



Farmer and Field Survey in Cassava-Growing Districts of Rwanda Reveals Key Factors Associated With Cassava Brown Streak Disease Incidence and Cassava Productivity

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Cassava (*Manihot esculenta* Crantz) is a vital crop in Rwanda where it ranks as the third most consumed staple. However, cassava productivity remains below its yield potential due to several constraints, including important viral diseases, such as cassava brown streak disease (CBSD). Because various factors can be addressed to mitigate the impact of viral diseases, it is essential to identify routes of virus contamination in the cassava agrosystems from the seed system to farmer's practices and knowledge. The present study aimed at (1) assessing the current cassava seed system and farmers' practices and their knowledge of the biotic constraints to cassava production, (2) determining the status of CBSD as well as critical factors associated with its spread through the seed system channels, and (3) determining factors that influence cassava productivity in Rwanda. A cross-sectional study was carried out from May to September 2019 in 13 districts of Rwanda. A total of 130 farmers and cassava fields were visited, and the incidence and severity of CBSD were evaluated. CBSD was detected in all cassava-producing districts. The highest field incidence of CBSD was recorded in the Nyanza district (62%; 95% CI = 56–67%) followed by the Bugesera district (60%; 95% CI = 54–65%), which recorded the highest severity score of 3.0 ± 0.6. RT-PCR revealed the presence of CBSD at the rate of 35.3%. Ugandan cassava brown streak virus was predominant (21.5%) although

cassava brown streak virus was 4% and mixed infection was 10%. An informal cassava seed system was dominant among individual farmers, whereas most cooperatives used quality seeds. Cassava production was found to be significantly influenced by the use of fertilizer, size of the land, farming system, cassava viral disease, and type of cassava varieties grown ($p < 0.001$). Disease management measures were practiced by a half of participants only. Factors found to be significantly associated with CBSD infection ($p < 0.05$) were the source of cuttings, proximity to borders, age of cassava, and knowledge of CBSD transmission and management.

Keywords: cassava, seed system, CBSD, field survey, cassava production, Rwanda

INTRODUCTION

Cassava (*Manihot esculenta* Cranz) ranks as the sixth most important food crop worldwide and the fourth after rice, maize, and wheat among developing and emerging countries (Otekunrin and Sawicka, 2019; Saranraj et al., 2019). In Rwanda, cassava is the third most important crop after banana and sweet potato (Night et al., 2011). Because of its importance in several tropical regions and its relatively good performance on marginal lands under suboptimal climatic conditions (Burns et al., 2010), cassava is recognized as a subsistence crop to overcome food insecurity for the fast-growing population in areas prone to important climatic changes (El-Sharkawy, 2004; Chavez et al., 2005; Lobell et al., 2008; Burns et al., 2010). Although cassava plays an important role as a food security crop in sub-Saharan Africa, it is also used as a cash crop in various cassava-growing regions (Spencer and Ezedinma, 2017; Munganyinka et al., 2018).

The yield potential of cassava under optimum conditions is about 90 tons of fresh roots per hectare, which is equivalent to 30 tons of cassava dry matter per hectare (El-Sharkawy, 2004). More than half (61%) of cassava production is taking place in sub-Saharan Africa; however, cassava yield in tropical countries is still far below its production potential. Indeed, in 2017, the world cassava yield was about 11.08 tons of fresh roots per hectare, and the top cassava producer (Nigeria) had an average yield of 8.75 tons per hectare, followed by the Democratic Republic of Congo with 8.14 tons per hectare (FAO, 2019; Otekunrin and Sawicka, 2019). Cassava production in Rwanda varied between 3,000 and 3,701 Mt of fresh roots per year from 2015 to 2018 with a reported average yield of about 14.5 tons per hectare (FAO, 2018; Rwanda Agricultural Board¹). Despite its resilience under adverse environmental conditions, the production of cassava remains constrained by several abiotic and biotic factors. The former includes postharvest deterioration, infertile soils, planting unimproved traditional varieties, and inadequate farming practices, whereas the latter includes green mites, mealy bug, cassava bacterial blight, and viral diseases (Bull et al., 2011; Kombate et al., 2017).

As a consequence of viral diseases and the lack of resistant varieties, cassava yields have drastically decreased in many countries. Cassava mosaic disease (CMD) and cassava brown

streak disease (CBSD) are the most economically important cassava diseases, causing yield losses of more than US\$1 billion yearly globally (Legg and Thresh, 2003; Legg et al., 2006; Patil et al., 2015; Rey and Vanderschuren, 2017).

CBSD has so far only been reported in sub-Saharan Africa. CBSD is particularly devastating because it negatively impacts cassava tuberous roots both quantitatively and qualitatively, causing important economic losses to African farmers (Mohammed et al., 2012). For decades, CMD has been managed through dissemination of resistant varieties, but unfortunately, the distributed CMD-resistant varieties were found to be sensitive to CBSD in Rwanda and in many other African countries (Legg et al., 2001; Thresh and Cooter, 2005; Bua, 2017; Nyirahorana et al., 2017). CBSD is caused by two species of single-stranded RNA viruses of the family *Potyviridae*, Genus *Ipomovirus*; Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) (Mbanzibwa et al., 2009). In Rwanda, CBSD was first reported in 2009 in the Southern province (Muhanga district). CBSD has since spread to reach most cassava-producing regions in the south (Kamonyi, Ruhango, Nyanza, Gisagara and Muhanga Districts) and east of the country (Bugesera, Nyagatare, Gatsibo, Kirehe, Kayonza and Ngoma Districts) (Nyirahorana et al., 2017). A study conducted in Rwanda in 2014 reported a distribution of CBSD incidence as follows: 74.2% UCBSV, 15.3% CBSV, and 10.5% mixed infection (Munganyinka et al., 2018).

The CBSVs are transmitted by either the whitefly *Bemisia tabaci* and/or exchange of infected planting materials between farmers. Plant pathologists and extension services recognize the importance of establishing a disease-free seed system to mitigate the spread of CBSD (Mbanzibwa et al., 2009; Patil et al., 2015; Maruthi et al., 2017). In Rwanda, formal distribution of clean planting seeds usually involves the whole production chain from the Rwanda Agricultural and Animal Resources Development Board (RAB) where researchers produce basic clean seeds. Basic seeds are then distributed to seed multipliers across different regions for further multiplication, and before their dissemination to farmers, a quality seed certification agency is involved to ensure the quality of the planting materials (Broek and van den Byakweli, 2014; Andrade-Piedra et al., 2016). Quality seed refers to the seed preferred by farmers and consumers with good health (virus free), genetic purity, appropriate physiological age, and physical quality (Andrade-Piedra et al., 2016).

¹ Available online at: <https://www.newtimes.co.rw/business/what-holding-back-cassava-yield>.

