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Potential and Stability of Promising Fine Flavor Cocoa Clones in Different Seasons: Yield, Quality and Resistance to Vascular Streak Dieback

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Abstract

The intensity of vascular streak dieback (VSD) disease is one of the major causes of decreased fine flavor cocoa production in Indonesia; therefore, it is essential to develop superior planting materials resistant to this disease. The purpose of the study was to conduct initial identification of yield potential and resistance level to VSD disease in several promising clones of fine flavor cocoa and to identify bean quality profile. This study was conducted at Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute, Jember, Indonesia, with an altitude of 45 m above sea level (asl.) and C climate type, according to the Schmidt-Ferguson classification system. The study design used a complete randomized block design consisting of 13 promising clones and 3 superior clones of fine flavor cocoa as control. Each treatment was repeated 4 times in blocks, and each plot consisted of 5 plants. The results showed that PNT 16 was potential to be developed as a superior promising clone of fine flavor cocoa with high yield potential (66.6 pods/tree/year equal to 2.52 kg dry bean/tree/year) and low VSD level (VSD score: 0.4) in first year of production (4 years old after planting). The superior promising clone of PNT 16 comprises of 35.1 beans per pod with good quality, weighed by 1.25 g/bean, and a higher percentage of white seeds above 80% (94.9%). Furthermore, the specifications of the main flavor attributes are similar to ICCRI 09 and dominated by the browned roasted, nutty and browned fruit aroma. Therefore, the clone has the potential to be developed as one of the special cocoa products.

Keywords: Yield, bean quality, vascular streak dieback, *Theobroma cacao* L., fine flavor cocoa

INTRODUCTION

Fine flavor cocoa, better known as Java fine flavor cocoa, is an Indonesian specialty cocoa product that has successfully entered the global cocoa market. The market share is around 5-7% of the total cocoa production, nonetheless it is continuously increasing in line with more fine flavor incentives and consumers' interest in specialty products. There are 11 countries in the list of fine flavor cocoa producers in the world, including Indonesia (ICCO, 2019). It has specifications of fresh white beans and specific flavor. As compared to the other countries, the uniqueness of fine flavor cocoa is bright color beans with a white color level of more than 85%.

Vascular streak dieback (VSD, *Ceratobasidium theobromae*) disease causes the decline of cocoa production in Indonesia.

Therefore, the fine or flavor cocoa area has been converted into other commodities. This disease is responsible for shedding in plants, yellowing of leaves, and inhibition of flowers and pods (Anita-Sari *et al*., 2012). The attack rate of VSD not only causes a decrease in yield but also may cause death in susceptible plants up to 50% (Febrianto *et al*., 2016). This disease is more severe than pests and other cocoa diseases because it can kill plants. Susilo (2001) stated that the use of resistant plants is an effective and efficient approach to control VSD disease. Meanwhile, fine flavor cocoa clones such as DR series clones that not yet resistant to VSD disease. The development of fine flavor cocoa as a unique cocoa product requires provision for the production of superior material in plants that is resistant to VSD.

Exploration and selection of fine flavor cocoa genetic materials resistant to VSD have been carried out since 2006 in the Trinitario population at the Penataran Plantation, Blitar, East Java (Figure 1). Exploration results obtained several promizing genotypes resistant to VSD and high productivity. Based on the initial selection on 40 fine flavor cocoa accessions with the PNT series at Penataran Plantation in 2009, eight genotypes were suspected of having high performance and production such as PNT 1, PNT 2, PNT 3, PNT 16, PNT 17, PNT 30, PNT 37, and PNT 39. Meanwhile in 2011, seven serial genotypes of PNT showed excellent performance, i.e., PNT 1, PNT 8, PNT 9, PNT 14, PNT 16, PNT 17, and PNT 37 (Anita-Sari & Susilo, 2014). The evaluation results in 2012 obtained nine genotypes with good vigor, high yield potential,

