RESEARCH ARTICLE

THE FIELD SCREENING OF THE SOMATIC EMBRYOGENESIS CULTURES DERIVED COCOA CLONE TREES FOR THE RESISTANCE TO VASCULAR STREAK DIEBACK (VSD) DISEASE

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Abstract: Vascular streak dieback (VSD) caused by the fungus *Oncobasidiumtheobromae* is a devastating pathogen of cocoa (*Theobromae cacao* L.). This disease effects both young seedlings and mature trees. Plant tissue culture technique viz. somatic embryogenesis has a potential to overcome this problem by the development of VSD disease resistant cocoa planting materials. To ensure the effectiveness of this technique, the field screening of resistant of the regenerated cocoa clone trees to VSD was evaluated. The method used was field observation based on visual scoring of VSD infection under normal planting conditions. Thirty cocoa plants derived from immature zygotic embryo and 30 cocoa plants derived from staminode explants of Trinitario varieties were planted in field condition. Pruning to remove the infected branches was carried out to determine the relationship between characteristics of sprouting ability and VSD scoring of the severity for each regenerated cocoa clone trees. At one year of planting, it was found that immature zygotic embryo cultures derived cocoa trees were resistant than staminode cultures derived cocoa trees to VSD disease. The MCBC1 cocoa clone trees either derived from immature zygotic embryo culture or staminode cultures showed the optimum characteristics of sprouting ability than other type of cocoa clone trees.

Keywords: *Theobroma cacao*, Tissue culture, Somatic embryogenesis, Field experiment, Vascular streak dieback

INTRODUCTION

Theobroma cacao L. or simply known as cocoa tree is one of the most important cash crop trees grown in the humid tropics. In the last decade, its consumption has increased as cocoa is cultivated for its fruit in which the seeds are used for the production of chocolates and confectionaries. Conventionally, cocoa can be propagated through seed, rooted cuttings of buddings from plagiotropic or orthotropic shoots, marcotting and grafting.However, cocoa propagation through seed generates high genetic variability due to its natural propagation system of allogamous. A high degree of yield variation often found in cocoa plantation of using seeds as the method of propagation (Maximova*et al*., 2002). To minimize the negative effectthrough seed propagation, regeneration of cocoa through rooted cutting, marcotting and grafting are frequently used. Though, rooted cutting, results in a sprawling, bush-like architecture lacking the normal dimorphic growth habit. The regenerated trees required extensive pruning to achieve a more convenient shape for harvesting and other farm operations.These methods of propagation has been described as inefficient and costly (Figueira and Janick, 1995).

Recently, plant regeneration through somatic embryogenesis offers an alternative approach to overcome these conventional propagation methods of cocoa. In cocoa, somatic embryogenesis is the most frequently adopted *in vitro*technique, which has been used not only for plant propagation but also for genetic engineering (Loyola and Vasquez, 2006), virus elimination (Quainoo and Dwomon, 2012; Edward and Wetten, 2016) and germplasm preservation (Maximova*et al.* 2002). The diseases have been eliminated from various crops such as oil palm (*Elaeisguineensis* L.), banana (*Musa* L. sp.), apple (*Malus Mill.* sp.), Barley (*HordeumVulgare* L.), cherry (*Prunusaviumxpseudoceracus* L.) and pear (*Pyruscommunis* L.) (Gorbarenko&Zhuk, 1972; Hartman, 1974; Grout, 1999) throughplant tissue culture technique of somatic embryogenesis.

In cocoa, VSD is a serious disease that destroyed most cocoa plantations worldwide. This disease was associated with the windborne basidiomycetesfungus known as *O. theobromae*. The infestation of this basidiomycetes pathogen begins by infecting the new leaves of young cocoa trees. The fungus then moves to the xylem, resulting in vascular browning among the veins of the lamina. After that, the disease spreads to the midrib and petiole of the leaves and finally reaching the branch. The symptoms of VSD disease include the leaf chlorosis with green spots, necrotic leaf scars from abscised leaves with 3 dots or as dark streak when stems are split open, rough bark as a result of swollen lenticels immediately below the petiole of the affected leaves, "broomstick" symptoms as a result of proliferation and subsequent death of axillary buds following

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leaves abscission (Keane, 1981; Guest and Keane, 2007; Samuels *et al.* 2012).

As *C. theobromae* is a vascular pathogen, control by fungicides is hampered with difficulty, and although effective systemic fungicides such as Triazole, Flutriafol, Hexaconazole, Propiconazole and Triadimenol have been used, they are generally too costly for smallholder farmers (Minimol*et al.* 2015). To date, cultural practices to control VSD are by using clean nursery stocks, canopy pruning of disease infected trees and through shade and canopy management to increase aeration and sunlight exposure to the infection sites. Among these cultural practices, canopy pruning to control VSD infection is much preferred. It was reported that canopy pruning to remove all VSD tissues of ±10 cm beyond the streaking infections of VSD carried out every two to three weeks able to control the disease (Susilo and Sari, 2014). The canopy pruning of the infected cocoa branches about ±10 cm below the discolored xylems inhibits further spreading of the infection and decreases inoculum level by removing potential sites of sporulation (Guest and Keane, 2007). It was studied that when VSD infected cocoa trees were left unpruned, the disease incidence rise from about 30 to 90% within 10 months period (Guest and Keane, 2007). Minimol*et al.* 2015 reported that the use of systemic fungicides are ineffective in controlling VSD diseases and the only viable solution is to regenerate resistant cocoa planting materials.

