

## FIRST REPORT AND PRELIMINARY EVALUATIONS OF CASSAVA BROWN STREAK-LIKE ROOT NECROSIS IN CONGO REPUBLIC

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### ABSTRACT

In the last 10 years, the Cassava Brown Streak Disease has spread across Africa from the east coast of Africa to central Africa. Similar root necrosis to cassava brown streak disease have also been identified in the Democratic Republic of Congo where the first symptoms were identified in 2002 in Kinshasa and Central Kongo province. In 2012, the presence of cassava brown streak disease was confirmed in eastern Democratic Republic of Congo. All attempts since 2002 in western Democratic Republic of Congo to identify the cause of these root necrosis failed. In 2017, a team of scientists surveying the Luozi Territory in the Kongo central province, identified the same root necrosis similar to cassava brown streak in several localities bordering the Republic of Congo. These unexpected results will foreshadow the presence of cassava root necrosis in Congo Republic. This preliminary investigation in Congo Republic was conducted specifically in Mfouati District, a District bordering Democratic Republic of Congo in order to verify, whether or not, these root necrosis are present in Congo Republic. Results obtained from this exploratory survey confirmed the presence of root necrosis similar to cassava brown streak disease in Congo Republic, in several fields of Mfouati District.

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### INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a starchy root plant, introduced to the African continent during the 16th century by Portuguese merchants (Abessolo, 2003). As with all cultivated plants, cassava is the victim of numerous attacks by enemies (diseases and pests). Damage caused by these attacks severely reduces yield.

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Cassava mosaic disease (CMD), cassava brown streak disease (CBSD) and cassava bacteria blight (CBB) are among, if not, the most severe attacks that cassava has experienced in recent years. Losses due to CMD attacks were evaluated between 30 and 90% in tuberous roots depending on the strain of the virus and the variety of cassava used. These losses can reach 100%, in case of virulent strain, for example, the Ugandan variant. During the last ten years in East Africa, cassava is under threat from a new disease: Cassava Brown Streak Disease (Alicai, 2017). It is a more destructive virus than previously known (Alicai, 2007). It is characterized by the alteration of green-clear or yellow on mature leaves, brownish longitudinal streaks on the stem, hence the name "brown streak" and by the

appearance of yellow / brown, dry necrosis, corky in tuberous roots. In fact, they cause not only quantitative losses of cassava, but also alter the quality of products that make them undesirable for cooking, marketing and processing (Buetusukina, 2017). Severity can induce significant reduction in tuberous root size and malformation. CBSD was first reported in the 1930s (Storey, 1936) from the northeastern part of what is now Tanzania but was later shown to occur throughout the coastal lowland of East Africa, including the lakeshore areas of Malawi (Bock, 1994; Legg and Raya, 1998; Munga and Thresh 2002; Nichols, 1950; Thresh and Raya 1998; Munga and Thresh, 2002; Nichols, 1950; Thresh and Mbwana, 1998). Prior to 20014, CBSD was considered to be limited in its distribution to areas below 1,000 m above sea level (m.a.s.l) (Hillocks et al.1996; Nichols, 1950), northwestern Tanzania (Legg and Raya, 1998), and Southern Uganda (Hillocks and Jennings, 2003) -all locations significantly above 1,000 m.a.s.l. Since 20014, CBSD has spread throughout the Great Lakes region of East and Central Africa, driven by unusually abundant populations of the whitefly vector (Legg et al.2011). CBS viruses are propagated through the use of cuttings from infected parent plants and are transmitted by the whitefly vector *Bemisia tabaci* (Maruthi et al. 2005).