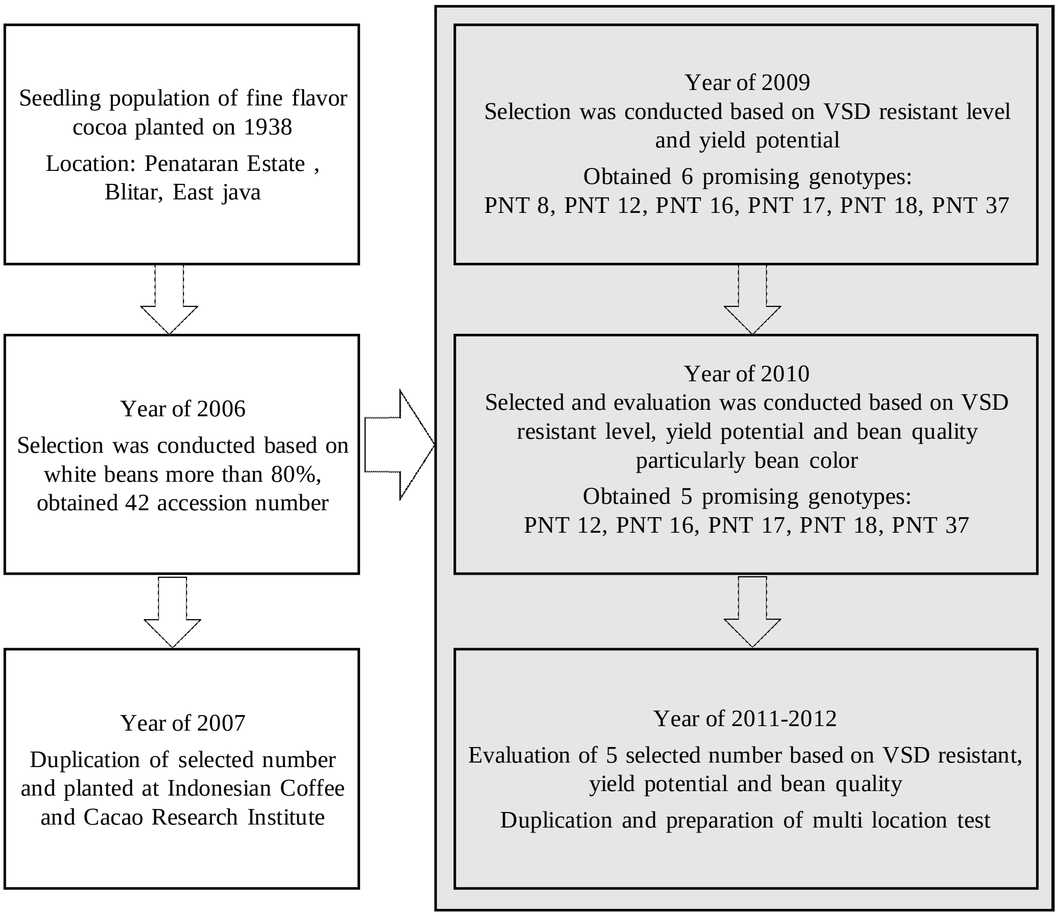

Figure 1. Genealogy of PNT series fine flavor cocoa clones

and good quality as well as having resistance to VSD.

The selection results of the identification of potential yield and quality from the promising fine flavor cocoa clones need to be carried out to obtain superior fine flavor cocoa clones with high yield and VSD resistance. In the tested clones, flush and flowering intensities were also observed to determine their development patterns. Flowering phenology is an essential aspect of the growth cycle of cocoa plants. The plant performance, especially flowering, has a relationship with the anthesis time, receptive length of the pistil, pod formation, crossing, and bean development, which are crucial in the plant breeding process (Adjaloo *et al.*, 2012). The target of cocoa breeding is still emphasized on the yield potential and plant resistance towards disease (Lopes *et al*., 2011). The purpose of this study was to obtain preliminary information on superior promising clones of fine flavor cocoa with high yield potential, good bean quality, and potential of resistance to VSD.

MATERIALS AND METHODS

The study used a complete randomized block design, which consisted of twelve superior promising clones of fine cocoa as treatments, and three superior clones of fine flavor cocoa as comparison (DR 1, DR 2, DR 38). Each treatment was repeated four times in blocks, and each plot consisted of 5 sample plants. According to the treatment clone, the plants were derived from young clonal seedlings with rootstocks from ICCRI 06H hybrid seeds grafted with plagiotropic branches. *Leucaena glauca* L2 clone shade plants were used with a spacing of 3 m x 3 m. The study was conducted at the Kaliwining Experimental Station (with an altitude of 45 m asl., C climate type according to the Schmidt-Ferguson classification), of Indonesian Coffee and Cocoa Research Institute in 2016-2019.

Table 1. List of fine flavor cocoa testing accessions

Clone	Description
DR 1	Control clone
DR 2	Control clone
DR 38	Control clone
PNT 8	Promising clone
PNT 12	Promising clone
PNT 16	Promising clone
PNT 17	Promising clone
PNT 18	Promising clone
PNT 18K	Promising clone
PNT 30	Promising clone
PNT 33B	Promising clone
PNT 37	Promising clone
PNT 38	Promising clone
KPC 1	Promising clone
KPC 2	Promising clone

Observations were carried out on 5 (five) plant samples per plot (genotype), which had consistent growth; therefore, the total samples were 20 plants per genotype. The factors to be observed including the intensity of VSD disease attack, level of flush intensity, flowering intensity, and number of pods. The character parameters of beans were observed to focus on the promising clones that potentially resistant to VSD disease.

