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RESEARCH ARTICLE

Field screening of elite Cacao (*Theobroma Cacao* L.) clones to vascular streak dieback (VSD) disease as the selection criteria for planting materials

Gibson Entuni[1*], Hollena Nori[1], Rebicca Edward[1] and Ahmad Kamil bin Mohammad Jaafar[2]

[1]*Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.*
[2] *Malaysian Cocoa Board, Cocoa Research and Development Centre, Lot 248, Blok 14, Biotechnology Park, 94300, Kota Samarahan, Sarawak, Malaysia.*

Edited by:

P. Nallathambi, ICAR-IARI, Regional Station, Wellington, Tamil Nadu, India

Reviewed by:

Fakhrusy Zakariyya,

Indonesian Coffee and Cacao Research Institute (ICCRI), Jember Street, East Java, Indonesia.

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*Corresponding author e-mail address: gib5181@gmail.com (Gibson Entuni)

ABSTRACT

The field screening for VSD disease infection in four elite cocoa clones (KKM22, KKM4, PBC230, and MCBC1) was conducted after two years of transplanting. This study aimed to determine the efficient selection criteria for planting material of cocoa-based on the intensity of VSD disease and new shoot regeneration characteristics such as number, length, and diameter of new shoots after removal of the infected VSD twigs. Around half population (60%) of the trees were infected by the disease but the infection was minor and observed only on the leaf and twigs of 150 mm in length along the jorquette branches. The MCBC1 clone with a disease severity score of 1.82 is classified under the VSD disease resistance clone whereas other clones with a score averaging 2.21 are grouped under the moderately resistance clone. The cultural practice via pruning of all infected twigs (150 mm) was efficient to control the disease from spreading. One month after pruning, the MCBC1 from disease resistance clone had the lengthiest (62.3 mm) and thickest diameter (15.6 mm) of the new shoot compared with other clones. This study indicates the need for the screening of VSD disease in cocoa plantations so that the disease can be managed and controlled at the early stage of infection. This study also reported the first finding on using new shoot regeneration after pruning for the selection criterion of cocoa planting material in the future breeding programme.

Keywords: *Theobroma cacao*, VSD disease, field screening, pruning

INTRODUCTION

Theobroma cacao L. or simply known as cocoa is an important commercial plantation crop mainly grown in most of the countries in Asia such as Indonesia, India, Malaysia, Philippines, Papua New Guinea and Vietnam. The cocoa bean is highly valued as a raw material for the chocolate making industry (Singh et al., 2020) and it was estimated that it contributes to approximately ~83 billion dollars annually for the chocolate industry in the world (Florez et al., 2015). Nevertheless, a reduction in cocoa production by the mass producer countries since 2001 due the recent spread of Vascular Streak Dieback (VSD) disease has been reported by several researchers (Anita Sari and Susilo; Tee et al., 2019). According to Pattoni et al., (2013), pathogens are often transmitted from the diseased to healthy cocoa plants including for those propagated from seeds or vegetative means such as grafting.

The production of healthy trees is crucial especially for the perennial crop species which have a long-life span in producing yield such as cocoa plant. For instance, when cocoa trees are infected by the disease, this will be resulted in the unproductive trees which can caused a severe long-term reduction in yield (Azhar and Lee, 2004). For cocoa, VSD disease is one of the devastating diseases affecting its production under the field condition. This VSD disease was first observed in Keravat, Papua New Guinea in 1960s before spread to Southeast Asia in 1970s and westward to Kerala State in India and Hainan Island, China (Keane, 1972). In Malaysia, the disease was first observed in Tawau, Sabah in mid-1980s (Chan and Syed, 1976).

