



# Effect of rootstock and scion on resistance of cocoa clones to vascular streak dieback caused by *Ceratobasidium theobromae*

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## ABSTRACT

Vascular streak dieback (VSD) disease, caused by *Ceratobasidium theobromae*, is one of the cocoa diseases responsible for decreasing cocoa production and declining cocoa plantation area in Indonesia. Planting cocoa clones with partial resistance to VSD has been useful in managing the disease. These have been produced by grafting or budding selected resistant genotype of cocoa onto unselected seedlings. The main objective of this study was to evaluate the effect of rootstock × scion combinations on VSD resistance through top grafting. The experiment used five rootstocks and scions selected from five clones, namely: MCC-01, Sulawesi 1 (S-1), M-05, RB, BB-01 and the disease evaluation was carried out under natural infection conditions for 18 months. The experimental area was surrounded by cocoa trees from a severely VSD-infested cocoa farm. M-05 performed well for suppression of VSD incidence and severity as a scion regardless of the genotype of the rootstocks followed by Sulawesi 1 (S-1). The rootstocks M-05 and S-1 did not significantly reduce VSD incidence or severity on the susceptible scions from different clones such as MCC-01, RB and BB-01. MCC-01 showed a higher incidence and severity of VSD as a scion regardless of the rootstock genotype. This research proves that rootstocks from cocoa genotypes considered resistant, moderately susceptible, or susceptible to VSD, have little effect on cocoa scion resistance to VSD. Cocoa scion genotypes play a crucial role in VSD resistance.

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## 1. Introduction

Cocoa plantations in Indonesia have experienced the invasion of plant pests and diseases, and their impact strongly influences the quantity and quality of produced cocoa in the area. Among important diseases, vascular streak dieback (VSD) causes the death of many young trees as well as dieback on mature trees. VSD is currently confined to SE Asia and parts of Melanesia. In Papua New Guinea, it is considered a minor disease because of planting more resistant genotypes of cocoa. In Sulawesi, an upsurge in VSD incidence since 2000 has caused many farmers to abandon cocoa as a crop (McMahon et al. 2018). VSD disease is caused by the basidiomycete fungus *Ceratobasidium theobromae* (formerly *Oncobasidium theobromae*) (Keane and Prior 1991; Samuels et al. 2012).

The pathogen spreads by the wind in the night and is thought to penetrate plant tissue through young leaves. Spores are not spread far away. Very few infections occur beyond 80–100 m from inoculum

source (Keane and Prior 1991; Guest and Keane 2007, 2018). The symptoms of the disease were described by Keane and Prior (1991), Guest and Keane (2007), and later by McMahon and Purwantara (2016). Necrotic lesions and chlorosis on the leaf as well as dark vascular discoloration, are observed when branches are split. When diseased leaves fall down from symptomatic branches, the three vascular bundles traces that are observed on leaf scars also show dark discoloration. Swollen lenticels on the bark of infected branches are causing the rough surface of the bark, another very typical symptom of VSD infection.

As *C. theobromae* is a vascular pathogen, control with fungicides is difficult, and fungicides have not been shown to be effective in the field. They are generally too costly for smallholder farmers (Holderness 1990). Currently, the most common control method applied by farmers is the sanitation pruning of diseased branches. However, sanitation pruning is sometimes impractical, especially on infected young cocoa trees and seedlings. In mature cocoa plantations, to maintain VSD occurrence below 1%, infected branches should be cut about 30 cm below the end of the brown streaking symptoms at 2-weekly intervals for nearly two years (Parwirosoemardjo and Purwantara 1992). However, the infected young cocoa trees and seedlings might die because of the diseased sanitation pruning. The use of VSD resistant genotypes is a promising

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method and has been promoted to farmers in Papua New Guinea, Malaysia, and Indonesia. Since the 1980s, planting of resistant genotypes has been successful in controlling the disease in Papua New Guinea, Malaysia, and Indonesia and many partially resistant clones have been selected and been widely planted. These improved genotypes combined resistance with high yield and quality (e.g. PBC123 - S1) (Efron et al. 2002; Keane 2015; Papua New Guinea Cocoa Extension Manual, 2017; Guest and Keane 2018). Delivery of the appropriate genotype to farmers' fields with a combination of high-yielding, VSD resistant clones with good bean characteristics remains a challenge in Sulawesi (McMahon et al. 2018). However, it has been done effectively in Malaysia and PNG since the 1970s, leading to a decline in the damage caused by VSD. Partial but adequate resistance has been combined with high yield and quality (Efron et al. 2002; Keane 2015).

