African Journal of Rural Development, Vol. 2 (3):July - September 2017: pp. 455-466. ISSN 2415-2838

Limits of phytosanitation and host plant resistance towards the control of cassava viruses in Uganda

R.S. KAWUKI,[1] G. ADIGA, [1] J. ORONE,[1] T. ALICAI,[1] M. EDIMU[2], T. OMARA,[1] A. PARIYO,[1] W. ESUMA,[1] C. OMONGO,[1] A. BUA,[1] E. KANJU[3] and BAGUMA, Y.[1]
[1]National Crops Resources Research Institute (NaCRRI), P.O. Box 7084, Kampala, Uganda
[2]Research and Education Agency, PO Box 54, Kampala, Uganda
[3]International Institute of Tropical Agriculture, P. O. Box 7878, Kampala, Uganda
Corresponding author: kawukisezirobert@gmail.com

ABSTRACT

Cassava (*Manihot esculenta* Crantz) and the viruses that infect it, notably cassava mosaic virus and cassava brown streak viruses, have a unique history of co-evolution and co-existence. While cassava originated in South America, both viruses and the diseases they cause have largely been limited to the East African region, where they have, and continue to be key yield-robbing stresses. For sustainable control, we assume that deployment of resistant varieties when carefully combined with phytosanitation will combat these viruses. We have thus generated empirical data and tested the limits, i.e., how long this strategy can last. This entailed the comparison of elite cassava varieties, one set of virus-indexed tissue culture plantlets, and the other set, re-cycled planting materials under farmer's cyclic propagation for 6-23 years. Trials were established at diverse sites in Uganda. We observed that both officially-released and unofficially-released cassava varieties are common in farmer's fields; these varieties have varying susceptibility levels to viruses. Worrisome was that some officially-released varieties like NASE 3 registered cassava mosaic disease (CMD) incidences of up to 71% (virus-indexed), which was not any different from its re-cycled counterparts. Other varieties like NASE 14 have maintained high levels of CMD resistance six years after official release. Predominant re-cycled cassava varieties notably TME 204, I92/0057, TME 14, and to a limited extent NASE 14, are key reservoirs for cassava brown streak disease (CBSD) associated viruses. These findings highlight the limits of phytosanitation, i.e., in areas like Kaberamaido associated with high CMD pressure, varieties NASE 1 and NASE 3 can not be recommended; on the contrary, these varieties can be deployed in Kalangala, where they can survive with phytosanitation. And for CBSD, the findings justify the urgent need for phytosanitation (community-led) and development of varieties with higher levels of resistance and/or tolerance, as no immune variety has so far been identified.

Key words: Cassava mosaic virus, Cassava brown streak virus, seed system, East Africa, Phytosanitation, Uganda

RÉSUMÉ

Le manioc (*Manihot esculenta* Crantz) et les virus qui l'affectent, plus précisément le virus de la mosaïque et les virus de la striure brune du manioc ont une historique unique de coévolution et de coexistence. Quand bien même que le manioc soit originaire d'Amérique du Sud, les deux types de virus et les pathologies causées ont été largement enregistrées en Afrique de l'Est, où ils sont, et continuent d'être, des facteurs clés de perte de rendement. Pour un contrôle durable, nous supposons que le développement des variétés résistantes, lorsque combinées sont soigneusement à la phyto-prophylaxie, permettra de lutter contre ces virus. Ainsi, nous avons généré des données empiriques et testé les limites, c'est-à-dire, pour combien de temps cette stratégie peut durer. Ceci a impliqué la comparaison d'excellentes variétés de manioc, d'un ensemble de plantules de culture tissulaire, et d'un autre ensemble de matériels végétatifs recyclés à travers une multiplication cyclique de 6 à 23 ans. Plusieurs sites d'essais ont été établis en Ouganda. Nous avions observé que les variétés de manioc officiellement et non officiellement acceptées sont communes dans les champs des

Received: 14 August 2016
Accepted: 24 September 2017
Published: 30 September 2017

agriculteurs; ces variétés présentent des niveaux variables de sensibilité aux virus. L'inquiétude a été de constater que certaines variétés officiellement acceptées, comme la « NASE 3 », enregistraient jusqu'à 71% des cas de pathologie de la mosaïque du manioc, ce qui n'était pas différent des autres recyclés. D'autres variétés comme la «NASE 14» ont présenté des niveaux élevés de résistance à la mosaïque du manioc six ans après leur mise en utilisation officielle. Les variétés prédominantes de manioc recyclées, notamment les TME 204, I92 / 0057, TME 14 et, dans une certaine mesure, la NASE 14, sont susceptibles aux virus associés à la pathologie de la striure brune du manioc. Ces résultats mettent en évidence les limites de la phyto-prophylaxie, par exemple, dans les zones comme Kaberamaido associée à une forte prévalence a la pathologie de la mosaïque du manioc, les variétés NASE 1 et NASE 3 ne peuvent pas être recommandées; cependant, ces variétés peuvent être utilisées à Kalangala, où elles peuvent survivre avec la phyto-prophylaxie. Et pour la striure brune du manioc, les résultats obtenus reflètent un besoin urgent de phyto-prophylaxie participative, et un développement de variétés présentant des niveaux élevés de résistance et / ou de tolérance, puisqu'aucune variété protectrice n'a été identifiée jusqu'à présent.

