





## Article

# Identifying New Resistance to Cassava Mosaic Disease and Validating Markers for the *CMD2* Locus

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**Abstract:** Cassava (*Manihot esculenta* Crantz) is a crucial staple crop, and provides carbohydrate energy to more than half a billion people in the tropics. Cassava mosaic disease (CMD) is the most important disease of cassava in Africa. Since Sri Lanka Cassava Mosaic Virus (SLCMV) was first reported in South East Asia in 2015, establishing sustainable solutions to CMD has become a top priority for the cassava program at the International Center for Tropical Agriculture (CIAT) and its partners. In the present study, we screened two populations for CMD resistance: VNM142, 142 clones collected from farms throughout Vietnam, and CIAT102, 102 clones resistant to CMD or mites, which were introduced from CIAT. High broad-sense heritability was observed in all the trials (>0.80). From the population VNM142, eight clones showed high CMD resistance with CMD severity scores less than 2.0. Two resistant clones had the same DNA fingerprinting with the accessions CR63 (PER262 or TAI9) and KM57 (VNM8) in the genebank, respectively. To our knowledge, this is the first report of CMD resistance in the genebank at CIAT. We also used the two populations to validate the CMD markers S12\_7926132 and S14\_4626854. Both markers explained 51% of the population variance in the segregating population CIAT102, but only 11% in the diverse population VNM142. Thus, we concluded that the two CMD markers could not be used to select for CMD resistance in diverse populations, but could predict the CMD resistance in segregating populations when the susceptible parents do not have resistant marker alleles and the resistance of the *CMD2* donors is confirmed.

**Keywords:** cassava; cassava mosaic disease; marker-assisted selection; *CMD2*

## 1. Introduction

Cassava (*Manihot esculenta* Crantz) provides the third-largest source of food carbohydrates in the tropics, after rice and maize. Cassava is usually grown by smallholder

farmers and feeds more than half a billion people worldwide [1]. It is a major staple crop in developing countries, especially in sub-Saharan Africa. In 2019, among the 304 million tons of global production, 63% were produced in Africa and 45% were in low-income, food-deficit countries [2]. Thus, cassava is considered as one of the high-priority staple crops, contributing to ending hunger and reducing poverty [3].

In South East Asia (SEA), cassava has become an importance crop for rural livelihoods and economic development [4]. It was estimated that more than 8 million farmers grow cassava in SEA, covering approximately 3.9 million ha and accounting for 25% of the global production [2]. Due to the strong market demand, cassava production has increased in the region with rapid expansion to Cambodia, Lao PDR, and Myanmar. The total export value of dried cassava was more than 1.3 billion US dollars on average every year in the past five years in SEA [2]. However, cassava mosaic disease (CMD) is spreading in the region, which caused a yield loss between 30% and 50% from the secondary infection [5].

CMD is caused by the cassava mosaic virus (genus, *Begomovirus*; family *Geminiviridae*). More than 11 species of cassava mosaic virus have been identified, nine of which from Africa; for example, *African cassava mosaic virus*, *East African cassava mosaic Cameroon virus*, and *East African cassava mosaic Kenya virus*. The two remaining species were reported in South Asia and SEA, namely, *Indian cassava mosaic virus* and *Sri Lanka cassava mosaic virus* [6]. The cassava mosaic virus family of geminiviruses has caused a widespread epidemic in Africa [7,8]. The CMD in SEA is caused by *Sri Lanka cassava mosaic virus* (SLCMV). SLCMV was first reported in Cambodia in 2015, and is now present throughout the major cassava production countries: Cambodia, Vietnam, Thailand, China, and Lao PDR [9–12].

With the support of the CGIAR Research Program on Roots, Tubers and Bananas (CRP-RTB) and Australian Center for International Agricultural Research (ACIAR) and the International Institute of Tropical Agriculture (IITA), the Cassava Program of the International Center for Tropical Agriculture (CIAT) is leading to establish sustainable solutions to cassava diseases in mainland SEA. Our aim is to enhance smallholder livelihoods and economic development in mainland SEA by improving the resilience of cassava production systems and value chains by addressing the rapidly evolving disease constraints.

Introducing and developing resistant varieties is a major component of the sustainable solutions. Two types of CMD resistance have been deployed in Africa: (1) quantitative and recessive resistance from *Manihot glaziovii* [13–15]; and (2) qualitative and dominant resistance, such as *CMD2* from cassava landraces collected in Nigeria and elsewhere in West Africa. Due to the dominant inheritance, *CMD2* became the predominant resistant source in African cassava breeding programs [16–19]. Collaborating with the cassava program at IITA, the CIAT cassava program has introduced and identified six source populations of CMD resistance in SEA: (i) C33, C39, and TME3 from CIAT—originally from IITA; (ii) five improved CMD-resistant clones from IITA; (iii) local resistant clones identified from 142 clones collected from farms in Vietnam—the population VNM142; (iv) 102 clones with CMD or mite resistance from CIAT—the population CIAT102; (v) >10,000 seeds produced from CIAT and IITA progenitors in Hawaii with the support of the NextGen Cassava project; and (vi) 474 seeds as the training population of genomic prediction derived from CMD donors and elite parents at CIAT. The populations (ii) and (v) might have the two types of resistance from Africa, and the populations (i), (iv), and (vi) mainly contained *CMD2* resistance, while the local population VNM142 might provide new type(s) of resistance to SLCMV.

Besides introduction and identification of CMD-resistant clones or varieties in SEA, conventional breeding is performed by crossing CMD-resistant clones with elite varieties in Vietnam, Thailand, and CIAT. To accelerate the selection cycles, and considering the challenges for trait introgression in cassava [20], we will use marker-assisted selection (MAS) to increase the selection efficiency and accuracy. MAS was mainly focused on the *CMD2* locus that conferred resistance to all cassava mosaic virus strains in Africa. Genetic mapping located the *CMD2* locus on chromosome 12 of the refer-

ence genome AM560-2 [17–19,21]. Three markers for CMD resistance were developed and are available on the Intertek genotyping platform. The Intertek platform is affordable for breeding programs and recommended by CGIAR Excellence in Breeding (EiB; <https://excellenceinbreeding.org/toolbox>, accessed on 26 August 2021.). Two tightly linked markers are on chromosome 12, S12\_7926132 and S12\_7926163, and one marker, S14\_4626854, on chromosome 14 [18,22]. These markers were routinely used in the African breeding programs. However, the cassava varieties in Asia belong to a different market segment, such as industrial use given plenty of stable dry matter; they also have an erect plant type and their genetic background is likely different from African breeding populations. Thus, the efficiency of the CMD markers should be validated before they are deployed in Asian breeding programs. Availability of reliable markers is essential since countries in SEA are taking measures to prevent further spread of the virus and, therefore, the availability of hotspots for phenotypic selection is limited.