Another option for effective long term solution for VSD control is through the introduction of the resistant cocoa planting materials of high quality. This can be potentially achieved through resistance plant breeding programme through application of somatic embryogenesis technique. The use of the disease resistant cocoa clone could decrease the usage of chemicals from pesticides for disease control thus beneficial to the environment, reduce the carbon emission if using machineries for the application of pesticides as well as useful for soil conservation. Besides, for the smallholder farmers, the cost of pesticides application can be minimized. Nevertheless, as not all plants regenerated *in vitro* are guaranteed free of diseases, therefore scoring and screening of the regenerated somatic embryogenesis cultures derived cocoa plants for the occurrence of disease such as VSD during early stage of field planting is crucial. In this study, the relationship between characteristics of sprouting ability and VSD scoring of the severity for each regenerated cocoa clone trees were evaluated. The characteristics of sprouting ability was evaluated by recording the pruned somatic embryogenesis cultures derived cocoa trees for the variables of the number of the regrowth shoot, shoot length, number of new shoots per pruned branch, shoot diameter and number of leaves per shoot.

MATERIAL AND METHOD

Experimental site and plant materials

This study was conducted from April 2016 toAugust 2017 in MCB Kota Samarahan, Sarawak, Malaysia involved 30 Trinitario variety cocoa clone trees fromimmature zygotic embryo cultures and also 30 Trinitario variety cocoa clone trees derived from staminode cultures. The soil in the area of the study was classified as Histosols and experiences an equatorial climate which is characterized by hot and humid conditions with a drier period during the middle of the year and high rainfalls at the end of the year (Mohamad Tarmizi*et al.* 2014). The area has an annual average temperature of 27.5°C, relative humidity of 88.0% and a mean monthly rainfall ranging between 19.1 to 310 mm.

Table 1. Total number of staminode cultures trees and immature zygotic embryo cultures trees assessed in the field for the scoring on VSD disease severity.

Type of somatic embryogenesis derived cocoa trees	Type of cocoa clone trees	Total
Staminode cultures derived cocoa trees	BR25	6
	PBC230	6
	KKM4	6
	KKM22	6
	MCBC1	6
Immature zygotic embryo derived cocoa trees	BR25	6
	PBC230	6
	KKM4	6
	KKM22	6
	MCBC1	6

Scale of assessment for VSD infection after one year of field planting

The data on the scoring for the severity of each regenerated somatic embryogenesis cultures derived cocoa trees was collected based on method described by Ahmad Kamil*et al.* (2016). The scale used for the scoring on VSD disease severity was ranging from 0 to 6 (Table 2) based on the cocoa VSD progressive infection on the cocoa branches in April 2017 after one year of field planting. These severity scale were used to classify the cocoa clone into four groups of resistance comprised of susceptible (severity score between 4.0 to 6.0), moderately susceptible (severity score between 3.0 to 3.9), moderately resistant (severity score between 2.0 to 2.9) and resistant (severity score between 1.0 to 1.9) (Susilo and Anita Sari, 2014; Ahmad Kamil*et al.* 2016). Four parts of plant canopies with the infected branches and leaves (Figure 1) were evaluated for the mean of the severity of VSD infections.

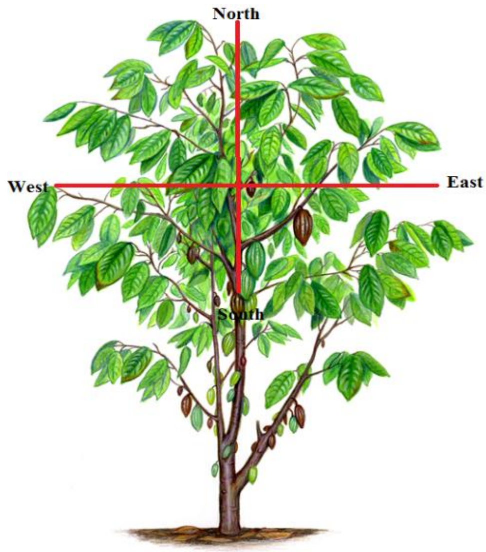

Figure 1. Diagram of the four part of plant canopies for the VSD disease assessment (Ahmad Kamil*et al.* 2016).

Table 2. Score of severity damage based on the primary symptoms due to VSD infection of somatic embryogenesis cultures derived cocoa trees for field screening (Adapted from Ahmad Kamil*et al.* 2016).

