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High-Throughput Field Screening of Cassava Brown Streak Disease Resistance for Efficient and Cost-Saving Breeding Selection

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Abstract: Cassava brown streak disease (CBSD) remains the most severe threat to cassava production in the Great Lakes region and Southern Africa. Screening for virus resistance by subjecting cassava to high virus pressure in the epidemic zone (hotspots) is a common but lengthy process because of unpredictable and erratic virus infections requiring multiple seasons for disease evaluation. This study investigated the feasibility of graft-infections to provide a highly controlled infection process that is robust and reproducible to select and eliminate susceptible cassava at the early stages and to predict the resistance of adapted and economically valuable varieties. To achieve this, a collection of cassava germplasm from the Democratic Republic of Congo and a different set of breeding trials comprising two seed nurseries and one preliminary yield trial were established. The cassava varieties OBAMA and NAROCASS 1 infected with CBSD were planted one month after establishment of the main trials in a 50 m^2 plot to serve as the source of the infection and to provide scions to graft approximately 1 ha. Grafted plants were inspected for virus symptoms and additionally tested by RT-qPCR for sensitive detection of the viruses. The incidence and severity of CBSD and cassava mosaic disease (CMD) symptoms were scored at different stages of plant growth and fresh root yield determined at harvesting. The results from the field experiments proved that graft-infection with infected plants showed rapid symptom development in susceptible cassava plants allowing instant exclusion of those lines from the next breeding cycle. High heritability, with values ranging from 0.63 to 0.97, was further recorded for leaf and root symptoms, respectively. Indeed, only a few cassava progenies were selected while clones DSC260 and two species of *M. glaziovii* (Glaziovii20210005 and Glaziovii20210006) showed resistance to CBSD. Taken together, grafting scions from infected cassava is a highly efficient and cost-effective method to infect cassava with CBSD even under rugged field conditions. It replaces an erratic infection process with a controlled method to ensure precise screening and selection for virus resistance. The clones identified as resistant could serve as elite donors for introgression, facilitating the transfer of resistance to CBSD.

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1. Introduction

In Sub-Saharan Africa, cassava serves as a staple and food security crop [1,2]. It provides household income and employment, and in many regions, it has reached a high commercial value due to its high carbohydrate content and gluten-free properties [3,4]. In the Democratic Republic of the Congo (DRC), cassava is the main food crop for more than 80% of the population. DRC grows almost all cassava for domestic consumption [5,6]. With an average 47 MT yearly production, DRC is the second-largest producer in the world and due to the utilization of both leaves and roots, cassava remains the first fighter against food insecurity and provides a major source of income for farmers [3,5]. However, the output capacity of most varieties remains by far lower than the predicted yield potential. The main diseases—cassava brown streak disease (CBSD), cassava anthracnose disease, cassava green mite, cassava mealybug, cassava mosaic disease (CMD), cassava root necrosis disease (CRND), and cassava bacterial blight [7–9]—are key production constraints. CBSD has become the major threat to cassava production, leading to food insecurity in Central, Eastern, and Southern Africa. In DRC, while CMD is endemic to the whole country, CBSD is confined to the eastern regions and remains the major threat to cassava, while CRND contests the mid-belt in the western region.

The most damaging disease, CBSD, causes over 50% yield loss and threatens farmers' livelihoods in the subregion [8], and losses can be up to 100% depending on the virus pressure and susceptibility of the cassava varieties. The main impact of the disease is the necrosis and destruction of edible roots, even when the plants otherwise show vigorous growth and the leaves and stems of the plants are asymptomatic [9,10]. To control CBSD and increase cassava productivity, the development, identification, and deployment of varieties with resistance to this disease remain the best option to provide improved cassava to the predominantly small household producers and medium-scale enterprises constituting the major stakeholders for the cassava breeding programs in Africa. Since the discovery of CBSD in Tanzania [10], millions of cassava genotypes have been evaluated subjecting cassava to natural virus infection under field conditions which is the most commonly used screening and selection method to date [11]. Field screening for CBSD resistance allows a quantitative evaluation yield and related traits but is challenged by soil heterogeneity, G X E interactions, and most importantly, by erratic virus infection processes relying on the presence of virus, virus-transmitting *Bemisia tabaci* whiteflies, and seasons of rain for high insect populations. In addition, the complex infection biology of CBSD viruses prolongs the process across several seasons.

To address these challenges, a high-throughput virus screening method for controlled virus infection was developed under glasshouse conditions [12]. The method shifts genotype evaluation from the field to the nursery and offers high precision of phenotyping and virus testing and short screening cycles. It allows rapid assessment during the early development stages in the screen house/glasshouse [12]. However, the method has limited scaling space and does not allow the evaluation of yield and further agronomic traits. Additionally, in a nursery, screening for virus resistance is associated with the uncertainty of genotype by environment interactions presented by field conditions of Central, Eastern, and Southern Africa. Consequently, disease evaluation, assessing cassava leaves and roots in the field, remains a crucial phenotyping process to identify virus resistance in cassava varieties adapted to each respective environment. It further resolves uncertainties associated with CBSD in cassava lines that do not show symptoms in leaves and in others that

develop symptoms but remain free of root necrosis. This study reports the work that has to be done to bring an effective virus screening protocol from the laboratory to the field. The goal is to provide a rapid and robust protocol for accurate identification of resistance against viruses causing CBSD.