CBSD symptoms are variable in terms of severity, onset of symptom expression and parts of the plant affected, depending on the viral strain, cassava cultivar, environmental conditions and age of the plant when infected (Nichols, 1950). In 2003, scientists reported for the first-time similar root symptoms to cassava brown streak disease in Democratic Republic of Congo (DRC) and more precisely in the provinces of Kinshasa (Batéké Plateau) and Kongo Central (INERA Research Center / Mvuazi) (Mahungu et al., 2003) but up to date all PCR tests did not detect any causative agent related to the observed symptoms in western DRC and the disease is still referred to as 'CBSD-like disease' (Bakelana, In press). The virus has been confined to the east coast of Africa, but over the last decade it has begun to spread rapidly across the continent, from east to west (Ndyetabula and al., 2016). CBSD is now one of the leading causes of cassava losses in East Africa and its ongoing spread threatens the major cassava-growing areas of central and west Africa (Legg et al., 2014). In 2012, Mulimbi et al. confirmed at the molecular level the presence of cassava brown streak disease in eastern DRC, precisely the UCBSD (Ugandan Cassava Brown Streak Disease) strain. In the Congo Republic, cassava is the main source of calories and covers about two thirds of the cultivated area. Cassava is consumed in the form of starch (foufou or chikwangue) twice a day on average and as such, it is estimated the average consumption per capita per year to 175 kg in cities and 425 kg in the countryside. It is also consumed in the form of leaves or saka-saka which constitutes a substantial protein intake (Mabanza et al., 2007).

As all cultivated plants in Congo Republic, cassava is the victim of numerous attacks by enemies (diseases and pests). Damages caused by these attacks has the first consequence, the yield reduction obtained in the field. Mosaic and bacteria blight are among, if not, the most severe attacks that cassava has experienced in recent years. Losses due to mosaic attacks were evaluated between 30 and 90% in tuberous roots depending on the strain of the virus and the variety of cassava used. These losses can reach 100%, in case of virulent strain, for example, the Ugandan variant.

In Congo Republic, the incidence of mosaic was evaluated at 86.7%, according to a cassava diagnostic study carried out in all 10 departments of the country, in 2003 (Ntawuruhunga et al., 2007). In 2017, a team of INERA-Mvuazi researchers surveying the Luozi Territory in the Kongo central province of DRC under the WAVE project (Western Africa Virus Epidemiology) , identified the same root necrosis similar to CBSD in several DRC localities (Ndandanga, Kintete and Kingoyi) bordering the Republic of Congo. These unexpected results will foreshadow the presence of CBSD-like root necrosis in Congo Republic. No studies to date have reported the presence of root necrosis symptoms similar to cassava brown streak disease in Congo Republic. It is in this context that an exploratory survey was initiated under the WAVE program, to search for symptoms similar to cassava brown streak disease in the District of Mfouati in Congo Republic which is along the DRC territory of Luozi.

## MATERIALS AND METHODS

The exploratory survey was conducted in the District of Mfouati, in the Bouenza Department. It is bordered on the North by the District of Mouyondzi, on the South by DRC, on the East by the Mindouli District and on the West by the District of Madingou and Bongho Songho (Anonymous, 2017).The District of Mfouati is part of Plateau des Cataractes which is a succession of mountains whose altitude varies between 177 and 800 meters. It has two staggered areas that stand out distinctly as one moves north to south on the border with DRC. The border area, south of the district, dominates the rest of the territory and rises between 672 to 753 meters. Materials used during this survey consisted of a motorcycle, a GARMIN Etrex 20 GPS, a digital camera, a machete, a questionnaire for interviewing farmers, data sheets for data collection, and illustrated images of brown streak root necrosis symptoms. The methodology used consisted of questioning farmers and recording data on maximum severity of root necrosis using a specific scale. The incidence of root necrosis was deduced from the data collected on maximum root severity. The sample of our study consisted of 12 localities selected from a list of 42 localities constituting the District of Mfouati. These localities were chosen due to their proximity to DRC and their accessibility. Due to accessibility, localities were selected in the lower (inner) and highest zones of the district. Selected localities were:

- Border area (highest zone) with DRC: Loufoua Loua Nzambie, Kingonda, Bikoumbi, Mouyonzi, Tala Nkoyi, Kindamba and Kinanga.
- Inner area (lower zone) of the district: Mfouati Center, Ngolonga, Louimbi (Loutete), Kinzaba and Bibondo pont.

The survey was conducted in two phases: farmer interviews and field observations. In order to learn about the situation of root necrosis on the ground, we started the survey by talking with farmers of different villages, through a pre-established questionnaire and presented to them, printed photos of CBSD symptoms. Interviews were based on key issues related to cultivated varieties, their origins, the year the symptoms appearance, and types of symptoms observed. Regarding field observations, data on maximum severity of root necrosis were recorded from various fields which were randomly selected in each locality. The same number of fields were selected per area.