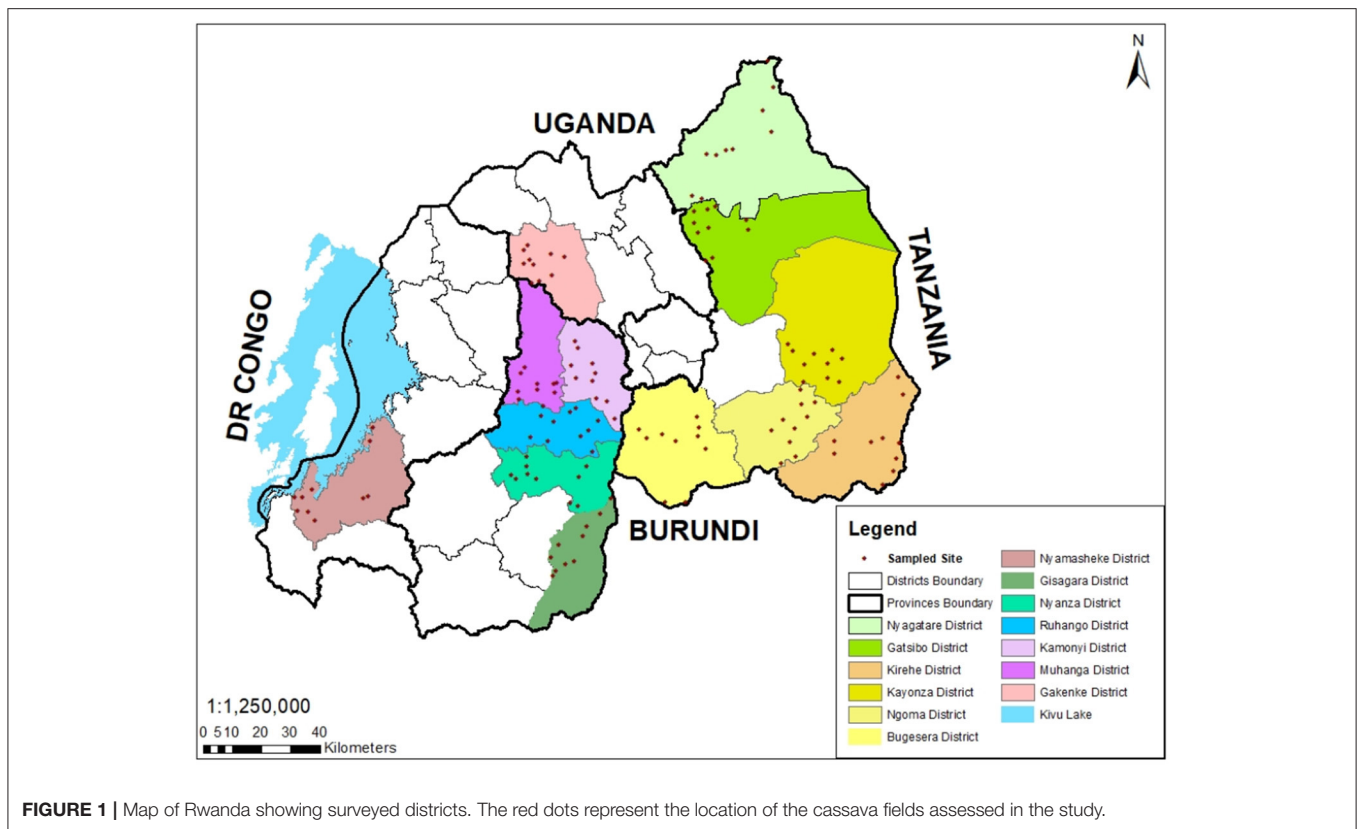


FIGURE 1 | Map of Rwanda showing surveyed districts. The red dots represent the location of the cassava fields assessed in the study.

The concomitant existence of an informal seed system involves production and dissemination of seeds by farmers usually without quality seed certification, which tends to favor disease dissemination and maintenance in cassava agrosystems (Muthoni and Nyamongo, 2008). Because of its prominence in most cassava agrosystems worldwide, the informal seed system plays a major role in the rapid spread of diseases (Andrade-Piedra et al., 2016; Maruthi et al., 2017).

Various management measures are applied to reduce CBSD impact, including investigation and breeding of CBSD-tolerant varieties (Masumba et al., 2017; Anjanappa et al., 2018; Sheat et al., 2019; Shirima et al., 2020) as well as the dissemination of disease-free planting material to farmers (Alicai et al., 2016). Conversely, farmers who reuse cuttings from their own fields will not escape the disease as this tends to maintain 30–50% of infection, especially in CBSD hot spots (Rwegasira and Rey, 2011; Patil et al., 2015). Unfortunately, the seed system sustainability remains fragile and needs to be consolidated to provide appropriate healthy planting cassava material to farmers. Despite the emergence of CBSD in cassava fields in Rwanda, there has been limited information about the CBSD distribution and factors associated with its transmission as well as factors that affect cassava productivity in the country.

The present study aimed at assessing the impact of farmers' practices and their knowledge of the biotic constraints on cassava production as well as determining the status of CBSD and the critical factors associated with its spread through the seed system channels.

MATERIALS AND METHODS

Study Area

The study was conducted in 13 cassava-growing districts of Rwanda during 2019. Districts in the southern and eastern provinces are considered to be major cassava-producing areas. In the south, five districts were surveyed, namely, Gisagara, Nyanza, Ruhango, Muhanga, and Kamonyi, whereas in the east, six districts were surveyed, namely, Bugesera, Nyagatare, Kayonza, Gatsibo, Kirehe, and Ngoma. In addition, two districts from the western and Northern provinces, Nyamasheke and Gakenke, respectively, were included in the study (Figure 1).

Farmer and Field Selection

A multistage sampling method was applied to select cassava farmers and fields. In the first stage, 13 districts, representing both major and minor cassava-growing areas, were selected. In the second stage, according to the information provided by district agronomists and RAB, five main cassava-growing sectors were purposely selected within each district. Sectors in which cassava is marginally cropped were not kept in the selection because they may not be representative of current limitations encountered by cassava growers. In the third stage, as cassava farmers are classified into two categories (either individual farmers or belonging to cooperatives) (Miklyaev et al., 2021), two farmers per sector, one individual farmer and one farmer belonging to a cooperative, were selected from a sampling frame provided by sector agronomists using a simple random-sampling approach,

making a total of 10 farmers per district and 130 interviewed farmers for the 13 districts surveyed. Selected participants were always heads of household, either a man or woman, depending on their availability. In case both were available, simple random sampling was applied to select one of them.

Furthermore, for each participant, a field with cassava plants older than 6 months was also visited for disease evaluation. The distance between two cassava fields visited was around 10 km. Within the selected fields, 30 plants were selected for leaf and stem CBSD symptom examination including five plants of the two diagonals and five of the four sides (Rwegasira et al., 2011). The field incidence per district was recorded as the percentage of symptomatic plants out of the total examined. The 10 plants examined at the two diagonals were further pooled and used for CBSVs indexing by RT-PCR.

