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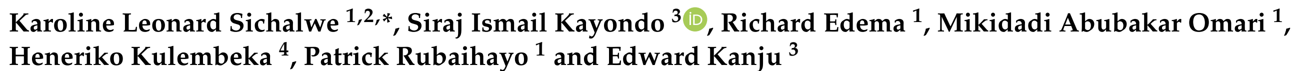
Unlocking Cassava Brown Streak Disease Resistance in Cassava: Insights from Genetic Variability and Combining Ability



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Abstract: Cassava brown streak disease (CBSD) threatens cassava production in sub-Saharan Africa despite the availability of resistant varieties. Extreme environmental factors weaken plant defenses, reducing CBSD resistance. This study examined CBSD inheritance in cassava populations, assessed genetic variability, and identified superior sources of resistance using F1, S1, and half-sib offspring populations derived from resistant sources. The offspring underwent field evaluation at two distinct sites from 2019 to 2021, and the symptom-free genotypes were analyzed using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Resistance to CBSD was categorized as most resistant, resistant, most tolerant, or tolerant based on symptoms and virus titers. The findings indicated that the resistance to CBSD is highly influenced by genotypes, F1/S1 types, and environmental conditions. An analysis of combining abilities revealed significant general combining abilities (GCAs) for CBSD, cassava mosaic disease (CMD), and traits associated with yield. The heritability estimates for resistance to CBSD varied between 43.4% and 63.2% for foliar symptoms and 14.6% and 57.9% for root necrosis across locations. The inheritance pattern involved a combination of additive and recessive genes with selfed (S1) populations displaying stronger and more effective resistance to the disease. The cassava brown streak virus (CBSV) was highly prevalent, and the Ugandan cassava brown streak virus (UCBSV) was not prevalent. Four genotypes were highly resistant to CBSD and could be key sources of resistance to this disease.

Keywords: resistance sources; CBSV; UCBSV; root necrosis; virus sources; heritability estimates

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1. Introduction

Cassava (Manihot esculenta Crantz) is a tropical root crop and an important source of carbohydrates for millions of people around the world [1]. This crop can be grown in marginal soils with limited rainfall, making it a food security crop for sub-Saharan African countries [2]. Cassava production is seriously threatened by several viral diseases, with cassava brown streak disease (CBSD) being a major concern [3]. CBSD has been a prevalent production constraint in East Africa for over seven decades [4]; its impact is steadily increasing in Central and Southern Africa [5,6] and emerging as a threat to West Africa, the continent's largest cassava producer [7].

CBSD is caused by two Ipomoviruses: the Ugandan cassava brown streak virus (UCBSV) and the cassava brown streak virus (CBSV) [8]. Their effects lead to significant crop yield reduction and economic losses of up to USD 1 billion [9]. CBSD profoundly impacts the economic stability of the affected regions, disrupting important economic activities and undermining the livelihoods of local communities. It poses a serious threat to

food security by affecting the availability and accessibility of this essential staple food and, thereby, the nutritional well-being of the population in the affected areas [10]. This disease manifests with various symptoms, including yellow chlorosis in the leaves, leading to a loss of green pigmentation and impaired photosynthesis. In the stems, brown stripes appear, and in severe cases, the disease can lead to dieback, where stems and branches progressively wither and die [11]. In the tuberous roots, cork-like necrosis appears, resulting in dry and decayed tissue [11]. The variability of CBSD symptoms has led to different classifications depending on the specific screening method used, including resistance mechanisms such as insect vector avoidance, virus spread, virus replication, and immune response. Identifying and quantifying resistance to viral infection in selected cassava varieties has been made possible by reliable techniques such as molecular virus detection [12].

Using CBSD-resistant varieties is considered a sustainable and effective control strategy. However, challenges remain due to whitefly vectors, variations in virus strain, and environmental conditions that can impact the consistent and durable expression of resistance across generations [13]. Previous studies have indicated that CBSD resistance is influenced by additive and non-additive gene actions. Additive gene actions have been reported in several studies where the cumulative effects of individual alleles contribute to a trait's expression [14,15]. In addition, non-additive gene action also plays a significant role in controlling CBSD resistance, as highlighted in Nduwumuremyi et al. [16], and Zacarias and Labuschagne, [17]. These findings reveal the complex genetic architecture underlying CBSD resistance, indicating that it involves a combination of multiple genetic effects that contribute to the overall resistance observed in cassava populations. Other important, related cassava diseases and pests, such as cassava mosaic disease (CMD) have been reported to be influenced by additive [18] and non-additive [19] gene action; resistance to cassava green mites has been reported to be influenced by additive gene action [20] and seventeen candidate genes associated with its resistance [21]. The importance of both additive and non-additive gene effects in controlling the expression of agronomic traits has also been reported [16,20]. Conflicting reports on gene effects can be attributed to factors such as population type, mating design, analytical methods, study locations, and genotype by environment interactions [22,23].

This study was conducted to address a critical gap in understanding disease resistance in cassava, specifically focusing on CBSD. Despite ongoing research aimed at developing resistant varieties, limited information is available on the use of mixed populations to determine inheritance patterns and gene actions controlling CBSD resistance. The objectives of this study were to (i) investigate the inheritance of CBSD resistance in cassava populations biparental (F) and selfed (S1) lines derived from known resistant parents; (ii) explore the variability in resistance to CBSD levels within the populations; and (iii) identify superior sources of resistance.

2. Materials and Methods

2.1. Plant Materials

The breeding materials were obtained from the International Institute of Tropical Agriculture (IITA) in Uganda. Three resistant CBSD genotypes, MM06/0123, CBSD MM06/0130, and MM06/0128, along with one susceptible parent (TME14), were selected to develop biparental (F1) and selfed (S1) populations. The criteria for selecting these parents included their resistance to diseases, high fresh root yield, and high dry matter content (Table 1). A total of 703 cassava genotypes were developed, and the NASE13 and NAROCASS1 checks were included in the experiments. The NASE13 and NAROCASS1 checks are improved varieties with high yield, high dry matter content, drought tolerance, and resistance to both CMD and CBSD. Before planting the clonal evaluation trial in April 2019, the generated populations underwent screening for CBSD and CMD in a germplasm maintenance trial for two years at the IITA.

Table 1. Characteristics of parental materials.

Parents	CBSD Status	Other Traits
MM060123	Resistant	CMD resistant, High yield, High DMC
MM060128	Resistant	CMD resistant, High yield, High DMC
MM060130	Resistant	CMD resistant, High yield, High DMC
TME14	Susceptible	CMD resistant, High yield, High DMC

CMD, cassava mosaic disease; CBSD, cassava brown streak disease: DMC, dry matter content.

2.2. Experimental Sites and Design

The experimental trials were conducted at the National Semi-Arid Resources Research Institute (NaSARRI) and an IITA research station. In eastern Uganda, where the NaSARRI is situated, the weather is characterized by a tall savannah ecology with rainfall between 1000 mm and 1300 mm per annum, temperatures between 18 °C and 31 °C (Supplementary Figure S1A), and sandy loam soil with a PH of between 5.8 and 6.0 [24]. This place is at latitude 01°32′00′′ N and longitude 32°25′00′′ E and is one of the country's medium spot areas for CBSD screening. The IITA is in Namulonge in Central Uganda, which is characterized by a tropical rainforest ecology, rainfall of about 1500 mm per annum, a temperature average of 22.20 °C (Supplementary Figure S1B), and sandy clay soil with a PH of between 4.9 and 5.0 [24]. This place is at latitude 00°32′00′′ N and longitude 32°36′36′′ E. The place is known as a hotspot for CBSD screening.