Severity score	Primary symptoms	Other associated symptoms
0	Uninfected trees	Smooth bark
1	<25% of cocoa plant branches and leaves were infected by VSD but no effect on plant vigor	Smooth bark with or without swollen lenticels
2	>25-50% of cocoa plant branches and leaves were infected by VSD that tend to result in vigor declining such as leaves turn chlorosis	Moderately smooth bark with slightly swollen lenticels
3	>50-75% cocoa plant branches and leaves were infected by VSD which slightly affect vigor in which most of the infected leaves turn chlorosis and necrotic but still remain attached in the branches	Moderately rough bark with slightly swollen lenticels
4	>75% cocoa plant branches and leaves were infected by VSD which tend to seriously affect vigor where the infected leaves begin to abscise	Moderately to very rough bark with slightly swollen lenticels
5	Most of the cocoa plant branches and leaves were infected by VSD which significantly affect vigor as most of the infected leaves have abscised	Very rough bark with the presence or absence of fruiting bodies
6	The infected plants were seriously damaged by VSD, some of those infected plants were died. Dieback occurred and infected plant part was died	Very rough bark without proliferation of auxiliary shoots and with presence VSD of fruiting bodies

Pruning procedures

At the same time, pruning was carried out to remove the four parts of the infected branches of around ±10 cm beyond the streaking infections based on modified method from Susilo and Sari (2014). The pruning was done for the three moderately resistance cocoa clone trees both regenerated through immature zygotic embryo and staminode cultures such as KKM4, BR25 and KKM22 (Lee *et al.* 1993; Malaysian Cocoa Board, 2005) as well as for the susceptible cocoa clone, the PBC230 (Lee *et al.* 1993; Malaysian Cocoa Board, 2005) cocoa clone trees that have been infected by VSD disease. On the other hand, MCBC1 as the resistance cocoa clone trees (Lee *et al.* 1993; Malaysian Cocoa Board, 2005) was also pruned and used as a control tree. A total of 12 parts (North, East, West and South parts) of each cocoa clone trees types infected branches were removed and pruned in this study. The pruning was done during dry season to minimize the incidence of the disease which correlated with high rainfall rate as found in the wetter areas, the spreading of the VSD disease will be higher than those in the dry area. The systemic fungicide such as Bayfidan also applied after pruning.

Pruning for the characteristics of sprouting ability

Assessment on the characteristics of sprouting ability for the variables of the number of the regrowth shoot, the length of the longest shoot, number of new shoots per pruned branch, the diameter of the longest shoot and number of leaves per shoot of each somatic embryogenesis cultures derived cocoa clone trees were evaluated after three months of pruning. The standard agronomy practices were carried out during the period of the evaluation with the application of the complex fertilizer with NPK contents of 12:12:17+TE. The application of fertilizer of around 20 to 30 gram per tree was done after pruning.

Data collection and analysis

The data on the characteristics of sprouting ability for the variables of the number of the regrowth shoot, shoot length, number of new shoots per pruned branch, shoot diameter and number of leaves per shoot of each somatic embryogenesis cultures derived cocoa clone trees were assessed after three months of pruning. For these data, only the normal shoot without VSD infections were further evaluated. Mean of data collected for the characteristics of the sprouting ability for each of the cocoa clone trees were analyzed using analysis of variance (ANOVA) test and if there is a significant difference detected then it is further analyzed with Fisher Protected LSD test at $p<0.05$ significance level using SPSS Software Version 22.

RESULT AND DISCUSSION

Scale of assessment for VSD infection after one year of field planting

Four parts of cocoa plant canopies with the infected branches and leaves (Figure 2) were evaluated in April 2017 after one year of field planting for the incidence of VSD infections. From data collected (Table 3), MCBC1 cocoa clone trees showed the lowest mean of severity scale for assessment when compare to others type of cocoa clone trees. Though, the MCBC1 cocoa clone trees derived from immature zygotic embryo were found more resistant than the MCBC1 cocoa clone trees derived from staminode cultures (1.36) as the mean severity scale for VSD infection was the lowest (1.11). MCBC1 cocoa clone was one of the most VSD resistant cocoa clone and recommended for the used as planting materials with the high yielding capability (MCB, 2015). An overall for all of the cocoa clones, the mean severity scale of VSD infection for immature zygotic embryo derived cocoa trees were also lowest as compare to the mean severity scale of the staminode cultures derived cocoa clone trees. Susilo and Anita Sari (2014) reported that cocoa clone have varying degree of tolerance to VSD disease depending on their genetic background.

Among cocoa clone trees evaluated, PBC230 cocoa clone trees from both somatic embryogenesis cultures showed the highest mean severity scale of VSD infection after one year of field planting. The primary symptoms of the VSD infection in PBC230 cocoa clone trees during the field inspection including leaf turn yellow and chlorosis(Figure 3a), rough bark (Figure 3b) and swollen leaf lenticel (Figure 3c).Under International Cocoa Gerplasm Database (2017), the PBC230 cocoa clone is categorized as VSD moderately resistant nevertheless frequently planted for its promising high yielding and quality of seeds. For the moderately resistant cocoa clone trees, cultural practice such as pruning was carried out in order to open the canopy to increase air circulation and reduce the humidity. Reducing the humidity is crucial to avoid spore formation, sporulation and infection of *O. theobromae*(Vos*et al.* 2003).This study revealed that the staminode cultures derived cocoa trees presented the highest mean severity scale of VSD infection than immature zygotic embryo cultures trees. Hence, from this study it was proved that cocoa clone trees regenerated from immature zygotic embryos cultures were better than cocoa clone trees regenerated from staminode cultures in term of field performance against VSD infection after one year of field planting.