Flush intensity was observed every month by calculating the flush percentage that appears with the following classification:

Score 0 : no flush
Score 1 : 1-25% flush percentage
Score 2 : 26-50% flush percentage
Score 3 : 51-75% flush percentage
Score 4 : 76-100% flush percentage

Flowering intensity was observed every month by scoring on a scale of 0-6 as follows:

Score 0 : there is no flower
Score 1 : 1-25 flowers per tree
Score 2 : 26-50 flowers per tree
Score 3 : 51-100 flowers per tree
Score 4 : 101-150 flowers per tree
Score 5 : > 150 flowers per tree

Table 2. Score of plant damage due to VSD attack is used as a measure of plant resistance to VSD disease attacks

Score	Plant damage symptoms
0	Healthy plants, there is no VSD attack symptoms
1	Approximately <25% of the plant's branches have been infected with VSD, but the plant remains vigorous, and there are no symptoms of decreased plant production
2	Approximately 25-50% of plant twigs have been infected with VSD, and attacks have an impact on reducing plant vigor but have not caused a significant decrease in production
3	Approximately 50-75% of plant twigs have been infected with VSD, and attacks have caused a decrease in growth vigor and plant production levels, although relatively small
4	Most of the twigs (> 75%) have been infected with VSD, and attacks have an impact on decreasing vigor growth and production rates.
5	Most of the twigs have been infected with VSD and cause damage to plant branches. Therefore there is a significant decrease in plant vigor and production
6	Severely damaged plants and some even died

The intensity of VSD disease attacks was observed every month by using the scoring method, referring to Susilo and Anita-Sari (2011).

Pod number was observed every month by counting the number of pods per tree, that were small pods (pod length <10 cm), medium pods (pod length 10-15 cm), and big pods (pod length > 15 cm) are included.

Observation for bean characteristics includes the number of beans/pod and weight of dry beans. Flavor analysis was conducted to determine the clone flavor profile, including cocoa taste, bitterness, astringency, acidity, flowery, spicy, and others.

Data analysis was carried out using ANOVA with the Duncan test, while stability analysis was carried out by combined analysis and then assisted with AMMI (additive main effect and multiplicative interaction) biplot charts (Yan & Kang, 2003). Excel 2010 program is used to carry out the regression correlation analysis.

RESULTS AND DISCUSSION

Potential Yield and VSD Resistance

Results of the variance analysis showed the different characteristics in flush intensity, flowering intensity, VSD disease attack intensity, and number of pods formed in several fine flavor cocoa genotypes tested (Table 3). These differences indicated that each genotype has different potential in producing flush, flower, pod, and response to VSD. The potential genetic information on each fine flavor cocoa genotype is important in the development of fine flavor cocoa planting materials that are resistant to VSD attacks and generate high production.

The analysis showed that there were different characteristics of flush intensity. KPC 1 had the highest average flush intensity for the period of 2016-2019 and was significantly different from other genotypes. PNT 30 showed the lowest average flush intensity and was not significantly different to PNT 8, PNT 12, PNT 18, PNT 33B, and PNT 37 (Table 4). There was a negative relationship between the ability of branch rejuvenation and its mortality rates after pruning. Plants with high rejuvenation rates were more tolerant of pruning treatments (Susilo & Suhendi, 2001). The differences in the rejuvenation level of branches between genotypes tend to correlate negatively with VSD attack scores (Susilo & Suhendi, 2001).

There were differences in flowering intensity. PNT 16 and KPC 1 showed the highest, and significantly different to the control clones. Meanwhile, PNT 30 showed the lowest flowering intensity and was not

Table 3. The variance analysis on flush intensity, flowering intensity, VSD scoring, and the number of pods in several fine flavor cocoa genotypes for the 2016-2019 observation period

SV	df	Mean square (MS)			
		Number of pods	VSD intensity	Flush intensity	Flowering intensity
Genotype	14	501.07 *	0.42 *	1.39 *	2.08 *
Year	3	2680.23 *	22.05 *	3.54 *	1.39 *
Genotype x Year	42	228.32 *	0.13 *	0.20 *	0.19 *

Description: Real f-count test *) and not real ^{ns}) at α = 5%.