2. Materials and Methods

2.1. Cassava Trials

2.1.1. Land Preparation

Ploughing is compulsory in land preparation to obtain loose and residue-free soil for the growth of cassava plants. After clearing, the land was mechanically ploughed approximately 35 cm deep. After twenty-one days, a second ploughing eliminated the clods in the topsoil and leveled the cracks from the first plowing. The topsoil was manually prepared for uniformity using a hoe, ensuring optimal field establishment.

2.1.2. Field Conditions

The best time to plant and screen in the Ruzizi plain (Lat: S 02.48122°, Long: E 28.58569°, Alt: 934 m asl) is from March to May [11]. During this period, the weather is mostly good for cassava growth and development. From June to August, there is a dry season window that exposes the plant/clone and raises the evapotranspiration conditions, leading to the appearance of severe CBSD symptoms. Ugandan cassava brown streak virus (UCBSV) and cassava brown streak virus (CBSV), which cause CBSD are common in the area. Also, there are several species of East African cassava mosaic virus, which cause CMD.

2.1.3. Planting Materials

For this study, true botanical seeds and stem cuttings were obtained from various cassava breeding programs of national and international institutions in Africa and South America. Seeds from cassava crosses (Table S1) made at the International Institute of Tropical Agriculture (IITA) in Uganda and the International Center for Tropical Agriculture (CIAT) in Colombia were grown in a seed nursery (SN) trial for evaluation. Cassava stem-cuttings were obtained from a preliminary yield trial (PYT) in Nigeria and a cassava germplasm collection in DRC. The latter was sourced from a CBSD disease-free area at the Institute National pour l'Étude et la Recherche Agronomiques (INERA) in Mulungu, DRC. Seeds received from the IITA cassava breeding program in Nigeria for clonal evaluation and cuttings from INERA Mulungu were planted on the Ruzizi plain presenting the CBSD hotspot (Table 1).

Table 1. Resistance screening trials (2022/2023) at the Ruzizi plain in DRC.

Cassava Materials	Number Plants/Clones	Experimental Design	Date of Planting	Number of Plants/Clones Grafted
Seed Nursery—Uganda	473	Unreplicated	3 December 2022	143
Seed Nursery—CIAT	637	Unreplicated	3 March 2023	96
Preliminary Yield Trial—Nigeria	26	RCBD 3 replications	3 March 2023	11
Germplasm collection—DRC	375	Alpha lattice 2 replications	5 March 2023	150
Total plants	1511			400

2.2. Virus Infections

The main goal of grafting was to clear up any confusion about the virus inoculum and the infection process. It was meant to quickly rule out a large number of susceptible candidates, which would speed up the field screening.

In this study, preliminary evaluations were carried out in various trials to identify non-infected plants and clones at 2- and 3-month ages, respectively, using CMD and CBSD. All the plants with CMD scores greater than 3 and CBSD scores greater than 1 from the grafting candidates were discarded. CBSD is known to be caused by two distinct strains: Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV). Both CMD and CBSD are transmitted by *Bemisia tabaci*. Cassava plants from the established trials (Table 1) were grafted 2 months after planting (MAP), for cassava plants developing from stem cuttings, and at 3 MAP for infection of cassava seedlings, using scions from infected plants essentially as described by Storey and Sheat et al. [12,13]. Infected cassava plants from the varieties OBAMA (TME 419) and NAROCASS 1 [11] served as virus sources to provide scions as inoculum for the grafting experiments. Stem cuttings encompassing 2–4 buds were excised from infected plants and cut into a wedge shape for insertion into the rootstock. An oblique cleft cut of 1–2 cm into the stem cambium of the rootstock plant was done and the wedge-shaped scion was inserted (Figure 1a). Care was taken that the vascular tissues of scion and rootstock joined in order to guarantee transfer of virus through phloem connections. The wound region was tightly closed with parafilm at the graft union to prevent desiccation, and the parafilm was removed after approximately 10 days. Leaving 1 to 2 leaves above the graft union, the rootstock was decapitated to force symptom development on fresh leaves developing from newly sprouting buds (Figure 1b). Plants were routinely indexed for symptom expression. Plants developing symptoms on newly expanding leaves were considered to be virus susceptible, while plants with CMD scores greater than 3 and CBSD scores greater than 1 were discarded. All cassava plants that did not show CBSD symptoms were subjected to RT-qPCR for virus detection.

(**a**) (**b**)

Figure 1. Introducing virus infection: (**a**) by side-grafting of scions from infected source plants to healthy cassava rootstocks; (**b**) observing development of symptoms on newly developing leaves of sprouting buds 3 weeks after grafting.

2.3. Symptom Assessment

A scoring system was used to assess and categorize symptoms on leaves and roots for CBSD resistance screening. A scale of 1 to 5 was used: 1 indicates no symptoms on leaves; 2 indicates slight leaf symptoms but no stem lesions; 3 indicates mild symptoms on leaves and moderate lesions; 4 indicates severe symptoms on leaves; and 5 indicates very severe symptoms on leaves and dieback. A similar scale was used to evaluate East African cassava mosaic virus (EACMV) symptoms and only leaf symptoms were recorded: 1. no symptoms on leaves; 2. inconspicuous yellow symptoms on leaves; 3. moderate mosaic symptoms on leaves; 4. severe mosaic symptoms on leaves; and 5. severe mosaic symptoms with leaf distortion.