In the present study, we evaluated two populations, CIAT102 and VNM142, in the CMD hotspot Tay Ninh in Vietnam and genotyped the two populations using CMD markers. The objectives of the study are (1) to identify CMD resistance in the introduced materials and the local germplasm; (2) to understand the frequency of the resistant marker alleles in the populations; and (3) to validate the effectiveness of the CMD markers in predicting CMD resistance.

## 2. Materials and Methods

### 2.1. Plant Materials

The population CIAT102 includes 102 clones introduced from CIAT in 2019. The 102 clones were the progeny of the CMD-resistant progenitors, C-4, C-18, C-33, C-39, C-127, C-243, C-377, and C-41. These C- clones were derived from the crosses between TMS30555 (NGA5 in CIAT genebank) and the CMD-resistant clone, TME3, with the *CMD2* gene [16]. Other progenitors of CIAT102 were derived from crosses with the wild relative, *M. esculenta* ssp. *flabellifolia*. The pedigree of CIAT102 is available in CassavaBase [23]. The population VNM142 contained 142 clones collected from farms in Vietnam in 2015 [24,25]. From VNM142, we identified 93 unique clones based on the DNA fingerprinting results using a SNP-chip with 96 SNP markers at CIAT (SNPY-Array; Fluidigm®, South San Francisco, CA, USA) [26,27]. The released varieties KM419 and KU50 were present in the population VNM142.

### 2.2. Field Trials for CMD Screening

All the CMD screening was performed in Tay Ninh, where whiteflies are active and quickly build up populations. The elevation is 18 m above sea level, and the monthly temperature ranges from 26.5 to 29.2 degrees Celsius. The population VNM142 was screened for CMD resistance in the 2018–2019 growth season (October 2018–August 2019). The trial was designed following a random complete block design (RCBD) with three replications. A total of 16 or 12 plants were planted in each plot depending on the availability of stem cuttings. The CMD data were collected plant-by-plant in each plot at 6 weeks, 3 months, 6 months, and 10 months after planting (MAP). We used the CMD score, 1–5, to record the CMD severity, where 1 is non-symptomatic and 5 is the worst infection with very severe chlorosis and heavily reduced leaf area [28]. The plots with less than 5 germinated plants were removed before data analysis.

Nine VNM142 clones with good CMD resistance were evaluated again in Tay Ninh in the 2019–2020 growth season (September 2019–August 2020). HLS11, KM419, and KU50 were used as the elite local checks. RCBD was used with three replications for all clones, except for HLS11 with 15 replications used as the virus spreader in the trial. There were 56 plants per plot with 7 rows and 8 plants per row. Due to the limited availability of stem cuttings of three VNM clones, VN19-340 (UNQ-45), VN19-390 (UNK-CH), and VN19-320 (UNQ-44) and C33, a new trial was established in the same field following RCBD with 3 replications and 20 plants per plot. HLS11, KM419, and KU50 were checks and HLS11 was

used as the spreader. The CMD severity was observed plant-by-plant every month, from 1 to 10 MAP. Because of the low germination caused by the drought stress, no agronomic data but only CMD scores were reported in the present study.

The population CIAT102, five CMD-resistant clones introduced from IITA (TMEB419, IITA-TMS-IBA920057, IITA-TMS-IBA972205, IITA-TMS-IBA980505, and IITA-TMS-IBA980581), and the elite varieties, HLS11, KM419, and KU50, were evaluated in Tay Ninh in the 2019–2020 growth season (October 2019 – September 2020). The yield trial was carried out using RCBD with three replications. There were 16 to 36 plants in each plot depending on the number of seedlings available for individual clones. The CMD data were collected plant-by-plant in each plot at 1 and 3 MAP using the CMD score, 1–5, for severity. Since the resistant clones did not show symptoms throughout the growth season, no CMD data were collected after three months. Five IITA clones were used as controls for both CMD scoring and CMD markers, and HLS11, KU50, and KM419 were used as the elite local checks. The planting materials were from tissue culture, rather than typical stem cuttings used by farmers, so the yield and dry matter data might not be accurate and were not reported here. Advanced yield trials were established in May 2020 to evaluate the agronomic performance of the resistant clones. The phenotypic data of these trials are available in CassavaBase.

### 2.3. Genotyping

Three CMD markers, S12\_7926132, S12\_7926163, and S14\_4626854, designed by Dr. Ismail Rabbi [18,22] on chromosomes 12 and 14, were used for the validation. Leaf tissues of the two populations were sent to Intertek following the protocol developed by CGIAR Excellence in Breeding (EiB; <https://excellenceinbreeding.org/toolbox/services/low-density-genotyping-service>, accessed on 26 August 2021).

### 2.4. Data Analysis

Among the 93 unique clones in VNM142, 78 clones had both CMD field scores and the genotypic data of three CMD markers. Among the clones from the population CIAT102, 82 clones had both CMD field scores and genotypic data for validating the CMD markers. Since there are missing data among the replications due to low germination or limited availability of planting material, a linear mixed model was used with replication and clone as the random effects, and BLUP was calculated using the R package *lme4* [29]. A t-test was used to assess the significance of the individual markers on the reaction to CMD. Linear regression was used to calculate the percentage of variance explained by the CMD markers. All the statistical analyses were performed using R [30].

## 3. Results

### 3.1. CMD Resistance Identified from the Populations VNM142 and CIAT102

The CIAT102 population showed high heritability, 0.91, at 3 MAP (Table 1). In total, 34 clones from CIAT did not show any CMD symptom during the growth season, and the elite varieties KU50 and KM419 showed severe CMD symptoms with CMD scores higher than 2.7.