The clonal evaluation trial used an augmented design comprising test genotypes and checks planted with parental genotypes in eight to ten blocks depending on the available genotypes in the specific year and location. The field experiments were planted for two cropping seasons from 2019 to 2022. The plot size was 5 m^2, and the spacing was 1 m × 1 m. No fertilizer or irrigation was applied in the experiments, and weeds were controlled using a hand hoe.

2.3. Phenotypic Data Collection

2.3.1. Disease Assessment

Disease assessment was conducted by collecting data on CBSD and CMD, as presented below (Table 2).

Table 2. Virus disease data collection parameters, description, and collection period.

Parameter.	Description	Period
CBSDLI	Cassava brown streak disease leaf incidence measured as a proportion of plants within a plot showing symptoms to the total plant population	3, 6, and 9 months after planting
CBSDLS	Cassava brown streak disease scored for severity of leaf symptoms on a scale of 1 (clean/no infection) to 5 (severely diseased)	3, 6, and 9 months after planting
CBSDRI	Cassava brown streak disease root incidence as a proportion of roots within a plot showing symptoms to the total plant population	At harvest (12 months after planting)
CBSDRS	Cassava brown streak disease scored for severity of root symptoms on a scale of 1 (clean/no necrosis) to 5 (severe lesions)	At harvest (12 months after planting
CMDI	Cassava mosaic disease incidence measured as a proportion of plants within a plot showing symptoms to the total plant population	3 and 6 months after planting
CMDS	Cassava mosaic disease severity scored for severity of leaf symptoms on a scale of 1 (clean) to 5 (severely diseased)	3 and 6 months after planting

2.3.2. Assessment of Yield and Yield Components

The following yield component data for each genotype were collected at harvest, twelve months after planting (12MAP): number of fresh storage roots per plot; the fresh storage root weight (kg/plot) converted to fresh root yield (t/ha); and the shoot weight (kg per plot). Other variables, such as harvest index (HI), were calculated using the formula in

Equation (1), and dry matter content (DMC in %) was determined using specific gravity, calculated using the formula in Equation (2) [25].

$$\text{HI} = \frac{\text{Weight of fresh storage root}}{\text{Weight of fresh storage root} + \text{Weight of the above ground biomass}}, \quad (1)$$

$$\text{Dry matter content } (\%) = 158.3\left[\left(\frac{\text{Wa}}{\text{Wa} - \text{Ww}}\right)\right] - 142, \quad (2)$$

where Wa = mass of roots in air (kg), and Ww = mass of roots in water (kg).

Samples were chosen for virus detection and quantification based on observable foliar CBSD symptoms with scores of 1, 2, or 3 for leaf severity and a score of 1 only (asymptomatic) for root necrosis. Root slices of 2 cm were cut and covered in aluminum foil, immediately stored on ice, refrigerated at −20 °C for short storage in the field, and transferred to a −80 °C freezer for long-term storage at the TARI Kibaha laboratory. Before extraction, samples were removed from the −80 °C refrigerator and returned to the −20 °C refrigerator. Total RNA was extracted using a cetyl trimetyl ammonium bromide (CTAB) protocol [26]. The RNA quality and purity were determined using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The RNA samples were used to test for the presence or absence of the CBSD virus using a Taqman assay. The tests for CBSV and UCBSV were independently performed [27]. Specific primers that anneal CBSV and UCBSV coat proteins were used (Table 3) for virus detection.

Table 3. Primers and probes targeting CBSV and UCBSV for real-time RT-qPCR.

Primer Name	Sequence (5′ to 3′)	Annealing Site	Source
CBSV-CP-Fer2	GAAGGGATTGGAYTRGAAGGA	7390–7410	Shirima et al. 2017 [27]
CBSV-CP-R1-1	GAACGCGGTATCCACACATA	8197–8216	Shirima et al. 2017 [27]
UCBSV-CP-F1−1	AGAGATCTGGAAAGGAAGT	7981–7999	Shirima et al. 2017 [27]
UCBSV-CP-R1-1	CTCGCCAYGACTTCTCATT	8403–8421	Shirima et al. 2017 [27]
COX-R	CAACTACGGATATATAAGRRCCRRAACTG		Adams et al. 2013 [28]
COX-F	CGTCGCATTCCAGATTATCCA		Adams et al. 2013 [28]
COX probe	[HEX]-AGGGCATTCCATCCAGCGTAAGCA-[BHQ1]		Adams et al. 2013 [28]

R represents A or G; Y represents C or T. Designations with -F denote forward, and those with -R denote reverse primers. Those with -probe represent TaqMan probes.

Complementary DNA (cDNA) was prepared from 1 µg of template RNA using first-strand cDNA synthesis (quick protocol). The complete reverse transcriptase reaction (2 µL) contained 50 µM of Oligo dT18 (New England Biolabs, Ipswich, MA, USA), 10 × M-MuLV buffer, 200 U/µL of MuMLV reverse transcriptase (RT), 10 mM of dNTP Mix, 40 U/µL of RNase inhibitor, and nuclease-free water. Samples detected with the virus were analyzed with an absolute quantification qPCR reaction using a Taqman assay specific to CBSV and UCBSV. Quantification was performed by measuring 6PK01-Fer2 plasmid concentration using a Qubit 3.0 fluorometer followed by ten-fold serial dilution starting with an original concentration of 21.5 ng/µL. A quantification reaction was prepared using 25 µL of Mg, 10 × PCR buffer, 25 Mm of $MgCl_2$, 10 mM of dNTP, 7.5 µM primers, a 5 µM probe, 1× reference dye, 5 U/µL of Taq DNA polymerase, and plasmids. The PCR program was set to 95 °C for 10 min, 95 °C for 15 s, and 60 °C for 1 min for 40 PCR cycles. The reaction was performed using the Stratagene Mx3000P qPCR system (Agilent Technologies, Santa Clara, CA, USA), and data were acquired using the Mx3000P qPCR software (Agilent Aria Software v1.71). RNA amplification during the PCR process ranged from 15 to 35 cycles with amplification efficiency between 100% and 104.8%, a standard coefficient of correlation (R2) curve of 0.99 and a slope of 3.21 (Supplementary Figure S2). The data were assembled in Microsoft Excel and analyzed for detected genotypes with their specific viruses and concentrations.

2.4. Data Analysis

Data analysis was performed at different phases. Phase one comprised analysis of variance using the lmer package in R (R.4.1.2) (R Core Team. 2021): all individual F1 and S1 genotypes were combined, and Best Linear Unbiased Prediction (BLUP) estimated the mixed model's random effects. In phase two, the crossed F1 and S1 genotypes were analyzed to determine variabilities using the lmer package in R.4.1.2. Finally, genotypes were averaged to assess family performance. Analysis of variance was performed using the model in Equation (3):

$$Yijk = \mu + Gi + Ej + GEk + \in ij, \quad (3)$$

where Yijk is the observed phenotypic value, μ is the overall mean, Gi is the genotype effect, Ej is the environmental effect, GEk is the interaction effect between genotype and environment, and ∈ is the residual error term assuming a normal distribution.

2.4.1. Analysis of Variance for Parents and Families

The combined data were analyzed using the ASReml package in R (R.4.1.1.) Genotypes were fitted as fixed factors; environments were treated as random factors; and the mean squares for SCA, GCA1, GCA2 and the interactions between environments and seasons were fitted in the model in Equation (4):

$$Y_{ijk} = \mu + G + F_i + M_j + FM + E_k + GE + \in, \quad (4)$$

where Y is the observed phenotypic value of the progeny of the ith female crossed with jth male in the kth environment, μ is the overall mean, G is the genotype effect, F is the GCA of the ith female, M is the GCA of the jth male, SCA is the cross between the ith female and the jth male, Ek is the environmental effect, GE is the interaction effect between the genotype and environment, and ∈ is the residual error term assuming a normal distribution.