Grafting is a popular and old technique to propagate plants vegetatively by combining two segments of a plant, rootstock and scion; the technique allows the growers to combine desirable properties of both the scion and rootstock, including resistance properties to pest and disease. Grafting has an important role in the control of plant diseases, including epidemics from a variety of soil-borne diseases, foliar diseases and viruses. However, grafting can also cause the spread of many important plant diseases (Goldschmidt 2014; Asman et al. 2019). The rootstock can influence scion traits such as scion vigor, yield and quality. In *Hevea brasiliensis*, it was shown that the rootstock has a significant influence on graft growth and yield (Buttery 1961; Cardinal et al. 2007). In *Theobroma cacao*, the rootstock influences the vigor of the scion and yield in the first years from 3.5 years of study (Yin 2004). The rootstock may also modify scion's resistance to diseases. For instance, in *Poncirus trifoliata*, rootstocks influence citrus resistance against Tristeza virus (Mudge et al., 2009). In *T. cacao*, rootstocks confer tolerance to Witches' Broom Disease (WBD) to the scion (Ribeiro et al. 2016). A variety of mechanisms are involved in resistance effects linked to grafting, including non-host resistance, rootstock vigor effects and rootstock-induced systemic defense, such as defense-related phytohormones, long-distance signals, defense-related enzymes, or antioxidative defense mechanisms (Guan et al. 2012; Goldschmidt 2014; Ribeiro et al. 2016).

Based on the hypothesis that resistance to VSD varies in cocoa and that rootstocks can influence VSD resistance of scions through grafting, the present research aimed to assess the VSD-incidence on cocoa scions of Sulawesi local clones in the field after grafting onto rootstocks from cocoa resistant genotype trees, to evaluate the effect on VSD-incidence in the scions on various cocoa rootstocks and to study whether a clone with proved resistance to VSD will result in an equivalent resistance if it is used as a rootstock.

## 2. Materials and methods

### 2.1. Plant materials and growth condition

Cocoa seedlings were prepared under a nursery building located in Bupon sub-district, Luwu District, South Sulawesi Province, Indonesia. Five rootstocks and scions were selected. They were selected according to their range of resistant reactions to VSD and yield performance based on the Mars Inc. study. All selected clones were classified as interpopulation hybrids (Dinarti et al. 2015):

MCC-01: Originates from the North Luwu District of South Sulawesi Province, Indonesia and selected in the 2000s by local farmer selections Pak Muchtar and Mars Inc. MCC-01 is considered a high yield clone and known to be susceptible to VSD.

Sulawesi 1 (S-1): Originates from Malaysia. Also known as 'PBC123'. A popular clone in Sabah in the mid-nineties issued from a large clonal selection from local hybrid trees at Prang Besar plantation. Medium vigor and tolerant to 'Vascular Streak Dieback' disease (Yin 2004). The clone has known strong partial resistance to VSD (Chong and Shepherd 1986).

M-05: Originates from the North Luwu District of South Sulawesi Province, Indonesia. M-05 is considered a low-yield clone with high resistance to VSD.

RB: Originates from the East Luwu District of South Sulawesi Province, Indonesia. RB was ranked as moderately resistant to VSD and medium to high yield.

BB-01: Originates from the Luwu District of South Sulawesi Province, Indonesia. The clone was selected for potential yield performance and unknown resistance to VSD.

### 2.2. Preparation of seedling for rootstock and selection of scion

The seeds for rootstock production were harvested from the clonal trees of the five selected clones (MCC-01, S-1, M-05, RB, BB-01), washed, removing their placenta, and soaked overnight. The seeds were treated with 1% Dithane M-45 fungicide (a.i. mancozeb 80%) before being placed on the germination sacks. After 2–3 days, the germinated seeds were planted in polyethylene (PE) bags containing 1.0 kg soil and placed in a nursery where the temperature ranged from 27 to 31 °C and relative humidity ranged 76 to 90%. Rootstock seedlings were grown in a nursery shade house with UV plastic as a roof and were irrigated daily.

