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Testing local cacao selections in Sulawesi for resistance to vascular streak dieback

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ABSTRACT

Vascular streak dieback (VSD) causes serious losses for cacao (or cocoa) smallholders in Sulawesi, Indonesia. The disease is caused by a Cantharellales species, *Ceratobasidium theobromae*, which colonises the xylem, resulting in leaf chlorosis or necrosis, abscission and eventual branch dieback. The disease is most successfully managed by pruning infected branches and the propagation of resistant genotypes. In participatory trials located in three provinces in Sulawesi, 2.5-year-old trees, which had been clonally propagated from local genotypes or the hybrid progeny of resistant parents, were evaluated for disease severity from 2010 to 2012. Consistent resistance rankings were obtained for clones common to the trials; these were confirmed by re-evaluation in 2014. From plot averages of disease severity, broad-sense heritability was estimated as 0.67–0.92. Two progeny clones, KW617 and ICCRI03, from East Java, had similar levels of resistance in the trials as their respective (resistant) parental clones, PBC123 and Scavina 6. Among four clones monitored for 3 months in West Sulawesi, PBC123 had a higher proportion of healthy leaves on the branch tips and a more restricted spread of infection within the xylem. In contrast, disease symptoms reached the younger leaves in susceptible clones. Individual branches of KW617, monitored from an early stage of symptom development, had a significantly lower number of diseased leaves and higher ratio of new to infected leaves after 9–16 weeks than that in four other clones. Other cacao clones with a relatively high number of diseased leaves during this period overcame infections with the addition of new flushes. Resistance in farm selections did not usually co-exist with yield and bean quality. Deriving new genotypes from crosses between parents with VSD-resistance and high-yield and/or quality traits is required for the production of promising clones with good resistance, yield and quality.

1. Introduction

Vascular streak dieback (VSD) is a serious systemic disease of cacao (or cocoa, *Theobroma cacao* L.) with a distribution in the Southeast Asian and west Pacific regions (Keane and Prior, 1991; Guest and Keane, 2007). The disease is caused by a Cantharellales species, *Ceratobasidium theobromae*, syn. *Thanatephorus theobromae*, *Oncobasidium theobromae* (Talbot and Keane, 1971; Roberts and Royal Botanic Gardens, 1999; Samuels et al., 2012). VSD is characterised by either leaf chlorosis or necrosis, followed by abscission and, in susceptible genotypes, branch dieback. Severe impacts of this disease have been reported in Indonesia, including Sulawesi (Turner and Keane, 1985; Pawirosoemardjo et al., 1990; Pawirosoemardjo and Purwantara, 1992; Guest and Keane, 2007; Parawansa et al., 2013; McMahon and Purwantara, 2016). Indonesia produced 545,000 tonnes of cocoa beans in 2006/2007, but this had declined to 325,000 tonnes by 2014/15, with further declines predicted (http://www.icco.org/about-us/icco-annual-report.html). Among the factors contributing to lower farm productivity, including ageing trees, soil fertility decline and socio-economic trends such as the increasing average age of farmers, VSD has

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been one of the most important and has frequently influenced smallholders to replace their cacao with other crops. In their 2008/2009 annual report, the International Cocoa Organisation (ICCO) identified VSD as a major constraint to cacao production in the region.

Fungal isolates from infected cacao leaves and petioles in Sulawesi (Samuels et al., 2012) revealed the typical *Rhizoctonia*-like morphology of the VSD pathogen described in Papua New Guinea (Talbot and Keane, 1971; Keane et al., 1972) and Malaysia (Mainstone et al., 1983; Zainal Abidin et al., 1984). Furthermore, the morphology and dimensions of basidiospores in sporocarps are consistent with the descriptions of Talbot and Keane (McMahon and Purwantara, 2016). Internal transcribed spacer (ITS) and large subunit (LSU) sequences of Sulawesi isolates have 99–100% identity with isolates from VSD-infected tissues in Papua New Guinea (PNG), West Papua, Java, Malaysia and Vietnam (Samuels et al., 2012). Whilst the symptoms of VSD infection may become severe during drier periods, the disease spreads between trees in the rainy season, which provides the necessary conditions for sporulation. During wet periods, adherent sporocarps with monilioid hyphae and basidia are formed from mycelia emerging from vascular tissue on scars left by abscissed leaves or on cracked petioles or leaf mid-ribs. Basidiospores are produced at night under moist conditions (Keane et al., 1972; Dennis et al., 1992) and dispersed for up to 100 m by wind (Keane, 1981). Infection of flush leaves at the branch or seedling tip is followed by colonisation of the xylem. Hyphae then spread through vascular tissue to adjacent leaves, causing leaf drop and eventually killing the seedling or the branches of more susceptible cacao genotypes (Keane et al., 1972; Prior, 1978; Keane, 1981). Visible symptoms develop 3–5 months after infection. Branch death may occur around 6 months after the initial infection, although infected branches in cacao genotypes with partial resistance can remain alive for long periods and can even occlude infections and continue growing (Prior, 1979, 1985; Keane and Prior, 1991).

Until the 2000s, the most common VSD symptom resulting from infection of cacao trees was chlorosis, characterised by yellowing leaves with patches of green tissue, followed by rapid leaf abscission. In the last decade, a new set of VSD symptoms has become more common in cacao-growing areas throughout Southeast Asia and the west Pacific. Characteristically, distinct dark lesions beginning on leaf margins or tips expand until they cover all or most of the leaf lamina. Leaves with such necrotic symptoms also remain attached for a longer period of time than those with chlorotic symptoms. Moreover, sporulation may occur in cracks in the petiole or leaf and on leaf scars (Purwantara et al., 2009; Parawansa et al., 2013, 2014).

Management of VSD is achieved largely by the cultural methods of pruning diseased branches, raising seedlings in nurseries under UV-resistant plastic sheeting (to prevent infection by the wind-borne spores) and the use of resistant plant material (Keane and Prior, 1991). Some triazolic systemic fungicides have been shown to be effective in controlling VSD (Prior, 1987; Singh, 1989; Holderness, 1990), but their high cost makes them uneconomic in most cacao farming situations. Frequent and regular pruning programmes have been shown to be effective on estates in West Java (Pawirosoemardjo et al., 1990; Pawirosoemardjo and Purwantara, 1992) and Malaysia (Jayawaderna et al., 1978) but are extremely labour intensive. Branches are cut about 30 cm below the extent of visible infection – pruned branches can be left on the farm as sporulation only occurs on attached, living branches (Prior, 1985; Keane and Prior, 1991). Such widespread programmes are difficult to implement on smallholdings, in which investment in management may vary greatly from farm to farm. Lack of management (especially sanitation pruning) and the propagation of relatively susceptible genotypes results in a higher rate of sporulation and infection of neighbouring trees (Keane, 1981; and see Ploetz, 2007). Therefore, on smallholdings in particular, the use of resistant genotypes, combined with adequate cultural management, is the most promising strategy for reducing the impacts of this disease.