The pathogen known as *Oncobasidium theobromae* is responsible for the outbreak of VSD disease in cocoa plant trees. It starts the infection on the new leaves of the young cocoa tree because stomata are at its highest functionality for photosynthesis at this stage, thus giving the optimum size for the fungus entrance (Susilo et al., 2016). From the young leaves, the disease moves to xylem, initiating a vascular browning on the laminae. The disease then spreads to midrib and petiole before reaching the twig and branch of cocoa tress. The general symptoms of VSD disease are including leaf chlorosis with green spots, necrotic leaf scars with three dots, dark streak opened stem, rough bark and appearance of "broomstick" structure of tree resulted from proliferation and subsequent death of axillary bud and leaf abscission (Keane, 1972; Guest and Keane, 2007; Samuels et al., 2012). According to Samuel et al., (2012), in the cases of severe infection, the VSD disease reached into the main stem and killed the cocoa tree. A high humidity condition (20°C to 30°C temperature and 700 to 1000 mm annual precipitation) and a long period of wetness have been reported of favouring the formation of VSD fungus basidiomycetes (Guest & Keane, 2007).

Nevertheless, several strategies have been adopted to control the VSD disease in the field condition including using the systemic fungicides such as Triazole, Flutriafol, Hexaconazole, Propiconazole and Triadimenol (Minimol et al., 2016). However, these fungicides are too costly for the smallholder farmers and hazardous to the environments (Minimol et al., 2016). Hence, the cultural practice by using the clean nursery stocks and canopy pruning of the disease infected twig to increase aeration and light penetration to the infection sites were the only ideal solution to overcome the outbreak of the disease. When VSD disease infected parts were left without removal, the disease incidence rose from 30 to 90% within 10 months period (Guest and Keane, 2007). The usefulness of using cultural practise such as pruning and removal of the infected parts to control disease spreading in plants have been reviewed extensively by Sonowski et al., (2009).

In Indonesia and Malaysia, it was reported that the removal of VSD infected parts (±15 cm in the branches) was effective to control further spreading of the infection and minimize inoculum level by removing potential sites of sporulation (Susilo and Sari, 2014; Ahmad Kamil et al., 2016; Susilo et al., 2016). All cocoa trees produced healthy and clean shoots after one month of pruning but the characteristics of sprouting ability differed among resistant, moderately resistant and susceptible cocoa clones (Susilo and Sari, 2014; Ahmad Kamil et al., 2016; Susilo et al., 2016). Therefore, all VSD disease infected parts were removed from the cocoa tree by pruning in this study. The shoot regeneration was then assessed after one month from pruning. The objectives of the present study were to examine VSD disease severity among cocoa clones and to assess shoot regeneration following pruning to facilitate selection criteria for resistance to VSD disease for the use in the future cocoa breeding programme.

MATERIALS AND METHODS

Study area and general background

This study was conducted in 2019 at Malaysian Cocoa Board Research Station, Kota Samarahan, Sarawak, Malaysia. The experimental site was located at 1° 27'N, 110° 29'E, 27 m.a.s.l. The field experimental design was conducted based on a complete randomized design (CRD). The incidence and severity of VSD disease in cocoa trees were determined in four elite cocoa clones at two years old, namely Prang Besar Clone 230 (PBC230), Koko Klon Mardi 22 (KKM22), Koko Klon Mardi 4 (KKM4) and Malaysian Cocoa Board Clone 1 (MCBC1) regenerated from grafting. Around 32 cocoa trees (eight replicates from each cocoa clones) were evaluated in this study.

Evaluation on the intensity and classification to VSD disease

The VSD disease intensity was measured using a visual disease severity scale (**Table 1**). This scale uses a numeric score from 0 to 6 that represented the progression of the disease symptoms from chlorosis to dieback (Susilo and Anita Sari, 2011; Ahmad Kamil et al., 2016). In each tree, a non-destructive sampling was conducted on four segments of the plant canopy, such as north, east, south and west (**Figure 1**). Each plant segment consisted of the terminal three flushes of leaf and twig (~150 mm in length) per jorquette branch. The scores of disease severity were then used to classify the cocoa clones into four groups of VSD disease resistance, such as susceptible (severity score between 4.0 to 6.0), moderately susceptible (severity score between 3.0 to 3.9), moderately resistance (severity score between 2.0 to 2.9) and resistance (severity score between 1.0 to 1.9) (Susilo and Anita Sari, 2011; Ahmad Kamil et al., 2016).