Incidence and severity scores were taken for CBSD and CMD leaf symptoms at 3, 6, and 9 MAP and for CBSD root symptoms at 12 MAP. Disease incidence was calculated by dividing the number of infected clones/roots by the total number of plants in the plots or roots and multiplying by 100. Additionally, fresh yield root (yield) was measured from the net plot harvested to extrapolate the potential yield per hectare and to explore the relationship between the vegetative and reproductive stages.

2.4. Uganda Cassava Brown Streak Virus and Cassava Brown Streak Virus (U/CBSV) Detection Using a One-Step (RT-qPCR) TaqMan Assay

Dried leaf samples (02) from each plant/clone remaining symptomless for three months after grafting were collected and sent to the DSMZ Plant Virus Department laboratory in Germany for molecular analysis. Additionally, at harvest, cassava tuber sections were collected, dried, and sent along with collected leaves. At DSMZ, dried leaf and root tissues were excised and transferred into 2 mL Eppendorf tubes containing stainless steel beads, flash-frozen in liquid nitrogen (N2), and stored at −80 °C prior to RNA extraction. Samples were homogenized in a tissue lyser (Qiagen, Shildon, Germany) to ensure tissue disruption and RNA extraction using a commercial kit (Epoch, San Jose, CA, USA), essentially following the protocol by Sheat et al. [13]. PRX buffer (450 µL) provided in the kit, adjusted with 0.2% β-mercaptoethanol (Merck, Darmstadt, Germany), was added directly to the powdered tissue, vortexed, and transferred into a fresh Eppendorf tube for further processing. The quality and integrity of the extracted RNA were assessed in a 2% agarose gel electrophoresis and a NanoDrop spectrophotometer (NanoDrop ND-1000, PEQLAB, Erlangen, Germany).

Sensitive virus detection was achieved by reverse transcription quantitative PCR (RT-qPCR), proven superior to RT-PCR for its high sensitivity to detect ultra-low amounts of virus [14]. A one-step TaqMan assay (TaqMan Kit Maxima Probe/ROX RT-qPCR Master Mix, Thermo-Fisher Scientific, Waltham, MA, USA), combining reverse transcription and quantitative PCR to detect viral RNA [14] using primers for RT-qPCR detection of U/CBSV (Table 2), is described by [15]. A fragment of cytochrome oxidase (COX) was amplified, serving as a plant control to assess RNA quality and reaction efficiency, as previously described by [16]. Each RNA sample was analyzed in duplicate (technical replicate), and non-template reactions served as additional controls. RT-qPCR, reactions were performed in a qTOWER3 (Analytik Jena, Jena, Germany).

Table 2. Primers used to detect cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV).

Ugandan Cassava Brown Streak Virus (UCBSV)	UCBSV Forward	GATYAARAAGACITTCAAGCCTCCAAA	[16]
	UCBSV reverse	AATTACATCAGGRGTTAGRTTRTCCCTT	[16]
	UCBSV probe	FAM-TCAGCTTACATTTGGATTCCACGCTCTCA-TAMRA	[16]

Table 2. *Cont.*

Cassava brown streak virus (CBSV)	CBSV forward	GCCAACTARAACTCGAAGTCCATT	[16]
	CBSV reverse	TTCAGTTGTTTAAGCAGTTCGTTCA	[16]
	CBSV probe	FAM- AGTCAAGGAGGCTTCGTGCYCCTC-BHQ1	[16]
Cytochrome oxidase	COX forward	CGTCGCATTCCAGATTATCCA	[16]
	COX reverse	CAACTACGGATATATAAGRRCCRRAACTG	[17]
	COX probe	FAM-AGGGCATTCCATCCAGCGTAAGCA-TAMRA	[17]

2.5. Statistical Analysis

To select resistant germplasm and measure the heritability of the traits measured, single trial analysis was carried out on each measured trait using a linear mixed model that was set up to account for variability within the specific trial. The model includes random effects for germplasm and replicates within each trial:

$$Y_{ij} = \mu + G_i + R_j + e_{ij} \quad (1)$$

where, Y_{ij} represents the response variable, μ represents the overall mean, G_i represents the random effect of the ith germplasm, R_j is the random effect of the jth replicates, and e_{ij} is the error term. The best linear unbiased predictions (BLUPs) from each trial were obtained with their respective standard error. For each trait, the heritability was estimated using the [18] approach below:

$$H^2_{Cullis} = 1 - \frac{V_{\Delta}^{BLUP}}{2\sigma_g^2} \quad (2)$$

where, $V_{\Delta}{}^{BLUP}$ is the mean standard error of the germplasm BLUPs, and σ_g^2 is the germplasm variance. The estimated BLUP values were then used to generate the correlation plot among the measured traits. The raw data points were used to generate the plots showing the level of CBSD incidence and severity by month after planting for each germplasm, respectively. All mixed model analyses were carried out using the ASReml-R package [18], and all visualizations were conducted using the Tidyverse package [19], both in RStudio [20].

3. Results

3.1. Virus Infection of Cassava

The grafting method using scions of infected plants proved highly effective for the introduction of virus infections under field conditions. Even when the scions had been dried a few days after grafting, the vascular juncture was sufficient to transmit the virus from the scion to the rootstock. A high number of plants developed symptoms on the leaves consisting of chlorosis and vein clearing after 3 to 6 weeks post inoculations (Figure 2a). Leaf symptoms were uniformly distributed among grafted plants, indicating successful systemic spread of the infection. Severe necrosis on the tuberous roots upon harvest (Figure 2b) confirmed the CBSD infection process.

