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Identification and Distribution of Viruses Associated with Cassava Brown Streak Disease in North Kivu, DR Congo

Sabrine Y.Yasenge[1,2], HonoréS. Muhindo[2, 3], ClerisseM. Casinga[4], DidyO. Onausthu[1] andGodefroidT.K. Monde[2,3]
[1.]University of Kisangani, Faculty of Sciences, Laboratory of Microbiology and Phytopathology B.P. 2012, Kisangani, DRCongo
[2.] West African Virus Epidemiology (WAVE), Kisangani, DRCongo
[3.] Faculty Institute of Agronomic Sciences of Yangambi, Laboratory of Plant Pathology and Biotechnology B.P. 1232 Kisangani, DRCongo
[4.] International Institute of Tropical Agriculture, Kalambo Station, Bukavu, DRCongo

Abstract:- The objective of the present study was to identify the viral strains associated with cassava brown streak disease, to determine the cassava cultivars used, their status (local or improved), and the age of cassava crops in the field, and to study its distribution in the Province of North Kivu. The modified Cetyltrimethyl ammonium bromide method was used to extract RNA from samples of collected cassava leaves. A pair of primers was used in diagnosis to confirm the presence of viral strains on the samples. Cassava cultivars followed by their status and age in cultivation were determined. Georeferenced survey data and molecular analysis data were integrated into molecular incidence and field incidence maps. Our results show that the cassava brown streak disease is spread in all the territories of North Kivu, the most infected are the territories of Beni, Masisi, and Rutshuru while the least infected are Lubero and Nyiragongo. The improved cultivars are the most cultivated in Beni and Lubero (5 improved against 4 local and 5 improved against 2 local respectively). The average age of the crops is 6 months. Out of 380 samples tested, 74 (19%) reacted positively to the Cassava brown streak virus. Cassava brown streak disease is recognized as a major constraint in the cassava fields of farmers in North Kivu caused by Cassava brown streak virus and is spread in all the territories surveyed. The cassava cultivars used are improved and local with an average age of 6 months.

Keywords:- *Determination, cassava brown streak disease, Molecular incidence, Field incidence, North Kivu.*

I. INTRODUCTION

In the Democratic Republic of Congo (DR Congo), cassava is economically important as it is a basic food for 70% of population [1].

Cassava brown streak disease (CBSD) is a viral disease caused by Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) [2], [3], [4]. The two viruses (CBSV and UCBSV) are positive single-stranded RNA viruses in the Ipomovirus genus of thePotyviridae family [5], [6].

Currently, the two viruses (CBSV and UCBSV) remain recognized by the ICTV (International Committee of Virus Taxonomy), while[7] had suggested deep speciation within the UCBSV clade. Previous studies have shown that CBSV is more devastating than UCBSV; due to its heavy infection of sensitive and tolerant varieties, which leads to enormous crop losses.[8] found that CBSV has a faster evolution rate than UCBSV and these authors found that in CBSV, no substitution of non-synonyms predominated more than the substitution of synonyms and occurred throughout the genome.

Cassava brown streak disease poses a serious threat to food security in eastern and central Africa [9], [10], [11]. Cassava brown streak disease is already reported in DR Congo by [10], [11], [12], [13], [14] based on observations and confirmation by molecular analysis of suspicious symptoms. Viruses associated with CBSD are believed to exist in the province of North Kivu.

II. MATERIALS AND METHODS

The present study was conducted from September 2017 to December 2020 in the province of North Kivu, specifically in the territories of Beni, Lubero, Masisi, Nyiragongo and Rutshuru (Figure 1). North Kivu is located between 0 ° 58 "North latitude and 02 ° 03" South latitude and between 27 ° 14 "East longitude and 29 ° 58" East longitude. The altitude varies between 800 and 2500 m.