2.4.2. Estimation of Variance Components and Heritability

Phenotypic and genotypic variances were computed from the expected mean squares of the analysis of variance; the phenotypic coefficient of variation (PCV) and the genotypic coefficient of variation (GCV) were estimated using the formula below, expressed in percentage [29].

$$\sigma_g^2 = \frac{MSG - MSE}{r} \quad (5)$$

$$\sigma_p^2 = \sigma_g^2 + MSE \quad (6)$$

Here, σ_g^2 is the genotypic variance, σ_p^2 is the phenotypic variance, MSG is the genotypic mean square, MSE is the error mean square, and r is the replication number.

$$PCV = \left(\sqrt{\sigma_p^2}/\bar{x}\right) \times 100 \quad (7)$$

$$GVC = \left(\sqrt{\sigma\,_g^2}/\overline{X}\right) \times 100 \quad (8)$$

Here, PCV is the phenotypic coefficient of variation, GCV is the genotypic coefficient of variation, $\overline{X}$ is the grand average of the traits, σ_p is the phenotypic variance, and σ_g is the genotypic variance.

Population heritability was estimated at two levels: level one was heritability across environments, and level two was for specific years in the location. Heritability was computed according to Falconer and Mackay, [30] using the following formula:

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100, \quad (9)$$

where σ_g^2 is the genotypic variance, and σ_p^2 is the phenotypic variance.

2.4.3. Selection of Resistant Genotypes and Level of Resistance in Populations

Absolute CBSV and UCBSV quantification was determined by running the default settings of the MxPro qPCR software (Agilent Aria Software v1.71) on the Stratagene Mx3000P qPCR system (Agilent Technologies). The data were assembled in Microsoft Excel, and correlation analysis was performed. The resistance levels were categorized as most resistant (MR), resistant (R), most tolerant (MT), and tolerant (T) based on the foliar symptoms, root necrosis, and the virus detection test. The proportion of resistant and tolerant genotypes per family was computed by expressing the absolute number of resistant genotypes as a percentage of the total number of genotypes established in that family.

3. Results

3.1. Performance and Genetic Variation in Genotypes and Population Types

The descriptive statistics for genotypes across seasons and locations are presented in Table 4. The CBSD incidences had a minimum of 0% and a maximum of 100%, while CBSD severities had a minimum score of 1 and a maximum of 5 in CBSDL3S and CBSDRS, respectively. The lowest mean CBSDLI (55.79%) was observed at 3MAP (CBSDL3I) and the highest (65.14%) at 9MAP (CBSDL91). The same trend was observed for CBSD leaf severities (CBSDLS), where the lowest mean (1.95) was observed at 3MAP and the highest (2.28) at 9MAP. For CBSD root necrosis incidence (CBSDRI) and severity (CBSDRS), the mean CBSDRI was 37.84%, whereas the mean CBSDRS was 2.76. The cassava mosaic disease severity (CMDLS) trend followed a similar pattern, where the genotypes had the lowest (1) and the highest (5) at 3MAP and 9MAP, respectively. The CMD incidences exhibited fluctuations across different months after planting, with CMDL6I having the highest mean incidence at 19.31 ± 0.65 standard error. The CMD severities also varied but generally remained lower than the CBSD severities.

Table 4. Descriptive statistics for CBSD incidences and severities (3, 6, and 9 MAP), CMD incidences and severities (3 and 6 MAP), and yield traits across two seasons and locations.

Variable	Min	Max	Mean ± SE	STDEV	CV (%)
CBSDL3I	0	100	55.79 ± 0.8	42.75	76.64
CBSDL6I	0	100	63.35 ± 0.78	41.75	65.9
CBSDL9I	0	100	65.14 ± 0.78	41.45	63.63
CBSDL3S	1	5	1.95 ± 0.01	0.75	38.63
CBSDL6S	1	4	2.23 ± 0.02	0.89	39.88
CBSDL9S	1	4	2.28 ± 0.02	0.88	38.51
CBSDR12S	1	5	2.76 ± 0.02	1.32	47.8
CBSDR12I	0	100	37.84 ± 0.66	35.27	93.2
CMDL3I	0	100	18.33 ± 0.64	34.01	185.51
CMDL6I	0	100	19.31 ± 0.65	34.61	179.3
CMDL9I	0	100	13.11 ± 0.56	29.75	226.96
CMDL3S	1	5	1.5 ± 0.02	0.87	58.3
CMDL6S	1	4	1.52 ± 0.02	0.86	56.56
CMDL9S	1	5	1.36 ± 0.01	0.75	55.18
DMC	16.3	68.16	35.5 ± 0.11	5.03	77.63
FYLD	0	175	15.46 ± 0.34	17.98	116.27
HI	0	1	0.34 ± 0	0.17	48.51
TRTN	0	73	14.45 ± 0.21	11.22	14.16

CBSDLS, cassava brown streak disease leaf severity; CBSDRS, cassava brown streak disease root severity; CBSDLI, cassava brown streak disease leaf incidence; CBSDRI, cassava brown streak disease root incidence; CMDS, cassava mosaic disease severity; CMDI, cassava mosaic disease incidence; 3,6 and 9, months after planting; FRYLD, fresh root yield; HI, harvest index; DMC, dry matter content; TRTN, total root number; Min, minimum; Max, maximum; SE, standard error; STDEV, standard deviation; CV, coefficient of variation.

Other yield traits, such as DMC, Fresh Yield (FYLD), HI, and the total root tuber number (TRTN), showed considerable variation. The DMC ranged from 16.3% to 68.16%

with an average of 35.5%, while the FYLD had an average of 15.46 t/ha; few genotypes outperformed at 175 t/ha. HI ranged between 0 and 1, indicating different biomass proportions allocated to harvestable parts. The TRTN showed variability with a maximum value of 73.

The analysis of variance detected highly significant differences ($p < 0.001$) between genotype, environment, and genotype by environment interaction effects for CBSD incidences and severities at different infection stages (3, 6, and 9 months after planting). Significant differences were also observed for CBSDRS, CBSDRI, and CMD severities and incidences at different infection stages (Table 5). Similarly, the significance levels for MSG, MSE, and MSGxE indicated significant genetic, environmental, and genotype-environment interaction effects on FRYLD, HI, and DMC. A significant genotype and environment interaction (MSGxE) was observed in the TRTN ($p < 0.05$) (Table 5).

Table 5. Mean squares for genotype (G), environment (E), and GxE interactions for CBSD, CMD, and yield traits over two seasons in Namulonge and Serere, Uganda.

	Sources of Variation			
Trait	**MSG (672)**	**MSE (3)**	**MSGxE (1351)**	**MSR (127)**
CBSDL3S	0.82 ***	41.973 ***	0.382 ***	0.144
CBSDL6S	1.52 ***	64.66 ***	0.39 ***	0.227
CBSDL9S	1.46 ***	28.12 ***	0.47 ***	0.225
CBSDRS	2.3519 ***	23.52 ***	1.39 ***	1.09 ***
CBSDL3I	2677 ***	166236 ***	1171 ***	578
CBSDL6I	3513 ***	111414 ***	843 ***	414
CBSDL9I	3224 ***	124710 ***	934 ***	429
CBSDRI	1938.7 ***	10980.7 ***	784.4 ***	604.7 ***
CMD3S	1.40 ***	36.29 ***	0.49 ***	0.173
CMDS6S	1.58 ***	33.97 ***	0.36 ***	0.097
CMDS3I	2022 ***	99169 ***	734 ***	112
CMD6I	2350 ***	100955 ***	614 ***	47
FRYL (ta/ha)	530.3 ***	18006.4 ***	258.7 ***	258.7 ***
HI	0.029 ***	3.76 ***	0.01 ***	0.011
DMC	46.49 ***	535.59 ***	15.08 ***	9.27
TRN	204.9 ***	7480.0 ***	79.4 *	65.4

* and ***, significance levels at 0.05, and 0.001, respectively; MSG, mean square of genotype; MSE, mean square of the environment; MSGxE, mean square of GXE interaction; MSR, mean square of residual; number in parenthesis, degree of freedom; CBSDLS, cassava brown streak disease leaf severity; CBSDRS, cassava brown streak disease root severity; CBSDLI, cassava brown streak disease leaf incidence; CBSDRI, cassava brown streak disease root incidence; CMDS, cassava mosaic disease severity; CMDI, cassava mosaic disease incidence; 3,6, and 9, months after planting; FRYL, fresh root yield; HI, harvest index; DMC, dry matter content; TRTN, total root number.