Table 1. Visual VSD disease severity scale (numeric) outlining the primary symptoms of the infections on terminal flushes (Source: Susilo and Anita Sari, 2011; Ahmad Kamil et al., 2016)

Scale	Primary symptoms	Other associated symptoms
0	Uninfected, healthy	Smooth bark, glossy leaves
1	<25% of flushes were infected. One young leaf along the 150 mm twig turned chlorotic	Smooth bark with or without slightly swollen lenticel
2	>25-50% of flushes were infected. Most of the infected leaves turned chlorotic and necrotic but remain attached to the branch	Rough bark with swollen lenticel
3	>50-75% of flushes were infected. Some of the infected leaves abscised from the tree	Rough bark with swollen lenticel
4	>75% of flushes were infected. Majority of the infected leaves abscised from the tree	Very rough bark with swollen lenticel
5	Dieback occurred. Broom stick structure developed due to abscission of the leaves. No more production of axillary shoots	Very rough bark and swollen lenticels. VSD fungus fruiting bodies appeared on the tree
6	The cocoa tree died	When stem split open, infected xylem is visible because VSD dark streaks developed within the vascular tissue

Removal of VSD disease on the infected twigs

Following visual observation of VSD disease intensity, the infected leaves and twigs were removed from the trees by pruning the twigs at 150 mm length according to Susilo and Sari (2014). The aim of pruning was to control the disease from further spreading to the jorquette branch and trunk. Immediately after pruning, Hydrated Calcium suspension (30% w/v) was brushed onto the cut surfaces to avoid damage from the sunlight. Potassium Nitrate (NOP) (13-0-46) fertilizer (KEMAPCO®, Jordan) was then applied at the rate of 500 g per cocoa tree to support the cocoa trees in the recovering process.

Regeneration of shoots

The shoots regeneration as indicated by the number, length and diameter of new shoots were assessed after one month from the removal of infected twigs. In each tree, the measurement was conducted on three pruned twigs. The length of new shoot was measured by using measuring tape (McKenic fiberglass measuring tape, Malaysia) whereas the diameter was measured by using digital calliper (OEMTOOLS 25363, Malaysia).

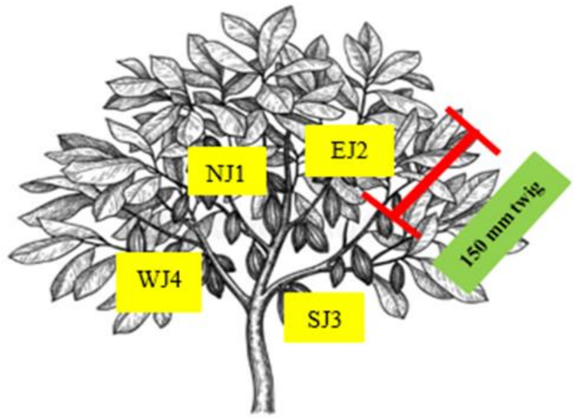

Figure 1. Four segments of terminal flushes of leaf and twig along the jorquette branches observed for VSD disease severity. The length of each twig is ~150 mm. J = Jorquette branch number, N = North, E = East, S = South, W = West.

Data analysis

The data for the intensity, classification of cocoa clones to VSD disease and new shoot regeneration's characteristics were recorded and analysed using one-way analysis of variance (ANOVA). The mean separation was conducted using Tukey Test at $p < 0.05$ to determine the resistance's classification of cocoa clones to VSD disease and assess which clones have the superior characteristics after the infection. All statistical data were analysed with SPSS Statistics Version 20.

RESULTS AND DISCUSSION

The intensity and classification to VSD disease

There was no clonal effect on the intensity of VSD disease among cocoa trees after two years of transplanting in the field condition (Table 2). Evaluation found that half (60%) of the proportion of the cocoa trees were infected by the disease. Through visual observation, the disease infection started at the leaves on the second and third flushes behind the growing tip. The fungus, *O. theobromae* entered the cocoa trees via the growing tip (shoot apex) and this could explain why only the flushes were affected by the disease (Marfu et al., 2016). The infected cocoa leaves showed a common symptom for the disease which is the presence of green spotted islets with a yellow background of about 2 to 5 mm diameter on the surfaces. From the flushes, the infections advanced to the twigs of the jorquette branches of the infected cocoa trees. During the assessment, around ±150 mm of the twig length was infected but the infection was mild and have no severe impact on the productivity of the cocoa trees. This is because, at the time of the assessment, all cocoa trees have produced their first flower and fruit.