Mots-clés: virus de la mosaïque du manioc, virus de la striure brune du manioc, système semencier, Afrique de l'Est, phyto-prophylaxie, Ouganda

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) and the viruses that infect it, notably cassava mosaic virus (Legg and Fauquet, 2004) and cassava brown streak viruses (Mbanzibwa *et al.,* 2009; Winter *et al.,* 2010), have a unique history of co-evolution and co-existence. While cassava's centre of origin is traced to South America (Olsen and Schaal, 1999; Olsen, 2004), both viruses and the diseases they cause have largely been limited to the East and Southern African region, where they have caused immense suffering to communities that primarily depend on cassava. In fact, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) were first reported in eastern Africa 122 and 86 years ago, respectively (Nichols, 1947; Jennings, 1957; Hahn *et al.,* 1979; Hahn *et al.,* 1980), but their outbreak in South America has not been reported. Both CMD and CBSD have remained key yield-robbing stresses that limit optimal cassava productivity on the African continent (Figure 1) (Legg *et al.,* 2014). Unfortunately, the vegetative nature of cassava complicates management of the viral diseases, as the planting materials (often referred to as stems or stakes) are carriers of both the inherent genetics (desired trait combinations) and the viruses that limit productivity (Ceballos *et al.,* 2004; McQuaid *et al.,* 2016). An ideal cassava variety should combine desirable commercial, agronomic and plant health traits (or genetics) in its seed, which for cassava is the stem.

Nonetheless, cassava remains an increasingly popular crop owing to its starch that can be subjected to diverse food and non-food uses. For instance, by 1961 cassava in Africa was established on 5,564,040 ha, which increased to 17,523,640 ha by 2014 (FAOSTAT, 2016). Similar production trends have been registered in Asia and South America, collectively attesting to the ever-increasing demand of cassava for either food or non-food products. It suffices to note that significant investments have been made to tackle cassava viruses in Africa (Taylor *et al.,* 2012). Accordingly, this has resulted into significant genetic gains in production and productivity that have luckily been attained with minimal global coordination. For example, it is commonplace for many African National Agricultural Research Systems to have uncoordinated cassava research and outreach activities that are often characterized by limited stakeholder categorization, inclusiveness, awareness and satisfaction. This needs to be addressed. Thus, the Global Cassava Partnership for the 21st Century (GCP21) instituted and formalized the "Global Alliance to Declare a War on Cassava Viruses in Africa"(Legg *et al.,* 2014).

Three fundamental issues were articulated and action points suggested by this alliance: 1) the

urgent need to prevent the spread of CBSD to Central and West Africa; 2) deliberate efforts to limit the effects of both CMD and CBSD wherever they continue to cause havoc; and 3) actions to prevent the spread of both viruses to other parts of the world (Legg *et al.,* 2014). Herein, we are providing empirical data and thoughts that are meant to guide the implementation of strategies tailored towards limiting the effects of both viruses wherever they occur. For sustainable control, we assume that deployment of virus/disease resistant cassava varieties along with community-led phytosanitation will combat these viruses where they are endemic. We have generated empirical data and tested the limits of this strategy. This entailed the comparison of elite cassava varieties: one set of virus-indexed tissue culture plantlets and another set of re-cycled planting materials under farmer's cyclic propagation for 6-23 years. We further propose a four-step seed system process that would be desirable whenever new cassava varieties are being deployed. Certainly, this information is equally relevant as a pre-emptive measure, where these diseases have not yet been reported, i.e., in central and western Africa for CBSD and Latin America forboth CBSD and CMD.

Empirical field data comparing virus-indexed and re-cycled cassava planting materials. We assumed that when cassava varieties with different levels of resistance to viruses (highly-resistant, resistant, moderately-susceptible and highly-susceptible) are deployed in areas of varying virus pressure, they will exhibit varying genetic, agronomic and commercial value. Thus, their usefulness (acceptance or rejection by stakeholders) will largely be limited to the rate at which their performance deteriorates as viruses buildup in the planting materials (McQuaid *et al.,* 2016). Thus, we set up experiments in contrasting parts of Uganda to generate empirical data to compare field performance of elite virus-free cassava clones and their counter-parts that had been under re-cycled propagation by the farming community for a period ranging between 6-23 years. We are best-placed to track re-cycled elite cassava varieties in farmer's fields because we are familiar and have custody to all of these varieties.

Test genotypes and virus-elimination procedures. We selected five elite cassava varieties: NASE 1 (released in 1993), NASE 3 (released in 1993), NASE 14 (released in 2011), NASE 18 (released in 2011) and NAROCASS1 (released in 2015). NASE 1 and NASE 3 have been in farmers' field under re-cycled propagation for no less than 23 years, while NASE 14, NASE 18 and NAROCASS1 have been in re-cycled propagation for at least six years. Pedigree information and historic agronomic datasets associated with these clones collected for the past 6-25 years (between 1990 and 2014) from different locations in Uganda is summarized in Table 1.

Figure 1. Cassava viruses associated effects. Left-characteristic root necrosis and root constricts associated with cassava brown streak disease; Right-leaf distortion and malformation associated with cassava mosaic disease in cassava fields at Kaberamaido, eastern Uganda.