Farmer Interviews

Primary data used in the study were collected using a structured questionnaire (**Supplementary Datasheet 1**), semistructured interviews and observations on five key subject areas (such as demography, social economics, agronomy, seed accessibility, and availability factors and disease aspect) relevant to cassava production. For farmers, quality seed was defined as seed certified by a seed-quality inspection agency (Rwanda Agriculture Board) that may be obtained from a professional seed multiplier. District and sector agriculture extension officers liaising with local community leaders were involved in the mobilization of farmers. Permission to conduct research in the area was sought from the administration of the study area (district and sector agronomists) through official communication by RAB authorities. Participants were told the purpose of the research and that participation was voluntary. Oral consent was given before starting the interview and field visit. All records were identified by study identification number to keep participant privacy and confidentiality.

Cassava productivity among participants was considered as cassava yield (kg fresh matter per hectare) in the present study, and income generated from cassava was estimated without considering price variation between districts. The price of cassava was estimated as the average of the cassava root prices recorded during the survey, which was 90 Rwandan francs (Rwf) per kilogram (Max = 95 Rwf, Min = 85 Rwf, std = 4.3 Rwf). Therefore, the income was estimated as the yield times the estimated price of cassava. To learn the factors influencing cassava productivity, the benefit generated from cassava per hectare was calculated as the total income minus the cost of production. Variables collected during the survey are listed in **Supplementary Table 1**. Biotic variables observed and measured in the visited fields are listed in **Supplementary Table 2**.

Disease Severity Assessment

A 1–5 CBSD symptom scale (Gondwe et al., 2003) was used to measure the degree of severity of CBSD aerial symptoms in the fields. The scale used was 1 = no apparent symptoms; 2 = slight leaf feathery chlorosis with no stem lesions; 3 = pronounced leaf feathery chlorosis, mild stem lesions, and no dieback; 4 = severe leaf feathery chlorosis, severe stem lesions, and no dieback; and 5 = defoliation, severe stem lesions, and dieback (Gondwe et al.,

2003). The average degree of severity was calculated omitting the score of 1, which represent asymptomatic plants to provide a true picture of the severity in the fields assessed (Sseruwagi et al., 2004). An average of disease severity per district was calculated based on the observation of $30 \times 10 = 300$ plants.

Sampling Test Materials for RT-PCR

Samples were collected from May to September 2019. In each cassava field assessed, 10 cuttings from 10 plants examined along the two diagonals were collected per field and established in the greenhouse. In total 1,300 plants were grown in the greenhouse from 130 fields. Five-month-old plants from collected cuttings were used for molecular analysis. In the laboratory, 20 leaf samples resulting from growth of cuttings collected on the same field were pooled (lower and middle leaves were used per plant); thus, a total of 130 samples were tested for CBSVs using RT-PCR.

Molecular Analysis

RNA Extraction

Total RNA was extracted from ~0.2 g cassava leaf using the cetyltrimethylammonium bromide protocol previously described (Abarshi et al., 2010).

cDNA Synthesis and RT-PCR

Synthesis of cDNA was done using a ProtoScript II Reverse Transcriptase kit (BioLabs, UK) following the manufacturer's instructions. Briefly, a Master Mix containing 1 μ l d(T)₂₃ (50 mM), 2 μ l of buffer, 1 μ l of 0.1 mM DTT, 0.5 μ l Protoscript II RT, 0.5 dNTP Mix, and 3 μ l of nuclease-free water was prepared. Then, 2 μ l of RNA template was subsequently added, making 10 μ l per reaction. The reaction mixture was incubated in a PCR thermocycler at 42°C for 1 h for primer annealing and cDNA synthesis, followed by 20 min at 65°C for inactivation of the ProtoScript II Reverse Transcriptase. The resulting cDNA samples were stored at –20°C.

The synthesized cDNA was subjected to polymerase chain reaction using a Taq G2 Hot Start Master Mix from Promega. The primer pair F: 5'-CCTCCATCWCATGCTATAGACA-3' and R: 5'-GGATATGGAGAAAGRKCTCC-3' that amplifies ~703 bp of CBSV and ~800 bp of UCBSV isolates was used (Elegba, 2018). The 10 μ l PCR reaction contained 5 μ l G2 Mix, 0.4 μ l each primer (0.4 μ M final concentration), 1 μ l cDNA and the volume was brought to 10 μ l with nuclease-free water. PCR conditions were as follows: predenaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30" and elongation at 72°C for 50" and final elongation at 72°C for 5 min.

An internal control gene from cassava called *Manihot esculenta* protein phosphatase 2A (*MePP2A*) was detected in parallel using a pair of primers F: 5'-TGCAAGGCTCACACT TTCATC-3' and R: 5'-CTGAGCGTAAAGCAGGGAAG-3' that amplifies 150 bp of *MePP2A* to ensure the accuracy of the PCR results by ruling out any false negative results (Moreno et al., 2011). PCR amplification was checked by loading 10 μ l of PCR products in 1% (w/v) agarose gel stained with Gel red in 1X Tris-acetate-EDTA (TAE) buffer for 1 h at 200 V to allow the separation of amplicons from the two isolates. The PCR

products were visualized on a UV gel documentation system and photographed.

Data Analysis

Raw data were transferred into Statistical Package for Social Sciences (SPSS) version 22 for analysis. Frequency and proportions for categorical variables were computed to describe the basic attributes of the respondents (farmers) as well as the occurrence of cassava infection (defined as the presence of CBSV or UCBSV after PCR) in sampled fields. A bivariate analysis with chi square tests was used to determine factors associated with cassava virus infections (categorical variable). Then, multivariate logistic regression analyses were performed by considering together all significant factors during bivariate analysis. The goodness of fit was assessed using Hosmer–Lemeshow test. In all statistical tests, differences were considered statistically significant at $p < 0.05$.

Similarly, descriptive statistics using mean and standard deviation for continuous variables were used. After testing for normality, cassava benefit was not normally distributed, and Sqrt transformation was used to ensure the normality of the cassava benefit distribution. Then, an ANOVA test was used to compare means to estimate the effect of sociodemographic, economic, and agronomic variables on cassava productivity. Multiple linear regression analyses were performed to assess the independent factors affecting cassava productivity. Ten factors were considered together, and upon fitting all against cassava productivity using multiple linear regression and specifying a “stepwise” method, five factors remained independently affecting cassava productivity. To validate the regression analysis, the histogram and P-P plot presented in **Supplementary Figure 3** were used to show the reasonable normality of data. Furthermore, multi-collinearity problems were assessed using tolerance and variance inflation factors (VIFs) as well as Durbin Watson was used to check for autocorrelation that the residuals from linear regressions are independent.