Only the underground part of the plant (roots) were taken into account during the survey because observations made from western DRC never show any relation between root necrosis symptoms with any leaf symptoms. For the assessment of root necrosis, sampled plants were uprooted. Roots were counted, and each plant was then cut cross-sectionally in five times at regular intervals along the length of the root. A symptom score was then recorded for each cut, using the 1-to-5 scoring of Hillocks and Thresh (2000).

The maximum root necrosis severity was assessed on each of the five sections using as follows:

- Apparently healthy root;
- Less than 2% of necrosis;
- Corresponds to 2-5% of necrosis;
- Correspond to 30-40% of root necrosis;
- Corresponding to more than 50% of root necrosis.

The incidence was deduced from data on severity and then calculated as follow:

Incidence = (number of diseased plants) / (number of plants observed) x 100

## RESULTS

Data collected during the survey were firstly analyzed from different questionnaires and forms. Data from questionnaires and forms were recorded in an Excel file in order to facilitate their statistical analysis using the Statistix 8.0 software. Observations, comments and different interpretations made from results obtained are going to be presented through the following lines.

**Root rot assessment:** Typical CBSD leaves and stems symptoms were not found in the Mfouati District and several cases of root rot were encountered on severe damaged plants

**Maximum severity and incidence of root necrosis in relation to varieties:** Data collected on the severity and the incidence of root necrosis in relation to varieties encountered on ground are presented in Table 1. Table 1 shows that the level of the maximum disease severity on cassava plants is a function of cassava varieties grown in the area. Among the 9 varieties encountered on the ground, two varieties performed better according to the disease incidence. Kinine and Moundele Mpakou varieties, which seem to be landraces, have shown less root necrosis.

These landraces appear to be resistant or tolerant to the disease and deserve further investigations on their genetic characterization. These varieties should also be considered in a cassava breeding program, as they could be used to develop resistant varieties. Table 1 shows too that there is a very high variability among cultivated varieties in Mfouati District in terms of incidence of the disease. The variety Ya leger, properly designated and known as RAV in DRC, has been shown as the most susceptible variety. The majority of varieties found in different localities showed an intermediate level of tolerance to the disease (incidence varying between 20.17 and 51.67%), while the Kinine and Moundele mpakou varieties showed very low incidence level. As previously indicated, these two local varieties appear to be resistant/tolerant to the disease.

**Maximum severity and incidence of root necrosis in relation to localities:** Data collected on the maximum severity of root necrosis in relation to localities are presented in Table 2. Table 2 reveals that different localities visited during the survey did not show the same level of the maximum severity of the disease. Bibondo pont is the most infested by the disease, while Bikoumbi, Loufoua, Nzambi, Tala nkoyi, Kindamba, Kinanga and Mouyonzi are not affected by the disease. These results show that there is a positive correlation between the maximum severity and the incidence of CBSD-like root necrosis in Mfouati district. Localities with high maximum severity are those where the incidence was also the highest and vice versa. The value of the correlation coefficient is 0.82. The incidence strongly varies between sites. Bibondo pont recorded the highest incidence (100 %) in comparison to others sites.

**Maximum severity and incidence of root necrosis in relation with the altitude (area):** Data collected on the maximum severity and the incidence of the disease in relation to the altitude are presented in Table 3.

## DISCUSSION

Root rot due to CBSD or CBSD - like attacks are very common in disease high pressure areas (hot spots) such as Mvuazi research center, Kintete and Lukuakua villages in Democratic Republic of Congo (Bakelana, personal communication, 2018) and in East Africa countries. A survey was carried out in Muheza district in Tanzania by the root / tuber team from Kibaha in April 2002. It was a response to farmers demand for action to control what they termed as 'cassava root rot' or Vidonda in swahili, reported by the district extension officer. Farmers said that the disease had become more serious after El Nino rains of 1997/98. Three villages were surveyed to examine the problem of cassava root rot. Informations on varieties / cultivars and crop age were gathered from individual farmers, and a destructive sampling on roots was performed in fields with mature crops of about 12 months. The root rot described by farmers was found to be CBSD. Crop loss as a result of the disease was 62% (Mtunda, 2002).