The analysis of variance for all traits evaluated among the population types (PTs), environments (Es), and their interactions (PTxEs) are presented in Table 6. Highly significant differences ($p < 0.001$) were detected between the PT for all the traits except for CBSDRI, HI, and DMC. The differences between PT were significant ($p < 0.005$) for CBSDL3I and CMD3S. Highly significant differences ($p < 0.001$) were detected between environments for all the traits recorded. Furthermore, highly significant differences ($p < 0.001$) were detected for the interactions between PT and E for all the traits except CMD3I, CMD6I, HI ($p < 0.01$), and CMD6S ($p < 0.05$).

Table 6. Mean squares for PT, environment Es, and PT × Es for disease resistance and yield traits evaluated across two seasons at Namulonge and Serere, Uganda.

Trait	Sources of Variation			
	MSPT (3)	**MSE (3)**	**MSPTxE (9)**	**MSR (2138)**
CBSDL3S	1.76 *	46.99 ***	2.06 ***	0.49
CBSDL6S	3.55 ***	67.71 ***	3.41 ***	0.71
CBSDL9S	3.87 ***	31.07 ***	2.32 ***	0.75
CBSDRS	10.02 ***	25.53 ***	4.62 ***	1.65
CBSDL3I	4332 *	19.933 ***	5629 ***	1551
CBSDL6I	9521 ***	11161 ***	7460 ***	1603
CBSDL9I	11298 ***	135177 ***	6052 ***	1573
CBSDRI	2157.3	18811.6 ***	2763 ***	1115.3
CMD3S	1.76 *	46.99 ***	2.06 ***	0.49
CMD6S	12.44 ***	30.71 ***	1.47 *	0.711
CMD3I	46803 ***	92391 ***	2795 **	1080
CMD6I	20097 ***	94448 ***	2934 **	1098
FRYLD (ta/ha)	1033 ***	530.25 ***	145.6 ***	33.15
HI	0.03	3.77 ***	0.045 **	0.02
DMC	35.73	768.78 ***	116.32 ***	23.83
TRTN	2421.1 ***	7863.7 ***	407.3 ***	112.8

*, **, and *** indicate significance levels at 0.05, 0.01, and 0.001, respectively; MSPT, mean square of population types; MSE, mean square of environment; MSPTxE, mean square of PT x E interaction; MSR, mean square of residual; number in parenthesis, degree of freedom; CBSDLS, cassava brown streak disease leaf severity; CBSDRS, cassava brown streak disease root severity; CBSDLI, cassava brown streak disease leaf incidence; CBSDRI, cassava brown streak disease root incidence; CMDS, cassava mosaic disease severity; CMDI, cassava mosaic disease incidence; 3, 6, and 9 months after planting; FRYL, fresh root yield; HI, harvest index; DMC, dry matter content; TRTN, total root number.

3.1.1. Frequency of CBSD Root Necrosis Categories within Crosses

The proportion of CBSD root necrosis in fresh roots within mapping population crosses was also compared (Figure 1). The classifications were as follows: resistant for a score of 1, tolerant for a score of 2, and susceptible for scores of 3–5 of CBSD root severity (Figure 2). Less than 10% of the resistant genotypes were observed among the F1 and S1 crosses. In a susceptible by susceptible cross (TME14XTME14), only 2% of resistant genotypes were observed. The resistant x resistant cross (MM060123) had slightly more susceptible genotypes at 71.7% compared with resistant × resistant F1 parents, including MM060123XMM060128 (58.6%), MM060123XMM060130 (59.5), and MM060128XMM060130 (66.3%).

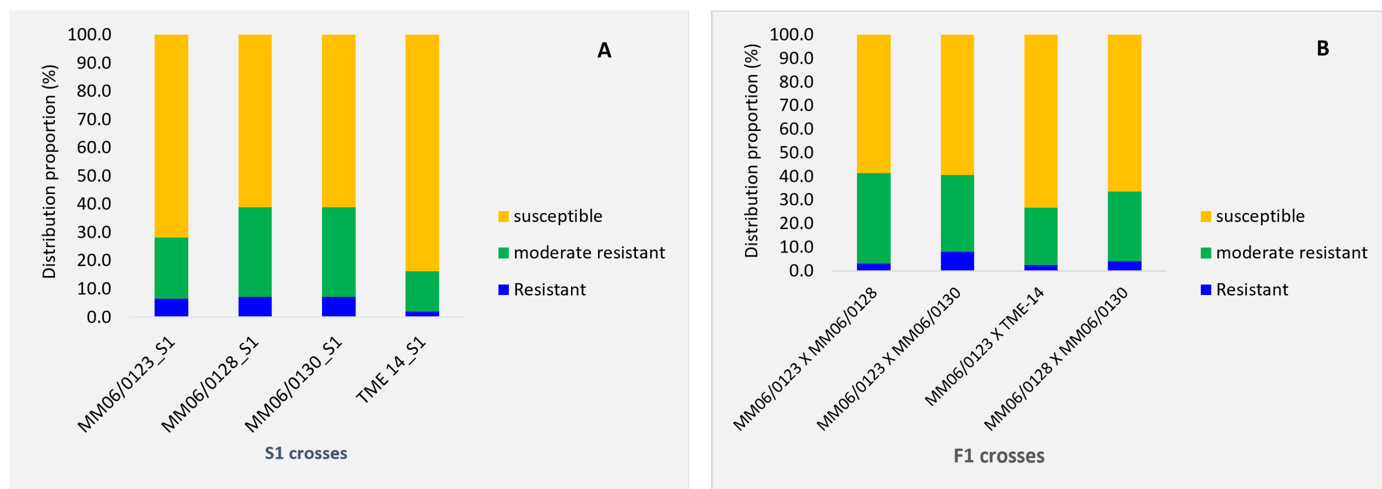

Figure 1. Proportion of CBSD root necrosis in biparental F1 and selfing crosses (S1). Score classification: score 1, resistant; score 2, tolerant; score 3–5, susceptible; (**A**), S1; (**B**), F1.

Figure 2. Score severity of cassava brown streak disease on root symptoms ranging from scale 1 (no visible symptoms, leftmost) to scale 5 (severely brown necrosis, rightmost).

3.1.2. Differential Performance of Controls, Parents, and Populations in Multiple Environments

The control lines had higher CBSD scores class than the parents and populations in the 2019 sendusu environment, and the lowest score (1) was observed in the 2021 serere environment (Figure 3a). In the F1 and S1 populations, the highest scores (2.5 and 2.6, respectively) were observed in the 2020serere environment. On the other hand, the S1 and F1 populations had the highest CMDS_6MAP scores (class 2) in 2019sendusu, while the controls and parents were class 1 (Figure 3b).