**Table 1.** Variation in CMD severity of the VNM142 and CIAT102 populations in two growth seasons, 2018–2019 and 2019–2020.

| Population | Trial         | Trait      | Mean | Median | Range     | V <sub>g</sub> | V <sub>e</sub> | H <sup>2</sup> |
|------------|---------------|------------|------|--------|-----------|----------------|----------------|----------------|
| VNM142     | 201801MDEAR   | CMD_1.5MAP | 1.56 | 1.43   | 1.00–4.00 | 0.10           | 0.21           | 0.50           |
|            |               | CMD_3MAP   | 1.87 | 1.75   | 1.00–4.00 | 0.18           | 0.22           | 0.63           |
|            |               | CMD_6MAP   | 2.23 | 2.20   | 1.00–4.00 | 0.27           | 0.19           | 0.75           |
|            |               | CMD_10MAP  | 2.82 | 2.86   | 1.19–4.00 | 0.40           | 0.19           | 0.82           |
| VNM142     | 201901MDEAR * | CMD_10MAP  | 2.65 | 2.33   | 1.00–4.12 | 1.30           | 0.04           | 0.99           |
|            | 201902MDEAR # | CMD_10MAP  | 2.83 | 2.63   | 1.70–4.10 | 0.62           | 0.11           | 0.94           |
| CIAT102    | 201903MDEAR   | CMD_3MAP   | 1.73 | 1.64   | 1.00–3.77 | 0.42           | 0.12           | 0.91           |

Note: V<sub>g</sub>, total genetic variance among unique clones; V<sub>e</sub>, the variance of the residue. The calculation of genetic variance was performed by using a linear mixed models by fitting replication and clone as random effects. MDEAR, cassava mosaic disease advanced yield trial. \* The trials with three clones from VNM142 and four checks, HLS11, KM419, KU50, and C33. # the trials with 9 clones from VNM142 and three checks, HLS11, KM419 and KU50.

The heritability of the reaction to CMD in the VNM142 population gradually increased from 0.50 to 0.82 from 1.5 months to 10 months after planting (MAP). The CMD pressure for the population was moderate, with 2.82 as the population mean of the CMD score (Table 1). Among the top three resistant clones, two were identical with the accessions in the genebank at CIAT based on the DNA fingerprinting data (Table 2). One clone was matched with CR63, PER262, and TAI9. The passport data showed that the common name of CR63 was Valencia from Costa Rica, PER262 was Valenka from Peru, and TAI9 was Hanatee from Thailand. The second clone was KM57, VNM8, and Xanh Vinh Phu (Table 2). The CMD resistance of the top seven clones was confirmed in the following year. Among them, the CMD scores of CR63 (PER262 or TAI9) and KM57 (VNM8 or Xanh Vinh Phu) were 1.9, while the elite varieties KU50 and KM419 were higher than 3.5 (Table 2). Different from the immunity (no symptoms) provided by CMD2 in CIAT102, the resistant clones in VNM142 showed a few symptoms, but significantly less than the susceptible elite varieties.

**Table 2.** The CMD score and genotype of the top resistant clones from VNM142 and the check clones.

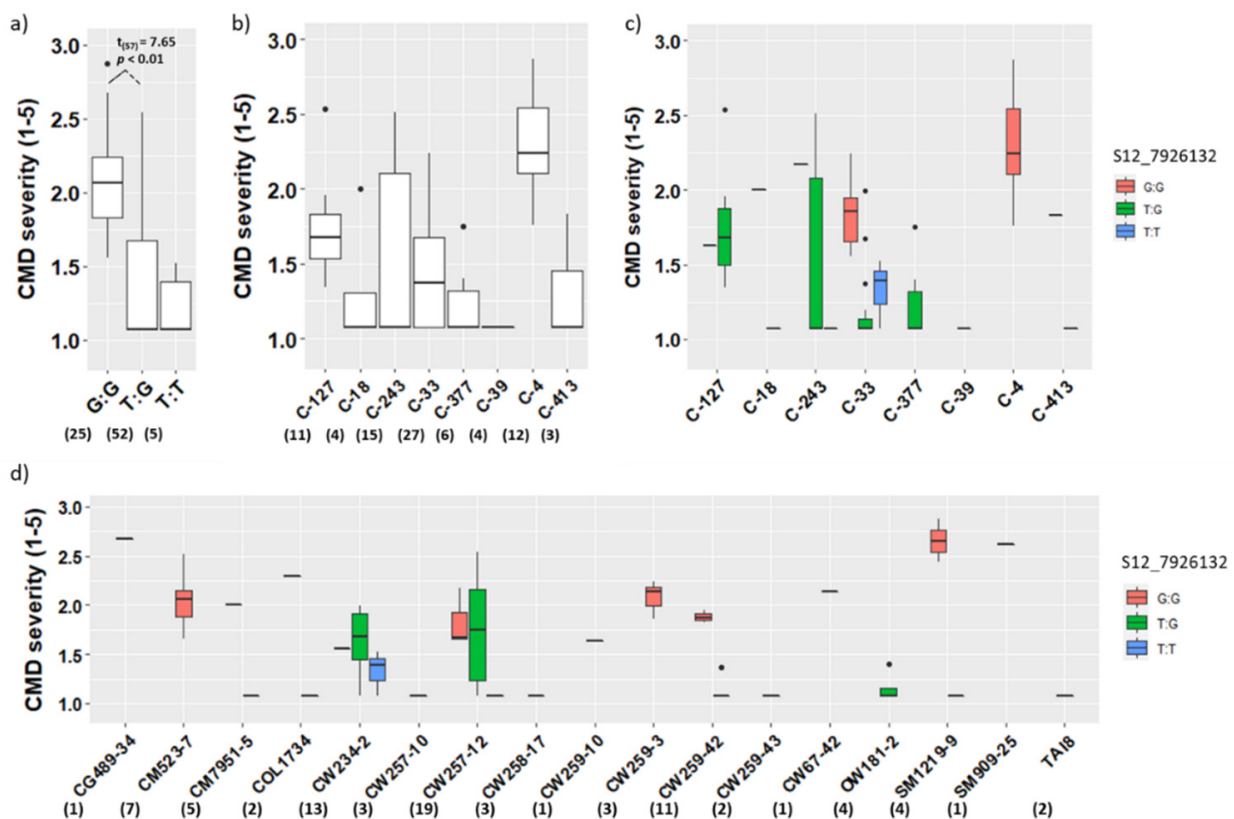
| Genotype/Group          | Clone                              | 2018–2019 | 2019–2020 | S12_7926132 | S14_4626854 |
|-------------------------|------------------------------------|-----------|-----------|-------------|-------------|
| UNK-CI-2                | VN19-442                           | 1.5       | 2.3       | T:G         | A:G         |
| CR63_PER262_TAI9        | VN19-1432, VN19-1556               | 1.6       | 1.9       | T:G         | A:G         |
| KM57_VNM8_Xanh Vinh Phu | VN19-1039, VN19-1050               | 1.6       | 1.9       | T:G         | A:G         |
| UNQ-115                 | VN19-773                           | 1.7       | 2.1       | T:G         | A:G         |
| UNK-F                   | VN19-1184, VN19-1194               | 2.0       | 2.6       | T:G         | A:G         |
| UNQ-44                  | VN19-320                           | 1.7       | 1.8       | G:G         | G:G         |
| UNK-AF-2                | VN19-1805                          | 1.8       | NA        | G:G         | A:G         |
| UNK-CH                  | VN19-390                           | 1.9       | 2.2       | G:G         | G:G         |
| KU50_KM94_TAI16         | 11 clone samples (e.g., VN19-1739) | 2.6       | 3.5       | G:G         | G:G         |
| KM140                   | 4 clone samples (e.g., VN19-2659)  | 3.6       | NA        | G:G         | G:G         |
| KM419                   | 2 clone samples (e.g., VN19-2202)  | 3.0       | 4.0       | G:G         | G:G         |
| C33                     | C33                                | NA        | 1.1       | T:G         | A:G         |