The nature of this experiment required that unquestionably confirmed virus-free planting materials are generated and used for comparisons with their counterparts under re-cycled propagation. Accordingly, 20 stems each bearing 5-7 nodes were collected from the National Agricultural Research Organization (NARO) cassava germplasm maintenance fields in Fortportal, western Uganda and shipped to Natural Resources Research Institute (NRI), United Kingdom for virus elimination using standard thermotherapy procedures (Wasswa *et al.,* 2010). Briefly, the production of virus-free plantlets using thermotherapy involved three key activities: 1) importing and establishing cassava varieties using standard procedures (IITA, 1990) at NRI for purposes of getting explants for tissue culture and virus samples for undertaking analyses; 2) optimizing a diagnostic multiplex protocols for simultaneous detection of both cassava mosaic viruses and cassava brown streak viruses (CBSVs); this was done to support the desired quality control measure to identify virus-free plantlets for micro-propagation; and 3) undertaking thermotherapy procedures on plantlets that have tested positive for either or both viruses, notably, the cassava mosaic germini viruses (CMGs) and CBSVs; this was done to ensure that only virus-free plantlets are deployed in the field. Only virus-free plantlets of the test varieties were micro-propagated following standard procedures. All this work was undertaken under the guidance of Dr. Maruthi Gowda of NRI. The generated virus-free plantlets were shipped to Uganda and initially multiplied in a virus-free location in western Uganda in September 2014 to generate sufficient planting materials for establishment of replicated experiments.

Field trials to evaluate performance of virus-indexed and re-cycled clones. Three sites were selected to evaluate field performances of virus-indexed tissue culture derived plants. Firstly, Kaberamaido, located in eastern Uganda; this is a traditionally known cassava growing area for a period of no less than 100 years; it has also witnessed overlapping CMD epidemics that are often a result of high whitefly populations. Secondly, Kalangala, an island located within Lake Victoria. Kalangala, is not a traditional cassava growing area and it is often characterized by low virus pressure and low whitefly populations. Thirdly, Namulonge located in central Uganda; this site was added for comparison purposes, as it is the site where rigorous early-selections trials are undertaken for at least three years prior to undertaking multi-locational trials. Thus, any officially released cassava variety will have to first be evaluated at Namulonge prior to subjecting it to further downstream evaluations at multi-locational sites. At each of these locations, virus-free planting materials of the five varieties were established during the second rains of 2015. At each site, each variety was represented by plots of 7 x 6 meters, giving a plot stand of 42 plants. The trial was replicated three times at each of the locations.

At each location, data were collected for both CMD and CBSD at three and six months after planting. For CMD, the 20 plants in the net plot were individually scored using a scale of 1-5 (IITA, 1990). For this scale; 1 = no symptoms observed, 2 = mild chlorotic pattern on entire leaflets or mild distortion at base of leaflets appearing green and healthy, 3 = strong mosaic pattern on entire leaf, and narrowing and distortion of lower one-third of leaflets, 4 = severe mosaic distortion of two thirds of leaflets and general reduction of leaf size, and 5 = severe mosaic, distortion of four-fifths or more of leaflets, twisted and misshapen leaves. For CBSD foliar symptoms, the 20 plants in the net plot were also individually assigned severity scores based on the standard five point scoring scale for CBSD (Gondwe *et al.,* 2003), where 1= no apparent symptoms, 2= slight foliar feathery chlorosis, no stem lesions, 3= pronounced foliar feathery chlorosis, mild stem lesions, and no die back, 4= severe foliar feathery chlorosis, severe stem lesions, and no die back, and 5= defoliation, severe stem lesions and die back.

At harvest, which coincided with 12 months after planting (MAP), all 20 plants in the net plot were uprooted and all roots cut transversally and assessed for CBSD root necrosis using the 1-5

scale. For this scale, 1= no necrosis, 2 = mild necrotic lesions (1-10%), 3 = pronounced necrotic lesion (11-25%), 4 = severe necrotic lesion (26-50%) with mild root constriction and 5 = very severe necrotic lesion (>50%) with severe root constriction as described in Kaweesi *et al.* (2014).

Data on re-cycled cassava varieties within Kaberamaido and Kalangala districts were collected from neighbouring farmer fields that were of the same age and separated by at least one kilometer. Additional data on re-cycled cassava varieties were collected from farmer fields in Pallisa (eastern Uganda), Nebbi (northern Uganda), Mutukula (western Uganda) and Mbarara (western Uganda). Within each of these locations, nine fields of sizes <1 acre were identified and used for collection of CMD and CBSD data for comparison purposes. At each site, farmers were engaged in discussion to establish variety names and how they accessed the planting materials.

We observed that most of the cassava varieties grown on their farms had been attained through informal exchanges amongst themselves, thus, they had been re-cycled for several years. Varieties being grown by farmers were verified using established descriptors. Within each field sampled, data were taken on both CMD and CBSD on individual plants as described earlier.

Field responses of re-cycled and virus-indexed cassava varieties. At the time of official release, the five varieties had varied responses to both CMD and CBSD (Table 1). NASE 1 and NASE 3 had average CMD incidences of <17% and severities of < 2. It was only NASE 1 that registered incidences of 58% at one location prior to release (Table 1). Other varieties notably NASE 14 and NASE 18 showed no CMD infection at time of release. On the contrary, NASE 14, NASE 18 and NAROCASS1, registered mean CBSD incidences of <10% and severities of <3.1. No CBSD records were made on NASE 1 and NASE 3 prior to their release, as CBSD was not present then (Table 1).

When these varieties were cleaned and re-deployed in the field, differential responses owing to the underlying genetics and virus pressure in the locations were observed. For example, CMD incidences for NASE 1 ranged from 100% at Kaberamaido to 10% at Namulonge, while CMD severities for the same varieties at both locations were 3.5 and 1.1, respectively (Table 2). For NASE 3, CMD incidence ranged from 71% at Kaberamaido to 9.4% at Namulonge, and the respective CMD severities at both locations were 2.2 and 1.1. Among the other three varieties, it was only NASE 14 that consistently had no CMD symptoms. Overall, after one year of evaluation, Kaberamaido registered highest CMD severity and incidence, while Kalangala registered the lowest CMD severity and incidence.