(**a**) (**b**)

Figure 2. Expression of cassava brown streak disease (CBSD) symptoms: leaves (**a**) and roots (**b**) of a susceptible variety.

The overall mean of the leaf scores for CBSD in cassava from the germplasm collection varied from 2.68 to 3.84, with a disease incidence ranging from 72.88 to 95.90%. In the preliminary yield trial at 3 MAP, the score varied between 1.91 and 2.6, with an incidence ranging from 0.0 to 74.07%. For CMD, moderate disease levels were observed, with a median severity score of 3 and an incidence ranging from 40% to 100%. Disease symptoms increased between 3 and 6 MAP and decreased at 9 MAP in the PYT. In contrast, the symptoms gradually increased in plants from the germplasm collection from 1 to 9 MAP. The same trends were noticed with the leaf severity score and the incidence in the two seedling nurseries (Figure S1).

Plants showing symptoms of CMD during the same period had average severity scores ranging from 1 to 4.58 and an incidence between 0 and 22.33% in the PYT, while the germplasm collection had a severity range of 1 to 3.73 and an average incidence increasing from 0 to 69.41%. The severity and incidence of the viral diseases in the clones were lower in PYT compared to the germplasm collection. Overall, the peak of disease expression in the leaf appears to occur 12 weeks after grafting, making it the most relevant period for symptom recording in the field.

All the twenty-six grafted PYT cassava clones expressed CBSD disease symptoms while only three of the 375 clones in the germplasm collection were asymptomatic. All grafted clones from PYT and the germplasm collection expressed CBSD symptoms in the leaves or roots except for the two wild cassava *M. glaziovii* (Figure S1) and the clone DSC260 (Table 2). Out of the 143 grafted seedlings in SN Uganda, 11 seedlings, including 6 biofortified ones, did not exhibit CBSD leaf symptoms, and 50 displayed both leaf and root symptoms. Similarly, the CIAT SN trial showed 12 seedlings solely exhibiting CBSD symptoms on leaves, 26 with leaf symptoms and root necrosis, 40 solely displaying root symptoms, and 18 plants remaining asymptomatic.

Observations at the seed nursery stage revealed that the clones/seedlings from three (03) out of the fourteen (14) grafted families (progenies/offspring from the same parents/crosses) did not exhibit CBSD symptoms. In contrast, the infected plants, particularly the crosses SM6778xGM10062-1, showed a high a severity score of 5 while SM6788 × PER221 and SM6431 × COL44 did not show resistance to root necrosis (Figure 3). In the Uganda population, many progenies from crosses showed resistance to root necrosis with a severity score of 1 (Figure 4).