Scions were taken from plagiotropic branches of 5 selected clones (MCC-01, S-1, M-05, RB, BB-01), 5–7 mm in diameter with a length of  $\pm 9$  cm of healthy scions. Only scions that showed green brownish to brownish buds were selected.

### 2.3. Grafting

Grafting was conducted in a nursery shade house with three-month-old seedlings. The process began with the rootstock being cut on top about 10–20 cm above the cotyledonary mark using a sharp grafting knife, leaving 3–4 leaves below the cut. The cut stem was split for 2–3 cm in depth. Scion was prepared by cutting the budwood in a wedge symmetrically in a V shape on the bottom with a length of 2–3 cm. The scion was inserted into the split top of the rootstock and wrapped with plastic from the bottom to the top. The graft was covered with a clear plastic bag for 20–25 days to maintain high atmospheric humidity around the scion (Asman et al. 2019).

### 2.4. Field trial establishment

A field trial was established in Masamba sub-district, North Luwu District, South Sulawesi Province, one of the areas with the highest infestation of VSD in South Sulawesi, Indonesia: latitude (2°37'32.9"S and longitude 120°20'48.9"E). Cocoa trees were planted three months after grafting in April 2009. There were 5 rootstocks and 5 scion clones, which resulted in 25 scion  $\times$  rootstock combination treatments. There were three replicate blocks with 25 treatments (combination) in each block. Each treatment combination consisted of 4 trees, providing a total of 300 trees (Fig. 1). Cocoa and shade trees were planted at a spacing of 4 m  $\times$  4 m (Fig. 2). *Gliricidia sepium* shade trees were planted three months before cocoa seedlings were planted. Shade trees were pruned within five to nine months after planting with light and heavy pruning, respectively. One hundred gram of NPK fertilizer (Phonska brand) with nutrient composition Nitrogen (N): 15%, Phosphate (P): 15%, Potassium (K): 15%, Sulfur (S): 10% and 100 g superphosphate (36%) was added to each tree in equal amounts at the time of planting. The following fertilization was repeated every three months. 100 g NPK (Phonska brand) was applied per tree in two rounds (three and six months after planting), whereas 200 g NPK (Phonska brand) per tree was applied at nine months and a year after planting, while 300 g NPK (Phonska brand) was applied in the next three months until 18 months after planting. Leaf pests were

|      |      |      |      |      |      |      |      |      |
|------|------|------|------|------|------|------|------|------|
| R4S3 | R5S2 | R5S5 | R1S4 | R1S5 | R3S4 | R4S1 | R4S5 | R3S2 |
| R1S1 | R2S5 | R3S3 | R4S2 | R5S4 | R1S3 | R2S3 | R1S2 | R2S4 |
| R2S2 | R4S1 | R1S5 | R5S1 | R5S3 | R4S4 | R3S5 | R2S1 | R1S1 |
| R1S2 | R3S5 | R5S1 | R1S1 | R2S3 | R4S5 | R3S3 | R4S3 | R5S2 |
| R1S3 | R1S4 | R2S1 | R3S2 | R3S1 | R2S5 | R5S4 | R4S2 | R5S1 |
| R4S5 | R2S4 | R5S3 | R5S5 | R2S2 | R1S2 | R1S4 | R3S4 | R5S5 |
| R4S4 | R3S2 | R4S2 | R5S2 | R3S3 | R2S4 | R5S3 | R4S4 | R3S1 |
| R3S4 | R3S1 | R5S4 | R2S1 | R4S3 | R4S1 | R2S5 | R1S3 | R2S2 |
|      |      | R2S3 |      |      | R3S5 |      |      | R1S5 |

Fig. 1. Trial design in the field; the number is a code for a combination of five clones used as rootstocks and scions.

controlled by the application of synthetic pyrethroid insecticides (a. i. lambda-cyhalothrin 25 g/l) when monitoring threshold values were exceeded. During three months after being planted, young cocoa was protected from sunlight using a fresh coconut leaf as a temporary shade. Cocoa trees were pruned for maintenance trees height control only.