For CBSD, it was evident that NASE 14 registered higher CBSD foliar susceptibility (with 26% foliar incidence) than the other varieties, and this was evident at Namulonge (Table 2). On the other hand, varieties NASE 1 and NAROCASS 1, registered lower CBSD root severities (<2) and incidences (<10%), as compared to varieties NASE 14, NASE 18 and NASE 3, which respectively had root incidences of 75%, 53% and 41%, with root severity scores >2 (Table 2). Overall, after one year of evaluation, Namulonge registered the highest CBSD root severity and incidence (Table 2).

Evidently, the datasets presented in Tables 1 and 2 do suggest that different strains of CBSVs, with varying diversity and/or aggressiveness, existed at the time of official release, when compared to those present in the fields when virus-indexed materials were evaluated (i.e., during 2016). The current CBSVs appear to be more virulent and thus inflicting higher CBSD severities and consequently lower yields as evidenced in datasets presented in Tables 1 and 2. Indeed, recent studies conducted in Uganda (Alicai *et al.,* 2016) using whole genome sequences of CBSVs have established that: 1) cassava brown streak virus (CBSV) has a faster rate of evolution than Uganda cassava brown streak virus (UCBSV); 2) for CBSV, non synonymous substitutions are more predominant than synonymous substitution; and 3) CBSV may be outsmarting the cassava immune system, making it more aggressive than UCBSV

Table 1. Pedigree and historic data associated with the five test genotypes during the time of official release in Uganda

Variety	Pedigree	Year of release	CMD		CBSD		Yield (t/ha)		
			Inc.	Sev.	Inc.	Sev.	Min	Max	Mean
NASE 1	TMS 60142; from IITA	1993	16.4 (58.7)	2.0 (2.9)	-	-	12.4	46.7	25.6
NASE 3	58308 x Branca de Santa Catarina; introduction from IITA	1993	3.0 (6.9)	1.5 (1.9)	-	-	18.7	29.0	24.4
NASE 14	192/0248 half sib; from IITA	2011	0	1	7.8 (24.2)	1.9 (3.0)	26.8	42.3	34.87
NASE 18	Half sib of TME 14	2011	0	1	2.0 (14.5)	1.6 (3.0)	12.5	55.4	32.3
NAROCASS1	Selection from Open pollinated seed introduced from Tanzania	2015	0	1	0.14 (0.7)	1.1 (1.3)	22.8	53.3	40.15

Data for NASE 1 and NASE 3 are summaries from replicated on-station and on-farm trials conducted during late 1980s to 1990s; these trials were conducted in different parts of northern region (Lira, Apac and Arua); western region (Bushenyi, Kasese, Hoima, Masindi and Kibaale) and central region (Luwero, Nakasongola, Wakiso, Mubende). On the other hand, data for NASE 14, NASE 18 and NAROCASS1 are summaries from replicated on-station and on-farm trials conducted between 2002 and 2015 in different parts of northern region (Lira, and Arua); central region (Luwero, Wakiso, Kayunga, Mukono, Nakasongola) and eastern region (Kumi, Busia, Soroti, Kamuli and Pallisa). Details of datasets associated with some of these trials are published elsewhere (Kawuki *et al.,* 2011). Empirical data on CBSD was first documented after 2004 (Alicai *et al.,* 2007; Kawuki *et al.*, 2016) and thus the absence of data for NASE 1 and NASE 3.

Table 2. Field response of indexed cassava clones to both CMD and CBSD at three locations in Uganda

Location	Variety	CMD		Foliar CBSD		Root CBSD		FRY
		Inc	Sev	Inc	Sev	Inc	Sev	
Kaberamaido	NASE 1	100.0 (99.61)	3.5 (3.52)	0.0 (-0.09)	1.0 (0.99)	0.5 (-4.55)	1.0 (0.88)	15.5 (15.90)
	NASE 3	71.0 (71.67)	2.2 (2.23)	0.0 (-0.90)	1.0 (0.98)	2.5 (0.74)	1.0 (0.97)	5.8 (5.40)
	NASE 14	0.0 (-0.61)	1.0 (0.99)	0.0 (0.07)	1.0 (0.99)	25.4 (19.74)	1.6 (1.47)	21.8 (22.30)
	NASE 18	7.1 (6.75)	1.1 (1.06)	0.0 (-0.09)	1.0 (0.99)	0.0 (-5.01)	1.0 (0.87)	3.4 (7.90)
	NAROCASS 1	6.3 (6.75)	1.1 (1.10)	0.0 (-0.74)	1.0 (0.98)	0.0 (-2.43)	1.1 (0.93)	19.8 (19.55)
Kalangala	NASE 1	12.7 (13.65)	1.2 (1.21)	0.0 (-1.06)	1.0 (0.98)	1.5 (3.80)	1.0 (1.09)	-
	NASE 3	5.6 (6.06)	1.1 (1.11)	0.0\ (-0.74)	1.0 (0.98)	13.2 (44.74)	1.2 (2.35)	-
	NASE 14	0.0 (1.17)	1.0 (1.02)	0.0 (-1.22)	1.0 (0.98)	19.6 (78.10)	1.2 (2.97)	-
	NASE 18	0.0 (0.95)	1.0 (1.01)	0.0 (-1.06)	1.0 (0.98)	1.6 (56.61)	1.0 (2.64)	-
	NAROCASS 1	0.0 (0.39)	1.0 (1.00)	0.0 (-0.66)	1.0 (0.98)	5.1 (7.24)	1.1 (1.15)	-
Namulonge	NASE 1	10.4 (10.03)	1.1 (1.12)	0.0 (0.68)	1.0 (1.02)	1.0 (0.40)	1.0 (0.97)	7.0 (6.93)
	NASE 3	9.4 (9.07)	1.1 (1.11)	0.0 (0.63)	1.0 (1.01)	41.8 (10.82)	2.3 (1.08)	6.9 (6.82)
	NASE 14	0.0 (-0.52)	1.0 (0.99)	26.6 (27.41)	1.4 (1.46)	75.8 (19.15)	2.9 (1.19)	9.3 (9.37)
	NASE 18	3.5 (3.13)	1.1 (1.05)	7.0 (7.70)	1.1 (1.09)	53.9 (0.48)	2.6 (0.97)	12.2 (12.13)
	NAROCASS 1	0.0 (-52)	1.0 (0.99)	0.0 (0.79)	1.0 (1.01)	4.9 (2.30)	1.1 (0.97)	15.3 (15.37)