Cacao genotypes surviving severe epidemics of VSD in PNG and Malaysia in the 1960s and 70s (Shaw, 1962; Bridgeland et al., 1966; Mainstone et al., 1983) demonstrated that VSD resistance occurred among diverse genotypes on farms (Keane, 1974; Prior, 1979; Chong and Shepherd, 1986; Keane and Prior, 1991; Efron et al., 2002). Resistant clones, such as KA2101, KEE1, PBC123 and KKM22, initially selected in PNG and Malaysia, are still propagated or used in breeding programmes (Chong and Shepherd, 1986; Efron et al., 2002). Two clones, PBC123 and BR25, were selected in the 1980s in Malaysia by screening the progeny of crosses for resistant, high-yielding types. These clones are presently the most widely planted clones in Sulawesi, Indonesia, where they have been renamed Sulawesi 1 and 2, respectively. Tan and Tan (1988) concluded the genetic basis of VSD resistance to be mainly additive and quantitative, in line with a horizontal type of resistance (Vanderplank, 1963; Keane, 2012). This is supported by the durability of resistance occurring in resistant genotypes (Prior, 1979; Keane and Prior, 1991).

In Sulawesi, a strategy of selecting and testing local cacao genotypes on smallholder farms, which enables the identification of genotypes with promising resistance and other characteristics, demonstrated the potential of a participatory farmer approach to select from genetic resources available on smallholdings (McMahon et al., 2009, 2010; 2015). The potential yield, quality characteristics and resistance to cacao pod borer (*Conopomorpha cramerella*) and *Phytophthora palmivora* of local clones tested in farm-based trials in three provinces in Sulawesi has been reported previously (McMahon et al., 2015; Purwantara et al., 2015). The clones tested in these trials included local farm selections and hybrid progeny identified with possible VSD resistance, in addition to susceptible clones. Here we report evaluations of the response of these clones to VSD infections transmitted under field conditions and identify cacao genotypes with potential resistance to the disease.

2. Materials and methods

2.1. Trial establishment

Trials were established in March 2008 at four sites in Sulawesi, each testing 11–12 local cacao clones. Clones were selected from working farms or from progeny of hybrid crosses. A few of these clones were common to two or more of the trials. Clones common to all four trials were the Malaysian clone, PBC123, also known as 'Sulawesi 1' (widely propagated for its VSD resistance and high yield) and the high-yielding M01, now officially released and renamed Masamba Cacao Clone 1 (MCC01). Three trials established on farms in early 2008 were located in West Sulawesi in the village of Beluak in Anreapi sub-district, Polewali-Mandar (Polman) District, South Sulawesi (Fakkie Village, Tiroang, Pinrang District) and Southeast Sulawesi (Pakue, North Kolaka District). The trial design, latitude/longitude coordinates and the clones tested in these trials have been reported previously (McMahon et al., 2015). A fourth trial, also established in early 2008, was located on a working farm in Bone-Bone subdistrict, North Luwu: latitude 02°38′587″S and longitude 120°37′163″E and an elevation of 67 m above sea level. The trial in North Luwu tested 11 cacao genotypes, including clones in common with the other trials, additional on-farm selections and a hybrid cross, ICCRI04 (see Table 1). To establish the trials, budwood obtained from mature trees of selected genotypes was grafted onto rootstock seedlings in a nursery and after 4–5 months planted at the trial sites. Each clone was propagated in 8-tree plots, with four replicate blocks, providing a total of 384 trees for the trials testing 12 clones and 352 trees in the North Luwu trial. In the North Luwu trial, some trees were lost to root diseases or had stunted growth so that finally 4–7 trees per plot were assessed for VSD severity. VSD, endemic to most cacao-growing areas in Sulawesi, was transmitted naturally under field conditions. As for the other trials, pesticides were not applied to the trees in North Luwu except for fungicidal treatment at the early stages of establishment after planting. No insecticides or fungicides were applied during the period of evaluation. Monthly rainfall

Table 1
Vascular streak dieback (VSD) severity in cacao (*Theobroma cacao* L.) clones evaluated in Sulawesi from 2010 to 2012. Clones were selected on farms in various districts in Sulawesi (second column) or from the progeny of hybrid crosses in East Java or Malaysia. Severity ratings shown are the means of four replicates. The same letter within a column indicates no significant difference.

Clone	Origin of selection	Disease severity[1]			
		Pinrang	N. Kolaka	Polman	N. Luwu
GeniJ[2]	E. Luwu	1.9[a]	1.3[a]	2.1[ab]	–
M05[2]	N. Luwu	1.8[a]	1.5[ab]	2.0[a]	–
HariJ	E. Luwu	–	–	–	1.9[a]
PBC123[2,3,4]	Malaysia	2.2[a]	1.6[b]	2.2[abc]	1.8[a]
ICCRI03[4]	E. Java	–	–	–	2.0[a]
M06	N. Luwu	2.7[b]	1.3[a]	–	–
KW523[4]	E. Java	–	–	2.5[bcd]	–
KW617[4]	E. Java	–	–	2.5[abcd]	–
KKM22[4]	Malaysia	–	2.6[c]	–	–
BR25[4]	Malaysia	–	–	2.6[bcd]	–
KW516	N. Sumatra	–		2.7[cd]	–
Moktar	N. Luwu	–	2.6[c]	–	–
PwPg	Pinrang	–	2.6[c]	–	–
MaPg	N. Luwu	–	–	–	2.7[b]
YD75	Luwu	–	–	–	2.7[bc]
Muhtar	Soppeng	–	–	2.8[d]	–
NR	Soppeng	–	2.8[c]	2.8[d]	–
Pat Gr	N. Luwu	–	–	–	2.8[bc]
RB	E. Luwu	2.9[bc]	–	–	–
ILH	Soppeng	–	3.2[d]	2.7[cd]	–
M01[3]	N. Luwu	3.2[cd]	2.7[c]	2.6[bcd]	3.0[cd]
Husbitori	Bone	–	–	3.0[d]	–
PCK	Mamuju	3.2[cd]	–	–	–
Terb Gr	N. Luwu	–	–	–	3.3[de]
DRC15	E. Java	3.3[cd]	–	–	–
Darno	N. Kolaka	–	3.4[d]	–	–
ICCRI04[4]	E. Java	3.4[de]	–	–	–
PanR	N. Luwu	3.6[de]	2.6[c]	–	3.3[de]
Tr01	N. Luwu	3.5[de]	–	–	–
SSrwg	N. Luwu	–	–	–	3.7[e]
M04	N. Luwu	3.9[e]	–	–	3.4[de]