RESULTS

Sociodemographic Characteristics of Participants, Source of Cassava Cuttings, Trust, and Disease Management

Among the 130 farmers interviewed, a majority of them were individuals (58.5%), men (64.6%), and married people (66.9%). The percentages of men and women in the participants also reflects the gender distribution for cash-crop growers in Rwanda (Gender Monitoring Office, 2017; Munganyinka et al., 2018). Most of the participants (72.3%) had attended primary school. The respondents' age was in three categories with 45 years and above being slightly higher (36.2%) than the other two categories (**Supplementary Table 3**). The majority of participants (62.1%) reported that they obtain planting material from their own fields and use the same materials over many seasons. A minority of interviewed farmers (25.1%) acquired planting materials from

seed multipliers every season. Among those, a large proportion (86%) were cooperative members (**Figures 2A,B**).

Although most farmers use seeds from their fields, about 45% questioned their quality and feared that their cassava fields may succumb to diseases. A gap in cassava viral disease management was noted through the study as 50.3% of interviewed farmers took no action to control viral disease and only 19.5% declared roguing out infected plants from their field (**Figures 2C,D**).

CBSD Incidence and Severity Score of Observed Symptoms

Field incidence of CBSD and severity of aerial symptoms were evaluated in the 13 districts. The highest incidence (62%; 95% CI = 56–67%) was recorded in the Nyanza district, and the lowest (12%; 95% CI = 8–16%) was observed in Gakenke district. Disease mean severity scores varied from district to district, ranging from 3.0 ± 0.6 recorded in Bugesera to 2 ± 0.2 recorded in Nyamasheke. **Table 1** shows field incidence and mean severity score of observed CBSD symptoms in the examined fields.

Molecular diagnostics was performed on a total of 130 samples collected from 13 districts. Samples were analyzed by RT-PCR for detection of CBSV and UCBSV. The overall incidence of CBSVs was 35.3%. Among the positive samples, 61% (28/46) were UCBSV whereas 11% (5/46) were CBSV, and 28% (13/46) had mixed infection of both CBSV and UCBSV (**Figure 3**), **Supplementary Figure 1** shows RT-PCR detection of CBSVs in field samples.

All 13 districts surveyed were found to be affected by CBSVs based on RT-PCR results, and the highest incidence (60%) was recorded in the south, in Nyanza district followed by Gisagara and Bugesera districts, both displaying an incidence of 50%. A single infection of UCBSV was found in all districts except Muhanga, whereas a single infection of CBSV occurred in Muhanga, Bugesera, Gatsibo, and Gakenke. Mixed infections were recorded in most districts except in Ngoma, Gatsibo, Gakenke, and Nyamasheke (**Figure 3**).

Bivariate Analysis of Factors Associated With CBSD Incidence

Fields From Farmers Working in Cooperatives Display Lower CBSD Infection Rate

Using data collected during the survey, we tested whether sociodemographic characteristics influence the level of cassava infection. Bivariate analysis (using Chi square test) of sociodemographic factors of 130 farmers (for whom cassava fields were visited) stratified by CBSV infection revealed that there is a significant association between category of respondents and cassava infection, and individual farmers had more infected fields than farmers in cooperatives ($p = 0.023$) (**Supplementary Table 3**). Farmer's age also significantly influenced the level of cassava infection of their field ($p = 0.043$). All the other sociodemographic factors had no influence on levels of cassava infection.

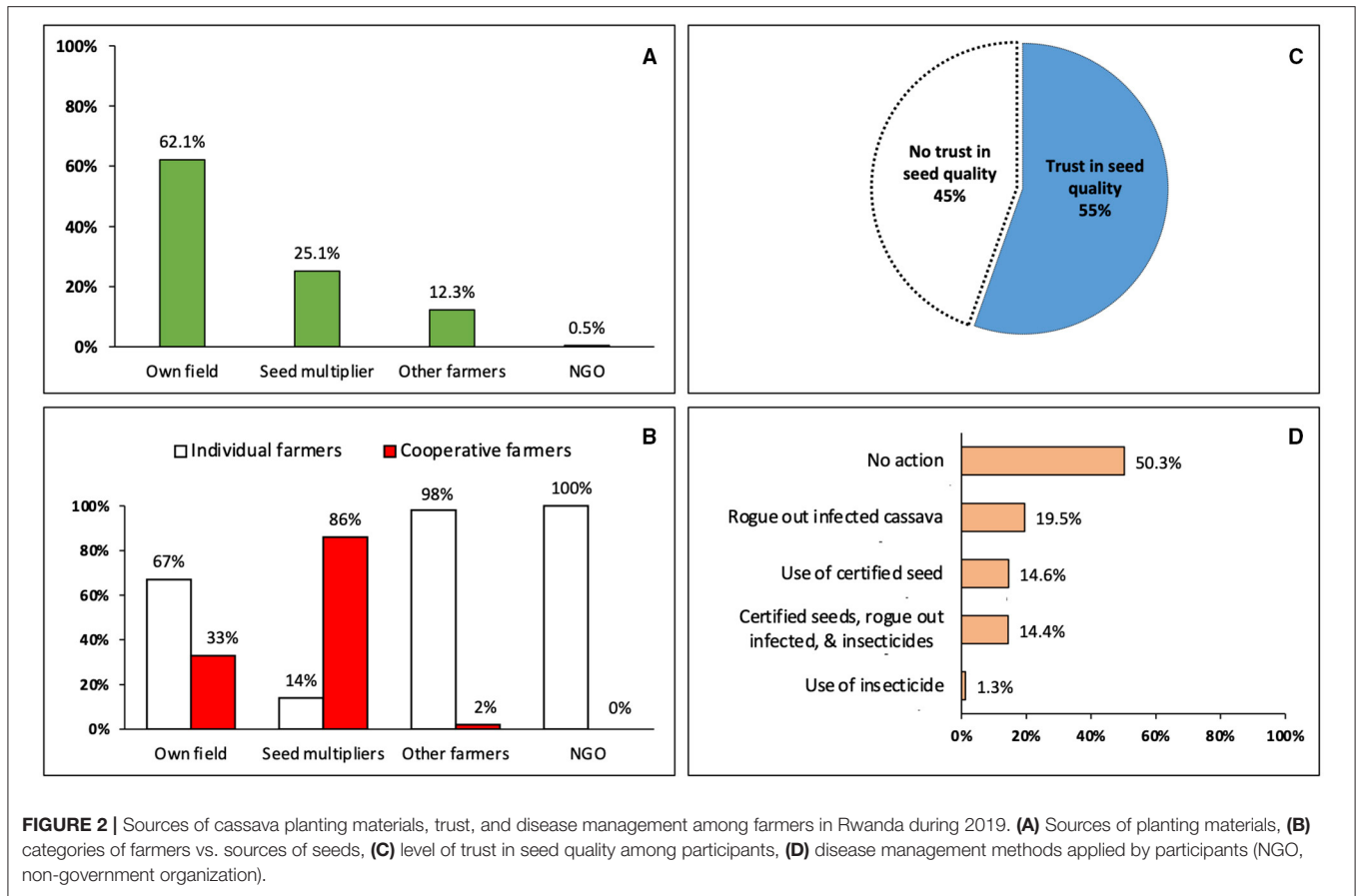


TABLE 1 | Field incidence, severity, and frequency of cassava plants showing aerial CBSD symptoms in Rwanda, 2019.