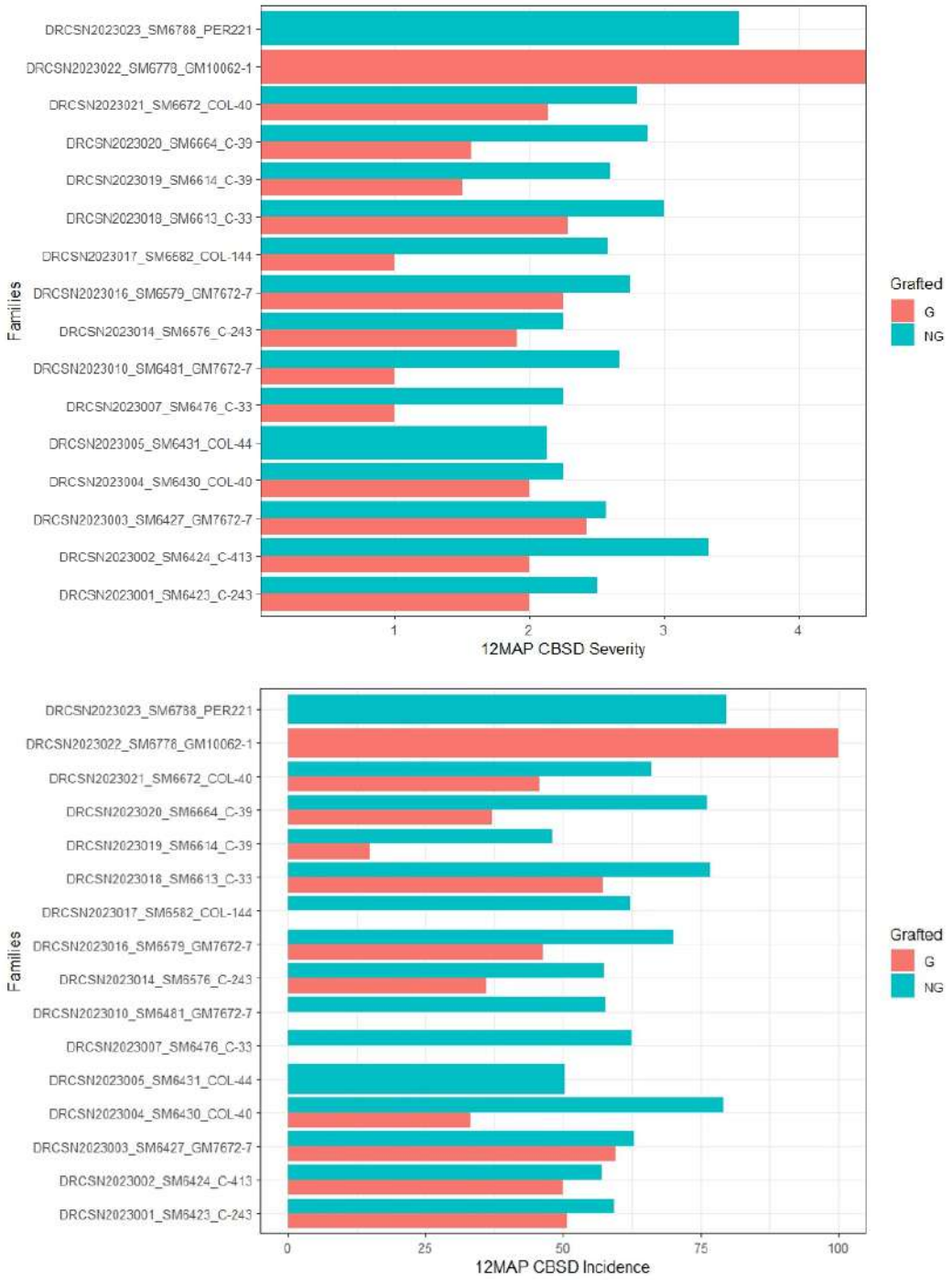

Figure 3. CBSD symptom evaluation in the root in the seed nursery after grafting CIAT population (G: grafted plants and NG: non-grafted plants).

Genotypes COL40, Narocass-1, KBH2016B-185, MM16-1487, MM16-0772, UG1703990, SM6424, C-413, SM6579, GM7672-7, SM6582, COL144, SM6614, and C-39 were reported as good donors to CBSD and/or CMD. To provide CBSD resistance, COL40 appeared to be the best clone (Figures 3 and 4).

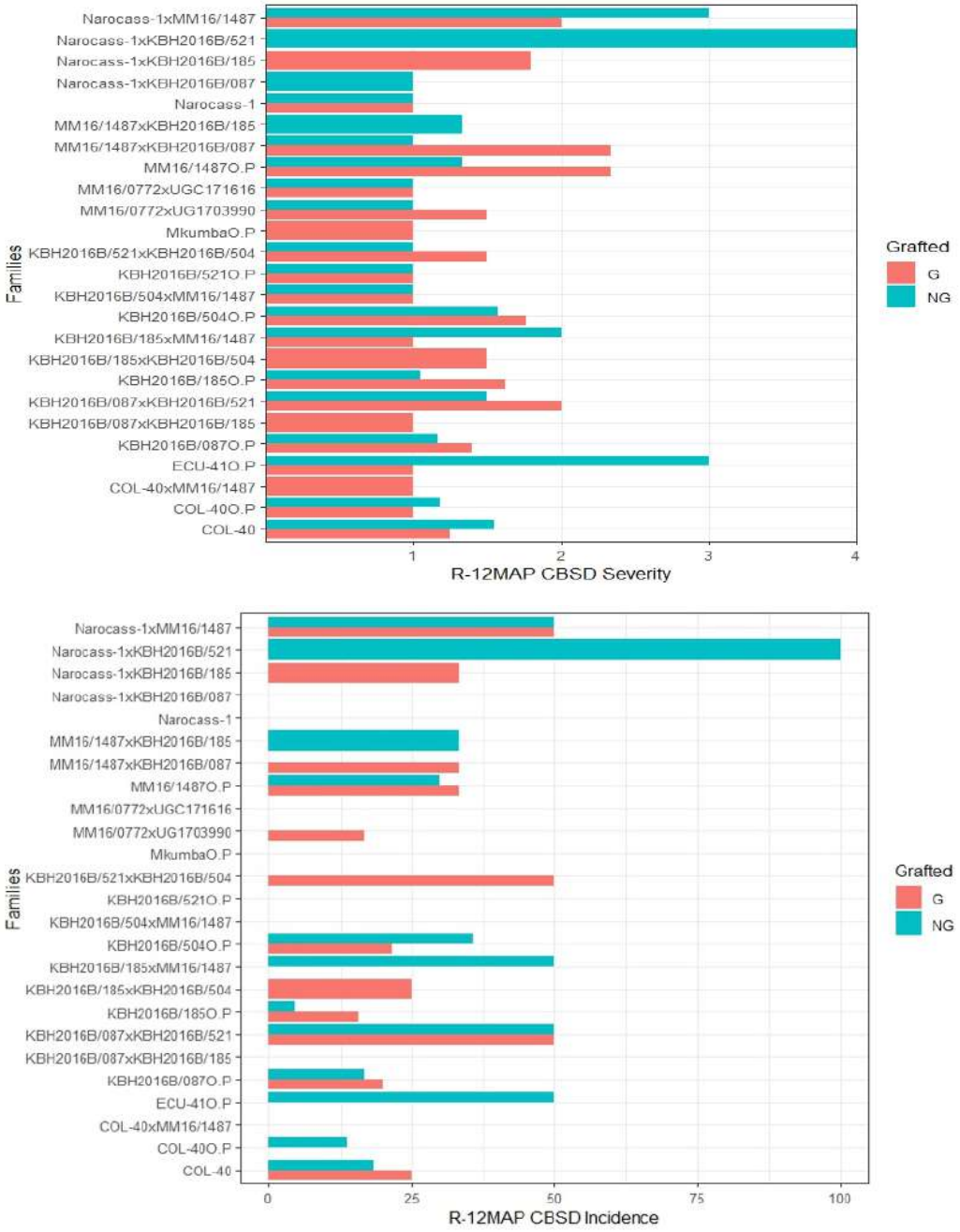

Figure 4. CBSD symptoms evaluation in the root in the Uganda SN after grafting at harvest (G: grafted plants and NG non-grafted plants).

