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Flush Characteristics of Several Cocoa Genotypes Different in Resistance to Vascular Streak Dieback

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Abstract

Vascular streak dieback (VSD) is one of the main diseases on cocoa that can cause a decrease in production and even death on susceptible plants. The use of selection criteria is very important in the selection process at the seedling phase, young plants and even mature plants in order to support the breeding process of resistant varieties. The aim of this study was to determine the characteristics of flush including flush color, stomata characters and duration of flush color change to green or towards mature leaves as one of the selection indicators for VSD resistance in cocoa. The research was conducted at Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute, Jember, Indonesia. Study of flush color and stomata characters was conducted using a randomized complete block design consisting of four cocoa genotypes with different levels of resistance, classified as resistant to VSD disease (Sulawesi 1, PNT 16) and as susceptible (BCL and BL 50). Each clone was repeated three times and each replication consisted of three plant samples. Duration of flush color change study was carried out on six cocoa genotypes as treatments, three genotypes with resistant to VSD (PNT 16, Scavina 12, Sulawesi 1) and three genotypes as susceptible to VSD (BL 50, BCL, Criollo 22). Each treatment was repeated three times and each replication consisted of three plant samples. The results showed that the resistant genotypes showed a tendency to have lower chlorophyll and anthocyanin content than the susceptible ones. The stomata character in the resistant genotypes was not different than the susceptible ones, however, the resistant genotype showed that the stomata density at flush tended to be lower. The duration of flush color change to mature leaves (green) in resistant genotypes was significantly faster than susceptible genotypes.

Keywords: duration of flush color change, flush color, stomata characters, *Theobroma cacao* L., vascular streak dieback

INTRODUCTION

In Indonesia, vascular streak dieback (VSD) disease is one of the factors that causes decreasing of cocoa production. This disease attacks the xylem of flush tissues and will show symptoms after three months of infection. VSD disease not only causes a decrease in plant productivity, but also causes death of susceptible plants. Utiliza- tion of resistant genotypes is the main strategy in controlling VSD disease effectively and efficiently (Susilo & Anita-Sari, 2011). The breeding of planting material which resis- tant to VSD is carried out through explo- ration, selection and crossing activities. An effort to support these plant breeding activi- ties, it is necessary to have selection criteria for early detection of the nature of plant resistance to VSD disease. The use of selec- tion criteria will be very useful in the selec- tion process for either the seedling phase, young plants or even mature plants.

The mechanism of VSD infection through flush takes time since the fungus begins to infect until the onset of disease symptoms. The period of flush color changes to green or mature leaves maybe related to the nature of plant resistance in response to this disease. Leaf flushing in cocoa is controlled both endogenously and environmentally. If envi- ronmental stresses are not apparent, it is mainly under endogenous control (Lahive *et al.*, 2019). The results of previous studies showed that leaf stomata character could be used as a selection indicator for VSD resistance (Anita-Sari & Susilo, 2014). Stomata is one of the selection criteria that can be used to detect the resistance of cocoa plants to VSD disease (Susilo *et al.*, 2016).

in form of chemical content or tissue structure
owned by plants to inhibit pathogen repro- duction (Collingborn *et al.*, 2000; Hulupi, 2008). The internal anatomy and surface features of the leaves often determine plant resistance to biotrophic pathogen infection (Pudjiwati *et al.*, 2013), such as stomata and trichome may influence disease resistance (Niks & Rubiales, 2002).

Indications of differences in color character and duration of color change from flush to mature marked by a change in color to green maybe related to resistance to VSD disease considering that VSD only enters plants through flush tissue. This study was conducted to examine the characteristics of flush in- cluding stomata character, flush color and

MATERIALS AND METHODS

Flush Color and Stomata Characteristics

The research was conducted at Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute, Jember, Indonesia. The study used a randomized complete block design (RCBD) consisting of four cocoa geno- types with different levels of resistance, classi- fied as resistant to VSD disease (Sulawesi 1, PNT 16) and susceptible to VSD (BCL and BL 50). Each clone was replicated three times and each replication consisted of three plant samples. Observation parameters include flush color and stomatal characters. Color analysis was carried out by measuring the content of chlorophyll and anthocyanins in the flush. The analysis of chlorophyll and anthocyanins was carried out at Jember the replica method referring to Anita-Sari & Susilo (2013) using transparent nail polish and stomata removal was carried out on the lower leaves. The stomata samples were then observed using a microscope and observed for number of stomata, width of stomata opening and density of the stomata. The leaf samples used were