Table 4. Flush intensity, flowering, VSD scores and the number of pod in several fine flavor cocoa genotypes

Genotype	Number of pods per tree / year	VSD score	Flush score	Flowering score
PNT 8	14.16 bc	1.07 a	0.79 de	0.75 b
PNT 12	1.30 c	0.77 ab	0.57 de	0.53 bc
PNT 16	66.58 a	0.40 c	0.92 cd	1.45 a
PNT 17	18.64 bc	0.74 ab	1.02 bc	0.85 b
PNT 18	14.7 bc	0.73 ab	0.87 de	0.77 b
PNT 18 K	5.88 bc	0.67 bc	1.31 b	0.63 bc
PNT 30	0.90 bc	0.63 bc	0.52 e	0.24 c
PNT 33B	16.55 bc	0.79 ab	0.81 de	0.80 b
PNT 37	5.08 bc	0.80 ab	0.72 de	0.60 bc
PNT 38	9.92 bc	0.87 ab	0.96 bc	0.92 b
KPC 1	27.37 b	0.73 ab	1.74 a	1.67 a
KPC 2	20.04 b	0.59 bc	1.09 bc	0.61 bc
DR 1	3.76 bc	0.62 bc	1.01 bc	0.52 bc
DR 2	6.85 bc	0.92 ab	0.95 bc	0.93 b
DR 38	9.12 bc	0.87 ab	0.92 cd	0.61 bc

Note: The numbers followed by same letter in the same column are not different based on the DMRT test at α 95% confidence level.

different to the control clones of DR 1 and DR 38. Flowering phenology is an essential aspect in the growth cycle of cocoa plants. The performance of plants, especially flowering, has a relationship with anthesis time, receptive time and duration of the pistil, pod formation, crossing, and bean development, which are vital in the process of plant breeding (Adjaloo *et al.*, 2012). Clarke (1974) stated that a good understanding of flowering phenology in plants would provide significant insight into plant growth and reproductive attributes.

The highest VSD disease intensity was shown in PNT 8 genotype and was not considerably different to the DR 2 and DR 38 as control. DR 2 and DR 38 are fine flavor cocoa clones that are not resistant to VSD, while DR 1 was a fine flavor cocoa clone, which is included in moderate resistance to VSD. PNT 16 showed the lowest VSD attack rate, which was not substantially different from the DR 1 as control clone but different from the DR 2 and DR 38. PNT 16 genotype had excellent potential for resistance to VSD; therefore, it can be developed as a fine flavor cocoa which resistant to VSD. The previous test results also showed that PNT 16 had a slight and lower stomata density as compared to the DR 2. It means that the low density of the stomata indicates the criteria of plant resilience because it provides a lower chance to the inclusion of spores into plant tissues (Anita-Sari & Susilo, 2014; Varquez *et al*., 1990). According to Susilo & Anita-Sari (2011), resistant planting material is useful and proficient in the control of VSD disease. The ability of plants to deal with pests and diseases depends on the resilience and vulnerability of plants (Anita-Sari *et al*., 2017). The severity of the disease is influenced by interactions between plants, pathogens and the environment, precisely agro-climate conditions (Ghini *et al.*,2008)

Moreover, having excellent resistance to VSD disease, the PNT 16 genotype displays a quite good yield potential, which was evidenced by the high quantity of pods per tree per year. In the 4 years old, a total of pod was 66.6 pods/tree/year was expressively different from other genotypes and control clones. Meanwhile, PNT 12, PNT 30, and PNT 37 presented the lowest potential yield.

Correlation and regression analysis displayed that the high flush intensity was associated with the flowering rate of the fine flavor cocoa genotype, as well as the number of pods formed (Figure 1.a.c.d). Besides, the high intensity of flush tends to specify the low intensity of VSD attacks (Figure 1.b).

Results of the analysis showed that the average flush intensity, flowering intensity, VSD attack rate, and the number of pods in all genotypes tested differed between years of observation (Figure 2). The highest flush intensity occurred in the period of 2018. The highest rate of flowering occurred in 2019 and was followed by the highest number of pods in the same year. Meanwhile, the highest intensity of VSD attacks occurred in 2017. Adjaloo *et al*. (2012) specified that flowering phenology significantly affects the functional features of plant growth and reproduction in cocoa. Synchronization between flowering phenology and pollination cycle is the key to enhance cocoa production. The increase of VSD attacks in 2017 might be due to the high number of dry months or less rainfall, because the total rainfall is lower in 2017 as compared to the previous year (Table 5). Correlation analysis displays that high rates of VSD attacks tend to occur in years with a high number of dry months. Other than genetic factors, environmental factors are also responsible for