The evaluation of disease intensity showed that MCBC1 clone with significant severity score of 1.85 can be categorised as the VSD disease resistant clone whereas PBC230, KKM22 and KKM4 clones are moderately resistant to VSD disease with score averaged 2.21 (Table 3). These findings are congruent with the International Cocoa Germplasm Database by Turnbull and Hadley (2018) and Malaysian Cocoa Board (2005) on the response of various cocoa clones towards disease pathogen. Similar with the present study, Marfu *et al*., (2016) and Santoso and Zakariyya (2019) also found variation in tolerance toward VSD infection among cocoa clones from both grafting and seed and explained that this was due to genetic factor. Thus, the selection of a resistant cocoa planting material is crucial to control the VSD disease.

Table 2. Percentage (%) and number of cocoa trees infected by VSD disease at 24 months transplanted based on primary symptoms of infections on the twigs (150 mm) and leaves.

Cocoa clones	Number of cocoa trees infected	Percentage (%) of cocoa trees infected
MCBC1	4.00±0.53[a]	50
PBC230	5.00±0.52[a]	63
KKM22	5.00±0.52[a]	63
KKM4	5.00±0.52[a]	63

Mean along the column followed by the same alphabet are not significantly different at $p \leq 0.05$ (Tukey Test)

Table 3. The intensity of VSD disease infection based on disease severity scale on four cocoa clones at two years after transplanting

Cocoa clones	VSD disease infection severity scores on the two years old cocoa trees
MCBC1	1.82±0.11[a]
PBC230	2.20±0.07[b]
KKM22	2.21±0.05[b]

KKM4	2.22±0.06[b]

In the several cocoa plant producing countries, the cocoa clones that are resistant to VSD disease have not been propagated in a mass quantity (Ahmad Kamil *et al.*, 2016). Therefore, it is suggested that cocoa clones were first screening for disease resistance before selected as the planting materials. This is to avoid losses in yield caused by the sudden outbreak of VSD disease especially for large scale cocoa commercial plantation. Nevertheless, the replacement of the VSD disease susceptible clones in the plantation through a rehabilitation programme have been practised by several cocoa producing countries around the globe (Susilo and Sari, 2014; Guest and Keane, 2018).

Regeneration of Shoots

No difference observed among cocoa clones for the number of new shoots produced after one month from the removal of the VSD disease infected twigs (Table 4). Around two new shoots were produced from the removal point of each twig of the cocoa clones trees. Nevertheless, the cocoa clones varied in the length and diameter of these new shoots. For instance, MCBC1 clone produced the longest (60.8 mm) and thickest diameter (15.4 mm) of the new shoot compared with the rest of the cocoa clones trees.

Table 4. The regeneration of shoots after one-month removal of VSD disease infected twigs.

Cocoa clones	Number of new shoots	Length of new shoots (mm)	Diameter of new shoots (mm)
MCBC1	1.70±0.41[a]	62.3±0.80[a]	15.6±0.20[a]
PBC230	1.80±0.42[a]	57.5±0.60[b]	12.1±0.12[b]
KKM22	1.70±0.41[a]	57.1±0.97[b]	11.9±0.14[b]
KKM4	1.80±0.41[a]	57.3±0.68[b]	11.8±0.20[b]

Mean along the column followed by the same alphabet are not significantly different at $p \leq 0.05$ (Tukey Test)