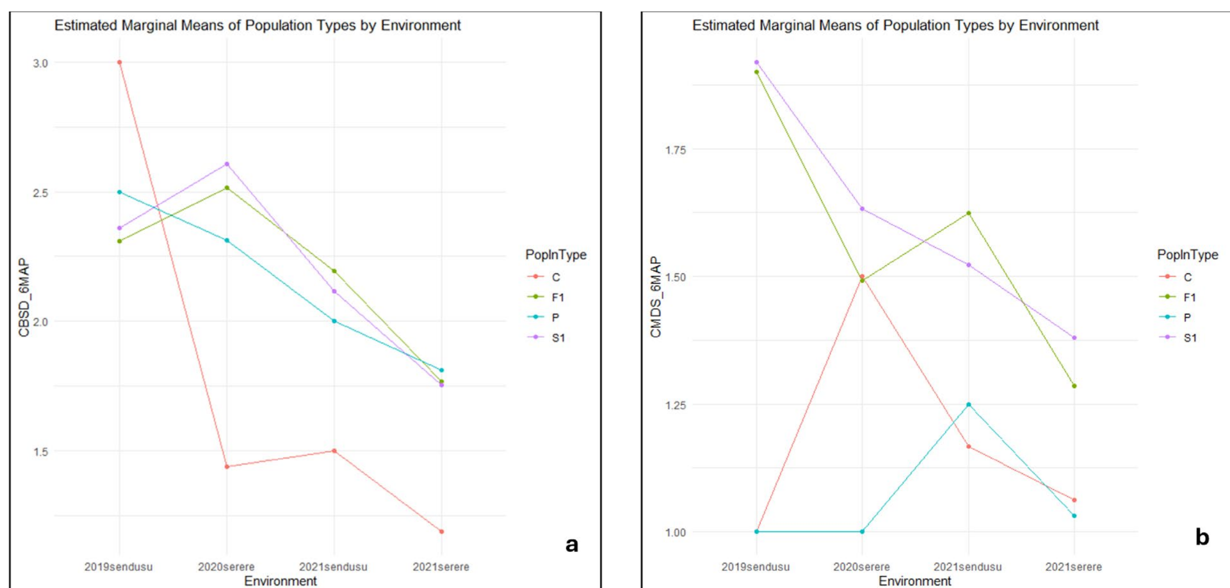

Figure 3. Estimated marginal means of population types by the environment for CBSD_6MAP (**a**) and CMD_6MAP (**b**).

The CBSDRS for the controls varied across all four environments, with the highest score at 3.5 and the lowest score at 2 in 2021serere (Figure 4a). The parent fresh yield was lower than that of the control and populations in 2019sendusu and was lowest in environments with less than 10 t/ha in 2020serere (Figure 4b). The highest fresh root yield was observed in the controls across all four environments.

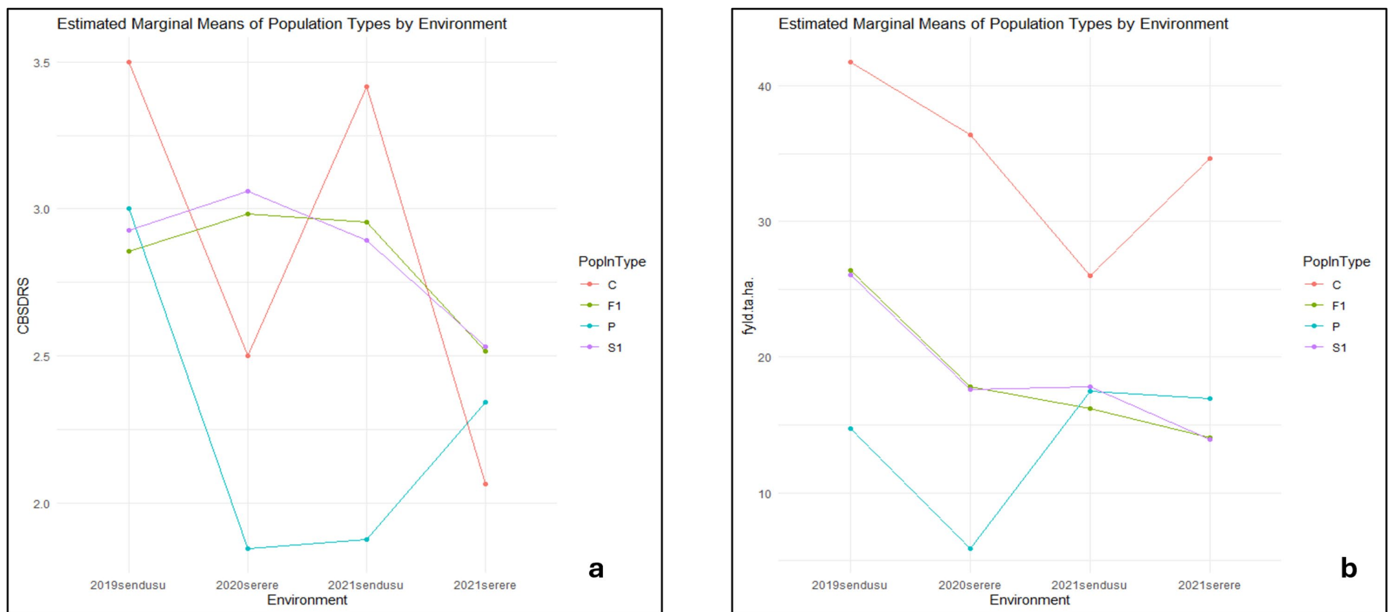

Figure 4. Estimated marginal means of population types by environments for CBSDRS (**a**) and fresh yield (fyld) (**b**).

DMC showed a substantial difference between populations and environments. The highest DMC in 2019sendusu was in the parents, while the lowest was in the controls (Figure 5a). There was only a small variation between F1 and S1 populations. The harvest index showed significant differences between the controls, parents, and the F1 and S1 populations, as well as across the 2019sendusu, 2020serere, 2021sendusu, and 2021serere environments. The highest harvest index (0.6) was observed in controls in 2020serere (Figure 5b). Plant establishment was higher than 95% for all population types in 2019sendusu, with the lowest observed in controls (82%) in 2021sendusu (Figure 5c).