Note: The BLUP of the CMD score at 10 months after planting was provided here for each unique clone (or group).

### 3.2. Validation of CMD Markers in the Population CIAT102

The genotypic data of markers S12\_7926132 and S12\_7926163 were the same, so only the results of S12\_7926132 are reported here. Among the 82 clones with both genotypic and phenotypic data, 52 clones had one resistant allele T, and five clones had the homozygous T alleles. All the clones with homozygous susceptible allele G showed CMD symptom, and the five clones with homozygous resistant allele T were resistant to CMD (Figure 1a). Although a significant difference in CMD severity was observed between genotypes G:G and T:G ( $t_{(57)} = 7.65, p < 0.01$ ), the heterozygous genotype, T:G, showed a large range of CMD severity scores, which was not consistent with the dominant inheritance of the CMD2 gene (Figure 1a).





**Figure 1.** The CMD severity of the population CIAT102 grouped by the genotype of the marker S12\_7926132 or family structure. (a) The CMD severity of the different genotypes at 3 months after planting. Among the 82 clones in CIAT102, 52 clones had the resistant allele T, and five clones are homozygous with the resistant allele. Significant difference among the clones with and without the resistant allele was observed (mean of CMD severity scores 1.4 vs. 2.1, respectively). All the 25 clones without the resistant allele T showed CMD symptoms. (b) The CMD scores of the CIAT102 clones grouped by the donor parents of CMD resistance. C-127 and C-4 did not produce progeny with CMD resistance. (c) The CMD severity of the CIAT102 clones grouped by their genotype and the donor parents of CMD resistance. Except for C-4, all other donor parents produced progeny with the resistant allele T, but the resistant allele T did not provide CMD resistance in the progeny of C-127. (d) The CMD severity of the CIAT102 clones grouped by their genotype and other progenitors. The progeny of CW234-2 and CW257-12 showed segregation in CMD resistance even though containing the resistant allele T.

The 82 clones were derived from eight *CMD2* donor progenitors, so we further looked at the CMD resistance of the progeny from these eight progenitors (eight half-sib families). All the progeny of C-127 and C-4 were susceptible to CMD (Figure 1b). We then analyzed the genotype of the progeny, and noticed that there was no T allele in the progeny of C-4, which was consistent with the susceptibility (Figure 1c). The progeny of C-127 had the resistant allele, but did not show CMD resistance, which indicated that the T allele did not link with the resistant locus and might belong to a different haplotype.

We then grouped and analyzed the progeny based on other progenitors (half-sib families), of which ten CW clones were derived from the wild crosses with *M. esculenta* ssp. *flabellifolia*. The progeny of CW234-2 and CW257-12 had the resistant allele, but showed segregation in CMD resistance (Figure 1d). The two progenitors might provide the haplotype with the T allele but without CMD resistance. We took a close look at the clones with the resistant allele T in full-sib families (the family name is part of the clone name, e.g., the family name of AR12-22 is AR12), and observed that the clones in full-sib families AR1 and AR7 were susceptible to CMD, even though they had the T allele (Figure 2). These two families were derived from C-127, CW234-2, and CW257-12, which was consistent with the susceptibility of the C-127 progeny. The other three full-sib families (AR9, AR12, and AR18) showing segregation also shared the two progenitors, CW234-2 and CW257-12, which was derived from the same genotype, FLA437-7 (*M. esculenta* ssp. *flabellifolia*; Figure 3). Thus,

the susceptible haplotype with the T allele in the CIAT102 population might come from the cassava wild relative *M. esculenta* ssp. *flabellifolia*, and the CMD donor, C-127, did not have the resistant T allele. Alternatively, the T allele coming from C-127 and FLA437-7 might link with a locus that inhibits or reduces the expression of the resistance of the CMD2 locus.