In Northern Mozambique, since 1997 farmers have been reporting major losses of cassava caused by a root rotting disease, which by 1998 were considerable, and had infected large areas of coastal Nampula province in the northern part of the country. As a result, many farmers started to turn to alternative crops. Farmers in the Zambezia Province, immediately to the south of Nampula were finding the same problem of cassava root rotting, but were using an avoidance strategy by harvesting roots before the crop had matured so as to reduce the amount of tuber rotting and losses. Two virologists from the Natural Ressources Institute (NRI) identified the disease as CBSD in 1999 (Mangana, 2002). The most important impact of CBSD to the farmers is on roots. Losses were well expressed by necrosis in roots. It was found in Tanzania even in Mfouati district that necrosis were present more on old plants than on younger plants. These results are in contradiction with those obtained by Mahungu et al. in 2003, where symptoms were also found on landraces in DRC. The situation is in line with results recorded in DRC (Mahungu et al., 2003) where the same variety also reached the 100% incidence and is among most susceptible varieties in DRC.

**Table 1. Effect of the cassava variety on the maximum severity and the incidence of the CBSD -like root necrosis in Mfouati**

N°	Variety	Max. severity	Incidence (%)
1	Jamais déçu	5a	51.67b
2	Ya leger	4b	100 a
3	V.0029	4bc	41.67 bc
4	Belle dame	3bc	31.67 bc
5	Ntsibisi	3c	20.17c
6	Marie madama	3c	23.67c
7	Mabounda	2c	25.00c
8	Kinine	1d	1.67d
9	Moundele mpakou	1d	1.67d
	Mean	2.24	22.51
	CV (%)	64.93	138.07
	LSD (5%)	**	**

**Table 2. Effect of locality on the maximum severity and incidence of CBSD-like root necrosis in Mfouati**

N°	Locality	Maximum severity	Incidence (%)
1	Bibondo pont	4a	100a
2	Ngolonga	3ab	26.66b
3	Louimbi	3ab	27.66b
4	Kinzaba	3ab	24.33b
5	Mfouati	2ab	5.66b
6	Kingonda	2ab	20b
7	Bikoumbi	1ab	10b
8	Loufoua loua Nzambi	1ab	10b
9	Tala nkoyi	1ab	10b
10	Kindamba	1b	1b
11	Kinanga	1b	0b
12	Mouyonzi	1b	1b
	Mean	2.24	22.51
	CV(%)	64.93	138.07
	LSD (5%)	*	**

**Table 3. Effect of the altitude on the maximum severity and incidence of CBSD-like root necrosis in Mfouati district**

N°	Area	Maximum severity	Incidence (%)
1	Low	3a	31.16a
2	High	3a	34.50a
	Mean	2,24	32.83
	CV (%)	64,93	138.07
	LSD (5%)	ns	ns