3.2. Field Screening

Almost all cassava clones from germplasm collections and the PYT that were grafted with scions from infected plants developed CBSD symptoms. Similarly, in the seed nurseries of genetic populations from CIAT, 78.57% of the grafted families and 60% of the seedlings from Uganda also developed CBSD symptoms. This high number of infected plants proved the efficiency of the infection method for field screening. Virus testing by RT-qPCR discriminated between CBSV and UCBSV (Table 3) and revealed that MVZ 2016290, with a severity score of 1 for CBSD was indeed negative for CBSV but tested positive for UCBSV. Vice versa, the cassava clone 9124 tested positive for CBSV but was negative for UCBSV, indicating a differential susceptibility in the cassava genotypes (Table 3).

Table 3. Virus indexing assessment using cassava leaf and root at harvesting.

Repetition	Clones	Tissue	# CMD Sev6MAP	* CMD Inc6MAP	# CBSD Sev6MAP	* CBSD Inc6MAP	RT-qPCR CBSV	RT-qPCR UCBSV	Decision to CBSD
Rep1	MVZ2016290	leaf	4	50	3	100	-	-	Non-selected
Rep2	MVZ2016290	leaf	1	0	3	100	-	+	
Rep1	DSC260	leaf	5	100	4	100	-	-	Selected
Rep2	DSC260	leaf	4	100	1	0	-	-	
Rep	DSC260	tuber	-	-	1	0	-	-	
Rep1	Kinuani	leaf	4	40	3	100	+	-	Non-selected
Rep2	Kinuani	leaf	3	60	1	1	-	-	
Rep	Kinuani	tuber	-		2	77.7	+	-	
Rep1	91204	leaf	1	0	5	0	+	-	Non-selected
Rep2	91204	leaf	1	0	3	0	-	-	
Rep	91204	tuber	-	-	1	0	-	-	
Rep1	Glaziovii20210006	leaf	3	100	1	0	-	-	Selected
Rep2	Glaziovii20210006	leaf	4	100	1	0	-	-	
Rep1	Glaziovii20210005	leaf	4	100	1	0	-	-	Selected
Rep2	Glaziovii20210005	leaf	3	100	1	0	-	-	
Rep1	MVZ2017095	leaf	1	0	3	100	-	+	Non-Selected
Rep	MVZ2017095	tuber	-	-	0	0	-	-	
Rep1	MLG2019095	leaf	3	100	3	100	+	-	Non-selected
Rep2	MLG2019095	leaf	4	100	3	100	-	-	
Rep	MLG2019095	tuber	-	-	3	100	+	-	

#: score of severity and *: percentage (%).

DSC 260 and *M. glaziovii* (Glaziovii20210006 and Glaziovii20210005) (Figure S1) from the DRC collection remained symptomless, tested negative, tested negative by RT-qPCR, and could be considered virus-free (Table 3). Similarly, progenies from crosses involving COL40 (IITA and CIAT programs; Tables S1 and S2) were free of symptoms and negative by RT-qPCR thus with proven resistance against CBSV and UCBSV.

3.3. Reliability of Field Screening

Seven traits were recorded for both disease resistant response and yield. Moderate to high heritability was recorded for all the traits, which varied from 0.22 to 0.97, respectively, for CBSD root incidence in the germplasm collection and in PYT. In this study, a very high heritability was recorded for leaf incidence, ranging from 0.63 at 9 MAP to 0.95 at 6 MAP for CBSD (Table 4). The same trend was recorded for the CMD severity score and incidence. Very high heritability was also recorded for CBSD incidence in the root (0.97) in PYT with few progenies compared to the germplasm collection (0.22), while low heritability was recorded for severity in both trials. On the other hand, both trials recorded high heritability for the fresh yield root.

Table 4. Mean CBSD and CMD severities and incidences in leaf and fresh root yield of cassava clones and its heritability (Diseases quality control assessment).

Parameters	CBSD_ Sev3MAP	CBSD_ Inc3MAP	CBSD_ Sev6MAP	CBSD_ Inc6MAP	CBSD_ Sev9MAP	CBSD_ Inc9MAP	CMD Sev3MAP	CMD_ Inc3MAP	CBSD_ SevRoot	CBSD_ IncRoot	Yield
DRC Germplasm Collection											
Mean	3.35	93.62	3.26	91.66	3.36	92.57	1.42	11.24	3.24	57.26	8.92
Min	2.68	72.88	2.39	57.20	2.53	77.52	1.0	0.0	2.40	36.25	3.28
Max	3.84	95.90	3.89	99.39	4.01	95.65	3.73	69.41	3.93	74.52	30.23
SE	0.33	7.75	0.36	10.62	0.37	7.06	0.49	13.31	0.56	14.43	3.99
H^2	0.43	0.89	0.42	0.89	0.41	0.88	0.78	0.69	0.23	0.22	0.48