Flush Color Change Duration

This study was also conducted at Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute. The study used a completely randomized block design consisting of six cocoa genotypes as treatment, three genotypes as resistant genotypes (PNT 16, Scavina 12, Sulawesi 1) and three suscep- tible genotypes (BL 50, BCL, Criollo 22). Each treatment was repeated three times and each replication consisted of three sample plants. The duration of flush changes was observed by marking the newly emerged flush with a size of 7-10 cm and exposed to direct sun-

Data Analysis

Data were analyzed using one-way ANOVA via STAR 2.0.1 (IRRI) and Duncan's test was further carried out when a significant difference was found. The histogram was illustrated using Graphpad program. Principal component analysis (PCA) was performed with a dendogram

RESULTS AND DISCUSSION

Characteristics of Flush Color and Stomata

The result showed that there were dif- ferences of anthocyanin and chlorophyll content in flush between resistant and clones (Figure 1). Sulawesi 1 and PNT 16 which were resistant to VSD showed lower chlorophyll and anthocyanin content than susceptible clones (BCL and BL 50). This result is different from the opinion of Tellez *et al*. (2016) that anthocyanins content in tropical crops protect young leaves from damage caused by fungal attacks during leaf development into mature leaves. Plants will protect themselves from fungal attacks through the production of phenolic compounds such as flavonoids that function as anti-fungal (Queenborough *et al*., 2013; Cheng *et al*., 2018). However, a comprehensive review on the adaptive function of anthocyanins concludes that the evidence for a defensive function against herbivores and pathogens is not very strong (Schaefer *et al*., 2008).

The tendency for the high content of anthocyanins and chlorophyll in the flush of susceptible clones is related to the inhibition of chlorophyll in the photosynthesis process so that it interferes with plant biosynthesis. Chlorophyll plays a significant role in plant development (Li *et al*., 2018), the accumu- lation of anthocyanins and chlorophyll is related to the regulation of plant development (Dai *et al*., 2016). The purple color component, anthocyanins, and their combination with chlorophyll can cause an adjustment of the ratio of the photosynthetic system and improve electron transport performance. Anthocyanins can cover chlorophyll more deeply and inhibit the photosynthesis process (Cooney *et al*., 2015; Li & Martin, 2015).

Leaf color is generally used as a morpho- logical marker in plant breeding programs because this character is an inherited character and can be used in important approaches to plant breeding programs