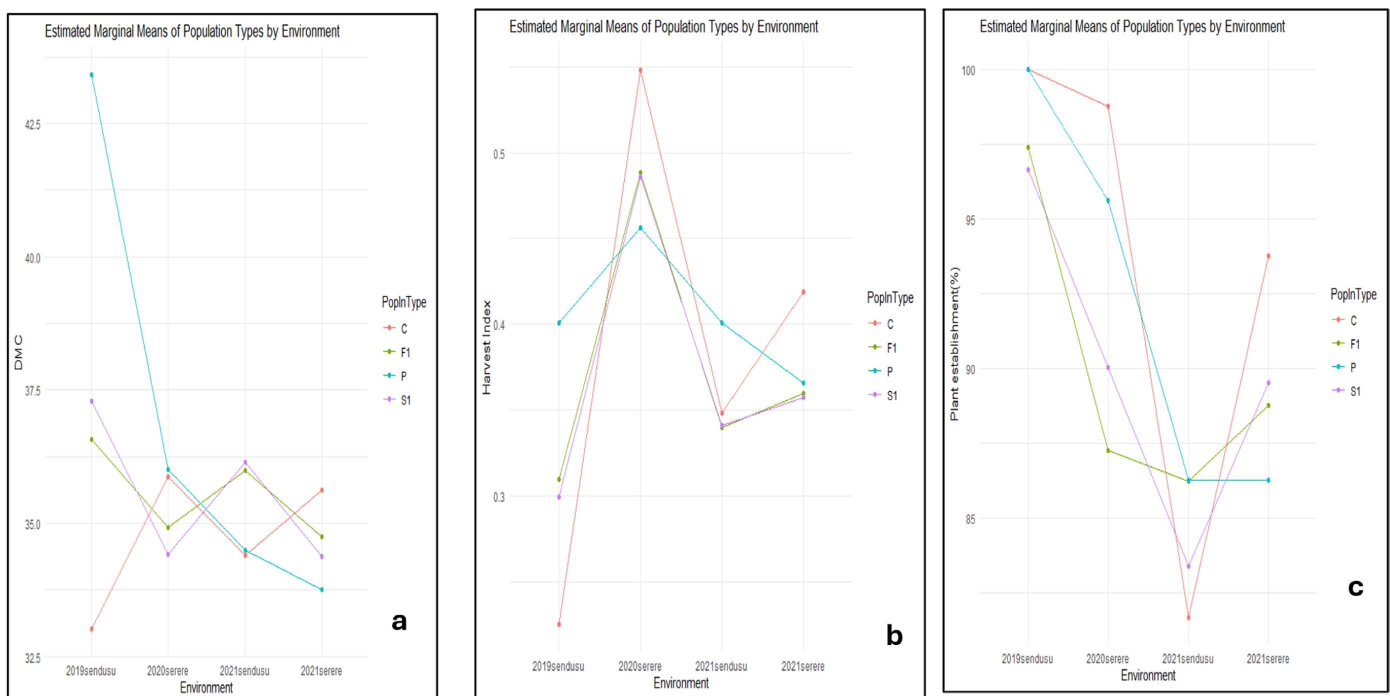

Figure 5. Estimated marginal means of population types by environments for dry matter content (DMC) (**a**), harvest index (HI) (**b**), and plant establishment (**c**).

3.1.3. Combining Abilities and Mode of Gene Action for Resistance to Cassava Brown Streak Disease

The analysis of variance for combining abilities showed that the environment was significant ($p < 0.05$) for all the traits measured (Table 7). The mean squares for GCA1, GCA2, and SCA represent genetic variability within the parents and families for each trait. The GCA1 (female) effects were significant ($p < 0.05$) for all CBSD incidence and severities on foliar, roots, and root yield traits. The GCA2 (male) effects were significantly different only in CBSDRI and CBSDRS; for the other traits, non-significant effects were observed. SCA (specific combining ability) represents the non-additive genetic effects resulting from interactions between parental genotypes. The SCA effects were not significant for all traits measured. The GCA/SCA ratio provides insights into the relative importance of additive (GCA) and non-additive (SCA) genetic effects in trait inheritance. The GCA/SCA ratio was high and positive. The percentage contribution to the Sum of Square showed that GCA1 had a higher contribution than its corresponding GCA2 or SCA for all traits under investigation.

Table 7. Mean square of parents and families for CBSD, CMD, and root yield traits.

SOV	CBSDLS	CBSDLI	CBSDRS	CBSDRI	CMDS	CMDI	FYLD	HI	DMC	TRTN
Environment	81.333 ***	141,870 ***	25.9728 ***	23,519 ***	50.116 ***	130,085 ***	23,491.5 ***	5.9935 ***	749.31 ***	11,551.2 ***
GCA1	7.061 ***	23,094 ***	14.7296 ***	13,209.4 ***	5.205 ***	7535 ***	5999.9 ***	0.2998 ***	178.08 ***	1988.7 ***
GCA2	8.159 ***	31,911 ***	8.398 ***	8952.5 ***	1.161 *	1903 *	2520 ***	0.1514 ***	34.77	1866.6 ***
SCA	1.385 *	3715 **	3.7492 *	1919.2.	0.403	349	1292.5 ***	0.029	9.62	784.1 ***
Environment:GCA1	2.06 ***	4237 ***	2.4087 *	2670.9 ***	0.693 *	1441 **	592.9 ***	0.0369 **	86.64 ***	277.4 ***
Environment:GCA2	0.422	2185	3.0499 *	2535.4 **	0.616	981	308.5	0.0095	5.8	89.3
Environment: SCA	0.481	998	1.0776	549.7	0.358	645	387.7	0.0045	8.47	135
Residuals	0.659	1438	1.6394	1145.1	0.471	754	268.3	0.0199	22.94	100.7
GCA/SCA ratio	9.3	12.1	6.03	10.9	17.6	29.7	6.8	15.5	24.9	4.3
% SS GCA1	61.76	59.47	69.84	72.08	86.87	87.87	74.61	78.17	89.18	59.14
% SS GCA2	28.55	32.87	15.93	19.54	7.74	8.88	12.53	15.78	6.96	22.2
% SS SCA	9.69	7.65	14.22	8.38	5.39	3.26	12.86	6.05	3.86	18.66

*,**, and *** indicate significance levels at 0.5,0.01 and 0.001 respectively; CBSDLS, cassava brown streak disease leaf severity; CBSDLI, cassava brown streak disease leaf incidence; CBSDRS, cassava brown streak disease root severity; CBSDRI, cassava brown streak disease root incidence; CMDS, cassava mosaic disease severity; CMDI, cassava mosaic disease incidence; FYLD, fresh root yield; HI, harvest index; DMC, dry matter content; DF, degree of freedom; GCA1, general combining ability for females; GCA2, general combining ability for males; SCA, specific combining ability.

The genetic parameters related to disease and yield traits in cassava populations are summarized in Table 8. The results showed that the phenotypic variances (PVs) for traits associated with CBSD, CMD, FYLD, and DMC were higher than the corresponding genotypic variances (GVs) and environmental variances (EVs). A high PCV (>20%) was recorded for all traits except DMC, whose PVC was moderate (12.39%). The highest GCV (67.05%) was observed for CMDI6 and the lowest for DMC (5.49%).

Table 8. Genetic parameters for diseases and yield-related traits in cassava populations.

Traits	Mean	SE	EV	GV	PV	ECV	GCV	PCV	H	GA	GAM
CBSDL6I	61.49	11.28	1018.6	310.47	1329.07	51.91	28.66	59.29	0.23	17.54	28.53
CBSDL6S	2.2	0.25	0.5	0.13	0.62	32.09	16.17	35.94	0.2	0.33	14.99
CMD6I	20.88	9.81	769.72	196.06	965.78	132.85	67.05	148.81	0.2	13	62.23
CMD6S	1.56	0.23	0.41	0.15	0.55	40.69	24.51	47.5	0.27	0.41	26.04
FYLD	19.24	5.92	280.26	30.96	311.22	87.01	28.92	91.69	0.1	3.61	18.79
HI	0.38	0.05	0.02	0	0.02	35.39	10.2	36.87	0.08	0.02	5.82
DMC	35.5	1.39	15.56	3.8	19.36	11.11	5.49	12.39	0.2	1.78	5.01
CBSDRI	20.72	10.48	878.08	9.62	887.7	143.04	14.97	143.82	0.01	0.67	3.21
CBSDRS	1.48	0.19	0.29	0.05	0.33	36.07	14.65	38.93	0.14	0.17	11.37

SE, standard error; EV, environment variance; GV, genotypic variance; PV, phenotypic variance; ECV, environmental coefficient of variation; GCV, genotypic coefficient of variation, PCV, phenotypic coefficient of variation; H, broad sense heritabilities; GA, genetic advance; GAM, genetic advance of mean.

The genetic advance (GA) ranged from 0.02 for HI to 17.54 for CBSDL6I, and the genetic advance as a percent of the mean (GAM) ranged from 3.21% for CBSDRI to 62.23% for CMD6I. The GAM was classified as low (below 10%), medium (10–20%), or high (above 20%). Low GAM (<10%) was observed for DMC, HI, and CBSDRI; medium for CBSDL6S, CBSDS9, FRYLD, and CBSDRS; and high for CBSDL6I, CMD6I, and CMD6S (Table 8). Low heritability estimates ($\leq$30) were observed across seasons and locations for all the traits presented, ranging from 0.01 (CBSDRI) to 0.27 (CMDS6).

The heritability estimates for CBSD traits were expanded to locations in specific growing seasons, and the results are presented in Table 9. Almost all broad sense heritability for CBSD severities and incidences at the IITA were higher than those at the NaSARRI. The highest (69.2%) was observed for CBSDL6I, and the lowest (27.3%) was observed for CBSDL3I in 2019/2022 (across all locations). The heritability estimates at the IITA were higher than those at NaSARRI. The broad sense heritability estimates ranged from 14.6 (CBSDRS at the NaSARRI) to 69.2 (CBSDI6 at the IITA).