Abbreviations: BR, Balong River; DRC, Djati Roenggo Clone; Geni J, Genin Jasi; Hari J, Hari Jasi; ICCRI, Indonesian Coffee and Cocoa Institute; ILH, Ilham; KKM, Klon Koko Mardi; KW, Kaliwining (E. Java); MaPg, Masamba Pongo; M01, Moktar 01, etc.; NR, Nasir Rauf; Pan R, Panimbu Red; PBC, Prang Besar Clone; Pat G, Patila Green; PCK, Puang Caddik; RB, Rahim Burau; PwPg, Polewali-Pinrang; SSrwg, Seleksi Rewang; Terb G, Terobok Green; TR01, Torea01.
[1]Disease severity based on scores: 1, none; 2, light; 3, moderate; 4, severe; [2] clones identified with VSD resistance across sites; [3]clones established at all four locations; [4] hybrid progeny selection.

records from 2009 to 2012 were supplied by the Meteorology Station, Majene, West Sulawesi and by Mars Inc. Indonesia field stations.

2.2. VSD severity and flush leaf production

Between April 2010 and May 2012, VSD severity was assessed in the four trials in Sulawesi. In the North Kolaka trial, VSD severity was evaluated each month for 20 months (April 2010 to November 2011), while in the other three trials, VSD was evaluated for 26 months (beginning in April 2010). VSD was evaluated once a month by assigning a severity score of VSD damage to the whole tree as follows:

1 (nil), no infection detected; 2 (low), < 10% branches on tree infected, infections generally slight, no significant leaf fall; 3 (moderate), 10–25% branches infected, moderate infection with some leaf fall; 4 (high), > 25% branches infected, some severe infection or imminent dieback apparent in which most leaves on some branches are infected or fallen.

For each clone, mean disease severity in each plot was calculated as follows:

$$\text{Severity} = (T_1*1 + T_2*2 + T_3*3 + T_4*4)/T_{total}$$

where T_1 = number of trees in the plot with a disease score of 1, T_2 = number of trees with a disease score of 2, and T_{total} is the total number of trees assessed in the plot.

In the Polman trial, West Sulawesi, production of flush leaves was assessed for a period of 15 months (February 2011 to May 2012). The degree of flushing in each tree was scored each month as follows:

1, (no flush); 2 (low flush), flush leaves on 1–5 branches; 3 (medium flush), flush leaves on 6–10 branches; 4 (high flush), flush leaves on > 10 branches.

For each clone, the average flush score was calculated as for VSD severity (see above). The mean, standard deviation and standard error of the scores for VSD severity and flushing of the four replicates were determined.

2.3. Disease progress

Branches of four clones in the trial in Polman, West Sulawesi (PBC123, M01, BR25 and Husbitori), showing signs of VSD infection were selected and evaluated on five occasions from 9 February to 10 May 2012, by which time an advanced stage of symptom development was reached. The clones monitored ranged from resistant (PBC123) to highly susceptible (Husbitori). Selected branches on each clone tree showing early symptoms of leaf chlorosis or necrosis were labelled with a tag. Branches were photographed and symptoms recorded on five occasions (with the final evaluation conducted at 91 days). Some branches were lost to pruning by the farmer, but finally, 3–4 replicate branches per clone could be evaluated for the full 91 days. Leaf position was defined from the first leaf or leaf scar (Leaf 1) at the base of the branch to the last fully expanded leaf or leaf scar. The presence of flush leaves, if any, was recorded. Each flush had an average of 7.5 leaves (see Section 2.5). For each leaf position, a record was made of the leaves that had fallen and attached leaves that showed early signs of infection (early chlorosis, indicated by a pale colour or slight yellowing), chlorosis (slight mottling or patchiness, or yellowing), advanced chlorosis (mottled, yellow and/or with green spots or small necrotic patches) and necrosis (browning of the leaf expanding generally from the leaf tip or margin to eventually encompass the whole leaf). In infected leaves with necrotic lesions, the proportion (%) of the leaf area that had become necrotic was estimated visually.

2.4. Xylem examination in infected branches

Infected branches of four clones, Husbitori, M01, BR25 and PBC123 (see Section 2.3), showing advanced stages of symptom development were collected, labelled, sprayed with water and placed in plastic bags with moist tissue for transporting back to the laboratory. The total length of each branch, number of leaves, leaf positions and number of symptomatic leaves was determined. Leaf position was determined from the base of the branch (Leaf 1) up to the last fully expanded leaf. Sections at approximately 5-cm intervals were cut from each branch using secateurs, keeping them wet, and then the xylem tissue was sliced lengthwise using a razor blade to prepare thin longitudinal sections of the tissue for microscopy. The lengthwise extent of the discoloured vascular tissue in the stem of each branch was measured. Longitudinal sections were mounted on microscope slides in lacto-cotton blue, covered with a coverslip and examined under a Nikon E100 compound microscope (Nikon Inc., Japan) for evidence of fungal hyphae within the xylem tissue. Cross-sections were also made and examined as above. Using this method, the extent of infection along the branch could be estimated.

2.5. Re-evaluation of selected clones in 2014

2.5.1. Determination of mean severity

Clones in two of the trials (at Pinrang and Polman, see Section 2.1) were evaluated again for VSD severity in March and July 2014. A

different evaluation method to the one described in Section 2.2 was employed. For each tree in a plot, 10 branches were randomly chosen and evaluated for severity of infection by assigning a score of 0 to branches with no infection, 1 for light infection (indicated by early signs of leaf yellowing), 2 for moderate infection and 3 for severe infection with disease symptoms reaching the branch tip. The scores for the ten individual branches in each tree were totalled and plot averages of these total scores determined. For each plot, the mean severity score of the two evaluations (March and July) was determined.