Districts	Field incidence % (95% CI)	Mean severity score	Frequency of severity score (%)				
			1	2	3	4	5
South							
Gisagara	58.3 (52–63)	2.7 ± 0.8	42	29	19	8	2
Nyanza	62 (56–67)	2.9 ± 0.8	38	18	33	7	4
Ruhango	35.6 (30–41)	2.1 ± 0.3	61	35	4	0	0
Muhanga	30 (24–35)	2.2 ± 0.4	70	24	6	0	0
Kamonyi	25.3 (20–30)	2.2 ± 0.6	72	23	2	3	0
East							
Bugesera	60 (54–65)	3 ± 0.6	37	11	44	5	3
Kayonza	32.3 (27–37)	2.1 ± 0.3	68	28	4	0	0
Gatsibo	18 (13–22)	2.4 ± 0.5	82	10	8	0	0
Nyagatare	30 (24–35)	2.5 ± 0.6	70	16	12	2	0
Ngoma	20.6 (16–25)	2.2 ± 0.5	80	16	3	1	0
Kirehe	50 (44–55)	2.6 ± 0.5	50	22	26	2	0
North							
Gakenke	12 (8–16)	2.1 ± 0.3	87	11	2	0	0
West							
Nyamashoke	14.3 (10–18)	2.0 ± 0.2	86	13	1	0	0

Three hundred plants per district were examined. CI, confidence interval.

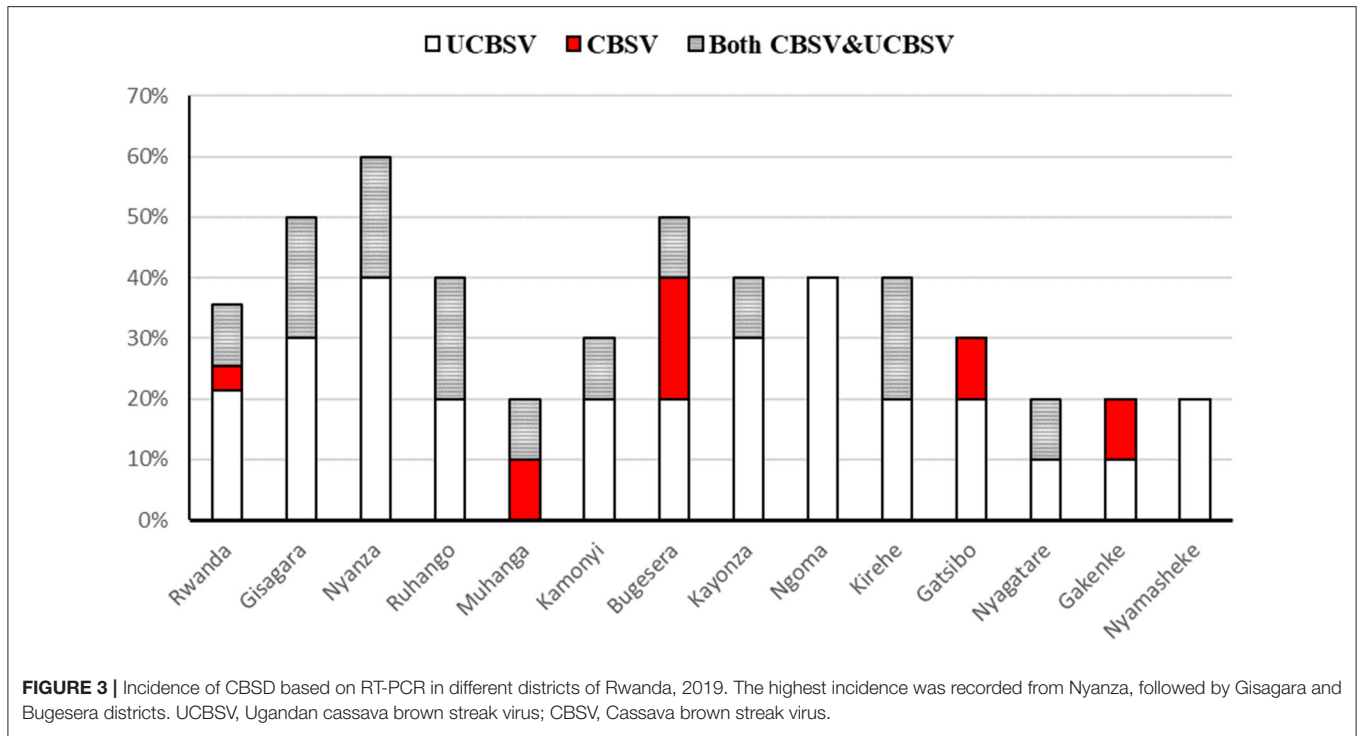


TABLE 2 | Impact of accessibility of planting materials on cassava infection.

Variables	Total, n (%)	CBSVs Positive, n (%)	Chi square value	df	p*
Source of cassava cuttings					
Seed multiplier	30 (23.0)	5 (10.9)	14.733	2	0.001
Own field	79 (60.7)	28 (60.9)			
Other farmers	21 (16.2)	13 (28.2)			
Distance to the source (Km)					
<1	70 (53.8)	26 (56.5)	4.032	3	0.258
1-4	28 (21.5)	7 (15.2)			
4-8	24 (18.5)	8 (17.4)			
>8	8 (6.2)	5 (10.9)			
Proximity to the tarmac road (Km)					
<1	14 (10.8)	4 (8.7)	1.732	3	0.630
1-4	24 (18.5)	11 (23.9)			
4-8	27 (20.8)	10 (21.7)			
>8	65 (50.0)	21 (45.7)			
Proximity to RAB (Km)					
<10	42 (32.3)	23 (50.0)	13.445	3	0.070
10-20	21 (16.2)	3 (6.5)			
20-30	21 (16.2)	4 (8.7)			
>30	46 (35.4)	16 (34.8)			
Proximity to the border (Km)					
<10	53 (40.8)	25 (54.3)	9.7	3	0.021
10-20	23 (17.7)	10 (21.7)			
21-50	10 (7.7)	3 (6.5)			
51 and above	45 (34.6)	8 (17.4)			

*Significant at $p < 0.05$ bolded; df, degree of freedom.

TABLE 3 | Impact of agronomic variables on cassava infection.