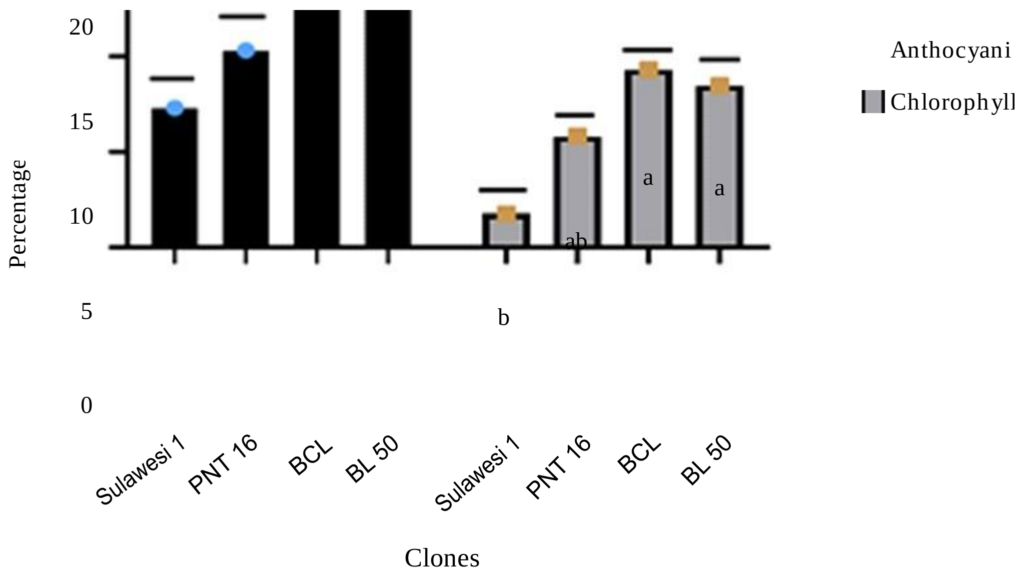

Figure 1. Anthocyanin and chlorophyll content in flush of some cocoa genotypes with different resistance levels

anthocyanin content exhibited high herita- bility in F2 generation (Jin *et al*., 2018). Variations in leaf color patterns are also important and necessary in the assessment of plant resistance (Plastino *et al*., 2006; Palmer & Mascia, 1980; Zhao *et al*., 2016).

The results of PCA analysis showed that there was a clear grouping between resistant and susceptible clones based on chlorophyll and anthocyanin content where BL 50 indi- cated one group with BCL and PNT 16 indi- cated one group with Sulawesi 1 (Figure 2). These results indicate that there are different patterns of anthocyanin and chlorophyll content in susceptible and resistant cocoa clones to VSD. Adaptation of plants to the environment can be induced by the propor- tion of colors that affect plant development, limiting the size of biological functions, espe- cially the photosynthetic system (Kerchev *et al.*, 2011) and the antioxidant

The characteristics of stomata in the flush did not show any difference between resistant and susceptible clones including its length, diameter, opening width and stomatal density (Table 1). The results of the analysis showed that there was no significant difference in stomatal flush density between resistant and susceptible genotypes, but resistant geno- types tended to have lower stomatal density than susceptible genotypes. The research results by Susilo *et al*. (2016) indicated that there was a correlation of stomata charac- teristics on young leaves and VSD disease resistance. The number of stomata and the width of stomata openings on old leaves showed a significant correlation to VSD disease resistance in cocoa. It showed that there were differences of stomata charac- teristics on different leaves age.

The low level of stomata density at flush provides a smaller opportunity for spores to enter the plant tissue. The higher the

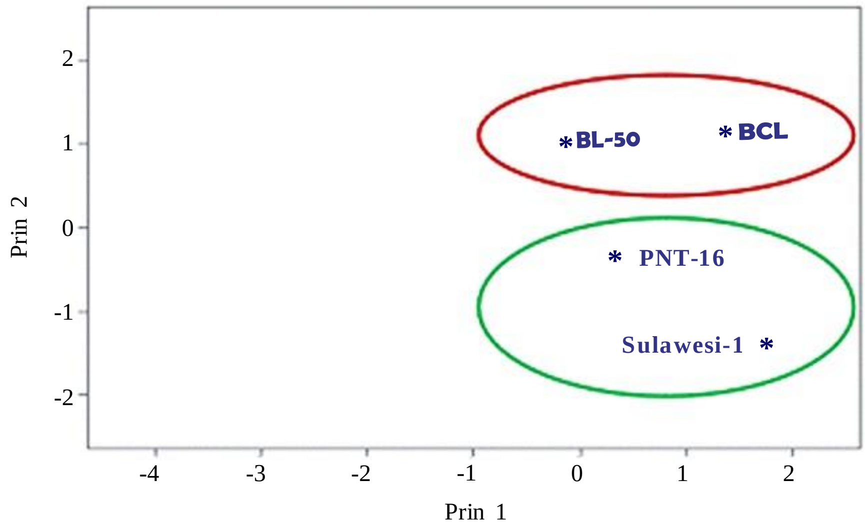

Figure 2. PCA biplot on anthocyanin and chlorophyll content of four cocoa genotypes

Table 1. Characteristics of stomata of flush in several cocoa clones

Clone	Width of stomata (μm)	Diameter of stomata (μm)	Stomata apperture width (μm)	Stomata density μm^{-2}
Sulawesi 1	14.5 a	5.23 a	1.15 a	242 a
PNT 16	14.5 a	4.96 a	1.06 a	317 a
BCL	14.4 a	5.33 a	1.09 a	430 a
BL50	14.3 a	4.99 a	1.06 a	386 a

tion process and also affect the capture of $_2$ from the air, so that the chance of entering