2.5.2. Determination of new growth on infected branches

In four locations, namely the Pinrang and Polman trials and two clone collections in East Luwu, South Sulawesi, one on a farmer's property in Cendana Hijau and the other at the Mars Inc. Cocoa Research Centre, Tarengge, branches of five selected clones (common to all the trials) with at least one infected leaf were tagged and monitored three times over 16 weeks. The trees included in the study varied in age. The trees in the Pinrang and Polman trials were planted in 2008 (see Section 2.1), while the trees in the East Luwu locations were younger, planted in late 2011 or early 2012. The number of branches monitored per clone varied from 10 to 19. The first evaluation was in February or early March and the final evaluation in June 2014. The mean number of leaves per flush on 106 branches in a range of cacao genotypes was determined to be 7.5. In each assessment, the number of flushes beyond the initial infection was determined. The number of new leaves was estimated by multiplying the number of additional flushes by 7.5 (the average number of leaves per flush). Diseased leaves were identified by typical symptoms of early yellowing, chlorosis or necrosis. These were counted with leaf gaps (or leaf scars) left by abscissed leaves to give a total number of diseased leaves per branch. The ratio of new to diseased leaves (number of new leaves/number of diseased leaves) was determined.

2.6. Statistical analyses

Data were subjected to ANOVA (one-way and two-way) using SPSS Statistics 19 (IBM Corp.) and Spearman's correlation tests. Means were separated by the Tukey b test or, in cases where Levene's test indicated non-homogeneity, by the Games-Howell test. Prior to ANOVA, ratios and leaf counts were transformed to their natural logarithms (after adding 1). Data were presented in their original format. Plot averages of VSD severity for clones common to three or four trial sites were used to determine the variance components of genotype, location, their interaction and the error. The proportion of the total variance attributable to a genotype effect provided an estimate of heritability.

3. Results

3.1. Disease severity

In the trials evaluated from 2010 to 2012, VSD infections were recorded in every cacao clone, which differed markedly in susceptibility to VSD (Table 1). Fig. 1 shows the mean severity in selected clones re-evaluated in 2014 in the Pinrang and Polman trials, resulting in a similar ranking for resistance. These evaluations showed that relative resistance/susceptibility was consistent between different sites (Table 1; Fig. 1).

Broad-sense heritability estimates, based on mean VSD severity between 2010 and 2012 in clones common to the trials, were high (0.67–0.92), confirming that most of the variation in mean severity between clones can be attributed to genotypic effects (Table 2). Nevertheless, location and the interaction between location and genotype also significantly affected the clones' response to VSD (Table 2).

Local clones indicating resistance were identified (Table 1, Fig. 1), particularly M05 (a farm selection from North Luwu) and Geni J and Hari J (both selected in East Luwu for potential VSD resistance). The data obtained in 2010–2012 indicate that the local farm-selected clone, M06 is moderately resistant to VSD (Table 1, Fig. 2). In contrast, the 2014 data identified M06 as one of the more susceptible clones.

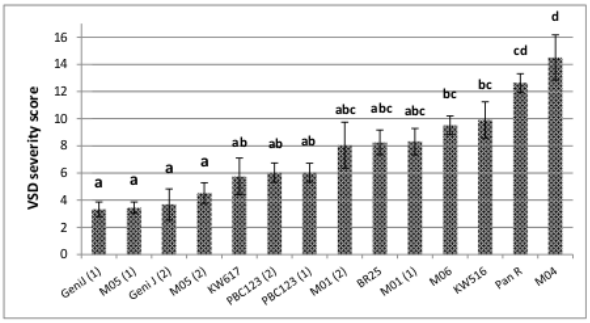


Fig. 1. Mean vascular streak dieback (VSD) severity evaluated in 2014 in selected clones in the Pinrang and Polman trials. Severity scores were assigned as described in Section 2.5. Locations of the clones tested in both trials are indicated in parentheses: site 1 is Pinrang and site 2 is Polman. Data shown are means of four replicates. The same letter indicates no significant difference ($p = 0.05$) in VSD severity.

Table 2
Heritability (H^2) based on plot averages of vascular streak dieback severity for clones tested in common at three or four trial sites. Variance components corresponding to genotype, (σ_g^2) location (σ_l^2) and their interaction (σ_{gl}^2) were determined by two-way ANOVA (**p = 0.001, *p = 0.05).

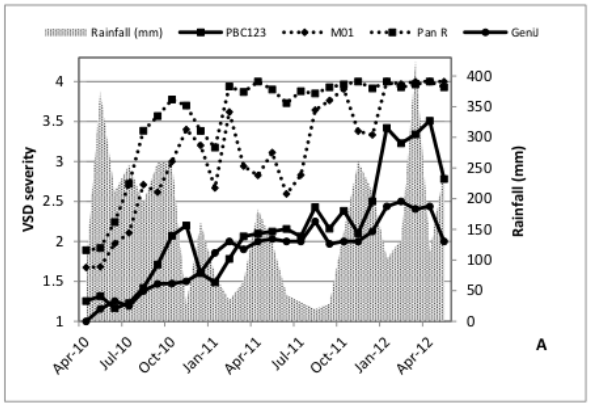
Source of VSD severity data	Variance component				H^2
	σ_w^2	σ_g^2	σ_l^2	σ_{gl}^2	
4 sites, 2 clones	0.035	6.849**	0.296**	0.242**	0.92
3 sites[a], 3 clones	0.031	5.951**	1.214**	0.122*	0.82
3 sites[b], 3 clones	0.129	2.644**	0.955**	0.240	0.67

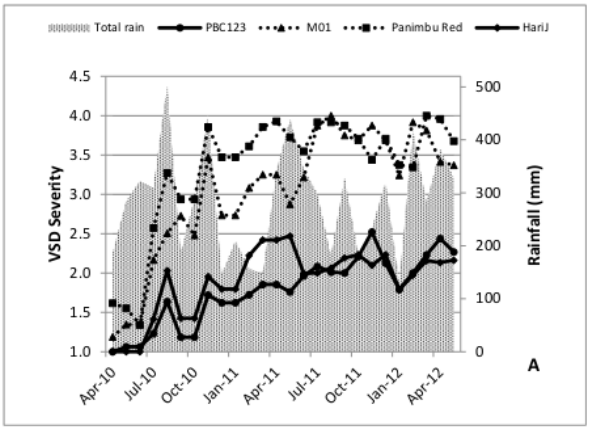
[a] Pinrang, N. Kolaka, N. Luwu.
[b] Pinrang, N. Kolaka, Polman.