Variables	Total, n (%)	CBSVs positive, n (%)	Chi square value	df	<i>p</i> *
Age of plants in months					
<8	57 (43.8)	9 (19.5)	19.65	2	<0.001
8–10	56 (43.1)	24 (52.2)			
>10	17 (13.1)	13 (28.2)			
Type of cassava varieties grown					
Improved	85 (65.4)	29 (63.0)	7.2	2	0.067
Local	9 (6.9)	0 (0.0)			
Both improved and local	36 (27.7)	17 (37.0)			
Farming system used					
Monoculture	60 (46.2)	16 (34.8)	3.7	1	0.054
Polyculture	70 (53.8)	30 (65.2)			
Using fertilizers to grow cassava					
Yes	75 (57.7)	18 (39.0)	10.05	1	0.002
No	55 (42.3)	28 (61.0)			
Access to extension services					
Yes	108 (83.1)	37 (80.4)	0.35	1	0.552
No	22 (16.9)	9 (19.6)			
Extension services benefited by farmers					
Visit of cassava field	42(32.3)	17(37.0)	2.06	4	0.725
Advice on diseases management	7(5.4)	3(6.5)			
Advice on farming practices	31 (23.5)	9 (19.5)			
None	22 (16.9)	9 (19.5)			
Field visit and advice on GAP	28 (21.5)	8 (17.4)			

*Significant at $p < 0.05$ bolded; df, degree of freedom.

Fields Established With Planting Material From Seed Multipliers Have a Lower Probability to Be CBSV Infected

We further investigated the link between cassava seed accessibility and CBSV infection. Our analysis showed that the source of cassava cuttings and proximity to the border had a significant impact on cassava infection with p values of 0.001 and 0.021, respectively (Table 2). Farmers who used seeds from their own field were more likely to have infected fields (60.9%) than those who got seeds from seed multipliers (10.9%). It was also noted that farmers near the country's border had more infected fields (54.3%) (Table 2).

A majority of cassava fields surveyed were more than 8 months old (56.2%). Most participants grew improved varieties (65.4%) and had access to extension services (83.1%). In fact, the analysis revealed that there was a significant association between age of the plant and cassava infection ($p < 0.001$) where the plants aged <8 months (57.1%) were significantly more likely to be healthy than to be infected (19.5%). Furthermore, a significant association was noted between use of fertilizers and cassava infection ($p = 0.002$) as the farmers using fertilizers (68%) were significantly more likely to have healthy than infected fields (39%) (Table 3).

Farmers' Awareness of Cassava Viral Diseases Is Associated With Lower CBSV Incidence

Although all farmers were aware of at least one cassava viral disease's existence, 34.6% of them did not know the symptoms of

cassava viral diseases. Symptoms of CMD were easily recognized by 31.5% followed by 20.8% who recognized both CMD and CBSV (Supplementary Figure 2 shows symptoms of CMD and CBSV). Farmers who were not aware that the viruses can be transmitted had more infected fields (65.2%) ($p < 0.001$), and likewise, those who did not know disease management had more infections in their fields (67.4%) compared with those who knew the management techniques ($p < 0.001$) (Supplementary Table 4).

Multivariate Analysis of Risk Factors Associated With CBSV

Eight factors that showed significant association ($p < 0.05$) during bivariate analysis (including source of cassava cuttings, proximity to the border, age of the plants, use of fertilizers, category of respondents, age of farmers, knowledge of cassava viral disease transmission, and knowledge of cassava disease management) were considered together in a multivariable analysis to identify the variables associated with cassava infections. Upon fitting the factors using multiple logistic regression and specifying the "backward conditional" method with removal at $p < 0.05$, five factors remained in the final analysis as shown in Table 4. After testing the goodness of fit using the Hosmer–Lemeshow test, the chi-square value was 4.80 with degree of freedom 6, and p value was 0.570, which indicates that the fitted model was adequate.

TABLE 4 | Factors associated with CBSD.

Variables	AOR	95% CI		p*
		Lower	Upper	
Category of respondents				
Individual farmers	0.43	0.12	1.52	0.191
Cooperative	Ref			
Farmers' age in years				
25–35	0.74	0.22	2.43	0.617
35–45	0.26	0.08	1.22	0.080
45 and above	Ref			
Source of cassava cuttings				
Seed multiplier	Ref			
Own field	7.31	1.52	35.06	0.013
Other farmers	10.1	1.73	58.81	0.010
Proximity to the border (Km)				
<10	4	1.33	12.05	0.014
10–20	4.24	1	17.97	0.050
21–50	1.7	0.27	10.65	0.571
51 and above	Ref			
Age of plants in months				
<8	Ref			
8–10	4.76	1.69	13.39	0.003
>10	18.47	3.93	86.78	<0.001
Using fertilizers to grow cassava				
Yes	Ref			
No	2.44	0.93	10.25	0.127
Knowledge of cassava viral diseases transmission				
Yes	Ref			
No	3.97	1.46	10.83	0.007
Knowledge of cassava viral diseases management				
Yes	Ref			
No	2.94	1.08	7.96	0.034

Ref, reference, *Significant at $p < 0.05$ bolded; AOR, adjusted odds ratio; 95% CI, confidence interval.

Farmers who use cuttings from their own fields or from other farmers' fields had a more than seven-fold higher risk than those who used cuttings from a seed multiplier ($p < 0.05$). Respondents located near the border had a four times higher risk of having CBSVs than those located far away ($p < 0.05$). Cassava plants <8 months old had fewer risks of infection compared with the older ones ($p < 0.05$). Similarly, those who were not aware of the disease transmission and management had nearly a three times higher risk of having the infected plants ($p < 0.05$) (Table 4).

Effect of Sociodemographic Variables on Cassava Productivity

Although diseases are among the main constraints to cassava yield and production (FAO, 2015; Rey and Vanderschuren, 2017), socioeconomic factors might also be associated with suboptimal cassava yields. Taking advantage of the farmer survey, we performed an ANOVA to assess the effect of different sociodemographic variables on cassava productivity.

The demographic variables that significantly affected cassava productivity were district, level of education and farmer category. The district of Ruhango had a significantly higher average of cassava benefit per hectare than other districts ($p < 0.001$). The mean of cassava benefit was significantly higher among respondents with a secondary level of education compared with illiterate farmers ($p < 0.001$). Similarly, cooperative farmers had significantly more average cassava benefit than individual farmers ($p < 0.001$) (Supplementary Table 5).

Effect of Economic and Agronomic Variables on Cassava Productivity

During bivariate analysis using ANOVA, all agronomic and economic factors tested showed that they significantly influence cassava productivity ($p < 0.001$). The mean cassava benefit was significantly higher among farmers with fields displaying less viral disease (after PCR), larger size of land, growing improved varieties, practicing monoculture, applying fertilizers, and using seeds from seed multipliers (Table 5).

