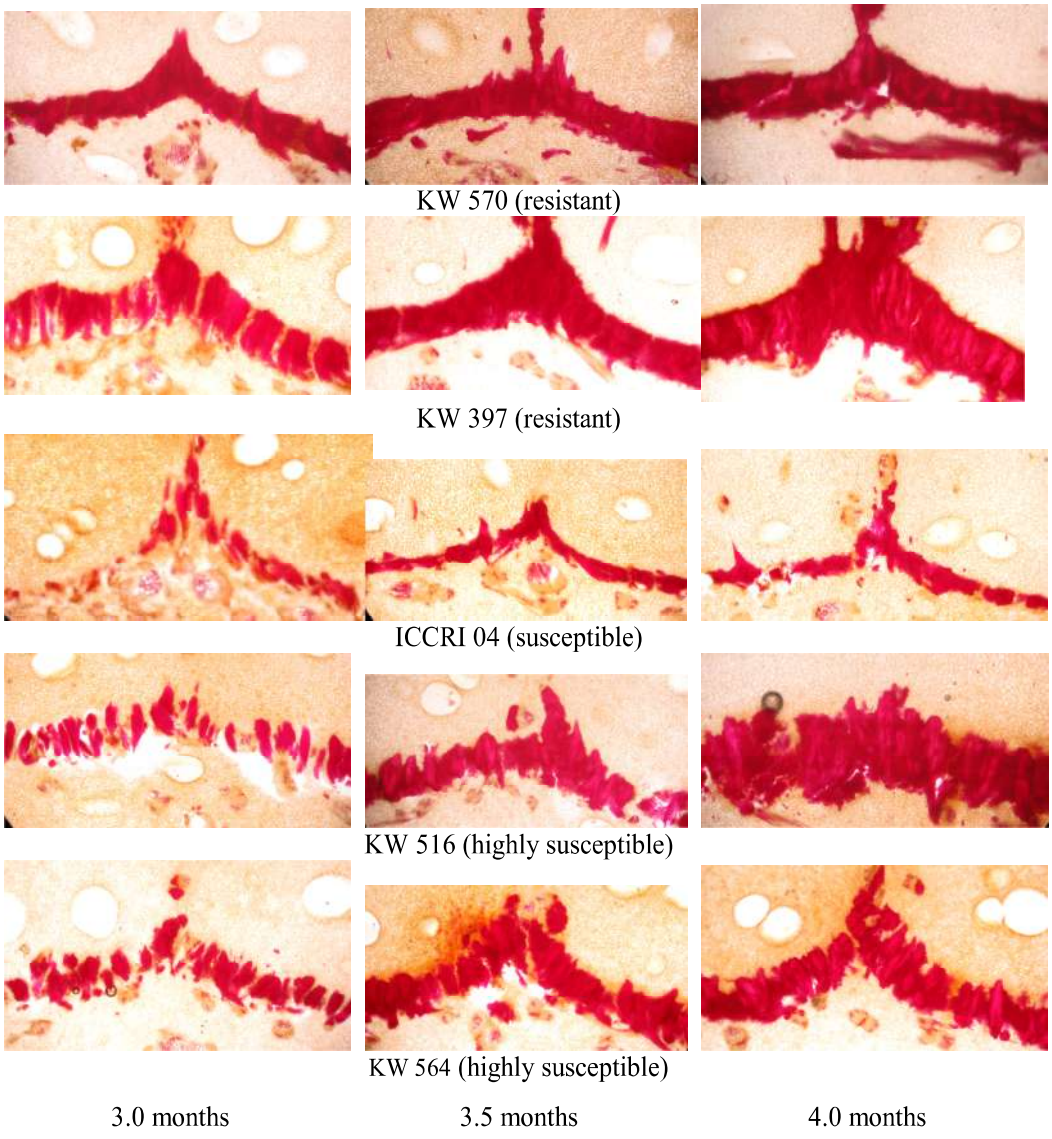

Figure 2. Comparison in lignification at the sclerotic layer of five cocoa clones having difference on CPB resistance at 400 X magnification