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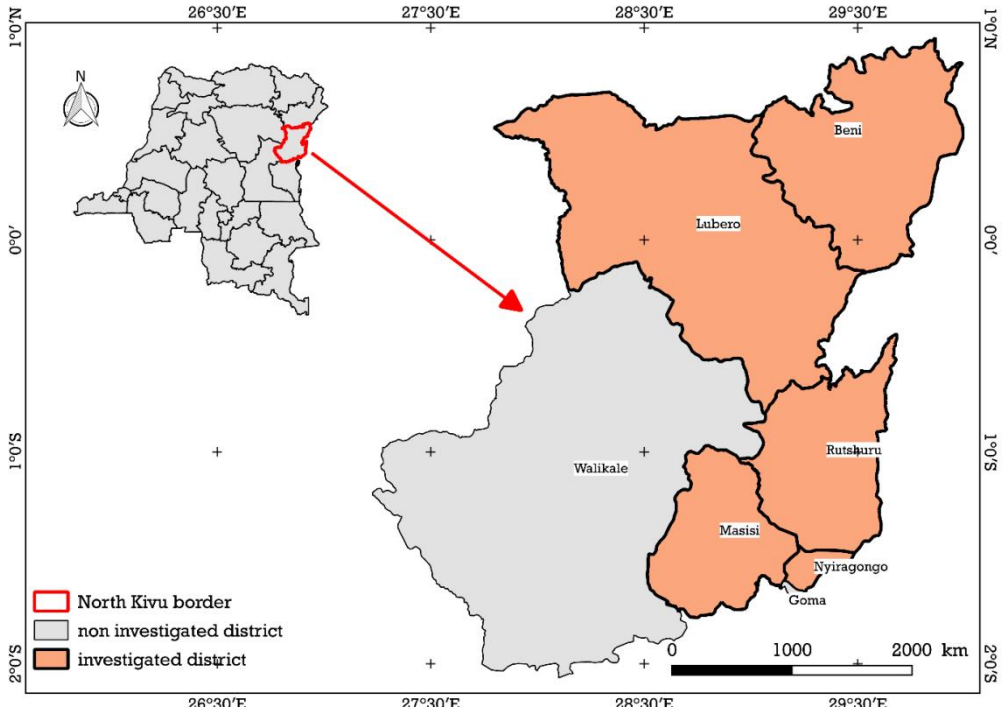

Fig. 1: Map of the North Kivu Province in eastern DR Congo showing the study environment

In the 5 territories mentioned above, the surveys were carried out on a road axis in which 46 fields of 3 to 9 months separated by 10 km were inspected. A total of 380 symptomatic and asymptomatic leaf samples were collected for diagnosis by RT-PCR [15]. The technique of interviewing field owners and direct observation were used to determine cassava cultivars, their status (local or improved) and the age of cassava in cultivation. Data from geo referenced field surveys and those obtained after molecular analysis were used to produce molecular incidence maps as well as field incidence maps.

The incidence was determined using the following formula:

$$\text{Incidence (\%)} = \frac{Numberofdiseasedplants}{Totalnumberofplantssampled} \times 100$$

Symptoms of CBSD on leaves and stems were assessed on these 30 plants using the rating scale of 1-5[16], where 1 corresponds to absence of symptoms and 5 the most severe symptoms including necrosis on the stem and die-back of the shoots.

The modified Cetyltrimethyl ammonium bromide (CTAB) method[17]has been used since DNA extraction from 380 samples of the collected cassava leaves. A pair of CBSV10 and CBSV11 primers were used in the diagnosis by the RT-PCR process to confirm the presence of cassava brown streak deseaseIpomovirus strains in the samples collected [18].

The preparation of the master mix followed the process of[2] which consisted of using 14.28 µl of sterile distilled water, 2 µl MmLV-buffer 10x, 0.6 µl of 2mM dNTPS, successively 0.4 µl of primers CBSV10 (ATCAGAATAGTGTGACTGCTGG) and CBSV11 (CCACATTATTATCGTCACCAGG) 10 µM, 0.16 µl of Taq DNA Polymerase 5 U / µl, 0.06 µl of MmLV-RT 200 U / µl, 2 µl of RNA which made a reaction mixture of 20.54 µl.

The 42 ° C cycle for 30 min (1x) was applied for RT. PCR followed the following cycle: 1x 94 ° C for 2 min followed by 35x 94 ° C for 30sec, 52 ° C for 30sec, 72 ° C for 40sec; 1x 72 ° C for 10 min.

The cDNA migration was carried out under an electrical voltage of 100 volts at 58mA for 40 min in the 1% agarose gel in 1x TAE buffer containing 0.5 µg / ml of ethidium bromide. The visualization took place under Ultra-Violet light at 312 nm in a darkroom using a 1kb DNA molecular weight marker which allowed the size of the amplicon to be determined.

The data obtained were analyzed with R software version 3.5.3.

The cartographic illustrations were produced with QGIS version 3.4 software.

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III. RESULTS

A. Identification of cassava brown streak viruses in North Kivu

The results relating to the detection of the viral strains (CBSV) responsible for the cassava brown streak disease are presented in Table 1.