The infected twigs were pruned off at the early stage of the illness to avoid ramification of the *O. theobromae* basidiospore to the jorquette branches and stem of the cocoa plant. Guest and Keane (2007) reported that the basidiospores of *O. theobromae* can survive for a week on the infected cocoa parts when they are left unpruned. Subsequently, delayed pruning of the jorquette branches due to late detection of the disease when the trees were severely infected was found to slow down the recovery of the cocoa trees (Riedel et al., 2019). Thus, the visual VSD severity scale is an important tool to facilitate cocoa growers to detect early symptom of the VSD disease for control measure in the plantation. The cultural practise of pruning to eradicate the VSD disease from further spreading to other parts of the trees have been adopted in many countries such as India (Uchoi et al., 2018), Nigeria (Adeyemi, 1999; Adejumo, 2005), Malaysia (Azhar and Lee, 2004; Ahmad Kamil et al., 2016), Indonesia (Susilo and Sari, 2014) and Papua New Guinea (Marfu et al., 2016).

The pruning of VSD disease infected twigs encourages the production of new and healthy shoots in cocoa trees. This production of lateral shoots is the outcome from the removal of apical dominance in many tree crops. This agrees with the work reported by Susilo and Sari (2014) in Indonesia. However, the regeneration of shoots after one month of pruning differed among cocoa clones in which MCBC1 clone produced the lengthiest (60.8 mm) and thickest (15.4 mm) new shoots compared with the rest of cocoa clones. Previous work conducted by Susilo and Sari (2014) concluded that the capacity of shoot regeneration was associated with the tolerance of cocoa clones to the VSD disease. For example, the resistance clones which had the lower VSD severity score showed better recovery for the regeneration of shoots after pruning of the infected parts. Indeed, MCBC1 clone which was categorised under a VSD disease resistance (Malaysian Cocoa Board, 2005; Turnbull and Hadley, 2018) showed superiority by having large shoots in comparison with moderately VSD disease resistance clones such as PBC230, KKM22 and KKM4.

CONCLUSION

This study revealed the efficiency of field screening method based on the disease intensity in twigs and leaf and new shoot regeneration after pruning for the selection criteria of planting material to VSD disease resistance in cocoa plant. MCBC1 clone which categorised as VSD disease resistance showed greater recovery by developing the lengthiest (62.3 mm) and thickest diameter (15.6 mm) of new shoot compared with the moderately VSD resistance clones such as PBC230, KKM22 and KKM4. It is recommended in the future to study the correlation between traits and VSD resistant such as in this study.

COMPETING INTERESTS

The authors declare that they have no competing interests

DATA AVAILABILITY STATEMENT

The raw data used to support the findings of this study are available from the corresponding author upon request.

REFERENCES

Adejumo, T. (2005). Crop protection strategies for major diseases of cocoa, coffee and cashew in Nigeria. *African Journal of Biotechnology, 4*(2), 143-150.

Adeyemi, A.A. (1999). Effective intercropping systems for young cocoa. *Tropical Science, 39*, 1-10.

Ahmad Kamil, M.J., Kelvin, L., Sapiyah, S., Lee, M.T., Shari Fuddin, S., Albert, L.A.F., & Bong, C.L. (2016). Evaluation of cocoa resistance to Vascular Streak Dieback in Malaysia. *Conference on Cocoa Germplasm Utilization and Conservation: A Global Approach*, The Netherlands.

Anita Sari, I., & Susilo, A.W. (2013). Investigation of different characters of stomata on three cocoa clones with resistance level difference to VSD (Vascular Streak Dieback) disease. *Journal of Agriculture Science and Technology, 3*, 703-719.

Azhar, I., & Lee, M.T. (2004). Perspective for cocoa cultivation in Malaysia: Re-look at the economic indicators. *Malaysian Cocoa Journal, 1*, 6-23.

Chan, L., & Syed, K. S. W. (1976). Vascular Streak Dieback of cocoa in Peninsula Malaysia. *Proceeding of Cocoa and Coconut for East Malaysia planter' Association*, Sabah.

Guest, D. I., & Keane, P. J. (2007). Vascular Streak Dieback: A new encounter disease of cacao in Papua New Guinea and Southeast Asia caused by the obligate basidiomycete *Oncobasidium theobromae*. *Phytopathology, 97*, 1654-1658.