Table 3. Mean VSD disease severity scale of the immature zygotic embryo cultures derived cocoa trees and staminode cultures derived cocoa trees after one year of field planting.

Type of somatic embryogenesis derived cocoa trees	Type of cocoa clone trees	Mean severity scale for VSD infection
Staminode cultures derived cocoa trees	BR25	1.88
	PBC230	3.88
	KKM4	2.04
	KKM22	1.67
	MCBC1	1.36
Immature zygotic embryo derived cocoa trees	BR25	1.60
	PBC230	3.22
	KKM4	2.00
	KKM22	1.44
	MCBC1	1.11

Figure 2. The white circles showed the four parts of cocoa plant canopies with the infected branches and leaves assessed for the incidence of VSD infections in which (a) PBC230 cocoa clone tree regenerated from staminode culture; (b) PBC230 cocoa clone tree regenerated from immature zygotic embryo culture.

Figure 3. The primary symptoms of the VSD infection in PBC230 cocoa clone trees during field inspection after one year of field planting in April 2017 where (a) leaf turn yellow and chlorosis; (b) rough bark; (c) swollen leaf lenticel.

Pruning for the characteristics of sprouting ability

Staminode cultures derived cocoa trees

The data on the characteristics of sprouting ability (Table 4) for staminode cultures derived cocoa trees was evaluated after three months of pruning in July 2017 after the removal of the VSD infected branches of around ±10 cm beyond the streaking infections (Figure 4a). The healthy shoots and healthy branches without any VSD infections on the cut surface were used for the evaluation (Figure 4b). BR25 and MCBC1 cocoa clone trees showed the highest percentage of the sprouting branches (100%). The PBC230 cocoa clone trees presented the lowest percentage of the sprouting branches of around 83.3%. It was found that there was a significant different between the mean number of the emerge leaves for MCBC1 cocoa clone trees with the other type of staminode derived cocoa clone trees. The MCBC1 cocoa clone trees recorded the highest mean number of the emerged leaves of 5.92±0.29 whereas KKM22 recorded the lowest mean number of the emerge leaves of 3.75±0.18. From this study, it was proved that the number of sprouting branches and the number emerge leaves were correlated with the mean severity scale to VSD infection. The mean number of emerge leaves were higher for the resistant cocoa clone tree with the lowest mean scoring of VSD such as the MCBC1 cocoa clone. The similar finding was also obtained by Susilo and Sari (2014)in Indonesia in which the VSD resistant cocoa clone trees have the optimum characteristics of sprouting ability than the moderately resistant cocoa clone trees after pruning.

In addition, for the mean number of shoot, the MCBC1 cocoa clone trees also recorded the highest mean number of shoot as compare to other type of cocoa clone trees in which the mean number was around 3.25±0.13. The KKM22 cocoa clone trees showed the lowest mean length of the longest shoot with the mean length of 6.68±0.22 whereas the MCBC1 showed the highest mean length of the longest shoot of around 15.38±0.33. Besides, there was also a significant different among mean diameter of the longest shoot for each of the cocoa clone trees derived from this staminode cultures. The mean diameter of the longest shoot was ranged from 1.20±0.55 to 2.31±0.92. The KKM22 cocoa clone trees mean diameter of the longest shoot was significantly different with other type of cocoa clone trees and this cocoa clone tree recorded the lowest mean diameter of around 1.20±0.55. In contrast, this mean diameter of the longest shoot was double in MCBC1 cocoa clone trees in which the mean diameter recorded was around 2.31±0.92. Based on this finding, it was proved that MCBC1 cocoa clone trees showed the superior characteristics of sprouting ability among other staminode derived cocoa clone trees based on the evaluation of the characteristics after three months of pruning to remove VSD infected branches.

Table 4. Pruning for the characteristics of sprouting ability among staminode derived cocoa clone trees after three months of pruning.

Type of somatic embryogenesis derived cocoa trees	Type of cocoa clone trees	Percentage of the sprouted branches (%)	Mean number of emerge leaves ± SE	Mean number of shoot ± SE	Mean length of the longest shoot ± SE (mm)	Mean diameter of the longest shoot ± SE (mm)
Staminode cultures derived cocoa trees	BR25	100	4.75± 0.22^{a}	2.42±0.14^{a}	12.17±0.43^{a}	2.26±0.12^{a}
	PBC230	83.3	4.17±0.32^{a}	2.42±0.14^{a}	11.10±0.27^{a}	2.01±0.98^{a}
	KKM4	91.6	4.67±0.26^{a}	2.17±0.11^{ab}	9.38±0.43^{b}	2.10±0.11^{a}
	KKM22	91.6	3.75±0.18^{a}	1.67±0.19^{b}	6.68±0.22^{c}	1.20±0.55^{b}
	MCBC1	100	5.92±0.29^{b}	3.25±0.13^{c}	15.38±0.33^{d}	2.31±0.92^{a}

Mean characteristics of sprouting ability among staminode derived cocoa clone trees after three months of pruning. Means with the same letter are not significantly different at p≥0.05 based on LSD test.