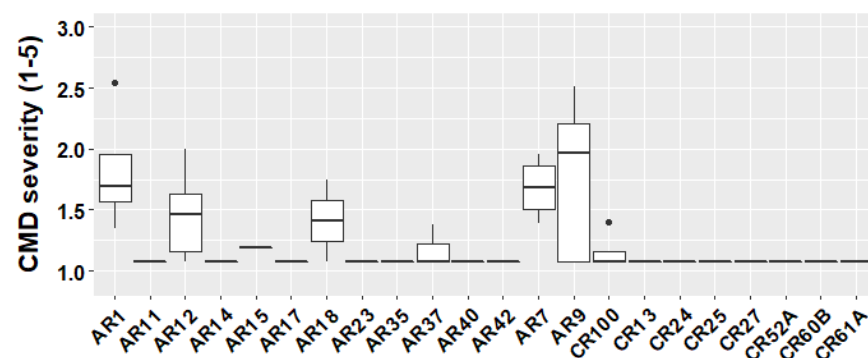
For the other CMD marker on chromosome 14, S14\_4626854, among 35 clones with the resistant allele A, 29 clones did not have any CMD symptom, and significant differences were observed between genotype A:G and G:G ( $t_{(79)} = -7.23, p < 0.01$ ; Figure 4a). The progeny of C-4 and C-127 did not have the resistant allele A and showed high CMD scores (Figure 4b). Similar with marker S12\_ S12\_7926132, the progeny of CW234-2 and CW257-12 showed variation in CMD scores even though they had the resistant allele A (Figure 4c).

Each marker explained 36% of the population variance in CMD severity, but their combined effect was 51%, which suggested the interaction between the markers on two chromosomes. Among the 25 clones with the G:G of S12\_7926132, 22 clones had the genotype G:G of S14\_4626854 (Figures 1a and 5a), but the 52 clones with the T:G of S12\_7926132 were divided into two groups by the marker S14\_4626854 (24 and 28; Figure 5b). The clones with T:G\_A:G were resistant to CMD, but the clones with T:G\_G:G mainly showed susceptibility (Figure 5a).

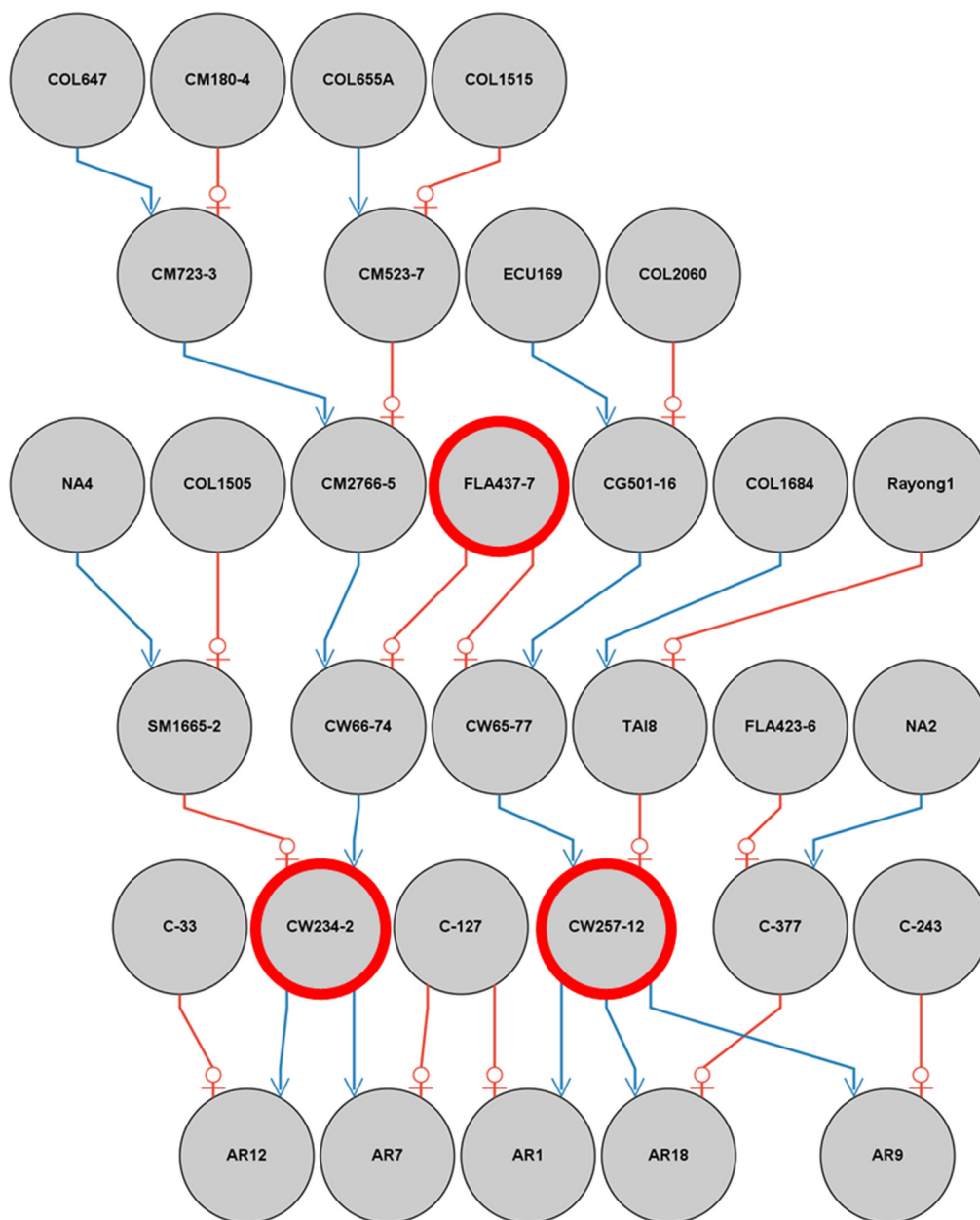
### 3.3. Validation of the CMD Markers in the Population VNM142

Among the 78 unique clones with both genotypic and phenotypic data, 20 clones had the resistant allele T of S12\_7926132, and 19 clones had allele A of S14\_4626854. Both the T allele of S12\_7926132 and the A allele of S14\_4626854 provided significant CMD resistance (Figure 6a,b). Among the top eight clones with a CMD scores less than 2, five clones had both the T allele of S12\_7926132 and A alleles of S14\_4626854, and the other three clones did not have the T allele, but one had the A allele. Two-year CMD evaluation confirmed their better CMD resistance than the elite varieties, KU50, KM140, and KM419 (Table 2).