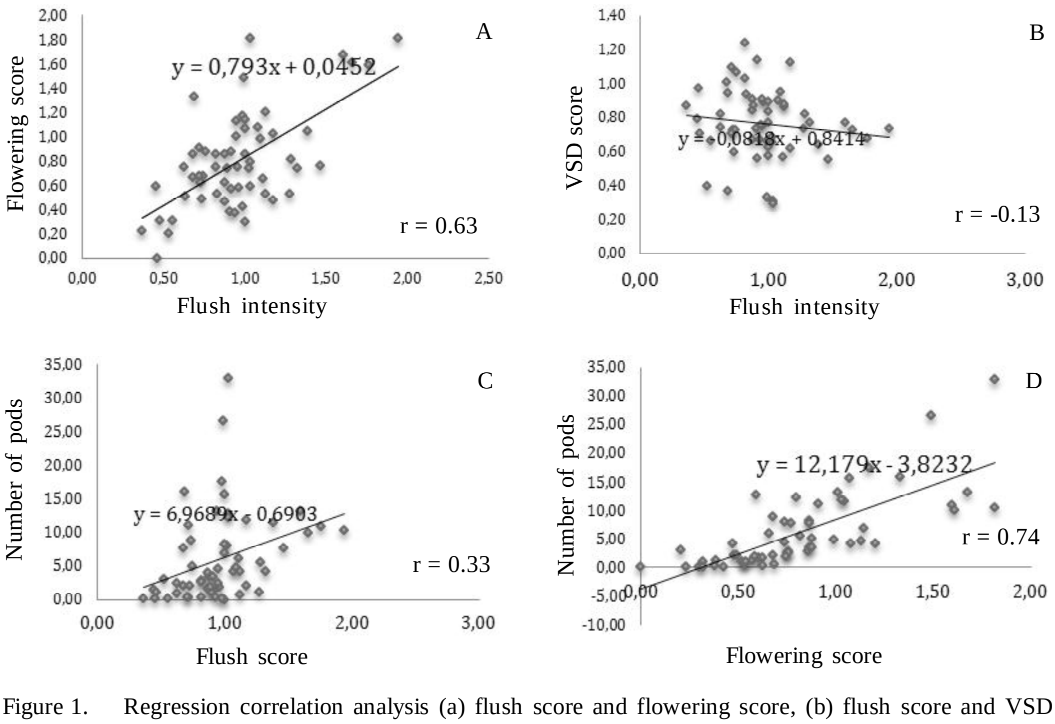

Figure 1. Regression correlation analysis (a) flush score and flowering score, (b) flush score and VSD score, (c) flush score and number of pods (d) flowering score and number of pods