Florez, S. L., Erwin, R. L., Maximova, S. N., Guiltinan, M. J. & Curtis, W.R. (2015). Enhanced somatic embryogenesis in *Theobroma cacao* using the homologous BABY BOOM transcription factor. *BMC plant biology, 15*, 121-121.

Keane, P. J. (1972). Aetiology and epidemiology of Vascular Streak Dieback of Cocoa *PhD. Thesis*, University of Papua New Guinea, p. 65.

Malaysian Cocoa Board. (2005). *Malaysia cocoa clone's manual booklet*. Malaysian Cocoa Board, Malaysia. p. 31.

Marfu, J., Efron, Y., & Epaina, P. (2016). Evaluation of resistance to Vascular Streak Dieback in Papua New Guinea. *Conference on Cocoa Germplasm Utilization and Conservation: A Global Approach*, Rome, Italy.

Minimol, J. S., Suma, B., Mahiya, U., & Chithira, P. G. (2016). Genetic improvement of cocoa by developing superior hybrids. *Journal of Tropical Agriculture, 53*(2), 157-165.

Riedel, J., Armengot, L., Kagi, N., and Scheinder, M. 2019. Effects of rehabilitation pruning and agroforestry on cacao tree and yield in an older full sun plantation. *Experimental Agriculture, 1*, 1-17.

Samuels, G. J., Ismaiel, A., Rosmana, A., Junaid, M., Guest, D., McMahon, P., Keane, P., Purwantara, A., Lambert, S., Carres, R. M., & Cubeta, M. A. (2012). Vascular Streak Dieback of cacao in Southeast Asia and Melanesia: In planta detection of the pathogen and a new taxonomy. *Fungal Biology, 116*, 11-23. https://doi.org/10.1016/j.funbio.2011.07.009

Santoso, T. I., & Zakariyya, F. (2019). Several physiological changes of cocoa (*Theobroma cacao* L.) in response to Vascular Streak Dieback disease. *Agrivita, 41*(1), 129-138. http://doi.org/10.17503/agrivita.v41i1.1668

Singh, L. H., Anok Uchoi, G. C., Acharya1, S., Apshara, E., & Das, A. (2020). Yield estimation in cocoa with partial harvest data. *Journal of Plantation Crops, 3*(1), 23-28.

Sosnowski, M. R., Fletcher, J. D., Daly, A. M., Rodoni, B. C., & Viljanen-Rollinson, S. L. H. (2009). Techniques for the treatment, removal and disposal of host material during programmes for plant pathogen eradication. *Plant Pathology, 58*(4), 7621-635. https://doi.org/10.1111/j.1365-3059.2009.02042.x

Susilo, A. W., & Sari, I. A. (2014). Relationship between the shoot characteristics and plant resistance to Vascular Streak Dieback on cocoa. *Pelita Perkebunan, 30*(3), 77-87.

Susilo, A. W., Arisandy, P., Sari, I. A., & Harimurti, R. (2016). Relationship analysis between leaf-stomata characteristics with cocoa resistance to Vascular Streak Dieback. *Pelita Perkebunan, 32*, 10-21.

Tee, Y. K., Balasundram, S. K., Ding, P., Hanif, A. H., & Bariah, K. (2019). Determination of optimum harvest maturity and non-destructive evaluation of pod development and maturity in cacao (*Theobroma cacao* L.) using a multiparametric fluorescence sensor. *Journal of the Science of Food and Agriculture, 99*(4), 1700-1708. https://doi.org/10.1002/jsfa.9359

Turnbull, C. J., & Hadley, P. (2018). International Cocoa Germplasm Database (ICGD). (http://www.icgd.reading.ac.uk). Accessed on 28 December 2018.

Uchoi, A., Shoba, N., Balakrishnan, S., Gopal, N. O., & Uma, D. (2018). Effect of different pruning levels and growth retardants on growth, yield and quality of cocoa (*Theobroma cacao* L.). *Journal of Pharmacognosy and Phytochemistry, 7*(4), 3354-3357.