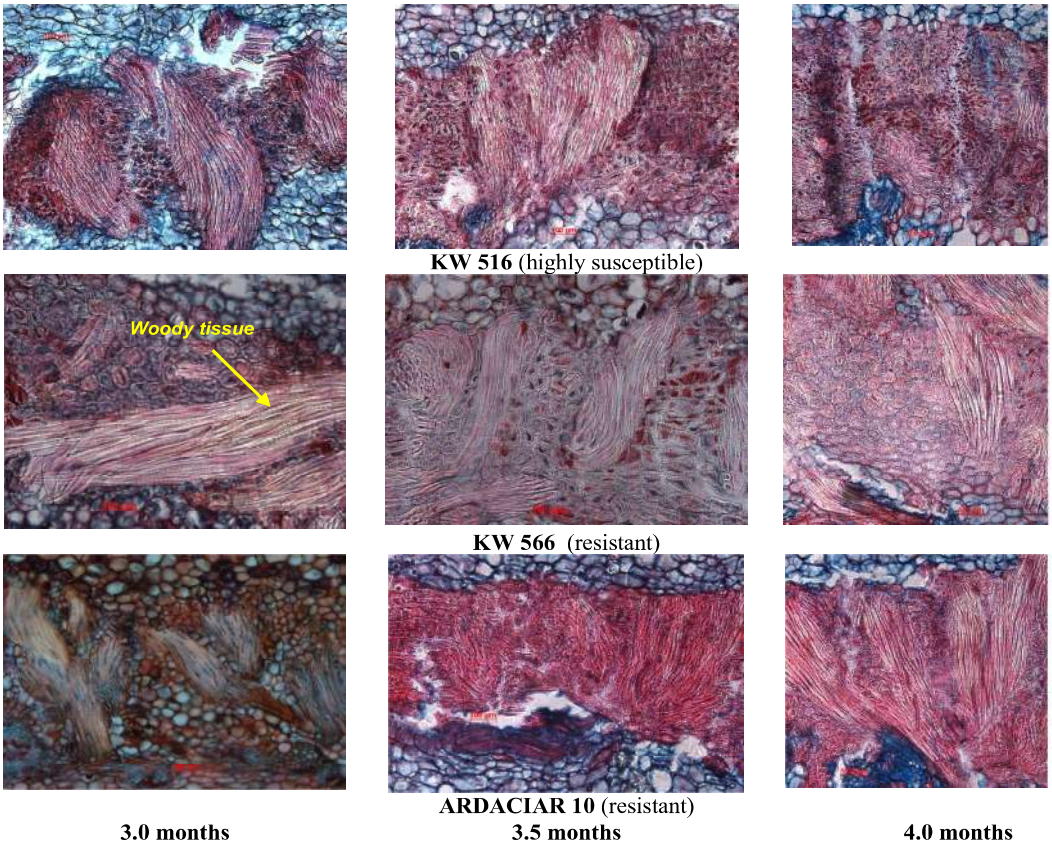

Figure 3. Histological comparison of the lignified sclerotic layer of three cocoa clones difference on CPB resistance at 200X magnification

locy (QTL) for lignin synthesis identified genes controlling on monolignol biosynthesis and polymerization (Chavigneau *et al*., 2012).

This result showed lignification at sclerotic layer as part of pod characteristics which is possible to be used as criteria for selection on CPB resistance. For further implementation of the lignification analysis it should be simplify the assessment method as lignification have to be evaluated using very complicated method that is not easy to be implemented for large number of cocoa genotypes in term of selection process. Further study have to be focused on the assessment of morphological characteristics of pod related to the lignification that assessment of the criteria selection will be more simple to be carried out in speeding up selection process.

CONCLUSION

1. The exploratory selected cocoa clones performed difference in the response to CPB infestation in which percentage of unextractable beans positively correlated with the number of larvae exit holes. All of the assessed characteristics of pod related to CPB resistance were significantly affected by clone factor confirming any genetic variation of the resistance among tested clones.
2. Lignification at sclerotic layer represented by the thickness at the secondary furrow on 3.0, 3.5 and 4.0 months of pod maturity performed higher portion of its contribution to represent resistant characteristics. This thickness positively

associated with the number of entry holes and the number of entry holes through sclerotic layer and negatively associated with ratio of the number of exit holes to the number of entry holes.

3. The thickness of sclerotic layer at the secondary furrow in 3.0; 3.5 and 4.0 months of pod maturity are genetic expression as having high value of broad-sense heritability, namely 0.75, 0.89 and 0.92, respectively.

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