Marker S12\_7926132 explained an 11% variation in the population VNM142, and S14\_4626854 accounted for 8%. When considering both markers, we observed that only 11% variation was explained, which indicated the strong interaction between the two markers. In this diverse VNM142 population, we found a high rate of co-segregation between the two markers. Among the 77 clones with the genotypic data of both markers, 72 clones (94%) had the same genotype for both markers (Figure 6c). The high rate of co-segregation between the two loci on different chromosomes seems abnormal, and the high rate of co-segregation (73%) was also observed in the CIAT102 population (Figure 5b).

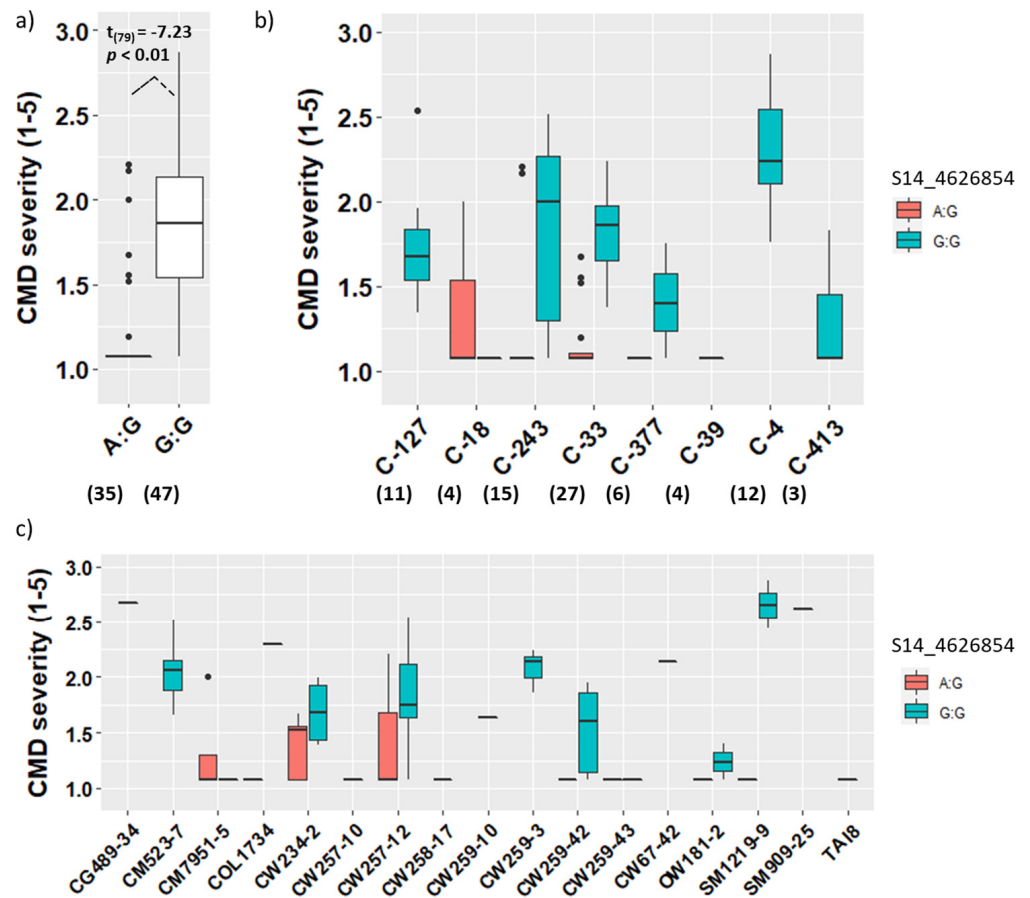


**Figure 2.** The CMD severity of 57 clones with the resistant allele T of S12\_7926132 in full-sib families of CIAT102. In the five full-sib families, AR1, AR7, AR9, AR12, and AR18, the clones with the resistant allele T showed segregation in CMD resistance.

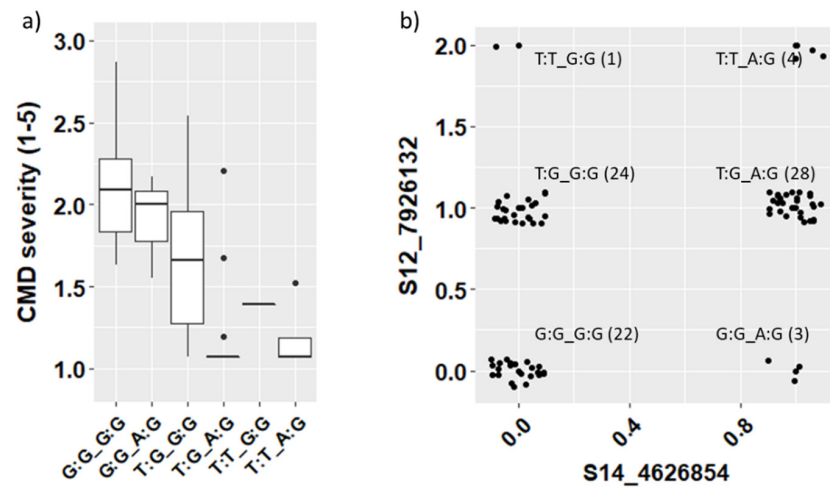


**Figure 3.** The pedigree of the five full-sib families, AR1, AR7, AR9, AR12, and AR18, which might have the T allele of S12\_7926132 without CMD resistance. Families AR7 and AR12 shared the progenitor CW234-2, and families AR1, AR9, and AR18 shared the progenitor CW257-12. CW234-2 and CW257-12 might be the sources of the T allele without CMD resistance. Both progenitors were derived from the wild crosses with FLA437-7 (*M. esculenta* ssp. *flabellifolia*).