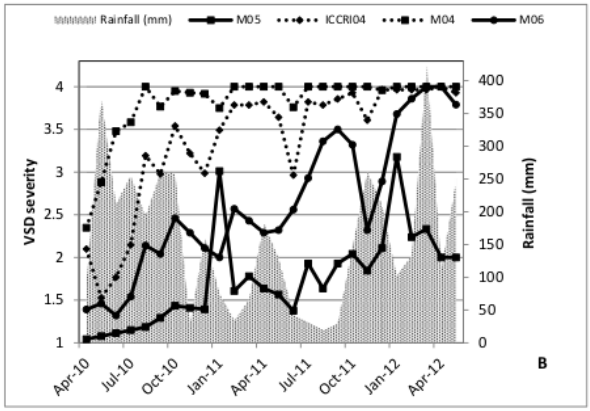
Figs. 2–4 indicate that while the mean disease severity fluctuated during the year, relatively low VSD scores were maintained in the more resistant clones in the wet and dry seasons over the 2 years (2010–2012) of evaluation. At the commencement of evaluation, when the trees were still quite young (two years after planting), the mean severity was low in all the clones but then increased substantially (Figs. 2–4). The data in Figs. 2–4 suggest that periods of high rainfall were associated with, or immediately followed by, declines in VSD severity, and in contrast, increases in mean severity occurred in the drier periods.

PBC123 (also known as 'Sulawesi 1'), selected by screening the progeny of parents with promising resistance characteristics in Malaysia (Chong and Shepherd, 1986), was one of the most VSD-resistant clone in the trials. However, BR25 (known locally as 'Sulawesi 2') and KKM22, productive selections from Malaysia, were ranked as moderately susceptible (Table 1).

ICCRI03 and KW617, selected from progeny of hybrid crosses in East Java, were identified as relatively resistant in the trials (Table 1, Figs. 1, 3 and 4). However, ICCRI04 (a clone obtained from crossing ICS60 (VSD susceptible) x Sca12 (moderately resistant)), which is rated with moderate resistance in East Java (personal communication, Agung Susilo), was susceptible in the Pinrang trial. The productive, farm-selected clone M01 (now officially released as Masamba Cacao Clone, MCC01) was consistently susceptible to VSD in the trials. Other clones with promising bean quality characteristics, including low bean count and shell content, and high fat content, such as M04, Pan R, TR01 and Husbitori (see McMahon et al., 2015) were among the most susceptible of the clones tested.







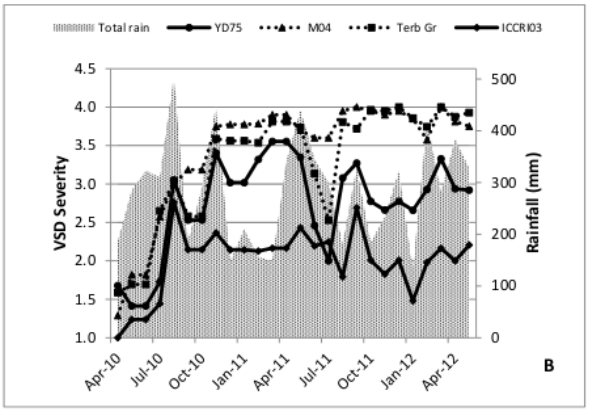


Fig. 2. Mean vascular streak dieback (VSD) severity (left axis) in relatively susceptible (dashed) and resistant clones (solid) in the Pinrang trial between April 2010 and May 2012: (A) Geni J, PBC123, M01 and Pan R; (B) M05, M06, M04 and ICCRI04. Total rainfall (mm) is shown by the shaded area (right axis) in both plots. VSD severity was evaluated as described in Section 2.2. Data shown are means of four replicates.

Fig. 3. Mean vascular streak dieback (VSD) severity (left axis) in relatively susceptible (dashed) and resistant (solid) clones in the North Luwu trial between April 2010 and May 2012: (A) Hari J, PBC123, M01 and Pan R; (B) ICCRI03, YD75, M04 and Terbrook Green. Total rainfall (mm) is shown by the shaded area (right axis) in both plots. VSD severity was evaluated as described in Section 2.2. Data shown are the means of four replicates. Note that VSD severity was low in April 2010, 2 years after planting, but infection severity then increased in all the clones.

3.2. Flushing and vascular streak dieback severity

Fig. 4 plots the changes in mean VSD severity in three clones and their flush cycles between February 2011 and May 2012 in the Polman trial. Flushing occurred in cycles that were not clone dependent (Fig. 4). A certain amount of rainfall was apparently needed for flushing since flush leaf production was delayed in the driest period (June–September 2011) in all the clones including those shown in Fig. 4. In times of adequate rainfall, however, a flush cycle occurring approximately every 2 months. Clones indicating relative resistance and susceptibility in the trial produced similar numbers of flush leaves. No correlation occurred for data obtained concurrently on flush leaf production and VSD severity in any of the clones in the trial. However, correlation coefficients for VSD severity and flushing with a lag of 3–4 months (for flush leaf data) were significant ($p = 0.05$) in KW617 ($r = 0.625$) and BR25 ($r = 0.683$). Such positive cross-correlation was not detected in the other clones in the trial.

3.3. Chlorosis, necrosis and leaf fall

The progressive development of symptoms was monitored for 91 days (between February and May 2012) in selected infected branches of four clones in the Polman trial. The leaf symptoms were predominantly necrotic in PBC123 and Husbitori and chlorotic in BR25. M01, however, expressed a mixture of both symptoms. Symptomatic leaves initially became pale green-yellow followed by either advanced chlorosis (with a patchy appearance) or the development of small necrotic lesions (usually on the tip or margin of the leaf), which then expanded. Leaves showing advanced chlorosis generally abscissed sooner than leaves with necrotic lesions, which in some cases remained attached for more than 75 days after the first symptoms were recorded.