CMD = cassava mosaic disease; CBSD = cassava brown streak disease; Inc= incidence; Sev= severity; FRY = fresh root yield (t/ha). Data in parentheses are least square (LS) means obtained from linear models that considered locations, variety and replicate effects.

and thus implicated in causing higher CBSD susceptibility.

Data on CBSD and CMD incidence and severity on the re-cycled cassava varieties across selected locations are presented in Figure 2. Predominant re-cycled cassava varieties sampled in farmer fields varied across the locations. For example, NASE 3 and NASE 14 were predominant in Pallisa; TME 204 and I92/0057 in Mutukula; TME 14, I92/0057 and NASE 14 in Nebbi; NASE 3 and NASE 14 in Kaberamaido; and local varieties notably Njule and Bukalasa in Kalangala. Cassava varieties TME 204, TME 14, and I92/0057, though highly resistant to CMD, are highly susceptible to CBSD (Abaca *et al.,* 2012). These varieties, though not officially released, are popular among farmers owing to their inherent desirable root qualities (Kawuki *et al.,* 2011).

Overall, it was evident that most variability in CBSD in farmer fields was observed in Nebbi followed by Mutukula. Indeed, Nebbi, had some fields showing foliar CBSD incidence of up to 40% with some fields with foliar severities >2 (Figure 2). We observed no foliar CBSD symptoms in cassava fields we sampled in Kalangala. The CMD incidence and severity were equally variable in most of the re-cycled varieties across the selected cassava fields. For example, in Kaberamaido and Palllisa, CMD incidence in some farmer fields approximated 80%, with severities >3 (Figure 2). Some fields in Kalangala had CMD severities >2 (Figure 2).

A number of factors are likely to explain these observed trends, notable of which include genotype susceptibility levels, predominant virus species in locality and/or season and climatic factors that either influence the abundance of whitefly vectors and/or the growth rate of the crop (Katono *et al.,* 2015). These factors have strong implications particularly towards optimization of cassava breeding efficiency, variety deployment for cultivation and/or for certified seed production. Based on these trends, it would be logical to suggest Namulonge and Kaberamaido as sites for conducting early-selection trials, whereas certified cassava seed production can, within limits, be restricted to the Kalangala islands.

Three key findings are apparent from these studies. Firstly, we observed that both officially-released and unofficially-released cassava varieties are popular in farmers' fields. Of the officially-released, NASE 3 and NASE 14 were the most predominant particularly in Pallisa, Kaberamaido and Nebbi. These varieties have been in cultivation for at least 23 years (for NASE 3) and/or for six years (for NASE 14). NAROCASS 1 has only been in cultivation for less than three years. The popularity of NASE 3 could in part be attributed to its ease of availability to farmers owing to the many years of propagation and/or its good traits, notably the high quality and quantity of starch that is desirable for processing. Cassava varieties TME 204, TME 14, I92/0057 and I92/0067, though popular among farmers owing to their desirable culinary root qualities and yield, were not officially-released because of their high susceptibility to CBSD (Alicai *et al.,* 2007; Abaca *et al.,* 2012). These varieties were introduced in the late 1990s and got quickly adopted by the farming communities prior to the outbreak of CBSD in 2004. Wherever these varieties are grown, they are potential sources of CBSV inoculum, and could be responsible for the spread of CBSD among farmers' fields. The low incidence of CBSD in Kalangala, which is rather isolated and thus associated with less germplasm exchange with mainland, further testifies to this. Thus, a combination of sustained concerted efforts on awareness campaigns and pytosanitation will enhance negative selection against these varieties (in areas where CBSD pressure is high) and thus, lower the CBSD inoculum in farmers' fields.

Secondly, after one year of evaluation, we observed varying CMD responses amongst the indexed virus-free tissue culture plants deployed in the field. Varieties deployed and evaluated in Kaberamaido had significantly higher CMD susceptibility, with NASE 1 and NASE 3 being most affected (Table 2). During the peak of CMD pandemic in the 1980 and 1990s, cassava varieties

NASE 1 (TMS 60142), NASE 2 (TMS 30337) and NASE 3 (TMS 30572) were widely disseminated particularly in northern and eastern Uganda to combat the disease (Otim-Nape *et al.,* 1998). Almost 23 years later, we still notice high CMD incidences in Kaberamaido, eastern Uganda. Certainly, this is a result of cyclic-propagation of infected planting materials (of NASE 3, a CMD tolerant variety), which when done several times leads to continued virus presence and build up in the environment. These viruses are efficiently transmitted from neighbouring infected cassava fields into clean fields (Maruthi *et al.,* 2005) as witnessed in this case.