**Figure 1. Root necrosis severity scores**

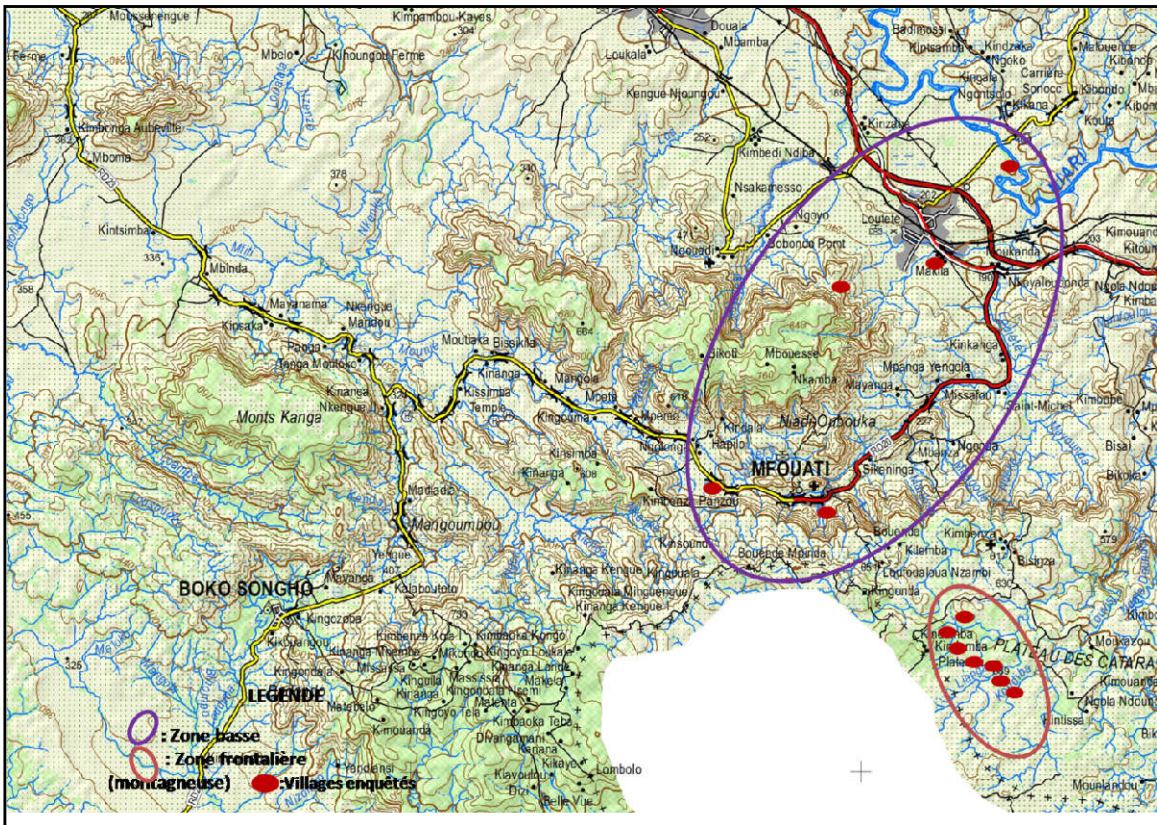


Figure 2. Map of surveyed area in Mfouati

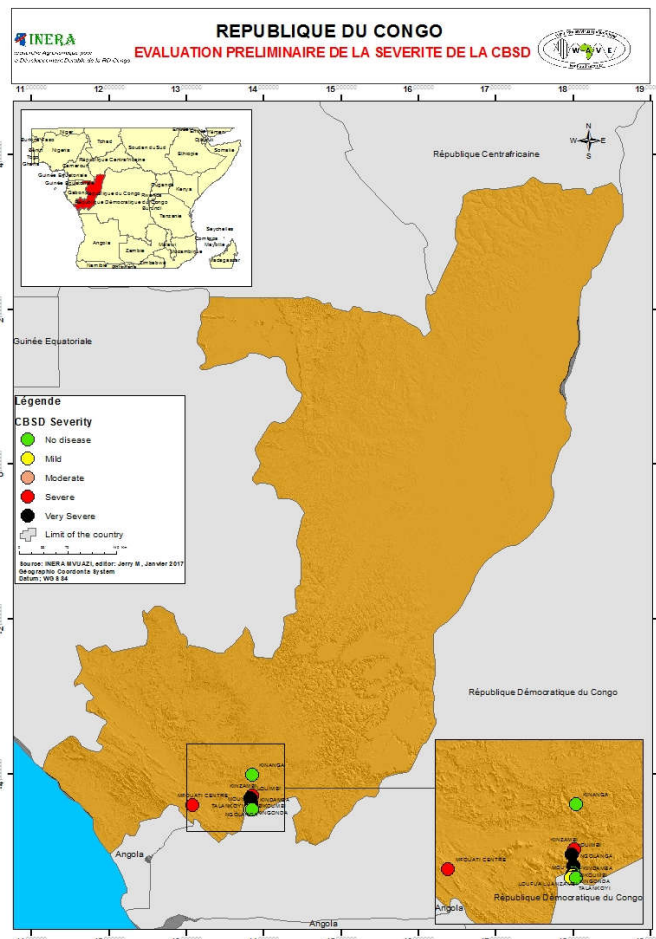


Figure 3. Map of root necrosis severity and distribution in Mfouati district



Figure 4. Photos of damaged cassava roots and chips with necrosis in Mfouati



Figure 5. Photo of typical cassava root necrosis in Mfouati

A number of local cultivars have been identified both in Tanzania and Malawi that show tolerance to root necrosis. That is to say that the onset of root necrosis is delayed. The cultivar Nanchinyaya was the first of these tolerant types to be identified and it is now widely grown in the Mtwara region of southern Tanzania. Root necrosis may begin show after 5-6 months in the more sensitive cultivars but it is delayed until 12-18 months in Nanchinyaya. CBSD management in both Tanzania and Malawi is based on the multiplication and distribution of these tolerant local cultivars (Hillocks, 2002). Variety susceptibility variability to the disease is demonstrated by previous research findings in East Africa. Two cultivars, Kitumbua and Kiroba, found between Dar es Salaam and the Rufiji River were observed to have some resistance to CBSD (Hillocks, 2000). Some local cultivars, e.g. Nanchinyaya, have exhibited a form of tolerance to CBSD in which foliar symptoms appeared but the development of root necrosis was delayed allowing the full yield potential to be realized others cultivars like Kigoma Cheusi, Gomani and Songolo were also