Territories	Samples analyzed by RT-PCR	Positive samples at CBSV	Negative samples at CBSV	Molecular incidence (%) CBSV+	Field incidence (%)
Beni	130	4	126	3	39
Lubero	140	17	123	12	20
Masisi	50	14	36	28	0
Nyiragongo	10	9	1	90	2
Rutshuru	50	30	20	60	39
Total	380	74	306	39	26

Table 1: Molecular incidence of CBSV in the different territories of North Kivu

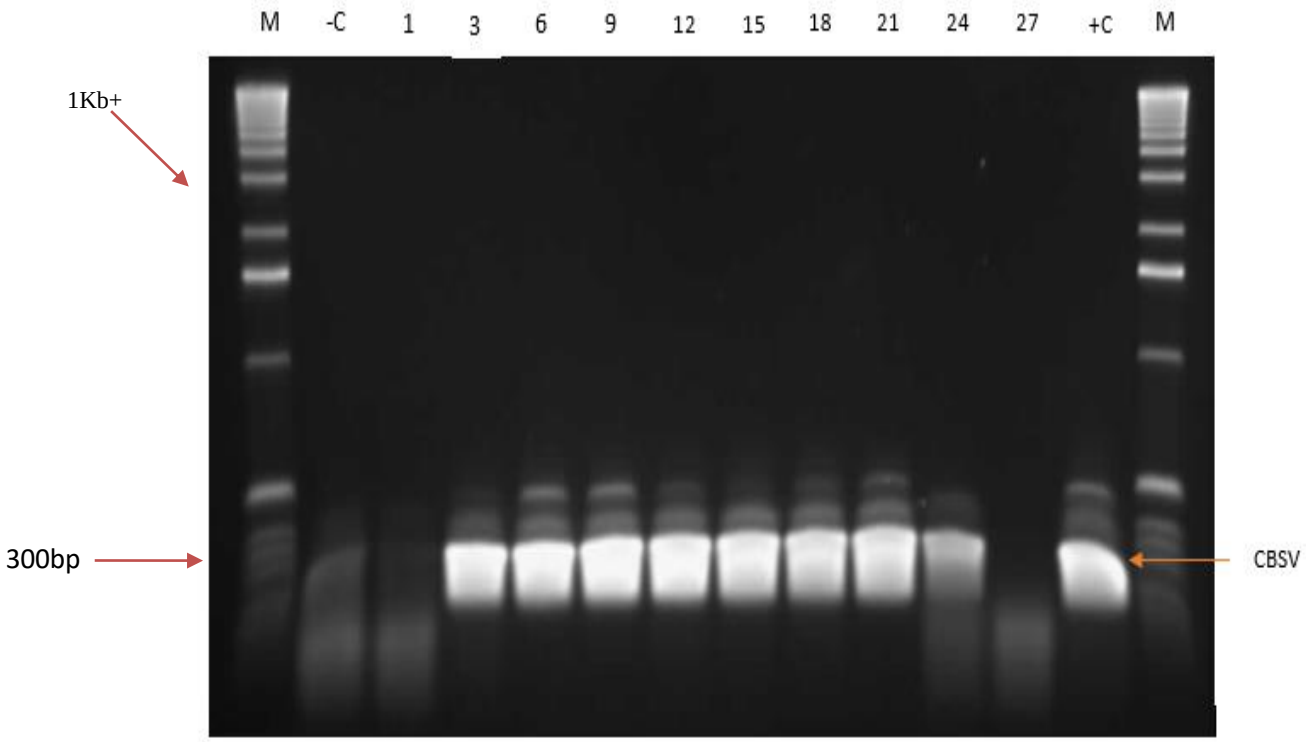

Fig. 2: Gel photo showing the amplification of CBSV in the samples tested

(M = DNA size marker 1Kb, -C = negative control, + C = positive control, CBSV = Cassava Brown Streak Virus and 1-27 are the samples tested).

The results of the analysis of the samples by RT-PCR (Table 1) show that the molecular incidence of CBSV is 3% in Beni, 12% in Lubero, 28 % in Masisi, 90% in Nyiragongo and 60% in Rutshuru. We notice that the territory of Nyiragongo is more infected than the other territories. Overall, we find that the molecular incidence is higher than the field incidence. This shows that cassava plants infected with CBSV virus do not necessarily show symptoms of the disease because some varieties may be resistant to symptom manifestation while the virus is present or the contamination is recent. This is why it is recommended to always use cassava plants that are tested at the laboratory level to actually confirm that they are healthy.

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B. Cassava cultivars and their status in the province of North Kivu

Table 2 gives the names of each cultivar by territory as well as its status in the cassava field in North Kivu.