## 2.5. Evaluation

VSD was evaluated by its incidence and severity each month for 14 months (September 2009 to October 2010), starting from five-month until 18 months after planting. VSD incidence was measured by calculating the proportion of plants with any infection symptom (the number of infected trees divided by all sampled trees). VSD severity was assessed once a month by scoring disease severity in individual trees as follows: 0 = No visible symptoms on leaves; 1 = ~10% of branches infected (Leaf shows necrotic lesions and chlorosis, or a mixture of both symptoms with three or more vascular bundles traces on leaf scars); 2 = 10–25% of branches infected; 3 ≥ 25% of branches infected.

Mean disease severity in each plot for each rootstock × scion combination was calculated using the formula (Mayee and Datar 1986):

$$I = \frac{\sum (n \times v)}{Z \times N} \times 100$$

where  $n$  represents the number of infected plants on each score,  $v$  is a score on each infestation category,  $Z$  is the highest score and  $N$  represents the total number of plants observed.

The area under the disease progress curve (AUDPC) was the second method of measurement of VSD severity and incidence development. AUDPC was calculated as the total area under the graph of disease severity and incidence against time, from the first evaluation to the last (from five-month until 18 months after planting) for each rootstock × scion and these were analyzed by analysis of variance (Madden et al., 2007; Shaner 1977):

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+2}}{2} \times (t_{i+1} - t_i)$$

where  $y_i$  is an assessment of disease severity or incidence (per tree) at the  $i$ th observation,  $t_i$  is the time at the  $i$ th observation, and  $n$  is the total number of observations.

## 2.6. Data statistical analysis

Analysis data was conducted by factorial ANOVA with the 5 scions and 5 rootstocks as factors 1 and 2, respectively, to determine the effect of every single factor and their interaction (scion × rootstock). When significant differences were detected, means were separated by Tukey's test at the level of significance ( $P < 0.05$ ) or 5% probability level.

## 3. Results

Rootstock × scion combinations showed highly variable of VSD incidence or severity. VSD disease symptoms emerged variably according to the combination of rootstock and scion from five until 16 months after planting. There is an effect of the scion (factor 1) on VSD incidence and severity where the influence was highly significant at the mid and final of evaluation (12,15,18 months after planting) whereas rootstock (factor 2) and interaction (scion × rootstock) effect were not significant in any evaluation event. However, at the beginning of the evaluation (six and nine months after planting), the mean incidence of the scion, rootstock and interaction (scion × rootstock) was not significantly



Fig. 2. Cocoa (13 months after planting) and shade trees position.

**Table 1**

Vascular streak dieback (VSD) incidence and severity in five cocoa genotypes that were used as rootstocks and also as scions evaluated 6, 9, 12, 15 and 18 months after planting in South Sulawesi (From September 2009 to October 2010). In all treatments, VSD symptoms were first observed 5 months after field planting.