Multiple Linear Regression for Cassava Productivity

A multiple linear method was used to determine which variables had the most significant impact on cassava productivity. The results show that 5 out of 10 factors predict cassava productivity. The 10 variables considered in the model were all those variables significant at the bivariate analysis using the ANOVA test (see Table 5; Supplementary Table 5). Upon fitting these 10 factors against the dependent variables using multiple linear regression and specifying the *stepwise* method, five variables (*using fertilizer, size of the land, farming system, cassava viral disease, and type of cassava varieties grown*) remained affecting the productivity of cassava (Table 6). There were no collinearity issues found in this study between the different outcome and independent variables as tolerance was above 0.1 and VIFs were below 10. Durbin Watson was also used to check for autocorrelation that the residuals from linear regressions are independent. Durbin Watson with zero indicated positive autocorrelation, and four indicated negative autocorrelation while around two indicated that the residuals are uncorrelated.

Using fertilizer was the main positive effect in the first place and explained 57% ($R^2 = 0.57$) of the changes in cassava benefit. In the second model, size of the land is added, which led to 67% ($R^2 = 0.67$) of the changes in the cassava benefit. In the third model, farming system is added, which led to 69% ($R^2 = 0.69$). In the fourth model, viral disease led to 71% ($R^2 = 0.71$), and type of cassava varieties grown in the fifth model led to 72% ($R^2 = 0.72$) variation in cassava benefit (Table 6).

DISCUSSION

The present study used a comprehensive cross-country survey to assess the current cassava seed system, farmers' practices, and their knowledge of the biotic constraints, the status of CBSD, and critical factors associated with its spread throughout the cassava

TABLE 5 | Effects of economic and agronomic variables on cassava productivity.

Variables	N	Mean (benefit/Ha)	Std. deviation	Std. error	95% CI for mean		F	ANOVA
					Lower	Upper		p
Cassava infection								
Positive	46	482.58	125.01	18.43	445.46	519.71	10.56	0.001
Negative	84	648.27	332.70	36.30	576.07	720.47		
Size of land used to grow cassava (Ha)								
<1	45	425.34	126.12	18.80	387.45	463.23	14.90	<0.001
1–5	31	556.81	96.95	17.41	521.25	592.37		
6–10	19	836.95	521.52	119.64	585.59	1,088.32		
>10	35	695.73	244.03	41.25	611.90	779.55		
Type of cassava varieties grown								
Improved	85	665.45	315.45	34.22	597.41	733.49	9.65	<0.001
Local	9	435.47	165.57	55.19	308.20	562.74		
Both improved and local	36	449.20	143.01	23.84	400.81	497.59		
Farming system used								
Monoculture	78	694.76	321.21	36.37	622.34	767.18	32.25	<0.001
Polyculture	52	431.98	109.14	15.14	401.59	462.36		
Using fertilizers to grow cassava								
Yes	75	710.33	317.06	36.61	637.38	783.27	40.68	<0.001
No	55	425.08	112.70	15.20	394.62	455.55		
Access to extension services								
Yes	108	620.19	300.65	28.93	562.84	677.54	7.54	0.007
No	22	439.71	144.64	30.84	375.58	503.84		
Source of cassava cuttings								
Seed multiplier	79	552.51	211.12	23.75	505.22	599.80	9.14	<0.001
Own field	30	768.35	433.75	79.19	606.39	930.32		
Other farmers	21	474.04	145.93	31.84	407.62	540.47		

*Significant at $p < 0.05$ bolded; Std, Standard; 95 CI, confidence interval; F, F test for continuous outcome.

seed system as well as factors that influence cassava productivity in Rwanda.

The current findings confirmed the occurrence of CBSD (both CBSV and UCBSV) in Rwanda. The disease was found in all 13 districts surveyed, indicating that it has spread out in all major cassava-growing regions, including Kirehe and Nyagatare, where CBSVs were not detected in previous studies (Munganyinka et al., 2018).

In our survey, we found that districts located near the border displayed a higher rate of CBSD incidence. The highest field incidences and severities were recorded in the three districts, namely, Nyanza, Bugesera, and Gisagara, bordering Burundi. In an earlier study carried out by Munganyinka et al. (2014), Nyanza and Gisagara districts also displayed the highest CBSD incidence, which confirms them as hot spots for CBSD (Munganyinka et al., 2018). This observation might correspond to the informal movement of cassava cuttings across countries that leads to the importation of infected cuttings or use of genetic material that is more susceptible to CBSVs. Furthermore, the high CBSD incidence in those districts could be because since its first report in 2009, the virus could have flourished in those areas season after season due to the relatively warm environments that favor proliferation of whitefly vectors (Campo et al., 2011).

A CBSD survey performed in Burundi previously reported an average incidence and severity of 15.3 and 2.3%, respectively (Bigirimana et al., 2011). A decade ago, UCBSV was the only viral species that was associated with the disease in Burundi although it was already present in Tanzania (Rwegasira et al., 2011).

Based on RT-PCR diagnostics, the overall CBSV incidence was found to be less than CBSV incidences reported elsewhere in East-Central Africa (Kenya, Tanzania, Malawi, Zambia) (Rwegasira et al., 2011; Mbewe et al., 2014; Koima et al., 2018; Mulenga et al., 2018). This difference might be due to a later introduction of CBSVs in Rwanda. UCBSV was prevalent across the country, indicating that it is the commonest cause of CBSD in the country. Similar findings were reported in the survey conducted in 2014 among major cassava-growing regions (Munganyinka et al., 2018). It should be noted that our study revealed an increased rate of mixed infections from 10% in 2014 to 28% in 2019, highlighting the dynamism of disease spread over time either through vectors or exchange of unhealthy cuttings among farmers.

Despite farmers being aware of the importance of quality seed to optimize output, it was observed that most farmers have difficulty identifying quality seed as was reported earlier (Minot et al., 2007).

TABLE 6 | Multiple linear regression for cassava productivity.

Model	Independent variable	Coefficients						Model summary and ANOVA test					Durbin Watson test
		Unstandardized Coefficients		t	Sig.	Collinearity Statistics		R	R ²	Adj. R ²	F	Sig.	
		B	Beta			Tolerance	VIF						
1	(Constant)	212.77		7.84	<0.001								
	Using fertilizers to grow cassava	212.31	0.75	12.92	<0.001	1.00	1.00	0.75	0.57	0.56	166.91	<0.001	1.347
2	(Constant)	198.98		8.29	<0.001								
	Using fertilizers to grow cassava	157.15	0.56	9.26	<0.001	0.73	1.38	0.82	0.67	0.66	127.04	<0.001	
3	Size of land	43.09	0.37	6.20	<0.001	0.73	1.38						
	(Constant)	156.56		5.95	<0.001								
	Using fertilizers to grow cassava	136.10	0.48	7.78	<0.001	0.63	1.58	0.83	0.69	0.69	95.25	<0.001	
4	Size of land	30.32	0.26	3.94	<0.001	0.5	1.83						
	Farming system used	65.92	0.23	3.35	0.001	0.5	1.97						
	(Constant)	227.38		5.86	<0.001								
	Using fertilizers to grow cassava	134.86	0.48	7.85	<0.001	0.63	1.585	0.84	0.71	0.70	75.75	<0.001	
5	Size of land	31.22	0.27	4.13	<0.001	0.54	1.83						
	Farming system used	52.71	0.19	2.63	0.01	0.47	2.12						
	Cassava infection	-36.80	-0.13	-2.45	0.016	0.87	1.14						
	(Constant)	177.62		4.07	<0.001								
	Using fertilizers to grow cassava	129.18	0.46	7.58	<0.001	0.61	1.61	0.85	0.72	0.71	63.90	<0.001	
5	Size of land	26.06	0.23	3.37	0.001	0.50	1.99						
	Farming system used	49.75	0.18	2.52	0.013	0.46	2.13						
	Cassava infection	-36.82	-0.13	-2.49	0.014	0.87	1.14						
5	Type of cassava varieties grown	29.23	0.13	2.35	0.02	0.74	1.34						