Figure 4. The PBC230 cocoa clone tree infected by VSD after one year of field planting. (a) The VSD infected branches of around ±10 cm beyond the streaking infections in April 2017; (b) The PBC230 cocoa clone tree new shoot sprouted after one month of pruning in May 2017.

Immature zygotic embryo cultures derived cocoa trees

All of the pruned branches of cocoa clone trees of the BR25, KKM22 and MCBC1 were found sprouted after three months of pruning for the removal of VSD infected branches. On the other hand, the PBC230 and KKM4 cocoa clone trees recorded the lowest percentage of the sprouted branches of around 91.6% respectively. The maximum mean number of emerge leaves was discovered in MCBC1 cocoa clone trees with around 6.83±0.72 whereas the minimum mean number of emerge leaves was found in KKM22 cocoa clone trees with mean number of emerge leaves of around 3.92±0.90. As obtained from LSD test, there was a significant different among cocoa clone trees in term of the mean number of the emerge leaves. The KKM22 showed the lowest mean number of shoot of around 1.75±0.22 while MCBC1 showed the highest mean number of shoot of around 3.50±0.15. The other cocoa clone trees recorded mean number of shoot of around 2.83±0.11 for BR25 cocoa clone trees, 2.58±0.19 for KKM4 cocoa clone trees and 2.25±0.13 for PBC230 cocoa clone trees.

The mean length of the longest shoot discovered were ranged around 6.73±0.10 to 16.47±0.20 in which the maximum mean of the longest shoot was found in MCBC1 cocoa clone trees whereas the minimum mean of the longest shoot was recorded in

KKM22. Bases on the LSD test, there was a significant different in term of the mean length of the longest shoot among these immature zygotic embryo derived cocoa clone trees. The mean diameter of the longest shoot was also studied in which it was found that the MCBC1 cocoa clone trees presented the highest mean diameter of the longest shoot of around 3.26±0.10. This followed by BR25 cocoa clone trees with mean diameter of the longest shoot of 2.82±0.58, PBC230 of around 2.91±0.07 mean diameter of the longest shoot and KKM4 with 2.71±0.07 mean diameter of the longest shoot. The KKM22 cocoa clone trees presented the lowest mean diameter of the longest shoot of around 1.62±0.05.

Table 5. Pruning for the characteristics of sprouting ability among immature zygotic embryo derived cocoa clone trees after 3 months of pruning.

Type of somatic embryogenesis derived cocoa trees	Type of cocoa clone trees	Percentage of the sprouted branches (%)	Mean number of emerge leaves ± SE	Mean number of shoot ± SE	Mean length of the longest shoot ± SE (mm)	Mean diameter of the longest shoot ± SE (mm)
Immature zygotic embryo derived cocoa trees	BR25	100	4.41±0.67^{ab}	2.83±0.11^{a}	13.20±0.31^{a}	2.82±0.58^{a}
	PBC230	91.6	4.50±1.17^{a}	2.25±0.13^{ab}	11.46±0.22^{b}	2.91±0.07^{a}
	KKM4	91.6	5.08±0.67^{a}	2.58±0.19^{a}	10.18±0.44^{c}	2.71±0.07^{a}
	KKM22	100	3.92±0.90^{b}	1.75±0.22^{b}	6.73±0.10^{d}	1.62±0.05^{b}
	MCBC1	100	6.83±0.72^{c}	3.50±0.15^{c}	16.47±0.20^{e}	3.26±0.10^{c}

Mean characteristics of sprouting ability among immature zygotic embryo derived cocoa clone trees after three months of pruning. Means with the same letter are not significantly different at p≥0.05 based on LSD test.

Hence, in this study the cocoa clone trees derived from immature zygotic embryos cultures showed the optimal characteristics of sprouting ability such as percentage of the sprouted branches (Figure 5), mean number of emerge leaves (Figure 6), mean number of shoot (Figure 7), mean length of the longest shoot (Figure 8) and mean number of longest shoot (Figure 9) as compare to cocoa clone trees derived fromstaminodecultures after three months of pruning.