Table 3 shows the mean proportion of total leaves of selected VSD-infected branches of the four clones that had developed symptoms, abscissed or were still healthy at the branch tip in late February and May 2012, an interval of 74 days. The data indicate that in PBC123, a smaller proportion of leaves were infected than in other clones, while a relatively large proportion were maintained as healthy terminal leaves. This demonstrates that growth was maintained at the branch tip despite infection with VSD. These observations are consistent with another longitudinal branch tracking conducted in the Pinrang trial: PBC123 trees in that trial also retained a larger proportion of their total leaves at the branch terminus (data not shown). In Polman, a high proportion of leaves became infected in the BR25 branches monitored, but this clone also demonstrated continued growth at branch tips despite VSD infection (Table 3). In contrast to PBC123 and BR25, less growth occurred at the tips of infected branches in the relatively susceptible clones M01 and Husbitori. Within 74 days, the branches of these more susceptible clones had suffered a high rate of leaf loss or necrosis and, in some

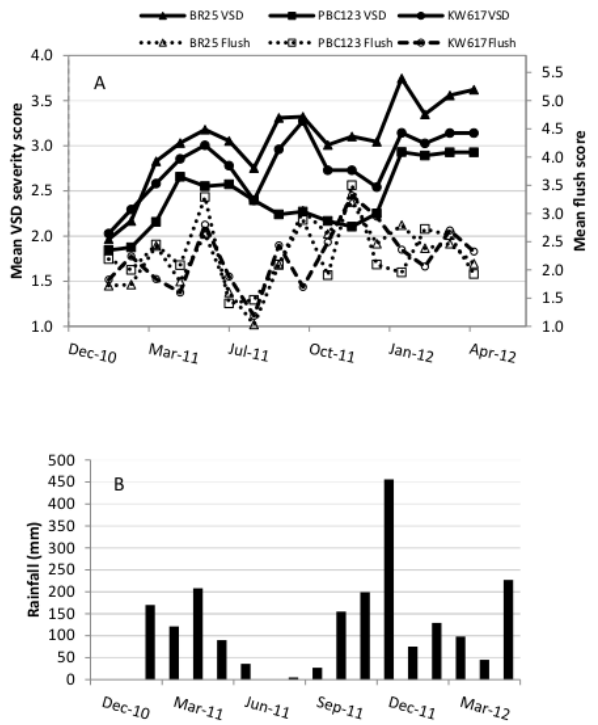


Fig. 4. A. Mean vascular streak dieback (VSD) severity (solid lines) and flush leaf production (dashed) in the clones BR25, PBC123 and KW617 each month from Feb 2011 to May 2012 in Polman, West Sulawesi. B. Monthly rainfall recorded in Majene, Polman, in the same period.

Table 3
Proportion of total leaves in vascular streak dieback (VSD)-infected branches of four clones in the Polman trial that had developed symptoms/abscissed or remained healthy at the branch tip in late February and May 2012.

Clone	Number of leaves on branch[a,b]		% leaves on branch[a]			
			Infected or fallen		Healthy on branch tip[c]	
	February	May	February	May	February	May
Husbitori	20.3 (1.6)	21.0 (2.3)	37.8 (11.0)	73.3 (6.5)	33.8 (3.7)	16.9 (1.5)
M01	19.0 (2.0)	21.0 (2.1)	40.4 (9.7)	69.0 (13.5)	27.0 (6.1)	18.7 (8.7)
BR25	22.0 (2.1)	31.3 (3.5)	46.8 (1.2)	56.3 (6.7)	46.0 (7.8)	40.4 (6.9)
PBC123	14.7 (0.9)	20.3 (1.2)	27.2 (6.8)	31.7 (9.0)	41.0 (8.7)	49.6 (4.4)

[a] Mean (and SE) of 3–5 replicates per clone.
[b] Determined as leaf positions (including leaf scars and attached leaves).
[c] Non-infected leaves beyond the most terminal infected leaf in the branch.

cases, dieback.

Table 4 shows the number of leaves infected in successive flushes over 3 months on evaluated branches of each clone. The data show that, after 91 days, infections in the branches of the most resistant clone (PBC123) were restricted to leaves of the same age (produced by a single flush), while in the other clones, infections moved more readily to older and/or younger leaves.

Microscopic examination of the xylem (see Section 2.4) also indicated a more restricted spread of infection along the branch in PBC123 than in the other clones. The data in Table 5 indicate that hyphae in PBC123 branches were restricted to two short sections of the

Table 4
The number of leaves infected with vascular streak dieback in successive flushes at 0 and 91 days in branches of four clones evaluated in Polman. In each flush, the number of infected leaves ranged from 0 (all healthy) to 8 (all infected).

Clone	Flush[a]	Branch 1		Branch 2		Branch 3	
		0	91 days	0	91 days	0	91 days
PBC123	1	1	8	1	8	0	0
	2	0	0	0	1	2	6
	3	yf	0	0	0	yf	0
	4				yf		0
M01	1	0	0	0	0	2	3
	2	0	5	1	2	1	5
	3	2	4	3	8*		0
	4						
Husbitori	1	0	8	0	0	4	8
	2	1	8	3	8	3	8
	3	yf	1	0	6	0	3
	4			yf	5		
BR25	1	5	5	8	8	8	8
	2	5	7	0	6	0	6
	3	0	1		0		0
	4		0				

*dieback occurred.
[a] Eight leaves per flush; yf, young flush leaves.

branch, while in the more susceptible clones, the extent of infection along the branch was much greater. In the Husbitori branch examined, staining of the xylem was observed over almost the entire length of the branch (Table 5). In some Husbitori and M01 branches, all or nearly all the leaves abscissed, and dieback and death of the branch tip occurred (personal observation). In one of the monitored Husbitori branches (data not shown), infection was first seen in leaf 11, which abscissed after 60% of the leaf had become necrotic, and in leaves 4 and 6, which remained attached to an advanced stage of necrosis. Following these earlier symptoms, the symptoms and progress of infection differed between clones. VSD infection in BR25 resulted in a substantial number of leaves with chlorotic symptoms and a high rate of leaf abscission. In contrast, leaf necrosis was more common in Husbitori, and leaves remained attached until an advanced stage of infection. Some leaves remained attached when over 80% of the leaf had become necrotic. In BR25, infections were concentrated at the base of the branch in leaves produced by the first or second flush (see Table 4), while in other clones, infections were commonly initiated in later flushes.