observed to have relatively high CBSD incidences (Hillocks et al., 2001). Six cultivars were examined for CBSD susceptibility or symptom expression. Cultivars Sheria, Albert and Nanchinyaya showed higher incidences of CBSD than Saranga, Limbanga and Mreteta. In some fields, for cultivar Sheria, the incidence reached 70%. There is considerable variation in susceptibility to CBSD among local cassava cultivars in Tanzania. (Mtunda, 2002). In Tanzania, the majority of local cultivars appear to be susceptible to CBSD (Rwegasira, et al., 2011). CBSD symptoms vary with cultivars of cassava, environmental factors and age of the plant. 3. Surveys conducted in Tanzania showed the variability of the disease incidence versus localities. Since the early 1990s, several surveys have been conducted to assess the status of cassava virus disease in Tanzania. A country-wide survey was carried out by Legg and Raya in 1993-94. Highest incidences were recorded in Mtwara and Lindi (36%), followed by Masasi and Nachingwea (25,2%) (Mtunda, 2002). A survey conducted along the Tanzania coastal plain from the Kenya border southwards again showed the disease incidence variability. Higher incidence of CBSD was recorded between the Rufizi

River and Lindi. Table 3 shows that the altitude did not influence the occurrence of root necrosis in Mfouati district, in accordance with results obtained by Mulimbi et al. (2012) who found symptoms of cassava brown streak in high altitudes in eastern DRC. These results also confirm those obtained by Alicai et al. in 2007 in Uganda and found symptoms of cassava brown streak on high altitudes in the Great Lakes region. Rwegasira et al. 2011, revealed that CBSD occurs throughout Tanzania, wherever cassava is grown. Earlier studies (Nichols, 1950; Hillocks, et al., 1999; Hillocks et al., 2002; Gondwe et al., 2002) reported the restricted distribution of CBSD to coastal areas, including Zanzibar and along the shores of Lakes Malawi. In 2004, the apparent restriction of CBSD to coastal lowlands changed with the re-emergence of CBSD at altitudes above 1000 masl (Alicai et al., 2007). In summary, the overall incidence of the disease is still low in the district of Mfouati even though some plants with severe symptoms have been identified. This situation can constitute a national risk and enable in a near future an outbreak of the disease as we are currently encountering in some areas of western DRC.

### Conclusion

CBSD-like root necrosis was found in the District of Mfouati, DRC and the overall incidence of the disease is still low even though some plants with severe symptoms have been identified. We have found that the occurrence of these symptoms is largely dependent on the cassava variety. The variety Ya leger (from DRC and known as RAV) is the most susceptible variety followed by varieties Jamais déçu, V.0029, Belle Dame, Mabounda, Marie Madama and Ntsibisi. The Kinine and Moundele Mpakou varieties have found more tolerant or resistant. They could be further investigated in the context of a breeding program. This situation can constitute a national risk and enable in a near future an outbreak of the disease as we are currently encountering in some areas of western DRC.

### Future plans for research work in Congo republic:

- Conduct an in-depth study in the same district to assess the current status of the disease by assessing variety yields and losses due to the disease;
- Carry out similar investigations across the country;
- Research needs to be conducted to allow the development of new resistant varieties.
- Need of Next Generation studies in order to identify pathogens that are causing root necrosis in the District of Mfouati with the option of using on ground for direct sequencing the real-time portable DNA sequencer that has been deployed in Tanzania, Uganda and Kenya to quickly identify plant viruses (Boykin et al., In press).
- Since the disease is likely not present in all areas of the country, quarantine may be of use and could be the first step in tackling the disease in Congo republic. The next step could be to identify high yielding resistant cultivars to root necrosis and others majors diseases and pests.
- Once resistant cultivars have been identified, they should be multiplied and distributed to farmers.
- There should be a need to organize campaigns and trainings for farmers as well as extension staff in field disease identification and application of control measures.

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