| Scion  | Rootstock | % VSD incidence<br>6 months after<br>planting | % VSD incidence<br>9 months after<br>planting | % VSD incidence<br>12 months after<br>planting | % VSD incidence<br>15 months after<br>planting | % VSD incidence<br>18 months after<br>planting | Mean disease severity<br>18 months after<br>planting | Mean AUDPC<br>disease severity for<br>14 months |
|--------|-----------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-------------------------------------------------|
| MCC-01 | MCC-01    | 8                                             | 8                                             | 39                                             | 100                                            | 100                                            | 67.6                                                 | 333.3                                           |
| S-1    | MCC-01    | 8                                             | 8                                             | 8                                              | 8                                              | 75                                             | 27.8                                                 | 87.5                                            |
| M-05   | MCC-01    | 0                                             | 0                                             | 11                                             | 22                                             | 50                                             | 16.7                                                 | 57.4                                            |
| RB     | MCC-01    | 8                                             | 8                                             | 28                                             | 100                                            | 100                                            | 58.3                                                 | 250.9                                           |
| BB-01  | MCC-01    | 8                                             | 17                                            | 50                                             | 100                                            | 100                                            | 72.2                                                 | 325.5                                           |
| MCC-01 | S-1       | 8                                             | 33                                            | 92                                             | 92                                             | 100                                            | 78.7                                                 | 294.9                                           |
| S-1    | S-1       | 0                                             | 0                                             | 8                                              | 19                                             | 56                                             | 29.6                                                 | 130.1                                           |
| M-05   | S-1       | 0                                             | 0                                             | 0                                              | 11                                             | 11                                             | 3.7                                                  | 32.9                                            |
| RB     | S-1       | 0                                             | 0                                             | 8                                              | 58                                             | 100                                            | 41.7                                                 | 179.2                                           |
| BB-01  | S-1       | 0                                             | 0                                             | 17                                             | 92                                             | 100                                            | 58.3                                                 | 233.3                                           |
| MCC-01 | M-05      | 17                                            | 17                                            | 42                                             | 83                                             | 100                                            | 70.4                                                 | 410.2                                           |
| S-1    | M-05      | 0                                             | 8                                             | 8                                              | 42                                             | 81                                             | 32.4                                                 | 76.9                                            |
| M-05   | M-05      | 0                                             | 0                                             | 0                                              | 8                                              | 36                                             | 12.0                                                 | 16.7                                            |
| RB     | M-05      | 0                                             | 0                                             | 8                                              | 81                                             | 100                                            | 56.5                                                 | 151.4                                           |
| BB-01  | M-05      | 0                                             | 8                                             | 25                                             | 92                                             | 100                                            | 50.0                                                 | 229.2                                           |
| MCC-01 | RB        | 11                                            | 11                                            | 31                                             | 92                                             | 100                                            | 77.8                                                 | 354.6                                           |
| S-1    | RB        | 8                                             | 8                                             | 8                                              | 42                                             | 92                                             | 30.6                                                 | 119.4                                           |
| M-05   | RB        | 0                                             | 0                                             | 0                                              | 0                                              | 33                                             | 11.1                                                 | 27.8                                            |
| RB     | RB        | 8                                             | 8                                             | 25                                             | 83                                             | 100                                            | 45.4                                                 | 202.8                                           |
| BB-01  | RB        | 0                                             | 8                                             | 33                                             | 92                                             | 100                                            | 61.1                                                 | 250.0                                           |
| MCC-01 | BB-01     | 0                                             | 0                                             | 50                                             | 83                                             | 100                                            | 58.3                                                 | 273.6                                           |
| S-1    | BB-01     | 8                                             | 8                                             | 8                                              | 50                                             | 67                                             | 25.0                                                 | 102.8                                           |
| M-05   | BB-01     | 0                                             | 0                                             | 0                                              | 0                                              | 17                                             | 5.6                                                  | 13.9                                            |
| RB     | BB-01     | 8                                             | 8                                             | 42                                             | 92                                             | 100                                            | 53.7                                                 | 236.6                                           |
| BB-01  | BB-01     | 8                                             | 31                                            | 67                                             | 100                                            | 100                                            | 68.5                                                 | 344.4                                           |

different. Similarly, although the rate of VSD severity and incidence progression varied among the rootstock  $\times$  scion combinations, only the scion significantly affected AUDPC. The rootstock and the interaction (scion  $\times$  rootstock) were not significant (Tables 1; 2; Fig. 3).

Irrespective of rootstock clones, MCC-01 (also known as “M-01”) was the most VSD susceptible scion in the study. On the final disease evaluation, VSD incidence reached its maximum extent (100%) in all combinations. Also, VSD severity was high (58.3%–78.7%), with BB-01 showing the lowest and S-1 showing the highest severity. AUDPC (incidence and severity) for MCC-01 scion was significantly higher than AUDPC for other scions. Moreover, VSD symptoms in MCC-01 showed

the fastest appearance in almost all the rootstocks (MCC-01, S-1, M-05, RB) (Tables 1; 2; Fig. 3).

BB-01 was the second most VSD susceptible scion in this trial. Similar to MCC-01, VSD incidence and severity were increased on the final disease evaluation, regardless of the rootstock clone. AUDPC (incidence and severity) for BB-01 scion was significantly different from that of S-1, M-05, RB scions. VSD symptoms appeared starting from five months (two rootstock genotypes) until 12 months after planting (Tables 1; 2; Fig. 3).