3.4. Evaluation of the number of new leaves

Individual branches of selected clones in four locations were monitored for new flushes and diseased leaves over 16 weeks in 2014 (see Section 2.5). The data in Table 6 indicate that by the second evaluation (at 9–10 weeks), KW617 not only had a significantly lower number of diseased leaves per branch but also had a higher new/diseased leaf ratio than the other clones, including PBC123. In contrast, although M05 had a lower number of diseased leaves per branch on average, the ratio of new/diseased leaves in this clone was not significantly higher than that of other clones.

4. Discussion

Resistance to VSD varied greatly among the tested cacao selections but was also relatively consistent in individual genotypes tested in different geographic locations. Seasonal fluctuations in mean severity were recorded (Figs. 2–4), with periods of relatively high rainfall associated with a decline in disease severity. Conversely, in drier periods, VSD symptoms became more severe, perhaps because of increased

Table 5
Length (cm) of stained vascular tissue in individual branches of four clones with moderately advanced vascular streak dieback infections. Infection was also indicated by symptomatic leaves and fungal hyphae in the xylem.

Clone	Length (cm)	Total number of leaf positions	Leaf position on branch		
			Symptomatic leaves	Vascular staining	Hyphae in stem xylem
Husbitori	45	13	5–12	1–12	1–12
M01 (specimen 1)	20	18	8-11, 15-18	5–11	4–13
M01 (specimen 2)	25	12	6, 8, 10-12	6–12	6–12
BR25	60	18	5-11, 13, 15, 17	1–17	1–13
PBC123	15	18	11	4–8	5-6, 11-12

Total branch length (cm): M01 (1), 50; M01(2), 45; BR25, 65; PBC123, 50.

stress as the fungus impeded xylem transport. However, new infections are initiated in wet periods (Keane, 1981). Despite these fluctuations, the mean VSD severity was generally lower in resistant than in susceptible clones during the period of evaluation.

Disease severity evaluated in various locations between 2010 and 2014 was consistent in clones such as PBC123 (resistant) and M01 (moderately susceptible), providing high broad-sense heritability estimates (Table 2). Heritability of VSD resistance is supported by the relative resistance of the progeny clones KW617 and ICCRI03 (Table 1; Figs. 1, 3 and 4), consistent with inheritance of a high degree of VSD resistance from the respective parental clones PBC123 and Scavina 6. Heritability of VSD resistance is also supported in the present Sulawesi study by the strong effect of genotype, compared to location, on mean VSD severity determined in clones common to the trials (see Table 2). Previous studies suggest that VSD resistance is likely to be polygenic and horizontal in nature, lending it durability (Prior, 1979; Tan and Tan, 1988; Keane and Prior, 1991; Keane, 2012). Durable resistance in PBC123 (now widely propagated in Sulawesi) and KA2-101 in PNG (Efron et al., 2002) has ensured that these clones have been useful to farmers for decades.

The study showed that VSD resistance did not generally occur in local cacao genotypes with high yield or bean quality. To replace VSD-susceptible genotypes on Indonesian farms, breeding programmes that combine VSD resistance traits and yield or quality are necessary. A number of local selections with promising yield and/or quality characteristics, such as M04 and Panimbu Red (McMahon et al., 2015), were highly susceptible to VSD (Table 1; Figs. 1–3). Therefore, while very promising, on-farm selection may be limited by the desired characteristics of yield, quality and resistance that are expressed by different cacao genotypes. For this reason, a number of government and company programmes are prioritising breeding programmes to produce hybrid crosses that combine VSD resistance with yield and quality traits. An additional justification for the incorporation of resistance traits into high-yielding (but susceptible) genotypes is that the propagation of susceptible genotypes can lead to increased inoculum and disease incidence on farms (Ploetz, 2007). Greater use of genotypes with VSD resistance traits would, in contrast, decrease pathogen spore loads on farms during wet conditions. Of the local farm selections identified with resistance in the evaluations between 2010 and 2012 (Section 3.1), only M06 could be considered useful for direct propagation on farms. The promising characteristics of M06, particularly its large bean size (McMahon et al., 2015), confirm its usefulness, and this clone is now widely used by Sulawesi cacao farmers. However, at times in this study, mean disease severity was high even in this clone (Figs. 1 and 2). Other local selections were identified with VSD resistance, but these clones would be of limited use. For example, Geni J is particularly susceptible to cocoa pod borer (with average infestation rates as high as 80%) and the bean size in M05 is too small to make this clone attractive for farm production (McMahon et al., 2015). These clones could potentially provide useful resistance characteristics in crossing experiments.

In Sulawesi, VSD evaluations of the clones produced from previous hybrid crosses gave mixed results. The trials included long-standing clones obtained from screening programmes in Malaysia, such as BR25 and PBC123. The latter clone was consistently among the most resistant of the clones tested, but BR25 was moderately susceptible. ICCRI03, the progeny of a cross between Scavina 6, a VSD-resistant clone, and DR2, a moderately susceptible clone with good yield and bean quality characteristics, proved one of the most VSD-resistant clones in the North Luwu trial (Table 1), confirming previous assessments in East Java (Agung Susilo, personal communication). Similarly KW617, obtained from a TSH858 × PBC123 cross in East Java, was determined as relatively resistant in the Polman trial, especially in the 2014 evaluation (Fig. 1; Table 6). Yields in this clone were among the highest in the Polman trial (McMahon et al., 2015). ICCRI04, also selected from the hybrid progeny of VSD-resistant and productive cacao genotypes, proved to be moderately susceptible in the Pinrang trial (Table 1, Fig. 2). ICCRI04 might have been more susceptible because of VSD susceptibility in its male parent (ICS60) (Agung Susilo, personal communication). However, the effect of location was shown to be significant for clones common to the trials in Sulawesi (Table 2), suggesting that while to a certain extent VSD resistance or susceptibility can be predicted by genotype, the effect of site (or environment) on resistance cannot be disregarded. A further example of inconsistency in different locations was provided by the clone DRC15, which was previously evaluated as moderately VSD resistant in East Java but as relatively susceptible in the Pinrang trial (Table 1).

Whilst being heritable and durable, the mechanism of VSD resistance remains unknown. Possible resistance mechanisms identified by Prior (1979) include restriction of the growth of the fungus within

Table 6
The ratio of new/diseased leaves and the number of diseased leaves in branches of selected cacao clones monitored from an early stage of infection with vascular streak dieback over 16 weeks (Section 2.5). Data shown are the means of n branches. Within each column, values followed by the same letter are not significantly different (p = 0.05).