B, beta coefficient; t, student t test for continuous variables; Sig., significance; VIF, variance inflation factor; R, correlation coefficient; Adj. R², adjusted correlation coefficient squared; F, F test for continuous outcome.

Our present study indicates that a majority of farmers (76.9%) use informal ways to get cuttings for free from either their own fields or from neighbors. Because the supplied planting material often suffers from a lack of quality control, farmers are more likely to plant virus-infected cuttings, which can lead to low yield and reduced benefits. The informal seed system might be further maintained by the lack of knowledge about cassava viral diseases as identified in the survey, highlighting the need to increase farmers' awareness of using quality seed and also to mobilize the private sector to invest in the commercial cassava seed business. Previous studies have already highlighted the need to promote farmers' awareness (Chipeta et al., 2016; Nduwumuremyi et al., 2016; Bentley et al., 2017) as farmers using cassava planting materials from appropriate sources (research institutions, NGOs, etc.) appear to have fields with reduced CBSV infection (Gondwe et al., 2003).

Despite differences in CBSV incidence between districts, farmers who used quality seeds kept having lower CBSV infection compared with those reusing seeds from their own field or from other farmers. This observation also highlights the importance of the human factor (transport and exchange of unhealthy cuttings), contributing to the propagation and dissemination of CBSV (Patil et al., 2015; Maruthi et al., 2017).

The ultimate goal of cassava farming is to optimize yields for food security and income. Taking into account that cassava is a climate-resilient crop essential in fighting food insecurity for the fast-growing population (El-Sharkawy, 2004; Chavez et al., 2005; Burns et al., 2010), crop-intensification programs have been launched in Rwanda and other African countries to improve agricultural production (FAO, 2016). Despite that cassava production is a profitable investment in Rwanda (Gasangwa, 2013), its production still remains constrained by several factors.

The present study indicates that high yield and income generation from cassava logically increase with the use of fertilizers, the size of the land, monoculture, and improved cassava varieties, whereas incidence of viral diseases is associated with a decrease in the income. Although the use of fertilizers is known to increase yields of cassava storage roots (Munyahali et al., 2017), cassava continues to be seen as a resilient crop with limited or no requirement for fertilization. Therefore, promoting adequate use of fertilizers in cassava should remain a priority in future crop-intensification programs.

The survey also indicates that cooperatives nearly double their benefits as compared with individual farmers. This observation could be explained by the fact that most cooperatives exploit bigger land and better comply with

good agriculture practices (including the use of fertilizer, improved quality seeds, and practicing monoculture) than individuals. A recent assessment of the benefits associated with cultivating improved high-yielding varieties shows that yields can be increased more than four-fold as compared with the cultivation of local varieties in Africa (Khonje et al., 2015). Thus, farmers should be encouraged to group into cooperatives and to practice good agriculture practices (GAP) to meet the high yield and food security objectives of crop intensification programs.

Following the emergence of CBSD in 2009, efforts made by governmental entities to combat cassava viral diseases, have helped reduce their impact on cassava production. However, CBSD remains a major challenge to cassava production despite past and ongoing efforts to breed for virus-resistant varieties, to distribute clean planting material, and to promote GAP (Catholic Relief Services, 2012). Although time and resource constraints prevented us from performing a more extensive survey, the random approach operated in the cassava-growing sectors makes our survey representative of the cassava-growing areas.

Our study reveals that viral diseases remain a constraint to cassava productivity with a disease prevalence that has increased to 35.3% in Rwanda. Therefore, there is a need to continue efforts to introgress virus-resistance traits into farmer-preferred varieties and to establish a cassava seed system enabling sustainable and affordable supply of clean planting material to farmers. The strengthening of the cassava seed system also requires the development of important capacities for virus diagnostics (Mukasa, 2015; Wossen et al., 2020). To sustain the implementation of GAP, there is also a need to increase farmers' awareness of cassava diseases and the immediate benefit of using quality seeds.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Ethical review and approval was not required for this study with human participants, in accordance with the local legislation and institutional requirements.

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AUTHOR CONTRIBUTIONS

HV, CBr, CBU, NG, CN, and JB contributed to the conception and design of the study. CN, JB, AN, VB, SM, CT, and HV contributed to the methodology design. HV, SM, CBr, CT validated the study. MH, YB, CT, CN, and JB performed the statistical analysis. CN and JB wrote the first original draft. YK, YB, CT, LL, SM, EM, AN, and HV revised and edited the manuscript. HV provided resources and supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2021.699655/full#supplementary-material>

Supplementary Figure 1 | Detection of CBSV and UCBSV by reverse transcription-PCR in samples collected from (A) Nyanza and (B) Bugesera districts. Expected PCR product sizes were CBSV (703 bp) and UCBSV (800 bp). From left to right, 100 bp DNA ladder; C+ correspond to positive control for both CBSV and UCBSV, whereas C− corresponds to negative control; lanes 1–10 represent pool of samples from fields 1–10; faster DNA ladder. (C) Detection of the internal control MePP2A for samples in (A); (D) detection of the internal control MePP2A for samples in (B); expected PCR product sizes was 150 bp. From left to right, 100 bp DNA ladder; Lanes 1–10: samples; internal control was detected in all tested samples.

Supplementary Figure 2 | (A) CBSD symptoms on cassava leaves; (B) CBSD symptoms on cassava stem; (C) CBSD symptoms on cassava roots; (D) CMD symptoms on cassava leaves.

Supplementary Figure 3 | Histogram and P-P plot normality tests for cassava productivity linear regression analysis.

Supplementary Table 1 | List of collected variables during the survey.

Supplementary Table 2 | List of biotic variables recorded or measured during the survey of the 130 visited fields.

Supplementary Table 3 | Socio-demographic factors stratified by cassava infection.

Supplementary Table 4 | Disease awareness variables stratified by cassava infection.

Supplementary Table 5 | Effects of demographic variables on cassava productivity.

Supplementary Datasheet 1 | Questionnaire.

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