RB was the third most VSD susceptible scion in this study. VSD incidence on RB scion was high (100%) on any rootstock genotype, but the

**Table 2**

ANOVA of Vascular streak dieback (VSD) incidence and severity in five cocoa genotypes that were used as rootstocks and also as scions evaluated 6, 9, 12, 15 and 18 months after planting in South Sulawesi (From September 2009 to October 2010). In all treatments, VSD symptoms were first observed 5 months after field planting.

| Scion/rootstock                | % VSD incidence<br>6 months after<br>planting | % VSD incidence<br>9 months after<br>planting | % VSD incidence<br>12 months after<br>planting | % VSD incidence<br>15 months after<br>planting | % VSD incidence<br>18 months after<br>planting | Mean disease severity<br>18 months after<br>planting | Mean AUDPC disease<br>severity for<br>14 months |
|--------------------------------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------------|-------------------------------------------------|
| Averages for each scion        |                                               |                                               |                                                |                                                |                                                |                                                      |                                                 |
| MCC-01                         | 8.89                                          | 13.89                                         | 50.56 <sup>c</sup>                             | 90.00 <sup>c</sup>                             | 100.00 <sup>c</sup>                            | 70.56 <sup>d</sup>                                   | 333.33 <sup>d</sup>                             |
| S-1                            | 5.00                                          | 6.67                                          | 8.33 <sup>a</sup>                              | 32.22 <sup>b</sup>                             | 73.89 <sup>b</sup>                             | 29.07 <sup>b</sup>                                   | 103.33 <sup>b</sup>                             |
| M-05                           | 0.00                                          | 0.00                                          | 2.22 <sup>a</sup>                              | 8.33 <sup>a</sup>                              | 29.44 <sup>a</sup>                             | 9.81 <sup>a</sup>                                    | 29.72 <sup>a</sup>                              |
| RB                             | 5.00                                          | 5.00                                          | 22.22 <sup>ab</sup>                            | 82.78 <sup>c</sup>                             | 100.00 <sup>c</sup>                            | 51.11 <sup>c</sup>                                   | 204.17 <sup>c</sup>                             |
| BB-01                          | 3.33                                          | 12.78                                         | 38.33 <sup>bc</sup>                            | 95.00 <sup>c</sup>                             | 100.00 <sup>c</sup>                            | 62.04 <sup>cd</sup>                                  | 276.48 <sup>d</sup>                             |
| Tukey's test                   | NS                                            | NS                                            | 24.36 <sup>(**)</sup>                          | 21.51 <sup>(**)</sup>                          | 16.10 <sup>(**)</sup>                          | 14.02 <sup>(**)</sup>                                | 63.77 <sup>(**)</sup>                           |
| Averages for each rootstock    |                                               |                                               |                                                |                                                |                                                |                                                      |                                                 |
| MCC-01                         | 6.67                                          | 8.33                                          | 27.22                                          | 66.11                                          | 85.00                                          | 48.52                                                | 210.93                                          |
| S-1                            | 1.67                                          | 6.67                                          | 25.00                                          | 54.44                                          | 73.33                                          | 42.41                                                | 176.85                                          |
| M-05                           | 3.33                                          | 6.67                                          | 16.67                                          | 61.11                                          | 83.33                                          | 44.26                                                | 174.07                                          |
| RB                             | 5.56                                          | 7.22                                          | 19.44                                          | 61.67                                          | 85.00                                          | 45.19                                                | 190.93                                          |
| BB-01                          | 5.00                                          | 9.44                                          | 33.33                                          | 65.00                                          | 76.67                                          | 42.22                                                | 194.26                                          |
| Tukey's test                   | NS                                            | NS                                            | NS                                             | NS                                             | NS                                             | NS                                                   | NS                                              |
| Analysis of variance (p-value) |                                               |                                               |                                                |                                                |                                                |                                                      |                                                 |
| Scion                          | NS                                            | NS                                            | **                                             | **                                             | **                                             | **                                                   | **                                              |
| Rootstock                      | NS                                            | NS                                            | NS                                             | NS                                             | NS                                             | NS                                                   | NS                                              |
| Scion $\times$ Rootstock       | NS                                            | NS                                            | NS                                             | NS                                             | NS                                             | NS                                                   | NS                                              |

Numbers in the same column followed by the same letter are not significantly different by Tukey's test analysis ( $p < 0.05$ ); \*\* and NS indicate statistical significance at  $p < 0.01$ , 0.05, and not significant, respectively; Mean disease severity as described on materials and methods, Section 2.5.

Abbreviations: MCC-01, Masamba Cocoa Clone 01; S-1, Sulawesi 1; M-05, Muchtar 05; RB, Rahim Burau; BB-01, Buntu Batu 01.