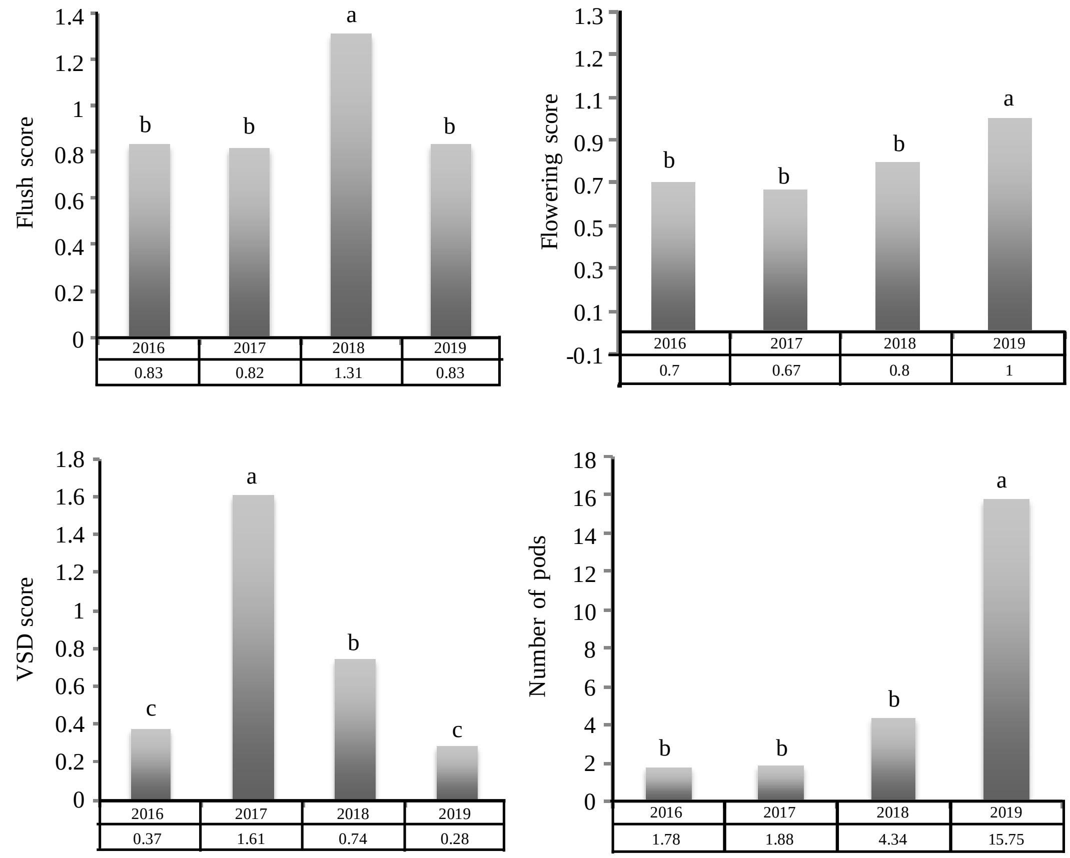

Figure 2. Average of flush score, flowering, VSD score and number of pods in several genotypes of fine flavor cocoa for the period of 2016-2019

Table 5. Rainfall and VSD scores in the Kaliwining Experimental Station for the 2016-2019 period

	2016	2017	2018	2019
Average VSD score	0.48	1.97	0.93	0.80
The amount of rainfall per year (mm)	3345.5	1892	1896.1	2523
Number of dry months	1	4	4	7
Correlation of VSD score and number of dry months	0.20			

severity of the disease because it is helpful in reproduce, spread, and carry out new infections. Sinaga (2003) stated that the environmental factors that significantly increase the severity of disease attacks include humidity, rainfall, wind, and temperature. Anita-Sari *et al.* (2017) described that the climatic factors such as rainfall, number of wet months, and altitude affect the severity of VSD attacks. Decreasing the number of wet months correlates with the rate of VSD disease. In dry conditions, there will be an inhibition of water transport; therefore, it results in disease severity. In high rainfall, the fungus will inhibit a portion of the water circulation in the xylem. Therefore the available water is not sufficient to carry out photosynthesis and transpiration as well as other physiological procedures. The genetic and environmental impacts can be examined through genetic testing in several different conditions, both environment and season (Trustinah & Iswanto, 2013).

AMMI analysis displays that in the 1-3 years old of plants (the period of 2016-2018), the fine flavor cocoa genotype has come into the pre-fertilization period evidenced by the initiation of pod production. In the 4th years old, PNT 16 genotype presented the highest potential yield as compared to other genotypes (Figure 3).

The analysis results exhibited that some of the taste fine flavor cocoa genotypes had relatively good VSD stability. Genotypes that displayed low resistance to VSD in the 2016-2019 observation period included PNT 8, PNT 12, PNT 30, and DR 2.