Table 9. Broad-sense heritability of CBSD across years and locations.

Trait	Year	Location	H (%)
CBSDL3I	2019 to 2022	IITA	65.0
CBSDL3I	2020 to 2022	NaSARRI	32.7
CBSDL3I	2019 to 2022	Multilocation	27.3
CBSDL3S	2019 to 2022	IITA	54.0
CBSDL3S	2020 to 2022	NaSARRI	32.5
CBSDL3S	2019 to 2022	Multilocation	29.2
CBSDL6I	2019 to 2022	IITA	69.2
CBSDL6I	2020 to 2022	NaSARRI	49.4
CBSDL6I	2019 to 2022	Multilocation	39.3
CBSDL6I	2019 to 2022	IITA	63.2
CBSDL6I	2020 to 2022	NaSARRI	43.4
CBSDL6I	2019 to 2022	Multilocation	36.4
CBSDL9I	2019 to 2022	IITA	53.3
CBSDL9I	2020 to 2022	NaSARRI	44.6
CBSDL9I	2019 to 2022	Multilocation	39.4
CBSDL9S	2019 to 2022	IITA	46.8
CBSDL9S	2020 to 2022	NaSARRI	58.4
CBSDL9S	2019 to 2022	Multilocation	42.4
CBSDRI	2019 to 2022	IITA	62.1
CBSDRI	2020 to 2022	NaSARRI	31.9
CBSDRI	2019 to 2022	Multilocation	48.2
CBSDRS	2019 to 2022	IITA	57.9
CBSDRS	2020 to 2022	NaSARRI	14.6
CBSDRS	2019 to 2022	Multilocation	36.1

CBSDLI, cassava brown streak disease leaf incidence; CBSDLS, cassava brown streak disease leaf severity; CBSDRI, cassava brown streak disease root incidence; CBSDRS, cassava brown steak disease root severity; 3, 6, and 9, months after planting.

3.1.4. Virus Detection and Quantification

Cassava genotypes that showed leaf symptoms and root necrosis (Figure 6) were excluded, while those with clean leaves and roots showing no observable CBSD symptoms (Figure 7) were selected for virus detection. Virus detection and quantification revealed the prevalence of CBSV and fewer UCBSV occurrences in both sites (Table 10). The roots chosen for virus detection were selected during the root assessment, and the sample with a score of 1 was selected.

Figure 6. CBSD-infected cassava leaves showing yellow chlorosis (**A**) and roots with brown lesions (**B**).

Figure 7. Uninfected cassava leaves (**A**) and roots (**B**).

Table 10. CBSV and UCBSV detection at the NaSARRI and IITA.

Sample Type	Location	Sampled	CBSV	UCBSV	CBSV & UCBSV	Negative
Roots	NaSARRI_1	175	59	0	0	116
	NaSARRI_2	61	37	1	0	24
	IITA	85	73	1	0	8
Leaves	NaSARRI_2	63	11	2	1	52
	IITA	74	36	0	0	38

NaSARRI_1, 2020/2021 harvest; NaSARRI_2, 2021/2022 harvest; IITA = 2021/2022 harvest.

UCBSV was observed on leaves at the NaSARRI and roots at the IITA. At the NaSARRI site, one sample had co-infection with both CBSV and UCBSV on its leaves. Root samples collected at the NaSARRI during season one had 66.3% uninfected genotypes, whereas, during season two, 82.54% of leaves and 39.3% of roots were not detected with the viruses. At the IITA, 51.35% of leaves and only 9.41% of root samples collected were virus-free.

Based on the observed leaf symptoms, root necrosis, and virus detection, nine genotypes were either most resistant (MR), resistant (R), most tolerant (MT), or tolerant (T) to CBSD. Their phenotypes (apart from a root necrosis class score of 1–2) had negative virus detection, low leaf incidence (less than 20%), and a maximum leaf severity score of class 2 (Table 11). The genotypes MM160145, MM160582B, and MM160089A tested negative (no cq) for leaf and root virus detection; however, they showed root symptoms (necrosis), implying that they were infected by a new strain that current primers cannot detect.

Table 11. Performance of nine of the most promising genotypes with $\leq$20% CBSD incidence and $\leq$2 CBSD severity, CBSDRS $\leq$ 2, CMD severity, and negative virus detection at the IITA and NaSARRI.

Genotype	Pedigrees	CBSDS3	CMDS3	CBSDS6	CMDS6	CBSDS9	CMDS9	CBSDRS	Status	Location
MM160145	S1_MM060130	1	1	1	1	2	1	2	MT	both
MM161487	S1_MM060123	1	1	1	1	1	1	1	MR	both
MM160227	S1_MM060130	2	3	1	1	1	1	1	R	both
MM160582B	S1_MM060128	1	1	1	1	1	1	2	MT	both
MM161113	F1_MM060123XMM060130	1	1	1	1	1	1	1	MR	NAS
MM161247	F1_MM060123XMM060130	2	1	1	1	1	1	1	MR	NAS
MM160089A	S1_MM060130	1	1	1	1	1	1	2	MT	IITA

Table 11. *Cont.*

Genotype	Pedigrees	CBSDS3	CMDS3	CBSDS6	CMDS6	CBSDS9	CMDS9	CBSDRS	Status	Location
MM160406	F1_MM060128XMM060130	1	3	1	3	1	1	1	T	IITA
MM160530	S1_MM060128	1	1	1	1	2	1	1	MT	IITA

CBSDS, cassava brown streak disease severity; CMDS, cassava mosaic disease severity; CBSDRS, cassava brown streak disease root severity; 3, 6, and 9, months after planting; MR, most resistant; R, resistant; MT, most tolerant; T, tolerant.

Sixteen asymptomatic genotypes, including the checks, were identified, with MM160031 exhibiting asymptomatic positivity for both foliar and root necrosis (Table 12). These observations were made at the IITA. The rest of the remaining genotypes displayed asymptomatic positivity for either foliar symptoms or root necrosis at both sites (Table 12). The NAROCASS1 (IITA) and NAROCASS 2 (NaSARRI) check varieties were also asymptomatically positive for root necrosis.

Table 12. Sixteen asymptomatic cassava genotypes (including two checks) at the IITA and NaSSARI.

Genotype	Pedigrees	CBSDL3S	CBSDL6S	CBSDL6S	CBSDRS	CBSV_L	CBSV_R	Location
MM160031	MM060130XMM060130	1	1	1	1	24.03	26.25	IITA
MM160371	MM060128XMM060130	1	1	1	1	No Cq	36.82	NaSARRI
MM160234	MM060130XMM060130	1	1	1	1	No Cq	31.92	IITA
MM161627	MM060128 HS	1	1	1	1	No Cq	29.52	IITA
MM160760	MM060123XMM060128	1	1	1	1	No Cq	34.27	NaSARRI
MM161145	MM060123XMM060130	1	1	1	1	No Cq	37.61	NaSARRI
MM160969	MM060123XMM060128	1	1	1	1		28.45	IITA
MM161030	MM060123XMM060128	1	1	1	1		29.26	IITA
MM160909	MM060123XMM060128	1	1	1	1		30.8	IITA
LTG 5	UNKNOWN	1	1	1	1		32.11	IITA
MM160668A	MM060128XMM060128	1	1	1	1		28.68	IITA
NAROCASS1	NDL9036	1	1	1	1		36.66	IITA
MM160069	MM060130XMM060130	1	1	1	1		34.96	NaSARRI
MM160877	MM060123XMM060128	1	1	1	1		35.76	NaSARRI
NAROCASS2	Kitumbua	1	1	1	1		36.68	NaSARRI
MM160602	MM060128XMM060128	1	1	1	1		25.37	NaSARRI

CBSDLS, cassava brown streak disease leaf severity; CMDS, cassava mosaic disease severity; CBSDRS, cassava brown streak disease root severity; 3 and 6, months after planting; CBSV_L, leaf samples quantified for CBSV; CBSV_R, root samples quantified for CBSV.