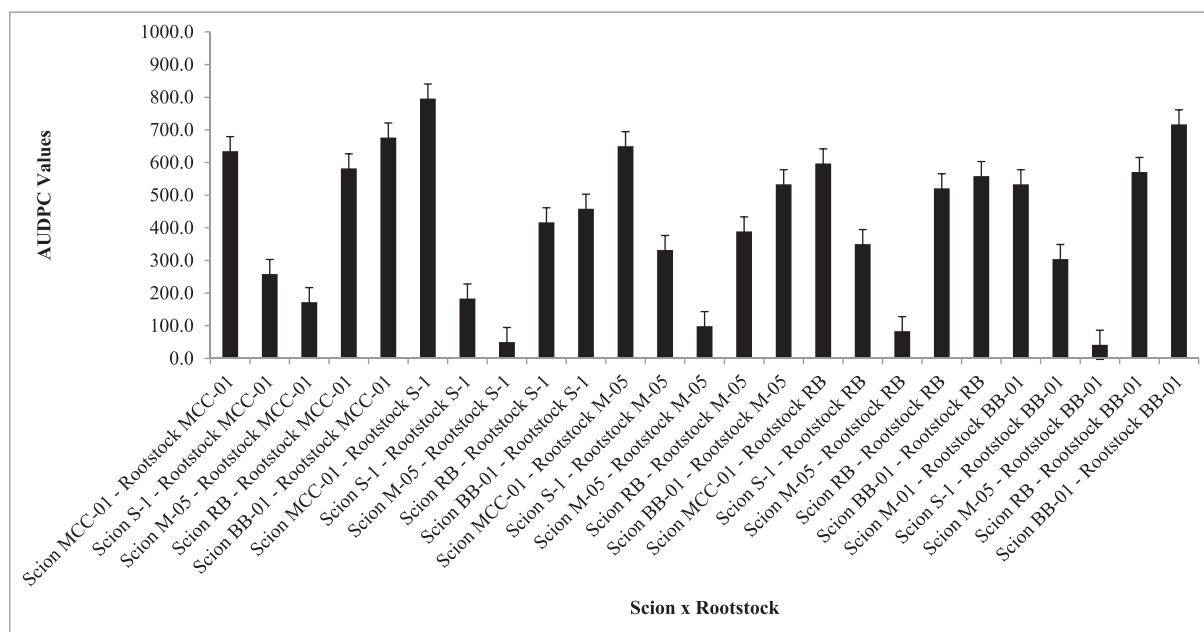


Fig. 3. Mean AUDPC values of VSD incidence on five genotypes that were used as rootstocks and also as scions for 14 months of observation.

range of VSD severity was moderately high (41.7%–58.3%), while the AUDPC (incidence and severity) for RB scion was higher than for M-05 and S-1 scion. The appearance of the first symptoms started at five months on the majority of rootstock genotypes until 12 months after planting (Tables 1; 2; Fig. 3).

M-05, selected by screening the progeny of parents with promising VSD resistance characteristics by Mars Inc., was the most VSD-resistant scion in the study, regardless of the rootstock clone. VSD incidence in M-05, as expected, was significantly lower than in other scions studied (11%–50%). Also, VSD severity was very low (3.7%–16.7%), with S-1 rootstock having the lowest and MCC-01 having the highest VSD severity. AUDPC for M-05 scion was significantly lower than AUDPC for the four other scions. Moreover, the time required for the VSD symptoms to appear was extremely long in all combinations. The combination with the fastest VSD development was MCC-01 and M-05 at 12 months after planting (Tables 1; 2; Fig. 3).

S-1 was the following VSD-resistant scion in the trial. S-1 scion showed the second-lowest VSD incidence (56%–92%) and VSD severity (25.0%–32.4%). Also, the AUDPC was moderately low, irrespective of the rootstock clone. However, the VSD symptoms began five and six months after planting on three rootstocks (Tables 1; 2; Fig. 3).

Scions of M-05 consistently showed to be the most VSD-resistant scion followed by S-1 based on the disease progression, confirmed by the lowest AUDPC values. AUDPC's reflect the development of VSD on the cocoa tree. As a rootstock, M-05 and S-1 showed various positive responses to their grafted scion. However, the influence was statistically not significantly different. Meanwhile, MCC-01 scion was consistently found to be the most VSD-susceptible scion, followed by BB-01 and RB (Tables 1; 2; Fig. 3).