Changes in species diversity of CMGs (Bruyn *et al.,* 2016) could also explain these observed findings meaning that the current strains of CMGs are highly virulent and limit the usefulness of varieties like NASE 1 and NASE 3 even when combined with phytosanitation. This highlights the limits of phytosanitation, i.e., it can not work with varieties that can be classified as tolerant to CMD. For example, in areas like Kaberamaido that are associated with high CMD pressure, varieties like NASE 1 and NASE 3 cannot be recommended for cultivation, as such varieties would be severely infected under the high disease pressures. Rather, such locations require highly resistant varieties deployed for sustainable production. On the contrary, when both NASE 1 and NASE 3 are deployed in Kalangala, they can do better with phytosanitation, implemented individually and/ or communally; this is possible because disease pressure is relatively low.

Thirdly, we observe complex "variety x disease x propagation cycle" interactions. Beginning with NASE 3, we observed that at the time of its official release in the 1990s, CMD incidence and severity were less than 10% and 3, respectively (Table 1). Twenty three years later, re-cycled NASE 3 on farmers' fields had CMD incidences of >60% and severities of >3 (Figure 2). When virus-indexed tissue culture plantlets were evaluated, we observed incidences of up to 71% within a single year (Table 2), a finding that illustrates its rapid degeneration in the face of CMGs.

On the other hand, NASE 14, which had been in

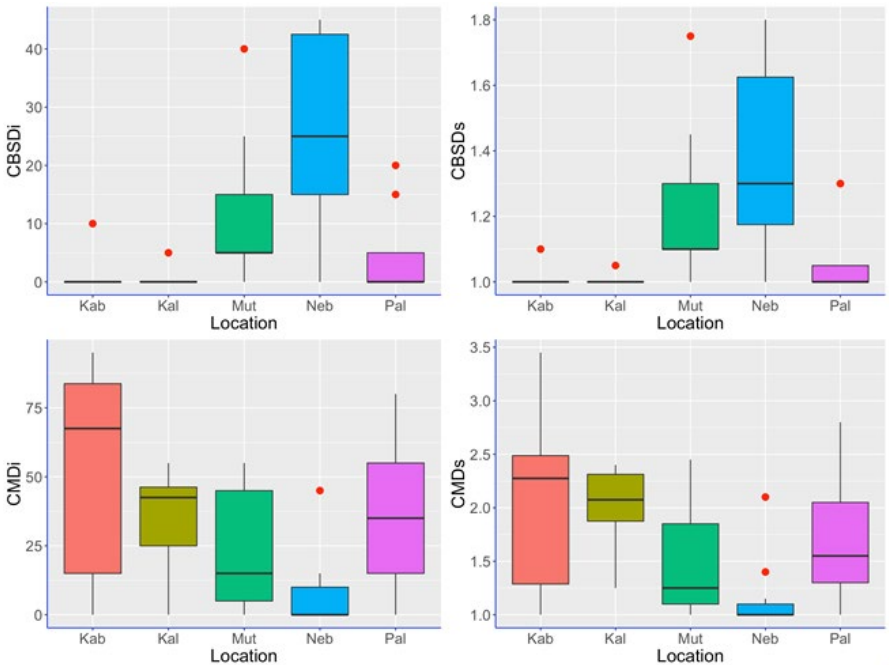

Figure 2. Prevalence of cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) in re-cycled cassava planting materials in selected locations in Uganda: CBSDi= foliar CBSD incidence; CBSDs = foliar CBSD severity; CMDi=incidence of CMD; CMDs = severity of CMD. Kab = Kaberamaido; Kal = Kalangala; Mut = Mutukula; Neb = Nebbi; and Pal = Pallisa.

cultivation for six years after its release in 2011 still maintained high levels of CMD resistance and thus, not vulnerable to degeneration as witnessed for NASE 3.

For CBSD, our discussion on "variety x disease x propagation cycle" interactions are limited to NASE 14, for purposes of emphasis; NASE 14 had more CBSD data points than the other varieties. At the time of its release, NASE 14 had average foliar incidence of <10% with limited root necrosis. Contrastingly, re-cycled NASE 14 on farmers' fields registered foliar incidence of up to 20%, particularly in disease hotspots. For the virus-indexed NASE 14, foliar incidences of up to 26% and root incidences of up to 75% were observed at Namulonge within a single year. This level of susceptibility highlights degeneration of NASE 14 due to CBSVs, with at least four species associated with CBSD confirmed as at the time of this writing (Ndunguru *et al.,* 2015). These finding further justify the need for phytosanitation (community-led) and the urgent need to develop varieties that express durable resistance to CBSD, an initiative that will require global alliance.

Working with schools to rapidly disseminate elite cassava varieties in endemic areas. Five factors have been identified as pivotal for sustaining access to clean cassava seed in the face of CBSD (McQuaid *et al.,* 2016). These factors include: 1) vector populations, which when high significantly increase disease pressure; 2) amount of disease in surrounding area, which when high increases disease pressure; 3) rouging practice, which if not optimally done, results into disease build up; 4) community field-based trainings, which if not done routinely, lead to increased disease pressure; and 5) promotion and/or development of seed multiplication innovations that increase quantity of planting materials generated per unit area. With that in mind, we worked with schools in Kaberamaido (a location associated with high disease pressure) to help with the dissemination of proven and elite cassava varieties to the nearby community. The datasets presented in Tables (1 and 2) and Figure 2 were the basis for selection of Kaberamaido.