4. Discussion

The genetic observed variation within and between populations in this study emphasized the role of the selfed populations in enhancing selection breeding. The results indicated that resistance to CBSD was primarily controlled by an additive mode of gene action, consistent with the previous studies by Chipeta et al. [16] and Nduwumuremyi et al. [14]. Furthermore, identifying the genotypes most resistant to CBSD across various locations highlighted the importance of utilizing different locations in the selection process [31].

The performance of cassava genotypes under varying environmental conditions revealed an essential dimension of their adaptability [32], enabling an assessment of the performance of the genotypes for a range of traits. The significant differences ($p < 0.05$) detected among the genotypes in response to both CBSD and CMD indicated a diverse array of resistance levels among the genotypes. These variations hold significant implications for programs seeking to breed resistance against these two economically undesirable diseases [33]. Consistent results were also observed for traits such as CBSDLS, CBSDRS, and FRYLD, further strengthening the validity and robustness of the results. The repetition of similar patterns across multiple traits underscored the stability of the findings and enhanced the validity of the observed trend. Identifying the quantitative loci associated with the resistance to CBSD will elucidate the genetic architecture of the resistance, paving the way for further investigation.

The interaction between genotypes and the environment, particularly in the context of CBSD, revealed the influence of quantitative traits [34], underscoring the multifaceted nature of genotype-environment relationships, emphasizing the need for a nuanced approach

in assessing and selecting genotypes for desired traits, and underscoring the complex interplay between genetics and the environment. This is crucial for informed decision-making in cassava genotype selection for superior resistance to disease and overall performance.

The performance of the resistant parents at the IITA in 2019 showed similar mean scores to the susceptible parents for CBSDLS, suggesting that prolonged drought might directly contribute to weakening plant defenses in some resistant varieties within the population [35]. This highlights the complexity of the interaction between drought stress and plant susceptibility to CBSD. Deeper insights into how environmental factors such as drought and soil conditions influence the expression of resistance traits are needed. This will enable the development of cassava varieties that are resilient to biotic and abiotic stresses. Additionally, developing environment-specific breeding strategies that account for genotypes with specific adaptations to some local environmental conditions will enhance resistance optimization and improve cassava yields.

The S1 and F1 results indicate that higher resistant gene accumulation might be attainable from crosses between lines. However, resistance rather than through biparental crosses of resistant and susceptible lines but resistant lines was also generated from a selfing susceptible line (TME14XTME14), suggesting that CBSD resistance is likely recessive, as reported by Sheat and Winter [36]. In addition, multiple genes conferring resistance to CBSD were indicated by segregation patterns in F1 and S1 populations through the distribution of the scores exhibiting similarities between crosses and S1s and indicating the complex genetic basis of CBSD resistance. Identifying more resistant progeny from the S1 generation of MM160123 suggested the strong expression of the recessive alleles for the resistance trait transferred from the parent MM160123.

The PV analysis demonstrated the allocation of total variation into GVs and EVs, providing valuable insights into the underlying sources of variability that contributed to the observed phenotypic variation. The high proportion of PVs indicated a strong environmental influence on the traits under study, and a relatively low proportion of Genotypic Variance Components (GVCs) was observed in some traits, particularly DMC, suggesting a comparatively weak genetic influence on their expression [37]. This observation highlights the potential for genetic improvement through hybridization, followed by rigorous selection, rather than relying solely on the phenotypic performance of individual genotypes. Similar conclusions were reported by Nduwumuremyi et al. [16]. The substantial differences observed between the PCV and its corresponding GCV highlighted the significant impact of environmental factors on the expression of these traits, indicating the sensitivity of genotypes to environmental variations. This suggests that selecting genotypes based solely on phenotypic performance may yield limited genetic improvements in traits due to the confounding influence of environmental factors [38].

The general combining ability (GCA) and specific combining ability (SCA) analysis provided valuable insights into the underlying gene effects and their interactions in the expression of the studied traits. The GCA results indicated additive gene effects and additive x additive interactions. On the other hand, SCA results suggested the dominance and epistatic effects. The significance of GCA across all traits underscored the predominance of additive genes in the expression of CBSDLS, CBSDRS, and FRYLD [16], CBSDLI [17], and HI trait [39]. The non-significance of SCA for CBSDRI, CMDS, CMDI, HI, and DMC indicated that parental interactions do not significantly influence the performance of their hybrids, suggesting that additive gene effects are more influential than non-additive effects. The high positive GCA/SCA ratio indicated that additive gene effects contributed to shaping the expression of the studied traits, underscoring the substantial role of additive genetic components in the variability of these traits.

The predominance of additive gene effects suggested that recurrent selection is an effective breeding strategy for increasing the frequency of favorable alleles for CBSD in cassava populations.

Across different seasons and locations, most heritability estimates were low, suggesting the pronounced influence of environmental factors [40]. However, at a specific location,

moderate to high broad sense heritability estimates were recorded for the CBSD trait, indicating a stronger genetic influence on the expression of this trait in that environment and implying that improvement through simple selection could be a viable approach for enhancing CBSD resistance, as suggested by Kayondo et al. [41].

We underscored the resistance level of several CBSD-resistant genotypes, including MM160145, MM161487, MM160227, and MM160582B, which exhibited minimal foliar symptoms and root necrosis ($\leq$2). These genotypes came from the full-sib population of CBSD-resistant, MM060123, MM060128, and MM060130 parents. The resistance observed in these genotypes was derived from S1 populations, surpassing the performance of their S0 parents. Similar results were reported by Pariyo et al. [42] and Kaweesi et al. [43], indicating the effectiveness of utilizing S1 populations for breeding resistance to CBSD. The stability of the four genotypes across contrasting environments indicates the potential for making genetic progress in CBSD resistance through inbreeding.

The root necrosis in genotypes that tested negative for the CBSD virus on roots and leaves indicated that root necrosis might be caused by a distinct virus biotype that could not be detected by the primers used. This study was limited by the inability to develop primers that matched the observed results. Additionally, the coexistence of genotypes that tested positive for CBSD on leaves but negative on roots indicated the virus concentration in the leaves, possibly because the virus had not yet reached the roots or the roots had developed resistance mechanisms. Conversely, genotypes testing positive for CBSD on roots but negative on leaves suggested differential resistance mechanisms between the two plant parts. Shirima et al. [27] reported similar observations. Understanding the molecular and physiological basis of these differential resistance mechanisms is important, as this knowledge can lead to more targeted breeding efforts and the development of cassava varieties with comprehensive resistance profiles.

5. Conclusions

This study finds that selfed populations exhibit strong recessive allele expression and greater resistance than their parental lines, making them valuable for enhancing resistance breeding. Additionally, the use of cassava genotypes MM161487, MM160227, MM170098, MM160582B, and MM160145 have demonstrated significant potential for high yield and resistance to CBSD and CMD, positioning them as key candidates for future breeding programs.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14092122/s1, Figure S1: The total monthly rainfall and average monthly temperature. Average monthly temperature (°C) and total monthly rainfall (mm) for Serere (A) and Sendusu (B); Figure S2: Amplification plots and standard curve. PCR amplification plots and the standard curve of serial dilution of linearized plasmid from Taqman assay probe chemistry. A: Amplification plots of CBSV clone (pFer2) and B: the standard curves of CBSV clone (pFer2). The efficiency of amplification was 104.8%, and the coefficient of correlation of 0.999 with a slope of −3.21 was achieved for CBSV absolute quantification.

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