#### 4. Discussion

This research is the first effort to evaluate a possible effect of rootstocks on VSD infestation levels of scions by testing five locally selected clones in Sulawesi as rootstocks and also as scions in all possible combinations. M-05 showed outstanding responses as clone resistant to VSD (McMahon et al., 2015, 2018), and consistently showed to be the scion with the lowest incidence, severity and progression of VSD on any clone onto which it was grafted. Additionally, M-05 as a scion showed the latest appearance of VSD symptoms. However, M-05 failed to

deliver resistance properties to the graft when used as rootstock. M-05 proved resistant to VSD. Similarly, S-1 showed low VSD-severity as a promising clone when used as a scion in highly VSD-infected areas (Chong and Shepherd 1986; Yin 2004; McMahon et al. 2018); As a rootstock, however, S-1 was unable to influence susceptible scions to be resistant to VSD.

The mechanism of VSD resistance remains unknown. According to Prior (1979), VSD resistance mechanisms may include restriction of the growth of the pathogen in the xylem vessels and/or inhibition of basidiospore germination by exudate release in young cocoa leaves. Additionally, scion VSD-resistance most likely depends on the production of physical barriers on the leaf such as leaf epidermis thickness, trichome density and stomata (Prawoto et al. 2013; Soesilo et al. 2016). Leaf-stomata characteristics, including stomata number, length and width of open stomata, play an important role in the resistance of the cocoa clone to VSD (Asman 2011; Soesilo et al. 2016).

MCC-01, one of the popular clones in Indonesia, had the highest VSD incidence and severity as scion regardless of which rootstock it was grafted. Even though MCC-01 was susceptible to VSD (McMahon et al., 2015, 2018), the clone did not significantly increase VSD incidence and severity to the graft when used a rootstock. The same conclusions can be made for BB-01 and RB clones that performed susceptible and moderately susceptible to the incidence and severity of VSD as the scion, respectively. However, both clones did not significantly convey either susceptibility or resistance to scion when it was used as rootstock.

The rootstock-induced systemic defense is considered to play a role in disease resistance on grafting, as described by Guan et al. (2012) and Ribeiro et al. (2016). Ribeiro et al. (2016) reported that cocoa genotypes SIC-876 rootstock provide higher tolerance to the CCN-51 scion to witches' broom disease. The tolerance is promoted by the increased ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) activities. However, in this study, we did not measure variables of systemic plant resistance. More importantly, this study did not indicate any rootstock effect. This means that rootstock-induced systemic resistance might play a less important role than assumed in VSD. Further research is needed to clarify the possible effects of rootstock-induced systemic resistance on VSD resistance.

The results show clear differences between the scions in their partial resistance to VSD, in line with previous knowledge about the clones, especially for M-05 and S-1 (Chong and Shepherd 1986; Yin 2004;

McMahon et al. 2018). Also, the study showed that VSD-resistant scions could occur on cocoa rootstock genotypes with different yield characteristics. M-05 is considered to be a low-yielding clone, whereas S-1 is considered to be a high-yielding clone. Furthermore, the rootstock from VSD susceptible (MCC-01) or resistant (M-05 and S-1) clones did not influence the susceptibility or resistance of scions grafted onto it. However, a larger experimental area to provide more cocoa trees and replication would be better for working with the VSD in natural infection conditions. Also, adding assessing more VSD characteristics is needed to evaluate VSD resistance in-depth in a further investigation, such as measuring the extent of vascular streaking at the end of the experiment. The use of rootstock seedlings has caused genetic variability in our rootstocks, which hampers the evidence of the rootstock genotypic effect on VSD resistance.

## 5. Conclusions

VSD resistance is linked with scion (branch and leaf) characteristics rather than with rootstock characteristics. VSD resistance breeding should focus on scions. There was no evidence that the VSD resistance of the rootstock affected VSD incidence or severity in the scion. However, the fact that seedlings derived from open-pollination were used as rootstocks probably increased the variability of the rootstocks, making it more difficult to show significant effects of rootstocks on the scions.

## Declaration of competing interest

The authors have no conflict of interest to declare.

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