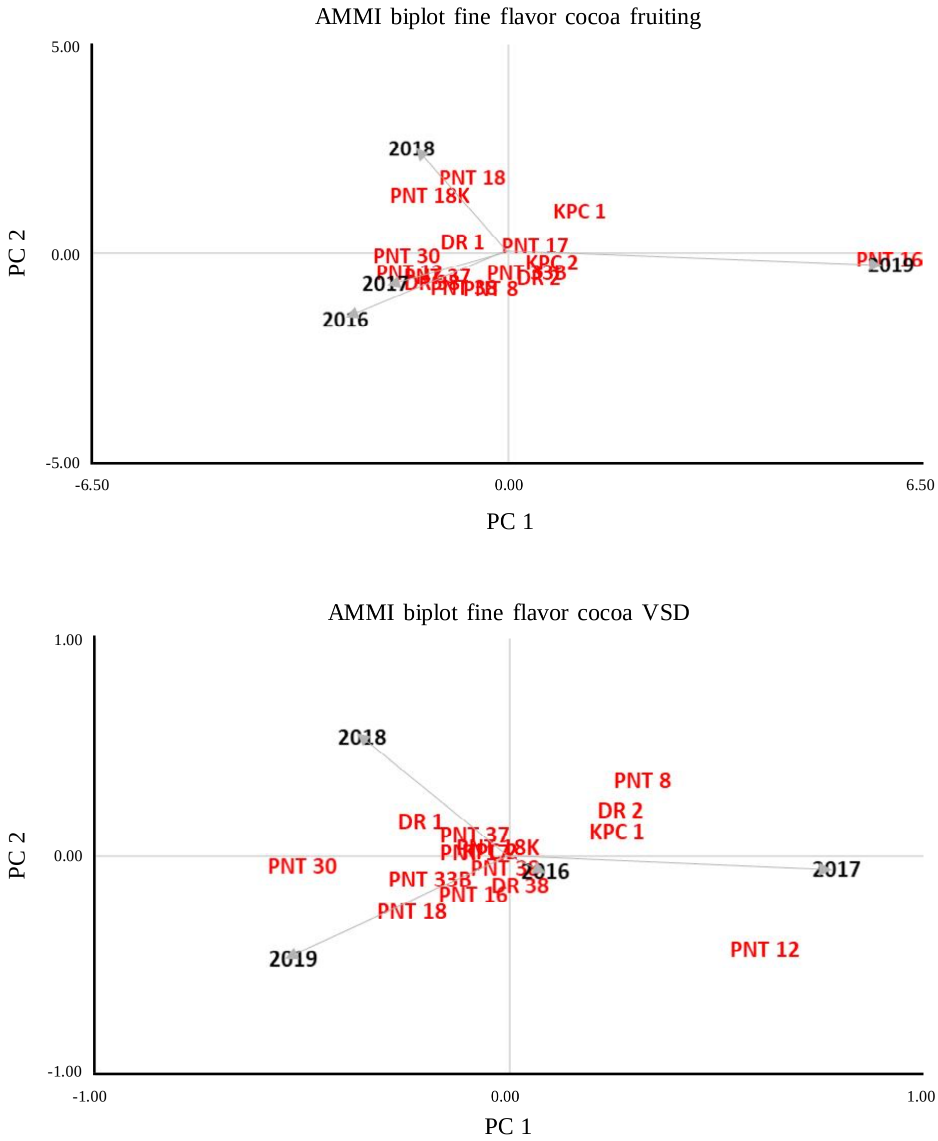

Figure 3. Stability analysis (AMMI) of several fine flavor cocoa genotypes period of 2016-2019

Bean Quality and Flavor

Beans and flavor quality analysis were focused on the superior promising clones that have the potential to produce VSD resistant varieties of fine flavor cocoa, such as PNT 16 and PNT 17. PNT 16 as a resistant clone to VSD showed the percentage of white beans more than the standard of fine flavour cocoa (> 80%), which was 94.9% with an 35.1 beans per pod (Table 6). According to the research on flush and fresh beans colors conducted by Devy *et al.* (2018), it displayed that PNT 16 has close relations with the fine flavor cocoa group of DRC 16 and DR 2.

The analysis of the physical quality of PNT 16 beans presented that this genotype had a good potential of dry bean, which is 1.25 grams per dry bean, although the weight is less as compared to clone DR 38. PNT 16 had a higher pod index (26.37) as compared to DR 38. However, if observed from the average number of pods per tree per year (Table 7), PNT 16 has a high yield potential of 2.52 kg of dry beans/tree /year.

PNT 16 has a good flavor potential with the main flavor profile, including cocoa, astringency, and bitterness similar to KW 617 (ICCRI 09) with higher acidity levels as compared to ICCRI 09 (Figure 4.A). ICCRI 09 is one of the superior clones of cocoa that have particular floral flavors. PNT 16 has a primarily attributed profile of different flavors with DR 2, which is more dominated by a bitter taste.

The analysis results exhibited that PNT 16 was dominated by the smell of browned roasted, nutty, and browned fruit (Figure 4B). This character was different from the smell of ICCRI 09, which was dominated by browned roasted, nutty, woody, and floral, and DR 2 was dominated by floral smell. The unique taste of both primary and additional attributes provides the opportunity for the clone to be developed into a specialty product.

Table 6. Character analysis of physical beans quality on several superior promising clones of fine flavor cocoa resistant to VSD

Genotype	Number of beans per pod	White bean (%)
PNT 16	35.60 ± 6.18	90.06 ± 7.14
PNT 17	29.80 ± 4.82	97.92 ± 9.84
PNT 18	29.00 ± 4.23	99.39 ± 1.05
DR 38	37.01 ± 2.54	99.41 ± 1.01

Table 7. Character analysis of physical bean quality on several superior promising clones of fine flavor cocoa resistant to VSD

Genotype	Weight per bean (g)	Nibs weight (g)	Shell content (%)	Pod index
PNT 16	1.25 ± 0.22	1.08 ± 0.21	13.43 ± 2.28	26.37 ± 0.18
PNT 17	1.10 +0.07	0.95 ± 0.16	13.53 ± 2.76	30.99 ± 1.71
DR 38	1.42 ±0.13	1.29 ± 0.13	9.65 ± 1.86	20.95 ± 1.08