Clone	n branches	Week 1		Week 9/10		Week 15/16	
		New/diseased leaves	No. diseased leaves	New/diseased leaves	No. diseased leaves	New/diseased leaves	No. diseased leaves
KW617	10	8.5^{a}	2.1^{a}	15.5^{a}	3.3^{a}	12.8^{a}	2.8^{a}
M05	12	4.2^{ab}	2.6^{a}	7.1^{b}	3.5^{a}	7.7^{ab}	4.2^{a}
PR	19	4.2^{ab}	4.2^{a}	5.6^{b}	7.1^{b}	2.4^{b}	8.5^{ab}
PBC123	16	3.4^{b}	4.1^{a}	5.1^{b}	5.1^{ab}	7.0^{ab}	6.7^{ab}
M01	18	3.5^{b}	4.5^{a}	3.3^{b}	7.2^{b}	4.0^{b}	10.9^{b}

the xylem and/or the release of exudates in young cacao leaves that inhibit germination of basidiospores. Flush leaf production might influence VSD infection. Spores generally infect young leaves, and, therefore, cacao flush cycles could influence VSD incidence and severity (Zainal Abidin et al., 1984; Keane and Prior, 1991). However, it is evident that flushing was as high in resistant as in more susceptible clones in the Polman trial (see Fig. 4). PBC123, for example, produced as many flush leaves as the other clones but had a low mean VSD severity (Table 1; Figs. 1–4). Furthermore, with the exception of two clones (see Section 3.2), no correlation was detected between the average flush leaf production and VSD severity. The timing of the flush may play a role. If peak flushes occur at the same time as sporulation, this would, in theory, increase the chances of infection. However, peak flush times did not usually differ among clones (Section 3.2), indicating that the clones would have been equally exposed to infection when sporulation occurred and further suggesting that the differences in VSD severity among clones were not dependent on flush leaf production.

In this study, observations of the progress of infection in selected branches (Section 3.3) demonstrated that in the susceptible local selections, Husbitori and M01, infections in different flushes apparently spread readily along the branch between leaves, while the spread of the fungus between leaves was more restricted in PBC123 (Tables 3–5). VSD resistance could be determined, in part at least, by the extent of spread of infection in branches. Prior (1979) reported that fungal growth in the xylem coincided with vascular streaking, which is consistent with the data in Table 5. The occurrence of VSD symptoms and vascular staining in the younger leaves of susceptible genotypes suggests that infections in young leaves are initiated by the spread of the fungus from the sites of initial infection. As the clones in these trials were top-grafted onto locally available seedlings (raised in nurseries from seed), the possibility that the rootstocks themselves influence resistance to VSD cannot be ignored. Further research is needed to elucidate the possible effects of rootstocks on resistance.

In some cases, infections that developed in younger leaves appeared soon after the initial infection of an earlier flush and appeared to bypass healthy leaves. This may be related to vascular anatomy because in order to spread to other leaves, the fungus needs to cross between primary xylem of leaf traces and the secondary xylem of the branch (Prior, 1979). Alternatively, the younger leaves or later flushes could be affected physiologically by earlier infections, suggesting some symptoms (chlorosis or necrosis) of VSD-infected leaves might be a result of nutrient deficiency. For example, nutrient deficiency symptoms in younger leaves (particularly calcium deficiency) could result from VSD infection (Keane et al., 1972). Keane (1981) reported that in seedlings inoculated when 3 weeks old, chlorotic symptoms first appeared on the second flush 7 weeks after inoculation and that this was followed, at 9 weeks after inoculation, by chlorosis in the leaves of the first flush, on which spores had been deposited. It appears that the younger flush was physiologically affected by the leaves of the initially infected flush. In the Polman trial, it is possible that in some cases, the symptoms observed in the younger leaves of the infected branches were a physiological result of infections in older leaves further down the branch rather than direct infection by the fungus. A blockage of xylem in the stem, for example, might lead to chlorosis or necrosis of younger leaves (sensitive to drought or nutrient stress) near the tip of the branch. In addition, the necrotic VSD symptoms are similar to those of some nutrient deficiencies, especially potassium deficiency, which causes marginal necrosis (Wessel, 1985).

Whilst restriction of spread of infection between leaves (Prior, 1979) was observed in this study, differential growth or vigour of genotypes clearly plays a role in overcoming infection. Evaluations conducted in 2014 showed that while the number of diseased leaves was significantly low in both M05 and KW617 (compared to the more susceptible M01), by the second and third evaluations, new growth was much higher in KW617 than in M05 (Table 6). This suggests that resistance in KW617 might be partly related to its capacity for new growth. The lower new/diseased leaf ratio in PBC123 and M05 could be explained by their comparatively slow growth, particularly that of M05. In addition, new growth enabled even susceptible clones to overcome VSD infection despite the higher number of infected leaves (Table 6). M01 and the Malaysian clone BR25, which are both high yielding in the absence of VSD but moderately VSD susceptible in the current trials (Table 1, Fig. 1), are propagated widely in Sulawesi. Despite its susceptibility to VSD, BR25 proved to have a relatively high proportion of uninfected leaves at branch tips (Table 3), suggesting that this clone can overcome VSD infections by new growth. Evaluations in 2014 of flush leaf production in branches at an early stage of infection in five clones grown in four locations indicated that some clones, including M01, with a high disease severity rating (determined by the number of infected and abscissed leaves) produced as many or more new leaves on average than clones with lower disease severity ratings (Table 6). Although a relatively high number of leaves became infected in M01, a high new leaf/diseased leaf ratio (3.3–4) ensured that the branches continued to grow.

5. Conclusion

The trials in Sulawesi enabled the identification of local cacao clones with promising yield and quality characteristics that were, unfortunately, also particularly susceptible to VSD. Other clones maintained VSD resistance in separate geographic locations, consistent with a high heritability for VSD resistance traits. In addition, clones obtained from the hybrid progeny of crosses between VSD-resistant and high-yielding parents were shown to have promising resistance characteristics, in addition to quality and yield. These observations suggest that in breeding programmes, selection for resistance followed by other less heritable traits, particularly yield (Lockwood et al., 2007), would be an appropriate strategy to incorporate VSD resistance into high-yielding cacao clones.

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Testing local cacao selections in Sulawesi for resistance to vascular streak dieback

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