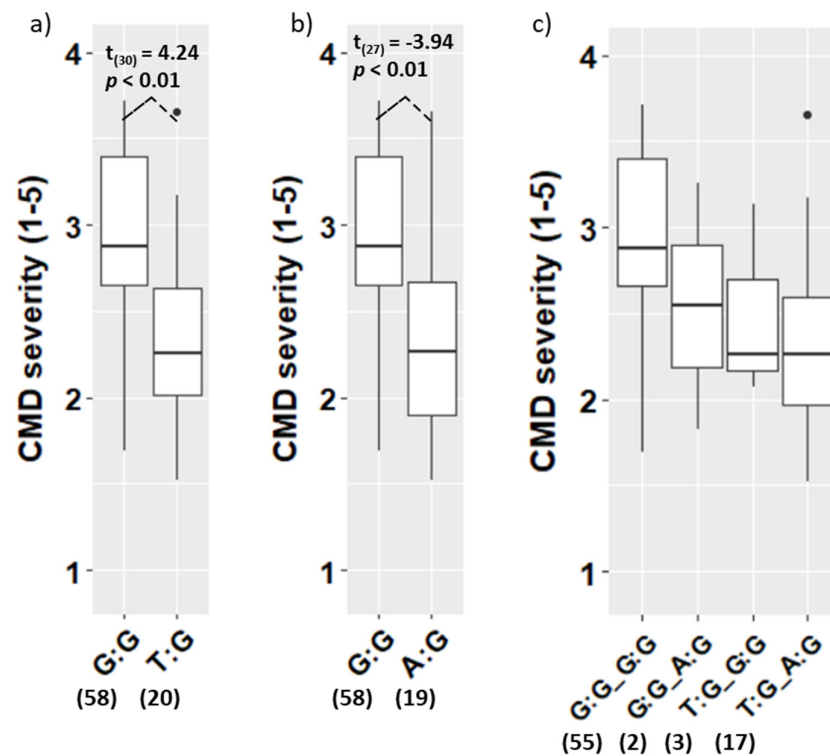




**Figure 4.** The CMD severity of the CIAT102 population grouped by the genotype of the CMD marker S14\_4626854 or family structure. (a) The CMD severity of the different genotypes at 3 months after planting. Among the 82 clones in CIAT102, 35 clones had the resistant allele A. Significant differences among the genotypes with and without the resistant allele were observed (mean of the CMD severity scores 1.2 vs. 1.8). Five clones without the resistant allele A showed CMD resistance, and six clones with the resistant allele A showed CMD symptoms. (b) The CMD severity of the CIAT102 clones grouped by their genotype and the donor parents of CMD resistance. C-127, C-4, and C-413 did not produce progeny with the resistant allele A. (c) The CMD severity of the CIAT102 clones grouped by their genotype and other progenitors. The progeny of CW234-2 and CW257-12 showed segregation in CMD resistance when containing the resistant allele A.



**Figure 5.** The combined effect and similarity of the markers S12\_7926132 and S14\_4626854 in CIAT102. (a) The CMD score of the clones grouped based on the two CMD markers. The x-axis showed the genotype combination. For example, T:G\_G:G means the clones had T:G alleles of S12\_7926132 and G:G alleles of S14\_4626854. (b) The genotypic similarity between the two markers S12\_7926132 and S14\_4626854. The two markers matched well for the susceptible genetics, G:G\_G:G (22 vs. 25), and the clones with T:G were divided into two groups (24 vs. 28). The 28 clones with both resistant alleles A and T showed high CMD resistance.



**Figure 6.** The CMD severity of the VNM142 population grouped by the genotype of the CMD markers S12\_7926132 and S14\_4626854. (a) The CMD severity of the different genotypes at 10 months after planting. Among the 78 clones in VNM142, 20 clones had the resistant allele T of S12\_7926132. Significant difference among the genotypes with and without the resistant allele was observed (the mean of the CMD severity scores 2.3 vs. 2.9). (b) Significant difference among the genotypes with and without the resistant allele A of S14\_4626854 was observed (the mean of the CMD severity scores 2.3 vs. 2.9). (c) The combination effect of the two markers. S12\_7926132 and S14\_4626854 co-segregated in 72 of the 77 clones (94%).

## 4. Discussion

### 4.1. CMD Resistance in the Introduced Germplasm from the CIAT and Asian Landraces

The introduced population, CIAT102, was derived from *CMD2* donors, C-clones, which were the progeny of the *CMD*-resistant clones in Africa, TME3 [16]. The *CMD2* resistance was confirmed in the CIAT102 clones because 34 clones were asymptomatic when exposed to the SLCMV. The siblings of the CIAT102 clones had been evaluated in Africa, and CR36-5, released as Ayaya, had good *CMD* resistance and high and stable dry matter (CGIAR news). Even though the family CR36 was not present in the CIAT102 population, we expect that some CIAT102 clones might have good agronomic performance and can be released as the first generation *CMD*-resistant varieties in SEA. Moreover, CR24-4, CR43-7, and CR43-2, introduced from CIAT, were released in India showing high resistance to the *CMD* strains there, which included SLCMV [4]. These resistant clones are being evaluated in new yield trials in Vietnam for their agronomic traits, especially the yield and root dry matter content, and further validation of their reaction to *CMD*.

Among the local clones from the farms in Vietnam, eight showed good *CMD* resistance with *CMD* scores less than 2.0 in two years of field trials. Two unique clones had the same DNA fingerprinting with the known landraces Hanatee and KM57 (Xanh Vinh Phu) in Asia. Hanatee (TAI9 in CIAT genebank) is a popular cassava landrace for human consumption in Asia and Latin America due to its low cyanogenic glucoside content and mealy texture after boiling [31,32]. However, Hanatee had a lower yield and starch content than the industrial varieties, KU50 and HB80 [33,34]. Breeding effort had been made in Thailand using Hanatee as parent. Two varieties, Pirun 1 and Pirun 2, were released in 2014 and 2015, but their reaction to *CMD* was unknown [4]. Little is known about the origin of the other seven resistant genotypes. Their agronomic performance is currently under evaluation in Vietnam. On the other hand, even though CR63\_PER262\_TAI9 (Hanatee) and KM57 had the resistant alleles T and A of the two markers, both clones still showed a few *CMD* symptoms. The resistance mechanism might be different from the immunity of *CMD2*. To our knowledge, this is the first report of the *CMD* resistance from the genebank at CIAT. It should be pointed out that a recent diversity study placed TME3 within the cluster of South American germplasms [35]. Genetic studies are on-going to understand the genetics structure of the *CMD* resistance in the resistant clones in VNM142. Furthermore, encouraged by the identification of resistance to *CMD* and cassava brown streak disease from the genebank at CIAT [36], we are screening the core collections and expecting to identify new sources of *CMD* resistance for the cassava community in Africa and Asia.