Territories	Number of fields surveyed	Cassava cultivars	Cultivar status
Beni	15	Ndoliro	Local
		Sidipe	Improved
		Kalinga	Local
		Nadizi	Improved
		Kavundayire	Local
		Kivere	Improved
		Kiyonga	Improved
		Balulu	Local
		Dieme	Improved
Lubero	15	Dieme	Improved
		Mwakamoya	Improved
		Mushukuzi	Local
		Mutsili	Local
		MM	Improved
		Mayumbe	Improved
		Sawasawa	Improved
Masisi	7	Ndakuoweya	Local
		Ndoliro	Local
		Mushikuzi	Improved
		Mulibwa	Local
		Karerenzi	Local
		Obama	Improved
Nyiragongo	4	Mulibwa	Local
		Muchukuzi	Local
		Nambiombio	Local
Rutshuru	5	Sukisa	Improved
		Muchukuzi	Local

Table 2: Different cassava cultivars used in the province of North Kivu and their status

The results of table 2 show that of the 20 cassava cultivars encountered in the province of North Kivu, 11 are improved against 9 locals.

In view of these results, we note that the territories of Beni and Lubero use improved cultivars more (5 improved against 4 premises in Beni and 5 improved against 2 premises in Lubero) than the other territories. While the territories of Masisi and Nyiragongo use more local cultivars (4 local versus 2 improved in Masisi and 3 local versus 0 improved in Nyiragongo). This proves that the 2 territories (Beni and Lubero) are sometimes beneficiaries of improved cultivars resistant to CBSD originating from NGDO disputes or humanitarian organizations compared to other territories used by local cultivars which do not undergo quality controls and are often susceptible to viruses.

The analyzes (Table 3) showed that it is the improved cultivars that are used the most, that is 63% for the whole province.

Cultivar states	Beni	Lubero	Masisi	Nyiragongo	Rutshuru	Total
Improved	60	87	43	0	80	63
Local	40	13	57	100	20	37
General total	100	100	100	100	100	100

Table 3: State of cultivars according to territory (%)

C. Age of cassava crops in North Kivu

Data on the age of cassava crops in the study area are presented in Table 4 below.

Territories	Number of fields surveyed	Age of crops (months)
Beni	15	5
Lubero	15	6
Masisi	7	7
Nyiragongo	4	6
Rutshuru	5	7
Mean	9	6

Table 4: Age distribution of cassava crops in the territories of North Kivu

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The results of table 4 show that the average age of cassava crops encountered in the province of North Kivu is between 5 to 7 months. These ages are distributed as follows: Territory of Beni (5 months), Lubero (6 months), Masisi (7 months), Nyiragongo (6 months) and at the end Rutshuru (7 months). In view of these results, we note that the average age of cassava found in the province of North Kivu is advanced (6 months) and it is often the age of infection and manifestations of the symptoms of CBSV.

D. The distribution of cassava brown streak in the North Kivu region

The results relating to the molecular and field incidences of CBSD in the different cassava fields prospected in North Kivu are presented in figure 3.