Table 4. *Cont.*

Parameters	CBSD_ Sev3MAP	CBSD_ Inc3MAP	CBSD_ Sev6MAP	CBSD_ Inc6MAP	CBSD_ Sev9MAP	CBSD_ Inc9MAP	CMD Sev3MAP	CMD_ Inc3MAP	CBSD_ SevRoot	CBSD_ IncRoot	Yield
Preliminary Yield Trial (Nigeria)											
Mean	2.17	20.08	2.99	73.02	1.58	21.19	0.44	2.14	2.83	35.01	13.87
Min	1.91	0.19	2.64	34.78	1.40	0.06	1.0	0.0	2.32	0.57	6.80
Max	2.6	74.07	3.43	95.37	2.54	96.08	4.58	22.33	3.5	99.48	22.88
SE	0.43	6.17	0.43	15.13	0.42	4.77	0.79	0.69	0.55	3.60	3.09
H^2	0.53	0.66	0.27	0.95	0.68	0.63	0.45	0.75	0.29	0.97	0.73

3.4. Relationship Between Yield and Cassava Diseases

The correlation between CBSD, CMD, and fresh root yield in the different trials is shown in Figure 5.

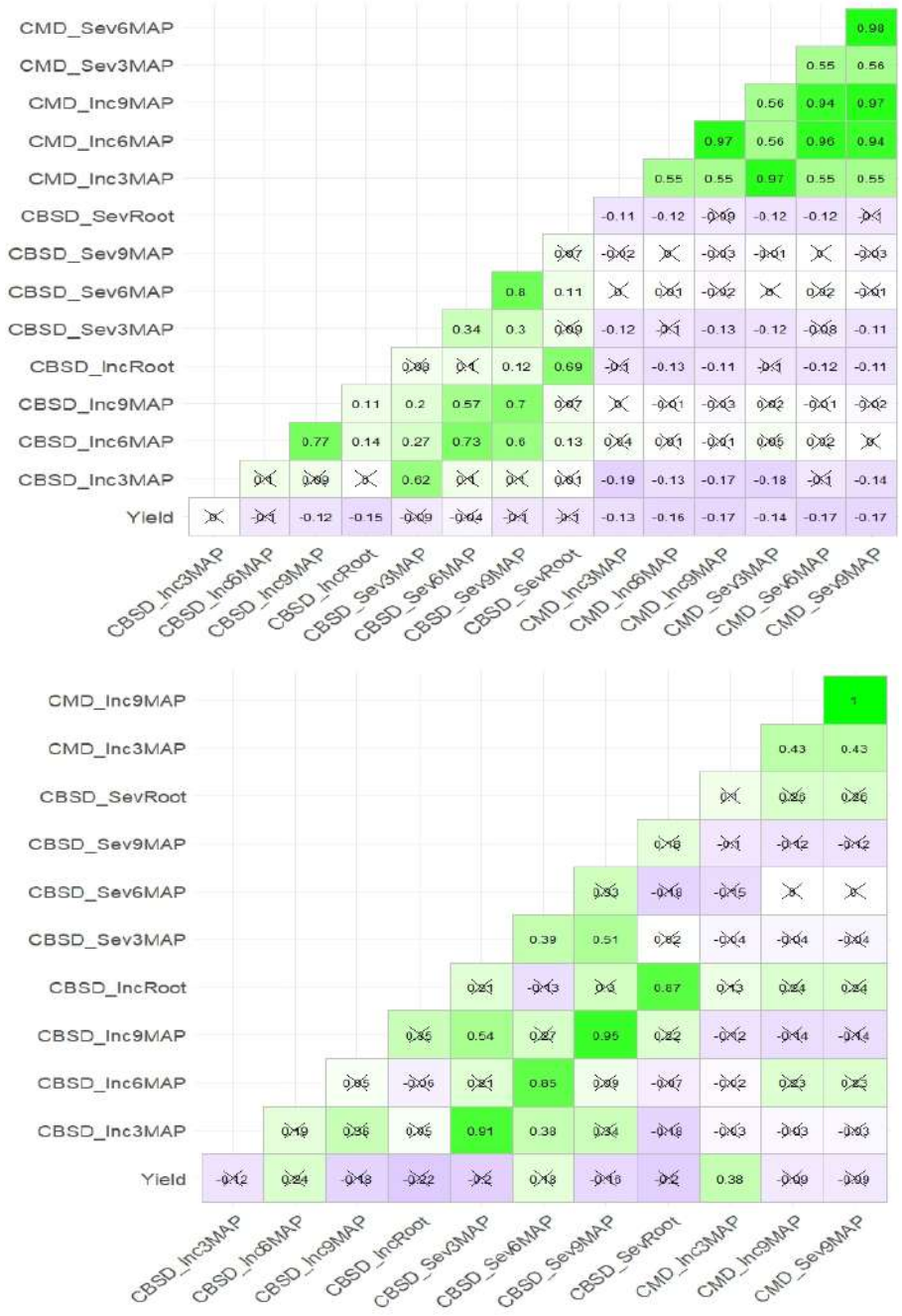

Figure 5. Correlation between CMD, CBSD, and yield in the DRC germplasm collection (**above**) and PYT Nigeria (**below**). Values in the figure represent the correlation coefficient.

A negative connection between yield and disease symptoms was found. A considerable rise in CBSD incidence was seen at 9 MAP and during harvest. However, there was no significant relationship between the severity of CBSD and the leaf yield. The CBSD severity may not be used as a secondary trait to predict the disease's root expression. CMD severity and occurrence have a significant to extremely significant positive link throughout the plant's growth phases, although there is a negative correlation with yield throughout.