Thus, from the introduced and local germplasm, we identified more than 40 genotypes showing a good level of *CMD* resistance. These clones were under intensive evaluation in Vietnam, and will be tested in Laos, Cambodia, and Thailand as soon as phytosanitary permits allow their introduction into these countries. Some of these resistant clones might have the potential to be released as varieties, because of their better performance than current varieties under moderate or high *CMD* pressure.

### 4.2. Effectiveness of the *CMD* Markers S12\_7926132 and S14\_4626854

The *CMD2* region on chromosome 12 has been reported from linkage mapping and genome-wide association mapping [17–19]. The susceptible allele G of S12\_7926132 was linked with the susceptibility in both the CIAT102 and VNM142 populations, but clones with the resistant allele T showed large variation in *CMD* resistance. In CIAT102, we observed the homozygous resistant alleles in five clones in the population CIAT102. Since no crosses had been made between the resistant clones, we assumed one of the T alleles was probably not linked with the *CMD2* locus. On the other hand, considering the large variation in the clones with T:G, we proposed that there was a T allele linked to the susceptible locus or an inhibitor to the *CMD2* locus in the CIAT102 population. After reviewing the *CMD* resistance of clones grouped by progenitors and the pedigree information, we concluded that the susceptible T allele was derived from two progenitors, CW2334-2 and CW257-12, which came from the wild crosses with FLA437-7 (*M. esculenta* ssp. *flabellifolia*).

In the VNM142 population, 20 clones had the resistant allele T, but only a few clones showed CMD resistance. Moreover, although a significant difference was observed between the clones with and without the resistant allele, the resistant allele of S12\_7926132 only explained 11% of the population variation. Considering no intended introduction of CMD resistance to Vietnam before 2011, we proposed that the T allele in VNM142 might be in the haplotypes different from that in TME3 in Africa. Similarly, non-significant marker effects were also reported in some families of the breeding populations in Africa [22].

For the marker S14\_4626854, among the 35 clones with the resistant allele A in CIAT102, 29 clones did not have CMD symptoms. The six remaining susceptible clones might result from the recombination between the marker and the resistant locus. Thus, the resistant allele A was linked with the resistant locus, but recombination could occur between the marker and the resistant locus and break the linkage. In VNM142, S14\_4626854 only explained 8% of the population variance, which was much smaller than that in CIAT102 (36%). We concluded that for the diverse population from Vietnam, S12\_7926132 and S14\_4626854 were not efficient in predicting the CMD resistance, which opened the possibility for new source(s) of resistance different from *CMD2*. For segregation populations such as CIAT102, with a known source of *CMD2*, the two markers worked well, but attention still needs to be paid to whether the resistant alleles (or *CMD2* inhibitor) are present in the susceptible parents (e.g., CW234-2 and CW257-12 of CIAT102).

#### 4.3. Co-Segregation of the Markers S12\_7926132 and S14\_4626854

In the CIAT102 population, both S12\_7926132 and S14\_4626854 explained 36% of the population variation, which is inconsistent with the smaller effect of the QTL on chromosome 14 than that on chromosome 12 [18]. Moreover, we observed a high rate of co-segregation between markers S12\_7926132 and S14\_4626854 in both CIAT102 and VNM142, 73% and 94%, respectively. Thus, we proposed that the two markers might be linked and located on chromosome 12, rather than on two chromosomes (12 and 14). The misallocation of the markers might result from the low quality of genome assembly in certain regions, the complexity of the *CMD2* locus [21], or the mismatch during SNP calling. The new haplotype-resolved de novo assemblies and annotations of the genomes for the African cassava varieties facilitate further investigation to figure this out [21,37].

If we considered the two markers were linked, it was easy to explain the segregation of CMD resistance in CIAT102. Marker S14\_4626854 helped to distinguish the resistant T allele from the susceptible T alleles of S12\_7926132. Among the 52 clones with T:G for S12\_7926132, 28 clones with the resistant allele A of S14\_4626854 showed high CMD resistance, but the 24 clones with the susceptible allele G of S14\_4626854 had CMD symptoms, in general. Thus, the T alleles in the 24 clones should be derived from the susceptible haplotype or the haplotype with the *CMD2* inhibitor, while the T allele in the 28 clones came from the haplotype with CMD resistance.

In CIAT102, all 25 clones with homozygous susceptible G alleles of S12\_7926132 are susceptible to CMD. The high CMD scores indicated that the link between the CMD susceptibility locus and the allele G was not broken in the CIAT102 population, even though the susceptible T allele was putatively introduced from the wild relative. In the case of S14\_4626854, six clones with homozygous susceptible G alleles did not have any CMD symptoms, so crossover might occur between the resistant locus and S14\_4626854. Thus, we concluded that the marker S12\_7926132 was tightly linked with the CMD-resistant locus, while S14\_4626854 might be a bit further from the resistant locus. This observation is consistent with the strong association signals at S12\_7926132 in three studies [17–19].

In the present study, we screened one introduced cassava population from CIAT and a local population collected from farms, and identified more than 40 clones showing a good level of CMD resistance, including eight clones from the farms. Moreover, two accessions in the CIAT genebank, CR63 (PER262 or TAI9) and VNM8 (or KM57), showed good CMD resistance because they had the same DNA fingerprints as the resistant clones in Vietnam. This is the first time to report the CMD resistance from the CIAT genebank and

local landraces in Asia. DNA markers S12\_7926132 and S14\_4626854 showed a high rate of co-segregation in both the CIAT102 and VNM142 populations, and we proposed that both markers might be linked and located on the same chromosome, chromosome 12. The markers did not work well for diverse populations (e.g., VNM142), but both markers can predict the CMD resistance from structured breeding populations with a known source of CMD2 (e.g., CIAT102). Before using the DNA markers, however, breeders should genotype the susceptible elite parents and make sure the elite parents do not have the susceptible haplotype with A or T alleles.

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