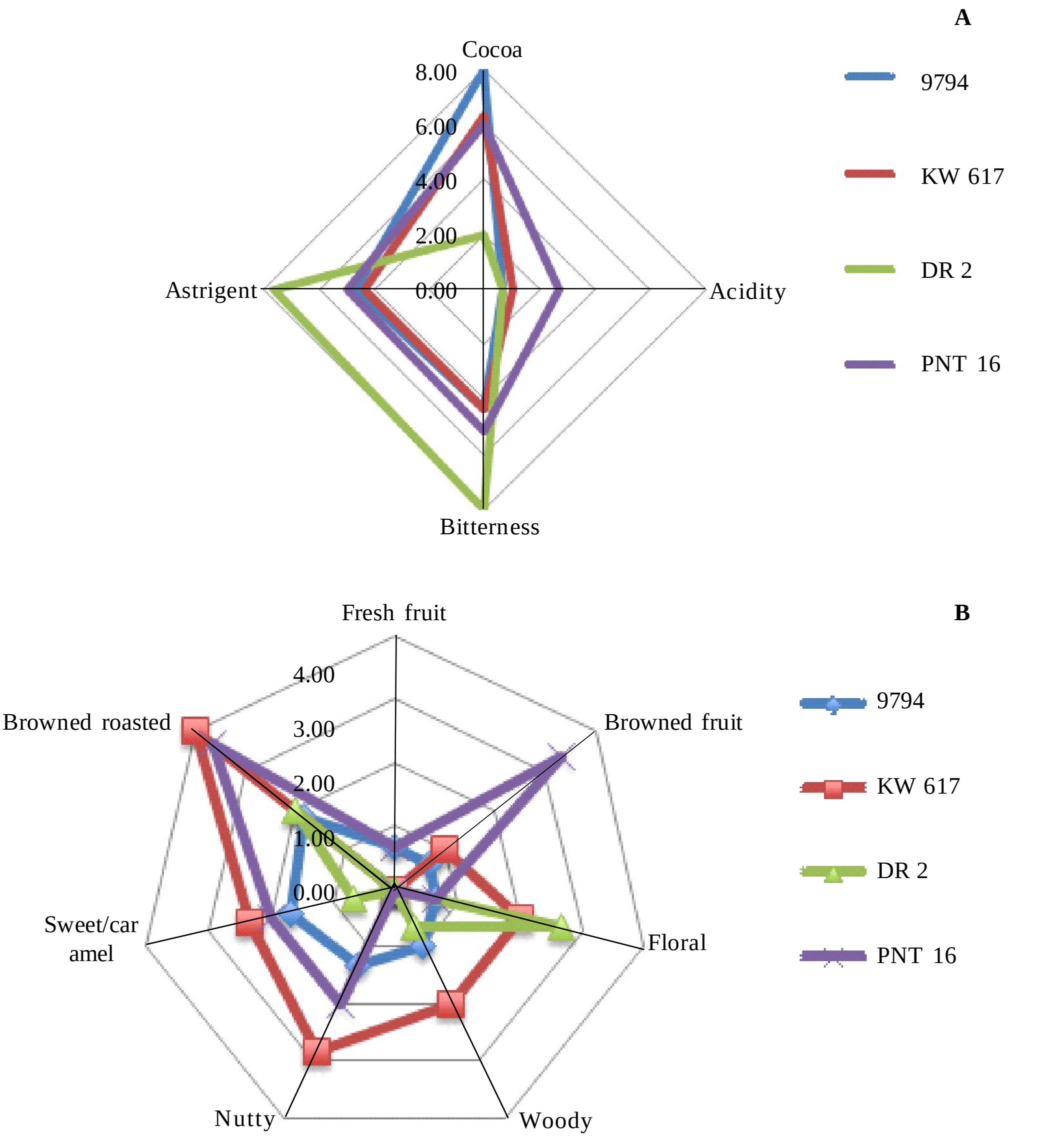

Figure 4. Flavor characteristics of fine flavor cocoa clones (A) main attributes and (B) additional attributes of flavor

CONCLUSIONS

PNT 16 has the potential to be developed as a fine flavor cocoa superior clone resistant to VSD as indicated by the high percentage of fresh white beans (94.9%); the initial yield was high (2.52 kg dried beans/tree/year) and exhibited the lowest VSD score in the period of 2016-2019. The main characteristics of PNT 16 flavors was similar to ICCRI 09, which was dominated by the aroma of browned roasted, nutty, and browned fruit; therefore, it had the potential to be developed as a specialty cocoa product.

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