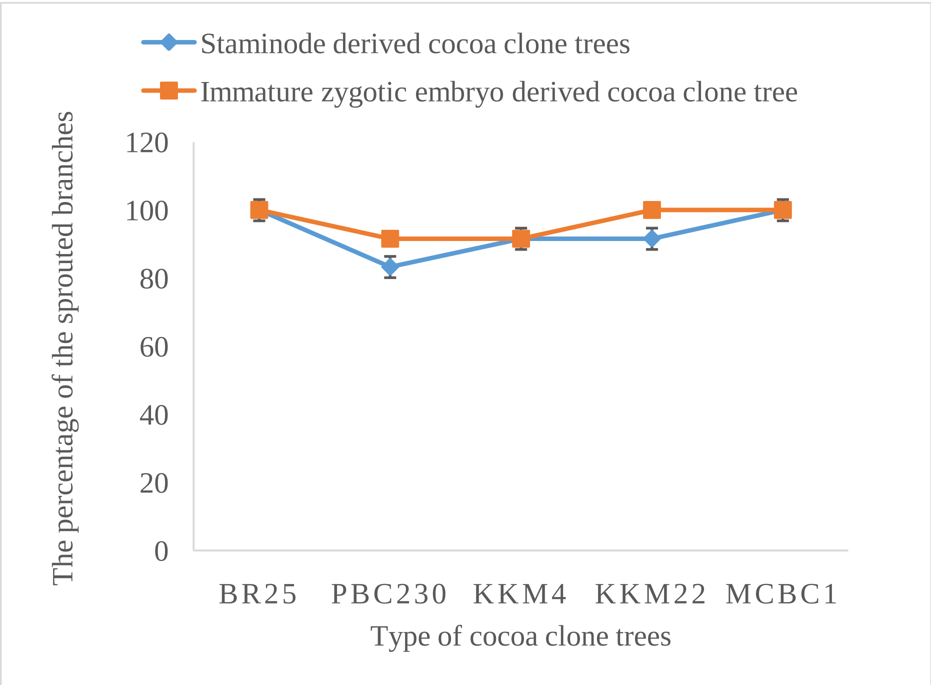

Figure 5. The percentage of the sprouted branches among cocoa clone trees derived from both staminode and immature zygotic embryo cultures.

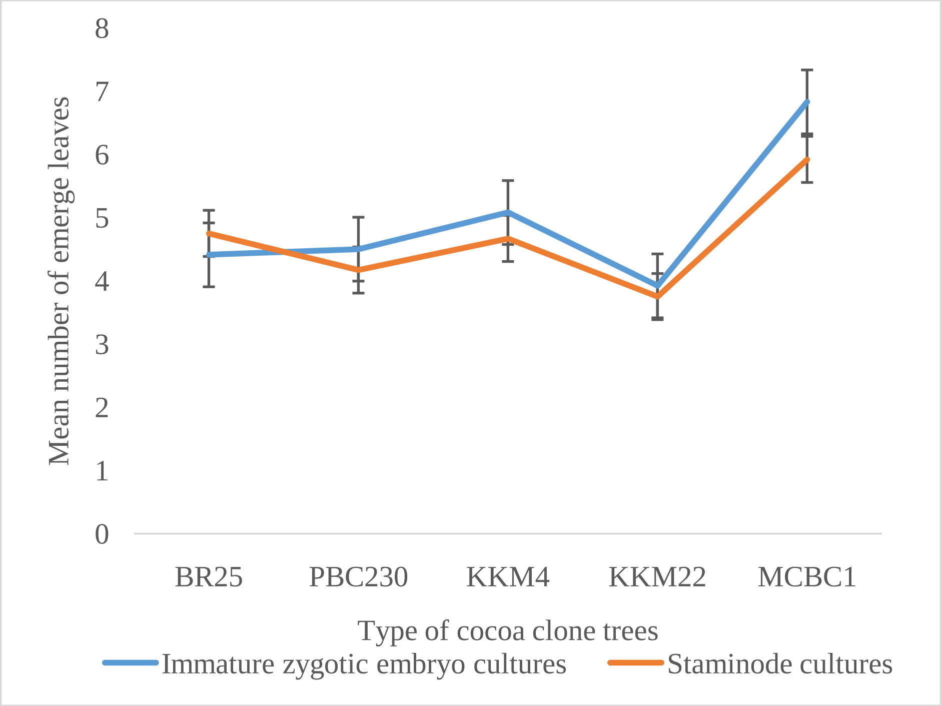

Figure 6. Mean number of emerge leaves of staminode and immature zygotic embryo cultures derived cocoa trees after three months of pruning.

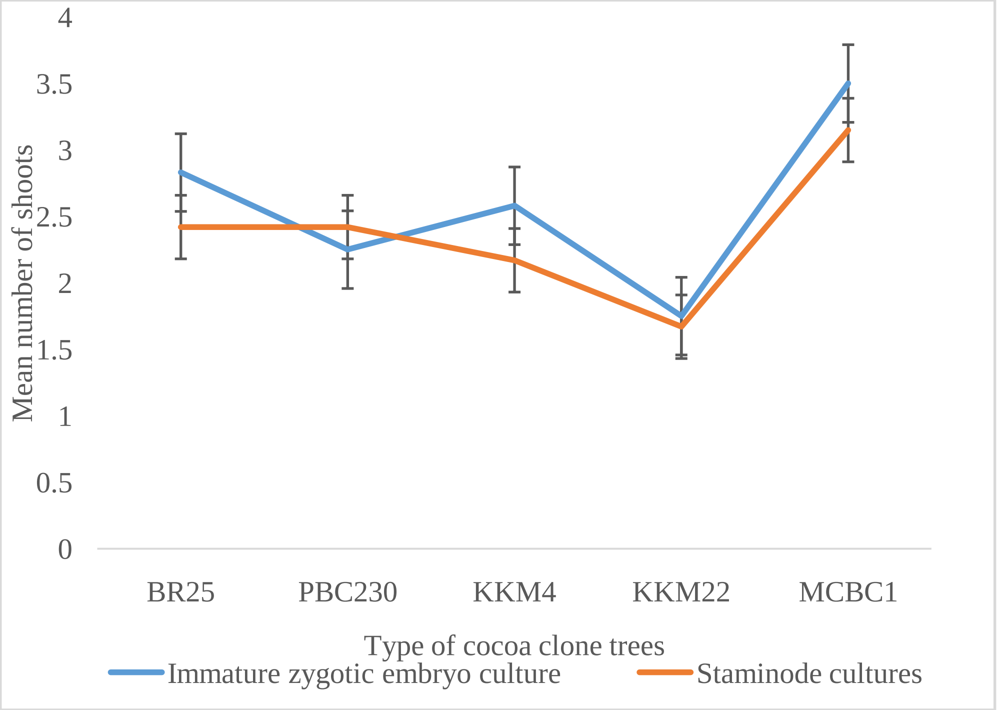

Figure 7. Mean number of shoots of staminode and immature zygotic embryo cultures derived cocoa trees after three months of pruning.