The incubation period is the time from inocu-
lation to the appearance of symptoms. Stomata density has been used as a selection indicator in breeding disease resistance in

Flush Color Change Duration

The results of the analysis showed that there was a difference in the duration of the flush color change to green in the cocoa geno- types tested. Criollo 22, BCL, and BL 50 clones required a longer duration and were signifi- cantly different than Sulawesi 1, Sca 12, and PNT 16 (Table 2).

The resistant clone group showed a rela-
tively faster duration of flush change compared to the susceptible clones (Table 2). The duration of time used to change the flush color to green in resistant clones ranged from
7-8 days, while for susceptible clones it was more than 10 days. The short period of time for flush discoloration indicates the least opportunity for spores to enter plant tissues. The length of time required for flush color change in susceptible plants provides a greater opportunity for spores to infect the flush so that the fungus can enter plant tissues.

Disease resistance is related to the

Table 2. Analysis of the variance of flush color change duration on several cocoa genotypes

Genotype	Duration of flush color change (days)	VSD resistance
Criollo 22	14.00 a	Susceptible
BCL	10.62 b	Susceptible
BL 50	10.11 b	Susceptible
Sulawesi 1	8.33 cd	Resistant
PNT 16	7.12 d	Resistant
Sca 12	7.00 d	Resistant

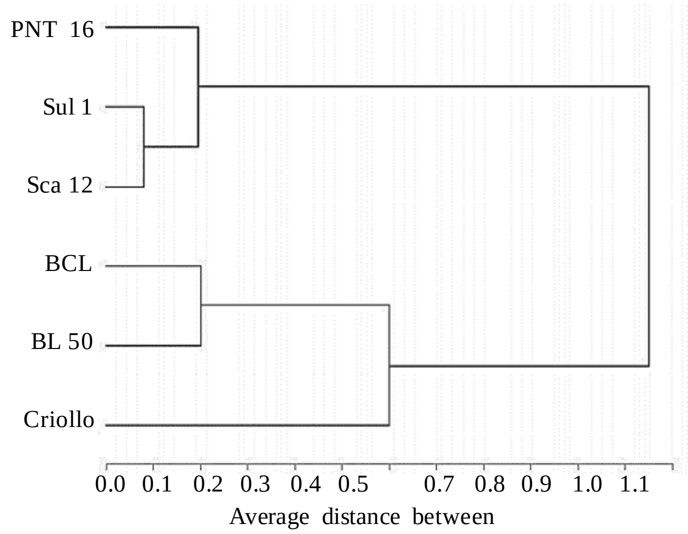

Figure 1. Cluster analysis based on duration of flush color change in some cocoa genotypes

leaves. Susceptibility will stop when the leaves are old because these conditions no longer support the growth of the pathogen (Coelho & Monteiro, 2003). Likewise, other plants including broccoli, show high susceptibility to older leaves because leaves that are already chlorotic are difficult for pathogens to grow and pathogens can no longer sporulate (Agnola *et al*., 2003).

In contrast, Kus *et al.* (2002) found that older plants or leaves showed increased resistance and decreased susceptibility to pathogens, this form of resistance is often referred to as age-related resistance (ARR). This result is similar to that of the resistance of cocoa plants to VSD, the faster the leaves turn green (mature) the plants

response is thought to be controlled by a single resistance gene expressed in mature plants (Roelfs, 1984). There is a positive correlation between increased leaf/plant age and glycoline production (Lazarovits *et al*., 1980).

The results of cluster analysis based on flush color duration and VSD scores showed that there were two groups, namely group I with longer duration category and high VSD level, namely Criollo 22, BL 50 and BCL and group II with fast duration

CONCLUSIONS

The content of anthocyanins, chlorophyll and duration of flush color change to mature can be used as indicators

resistance to vascular streak dieback disease
in cocoa. Resistant genotypes tend to have lower chlorophyll content, flush anthocyanins and stomatal density with the duration of flush color change to mature leaves faster

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