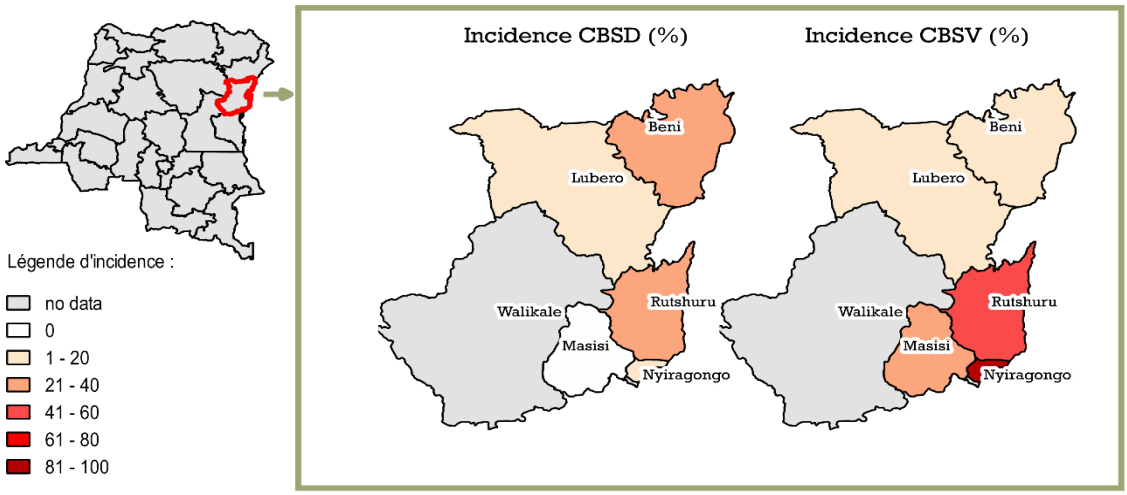

Fig. 3: Field and molecular incidence of CBSD in the Province of North Kivu

Figure 3 shows that the field spread of CBSD is higher in the territories of Beni and Rutshuru (21-40% incidence) and lower in the territories of Lubero and Nyiragongo (1-20% incidence). Our surveys revealed that Masisi territory is not yet affected by the disease (0% incidence). The expansive distribution of CBSV (Molecular incidence) is highest in the territory of Nyiragongo (81-100%), followed by the territory of Rutshuru (41-60%), Masisi (21-40%) and finally Lubero and Beni (1-20%). We note that the CBSV is more spread in the territory of Nyiragongo. These results confirm that cassava plants infected with CBSV virus do not necessarily show symptoms of the disease.

IV. DISCUSSION

Although CBSD has been recognized in coastal East Africa since 1930[19], its presence is recently reported in the Great Lakes region[20]. In these regions, the presence of two viral species UCBSV and CBSV has been reported [3], [21].

On the other hand, our study carried out in the Province of North Kivu in DR Congo highlights the presence of CBSV as a viral species associated with CBSD. The results we obtained do not correspond to those found by[11] who had confirmed the presence of UCBSV in the territories of Beni and Lubero. In view of these two studies, we can say that the two viruses CBSV and UCBSV are present in the province of North Kivu. Similar studies have been made by [18] in East Africa,[12]in North East DR Congo and[14]in Eastern province dismembered from DR Congo and they also found the presence of these two viruses.

The numerical differences in the field and molecular incidences observed between the prospected territories were significant (Chi-square = 121.94; dl = 4, p-value <2.2e-16).

We can say that the use of local cultivars carries the risk of contamination of CBSD disease,while the majority of improved cultivars undergo quality controls, which is why they are sometimes free and resistant to CBSV viruses. We observed this with the results obtained from the molecular impacts (See table 1) where the territories of Beni (3%) and Lubero (12%) which use the improved cultivars more were less infected than the territories of Masisi (28%) and Nyiragongo. (90%) that are used more by local cultivars. A similar study was made by[16] in the North East of the DR Congo precisely in Yangambi where the two cultivars were used (improved and local), the most used were also the improved cultivars (34 improved against 4 locals). The improved cultivars were also less infected (36.2%) than the local cultivars (48.9%) compared to the incidence in the field at the tuber levels.

The age of cassava crops found in North Kivu province is advanced, ranging from 5 months in Beni territory to 7 months in Masisi and Rutshuru territories. Compared to these territories, we find that the cassava crops found in the territory of Rutshuru were of advanced age (7 months) compared to the territory of Beni (5 months), so its molecular incidence (See table 1) was also high (60%) more than the territory of Beni (12%).

According to analyzes (Table 5), the average age of cassava crops is 6 months found in improved cultivars. The numerical age differences observed between cultivars were not significant.

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Cultivar states	Mean	Standard deviation
Improved	6	3
Local	5	2
All cultivars	6	3

Table 5: Age of cultivars according to states

The field incidence shows that the disease is more expansive in the territory of Beni and Rutshuru (21-40%) while the molecular incidence shows that it is more expansive in the territory of Nyiragongo (81-100%). We can say that in the fields the symptoms of the disease may be invisible due to some resistant varieties or the age of the crop when the virus is present at a high rate. We see this especially in the Masisi territories where the field incidence was 0% while the molecular incidence is (21-40%) and the Nyiragongo territory where the field incidence was (1-20 %) while the molecular incidence is (81-100%).

The numerical differences in the field and molecular incidences observed between the prospected territories were significant (Chi-square = 121.94; dl = 4, p-value <2.2e-16).

V. CONCLUSION

Cassava fields in North Kivu province are exposed to the devastating effects of cassava brown streak. Our study shows that the 5 territories investigated are infected with CBSD with a molecular incidence ranging from 3% to 90%. The cassava cultivars used are improved and local (Sensitive to CBSV). But the most used are improved in the territories of Beni and Lubero. The average age of cassava crops found in fields is 6 months. As for the spread of the disease, all the territories surveyed are infected. The areas most infected with CBSD disease are Nyiragongo and Rutshuru. Particular attention should be paid to the protection of the territories of Beni, Lubero and Masisi which appear to be even less infected with CBSD. The high molecular incidence recorded in the region by PCR diagnosis raises the question of early diagnosis of propagation material especially in seed fields.

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