4. Discussion

Screening for virus resistance by subjecting cassava to virus infection in disease hotspots is common practice in breeding. However, this method is not suitable for testing plant resistance against viruses that cause CBSD because of the erratic infection of viruses causing CBSD, inefficient insect vectors, seasonal variation in infection pressure, unknown virus populations, and highly complex viruses. As shown by Storey and Kaweesi et al. [10,17], grafting is a highly effective method to infect cassava. This report demonstrates the feasibility of grafting under the challenging conditions of African fields. In this study, wedge-grafting was used to achieve very high infection rates in the field to transmit viruses causing CBSD. This was almost as effective as grafting carried out in a glasshouse, where the environment is controlled. The high-throughput screening method to identify CBSD resistance [12] adopted for the field significantly reduced the time of evaluation and moreover, by precise phenotyping, reduced the number of clones to be evaluated in subsequent selection steps. Furthermore, it correlated infection and symptom types with the viral species CBSV and UCBSV [14] and the differential resistance responses of particular cassava clones already reported in earlier work [13,15].

A virus resistance background in cassava clones is a prerequisite to grow cassava throughout the World and is essential for effective disease management. The precise selection of resistant clones during breeding depends on accurate and reliable phenotyping which is supported by advanced virus detection tools to detect even traces of replicating virus in symptomless plants. As described in earlier studies [12,13,21–23], reverse RT-qPCR was utilized for its high sensitivity, outperforming conventional RT-PCR methods.

Results from the field screening confirmed the results from controlled glasshouse experiments and highlight the superiority of grafting over natural infections, providing consistency of infection and reproducibility. A very high proportion of grafted plants, 100% and 98% of grafted plants in PYT and DRC germplasm, respectively, exhibited CBSD symptoms. Graft infection seedlings in the seed nurseries of cassava from CIAT and Uganda resulted in 78.57% and 60% of infected plants with CBSD symptoms. In addition, high heritability was recorded for CBSD leaf incidence, ranging from 0.63 to 0.96 across the trials which means the disease in the field is well-spread. This implies that 63% to 96% of variation in CBSD is due to genetic differences while the remaining percentage is influenced by environmental factors. This means that the gene controlling CBSD is moderately to highly heritable, suggesting that there is a relatively good chance of improving resistance to CBSD through selection of genotypes exhibiting good resistance to CBSD. The experiments reported here confirm the feasibility of grafting under field conditions to achieve high infection rates and consistent symptom manifestation in cassava plants. The efficient transmission, and by following the systemic infection and establishment of CBSD in susceptible plants, underscores its value as a field tool for cassava breeding research and virus studies. Moreover, combining field testing with precise and sensitive molecular detection of the viruses method is a prerequisite to achieve a comprehensive and reliable assessment of differential resistance against virus species and tissue specific resistance. Furthermore, removing diseased plants to select non-symptomatic ones only for laboratory analysis significantly reduced the efforts of testing and subsequent evaluation in the field. Taken

together, the CBSD graft-infection method is robust, cost-effective, and feasible for breeding research to develop CBSD resistance.

A significant negative correlation was observed between CMD and yield, whereas no relationship was found for CBSD from all four trials in this study. This implies that symptom severity of leaves cannot be used as a good secondary trait for predicting root necrosis in CBSD. However, several researchers [9,24,25] reported a negative correlation between CBSD and fresh root yield cassava. Combining laboratory data with field screening using grafting revealed that two *M. glaziovii* species (Table S2) from the DRC germplasm collection did not exhibit CBSD leaf symptoms (Table 3), thereby creating a new avenue for CBSD research. Several studies report *M. glaziovii* as a good donor for CMD resistance by introgression to *M. esculenta* through backcrossing [9,26–28].

Nevertheless, 79 seedlings from CIAT and Uganda breeding programs are free of CBSD diseases and COL40 proved its superiority as a clone for CBSD resistance to confirm results from earlier crosses [12,21].

Field screening of cassava, comprising precise virus infection, symptom evaluation, and virus detection by RT-qPCR, is a comprehensive process to select virus resistance and identify asymptomatic clones. More importantly, it demonstrates the precise selection of clones that do not show symptoms but are still capable of replicating the virus. This integration not only enhances the accuracy of pathogen detection but also underscores the importance of identifying CBSV and UCBSV as key pathogens in cassava disease management and enables the selection of true resistant lines in asymptomatic plants. Overall, this study provides valuable insights into the screening for CBSD resistance. The study presents prospective targets for CBSD resistance in parent donors, facilitating population mapping and improving cassava germplasm to develop resistant varieties for end-users.

5. Conclusions

This feasibility study showed that grafting to transmit viruses to infect cassava under field conditions, combined with highly sensitive virus detection tools, is simple, dependable, and cost-effective to screen for resistance against CBSD. The experiments were conducted under real-world conditions and are reproducible and easily adoptable by African researchers. The two *M. glaziovii* (Glaziovii20210005 and Glaziovii20210006) and the cassava clone DSC 260 were identified as donors for CBSD resistance. Also, not many offspring from crosses were found to be possible candidates for CBSD resistance in the seed nursery trials. Therefore, candidate seedlings selected require further evaluation during the next stages of breeding; however, they present promising features to advance breeding programs with enhanced virus resistance and productivity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15020425/s1, Figure S1: Cassava M. Glaziovii (Glaziovii20210005) under CBSD hotspot in Ruzizi Plain at early and late stages; Table S1: Introduced families from IITA-Uganda; Table S2: Introduced families from CIAT-Colombia.

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