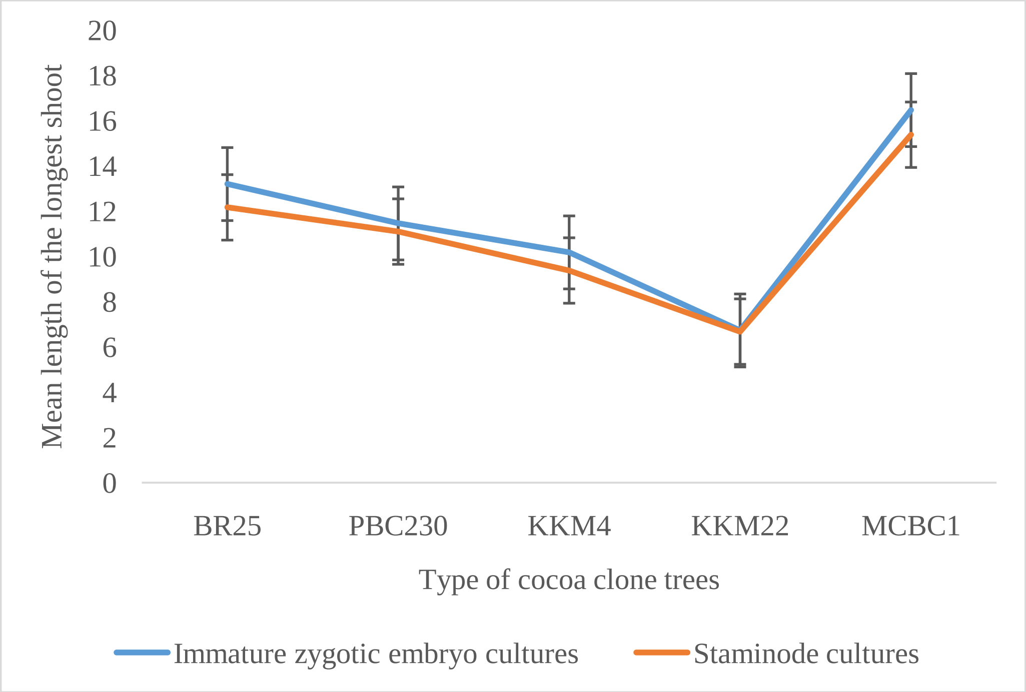

Figure 8. Mean length of the longest shoots of staminode and immature zygotic embryo cultures derived cocoa trees after three months of pruning.

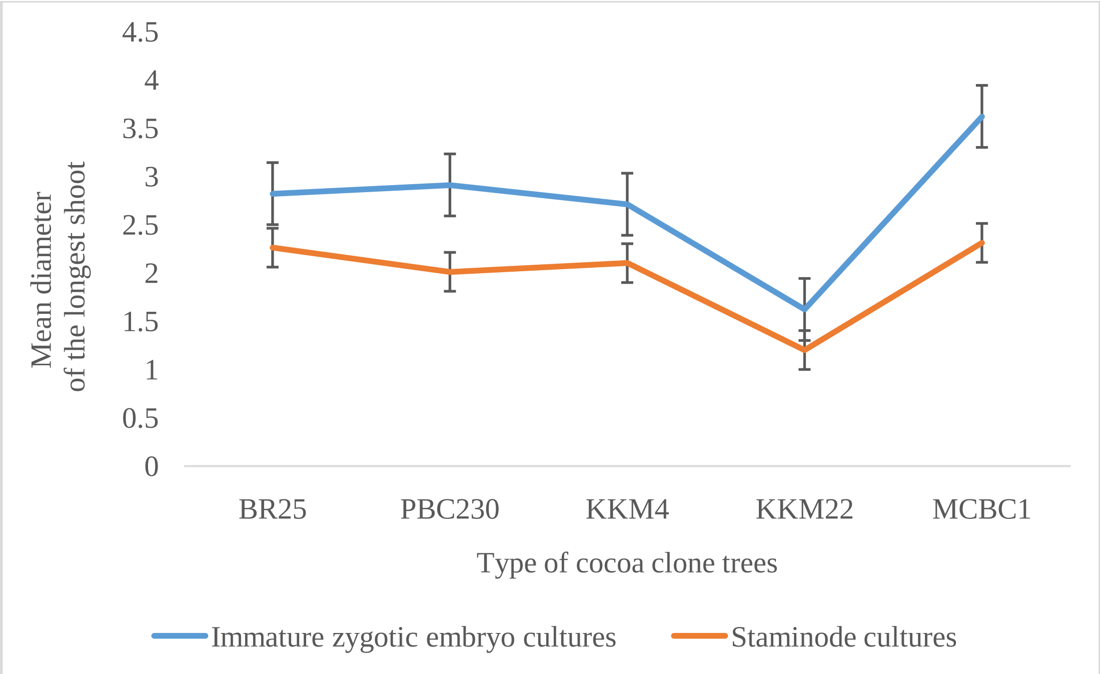

Figure 9. Mean diameter of the longest shoots of staminode and immature zygotic embryo cultures derived cocoa trees after three months of pruning.