Our forte for using the school platform was based on the premise that schools provide a foundation for educating future generations, which ensures sustainable transfer of knowledge and skills to the youth who will provide solutions to current and future challenges experienced by their communities where they reside. Results generated from this initiative will be used to guide strategies to utilize in other areas. For this to work, we acknowledge the promise of genetics and phytosanitation, mindful of their respective limitations. Thus, we propose a four-step process that would be desirable whenever new cassava varieties are being deployed. This proposed process is partly based on 1) our practice in the field, 2) published and unpublished datasets we have accumulated, 3) interactions with fellow scientists and farmers, and 4) literature review (Legg *et al.,* 2014; McQuaid *et al.,* 2016).

1) Step-1:Pre-basic seed production, where only virus-indexed and officially released varieties are micro-propagated at approved laboratories. A target of 100 to 500 tissue culture plantlets are desirable. Varieties that have degenerated in the field could be considered herein.

2) Step-2: Pre-basic seed bulking in screen houses, where 2-3 months tissue culture plantlets are cloned, i.e., used to generate at least two propagules that are hardened in the screenhouses. A target of 10,000 to 30,000 plantlets would be desirable depending on screen house space.

3) Step-3: Materials generated in step-2 are planted in the field to generate basic seed. The selected fields should be located in areas that are known to have low CBSD and CMD pressures. Such fields can be in non-traditional cassava growing areas or areas of low whitefly populations. Inspection at scheduled crop growth stages by qualified personnel is a prerequisite.

4) Step-4: Cassava stems generated in step-3 can be used to initiate a de-centralized certified seed production scheme that can be done in major cassava producing areas to enable easy access of planting materials by cassava farmers. Inspection

by qualified personnel as indicated in step-3 is a pre-requisite to ensure that all producers are certified. In addition, the producers should frequently undergo trainings on best practices. A cooperative arrangement should be advocated for.

Thus, the cassava stems we availed to Kaberamaido were sourced from cassava fields established in step-3. Accordingly, one variety (NAROCASS1) was established in Kaberamaido during the second rains of 2015, on a field ~0.5ha, as a joint activity with farmers following standard procedures (IITA, 1990). We selected this variety owing to its high level of resistance to CMD and tolerance to CBSD. In fact, this variety has superior yield attributes compared to NASE 3, which was predominant in the area and implicated in the continuous precedence of CMGs in the community for the last two decades. NAROCASS1 provides fresh root yields >25 t/ha with dry matter content >30%. This translates to approximately 7.5 t/ha of dry root yield, exceeding what would be obtained from NASE 3.

CBSD continues to be a major challenge in Uganda and thus the need for continued efforts to control the disease. The deployed varieties are only tolerant to CBSD (Kaweesi *et al.,* 2014), which means that phytosanitation is critical (McQuaid *et al.,* 2016). Practically, phytosanitation can be done in multiplication and/or cassava production fields by culling symptomatic plants at six months based on foliar symptoms, or at harvest, based on root necrosis (Kaweesi *et al.,* 2014). Such initiatives will certainly bring down the inoculum levels and subsequently lower the disease pressure, as better varieties are being developed and channeled through the proposed scheme. Therefore, we need to re-learn and also re-do an early-lesson learnt and later celebrated during the peak of the CMD epidemics of 1930s, and the initial outbreaks of CBSD in the 1940s (Jameson, 1964). During that time, phytosanitation was employed and it drastically reduced CMD losses and eradicated CBSD. What remains to be done are systematic and routine surveys to monitor the virus strains, their distribution and severity of symptoms they cause. This will inform decisions on control strategies and/or provide justification for other control strategies like use of genetic engineering. It is because of these threats that regional efforts by the International Institute of Tropical Agriculture (IITA) are currently evaluating the best-bet cassava genotypes from Uganda, Kenya, Mozambique, Tanzania and Malawi (all CBSD-affected areas), to identify outperforming clones for rapid dissemination. In Uganda, these evaluations are being done in seven locations including Kaberamaido and Pallisa. Thus, any outstanding clone will be taken through the proposed scheme and deployed in CBSD endemic regions to guarantee increased and sustained cassava productivity.

ACKNOWLEDGEMENT

We thank staff of the Root Crops Programme, National Crops Resources Research Institute (NaCRRI) who participated in data collection for the various multi-location cassava experiments undertaken during the period 1990 to 2014. We also thank Dr. Maruthi Gowda of the National Resources Research Institute, who helped with the virus indexing and generation of virus-free plantlets. Funds to undertake the multi-locational evaluations of the virus-indexed varieties were provided by IITA through the New Cassava Varieties and Clean Seed to Combat CBSD and CMD Project (5CP).

STATEMENT OF NO CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this paper.

REFERENCES

Abaca, A., Kawuki, R., Tukamuhabwa, P., Baguma, Y., Pariyo, A., Alicai, T., Omongo, C. A. and Bua, A. 2012. Evaluation of local and elite cassava genotypes for resistance to cassava brown streak disease. *Uganda Journal of Agronomy* 11 (3): 65-72.

Alicai, T., Ndunguru, J., Sseruwagi, P., Tairo, F., Okao-Okuja, G., Nanvubya, R., Kiiza, L., Kubatko, L., Kehoe, M. A. and Boykin, L. M. 2016. Cassava brown streak virus

has a rapidly evolving genome: implications for virus speciation, variability, diagnosis and host resistance. *Scientific Resports* 6 : 36164.