CONCLUSION

After one year of field planting, the immature zygotic embryo cultures derived cocoa clone trees were proved to beresistant to VSD disease than the staminode cultures derived cocoa clone trees. Among five of the cocoa clone trees evaluated, MCBC1 released for commercial planting as VSD resistant cocoa clone have the lowest mean severity scale to VSD infection either derived from staminode cultures or immature zygotic embryo cultures. From this finding, the characteristics of the sprouting ability of each cocoa clone trees were depending on the genotype in which the VSD resistance cocoa clone tree such as MCBC1 was more superior to moderately resistant cocoa clone trees such as PBC230 and KKM4 in term of sprouting characteristics after three months of pruning. The result revealed that pruning play an important roles on the management of VSD disease in cocoa.

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REFERENCES

Ahmad Kamil, M.J., Kelvin, L., Sapiyah, S., Lee, M.T., Shari Fuddin, S., Albert, L., Francis A. and Bong, C.L. (2016). Evaluation of cocoa resistance to vascular streak dieback in Malaysia. In Global *Approaches to Cocoa Germplasm Utilization and Conservation*, Final report of the CFC/ICCO/IPGRI project on "Cocoa Germplasm Utilization and Conservation: A Global Approach". The Netherlands/ICCO, London, UK/IPGRI, Rome, Italy. Pp. 191–199.

Anita Sari, I. and Susilo, A.W. (2014). Effect of genetic and altitudinal difference on stomata characters as resistance indicators to Vascular Streak Dieback (VSD) in cocoa (*Theobroma cacao* L.). *Journal of Agricultural Science and Technology*, 4*:* 157–163.

Edward, R. and Wetten, A. (2016). Virus detection and elimination in cocoa (*Theobroma cacao* L.) through somatic embryogenesis. *Journal of Plant Sciences*, 4(3): 52-57.

Figueira, M. and Janick, M.T. (1995). *In vitro* multiplication and plant regeneration of *Theobroma cacao* L. via stable embryogeniccalli. *10th IAPTC Congress in Plant Biotechnology*.

Gorbarenko, M.I. and Zhuk, I.P. (1972). Prevention of virus diseases in potatoes by meristem culture. *MikrobiolgyiaZhournal*, 34: 87-88.

Grout, B.W. (1999). Meristem tip culture for propagation and virus elimination. *Methods in Molecular Biology*, 111: 115-125.

Guest, D. I. and 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: 1654–1657.

Hartman, R.D. (1974). Dasheen mosaic virus and other phytopathogens eliminated from caladium, taro and cocoyam by culture of shoot tips. *Phytopathology*, 64: 237-240.

Keane, P.J. (1981). Epidemiology of Vascular Streak Dieback of cocoa. *Annals of Applied Biology*, 98*:* 227–241.

Loyola, V. and Vasquez, F. (2006). *Plant Cell Culture Protocols*. Second Edition. USA: Humana Press Incorpotation, p. 425.

Malaysian Cocoa Board (2005). Malaysian cocoa monitor. Vol. 3(2). Malaysian Cocoa Board, Malaysia. Pp. 1-8.

Malaysian Cocoa Board (2015). Cocoa planting manual. Malaysian Cocoa Board, Malaysia. Pp. 1-29.

Maximova, S.N., Alemanno, L., Young, A., Ferriere, N., Traore, A. and Guiltinan, M.J. (2002). Efficiency, genotypic variability and cellular origin of primary and secondary somatic embryogenesis of*Theobroma cacao*, *Invitro Cellular Developemental Biology*,38: 252-259.

Minimol, J., Shuma, B., Ummer, M. and Chitira, P.G. (2015). Genetic improvement of cocoa by developing superior hybrids. *Journal of Tropical Agriculture*, 53(2): 157-165.

Mohamad Tarmizi, M.Z., Tham, F.N., Wan Hasiah, A. and Mohammad Aqeel, A. (2014). Geochemical characteristics of a tropical lowland peat dome in the Kota Samarahan-Asajaya area, West Sarawak, Malaysia. *Environmental Earth Sciences*, 73(4): 1443-1458.

Samuels, G.J., Ismaiel, A., Rosmana, A., Junaid, M., Guest, D.I., McMahon, P.J., Keane, P.J., Purwantara, A., Lambert, S., Rodriguez-Carres, M. and 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.

Vos, G. M., Ritchie, B. J. and Flood, J. (2003). À la découverte du cacao: un guide pour la formation des facilitateurs. *CABI Bioscience*, 7(4): 14-18.