Alicai, T., Omongo, C. A., Maruthi, M. N., Hillocks, R. J., Baguma, Y., Kawuki, R., Bua, A., Otim-Nape, G. W. and Colvin, J. 2007. Re-emergence of cassava brown streak disease in Uganda. *Plant Disease* 91 (1): 24-29.

Bruyn, A., Harimalala, M., Zinga, I., Mabvakure, B. M., Hoareau, M., Ravigne, V., Walters, M., Reynaud, B., Varsani, A., Harkins, G. W., Martin, D., Lett, J. and Lefeuvre, P. 2016. Divergent evolutionary and epidemiological dynamics of cassava mosaic gemini viruses in Madagascar. *BMC Evolutionary Biology* 16 (1): 182.

Ceballos, H., Iglesias, C., Pérez, J. C. and Dixon, A. G. O. 2004. Cassava breeding: opportunities and challenges. *Plant Molecular Biology* 56 (4): 503-516.

FAOSTAT. 2016. Food and Agriculture Organization of the United Nations Statistics. Retrieved from http://data.fao.org/ref/262b79ca-279c-4517-93de-ee3b7c7cb553

Gondwe, F. M. T., Mahungu, N. M., Hillocks, R. J., Raya, M. D., Moyo, C. C., Soko, M. M. and Chipungu, F. B., Benesi, I. R. M. 2003. Economic losses experienced by small-scale farmers in Malawi due to cassava brown streak virus disease. pp. 28–35. In: Legg, J. P. and Hillocks, R. J. (Eds.), Cassava Brown Streak Virus Disease: Past, Present and Future UK: Aylesford.

Hahn, S. K., Terry, E. R. and Leuschner, K. 1980. Breeding cassava for resistance to cassava mosaic disease. *Euphytica* 29: 677-683.

Hahn, S. K., Terry, E. R., Leuschner, K., Akobunda, I. O., Okali, C. and Lal, R. 1979. Cassava improvement in Africa. *Field Crops Research* 2: 193-222.

International Institute of Tropical Agriculture (IITA). 1990. Cassava in Tropical Africa: A Reference Manual. Ibadan, Nigeria: International Institute of Tropical Agriculture.

Jameson, J. D. 1964. Cassava mosaic disease in Uganda. *East Africa Agriculture and Forestry Journal* 29 (3): 208-213.

Jennings, D. L. 1957. Further studies in breeding cassava for virus resistance. *East African Agricultural Journal* 22 (4): 213-219.

Katono, K., Alicai, T., Baguma, Y., Edema, R., Bua, A. and Omongo, C. A. 2015. Influence of host plant resistance and disease pressure on spread of cassava brown streak disease in Uganda. *American Journal of Experimental Agriculture* 7: 284-293.

Kaweesi, T., Kawuki, R., Kyaligonza, V., Baguma, Y., Tusiime, G. and Ferguson, M. E. 2014. Field evaluation of selected cassava genotypes for cassava brown streak disease based on symptom expression and virus load. *Virology Journal* 11 (1): 216.

Kawuki, R. S., Pariyo, A., Amuge, T., Nuwamanya, E., Ssemakula, G., Tumwesigye, S., Bua, A., Baguma, Y., Omongo, C., Alicai, T. and Orone, J. 2011. A breeding scheme for local adoption of cassava (*Manihot esculenta* Crantz). *Journal of Plant Breeding and Crop Science* 3 (7): 120-130.

Legg, J. P. and Fauquet, C. 2004. Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology* 56 (4): 585-599.

Legg, L., Somado, E. A., Barker, I., Beach, L., Ceballos, H., Cuellar, W., Elkhoury, W., Gerling, D., Helsen, J., Hershey, C., Jarvis, A., Kulakow, P., Kumar, L., Lorenzen, J., Lynam, J., McMahon, M., Maruthi, G., Miano, D., Mtunda, M., Natwuruhunga, P., Okogbenin, E., Pezo, P., Terry, E., Thiele, G., Thresh, M., Wadsworth, J., Walsh, S., Winter, S., Tohme, J. and Fauquet, F. 2014. A global alliance declaring war on cassava viruses in Africa. *Food Security* 6 (2): 231-248.

Maruthi, M. N., Hillocks, R. J., Mtunda, M., Raya, M. D., Muhanna, M., Kiozia, H., Rekha, A. R., Colvin, J. and Thresh, J. M. 2005. Transmission of Cassava brown streak virus by *Bemisia tabaci* (*Gennadius*). *Journal of Plant Pathology* 153 (5) 307–312.

Mbanzibwa, D. R., Tian, Y. P., Tugume, A. K., Mukasa, S. B., Tairo, F., Kyamanywa, S., Kullaya, A. and Valkonen, J. P. 2009. Genetically distinct strains of Cassava brown streak virus in the Lake Victoria basin and the

Indian Ocean coastal area of East Africa. *Archives of Virology* 154 (2): 353-359.

McQuaid, C. F., Sseruwagi, P., Pariyo, A. and Boscha, F. 2016. Cassava brown streak disease and the sustainability of a clean seed system. *Plant Pathology* 65 (2): 299-309.

Ndunguru, J., Sseruwagi, P., Tairo, F., Stomeo, F. and Maina, S. 2015. Correction: analyses of twelve new whole genome sequences of cassava brown streak viruses and Ugandan cassava brown streak viruses from East Africa: diversity, supercomputing and evidence for further speciation. *PLoS One* 10 (10): e0141939.

Nichols, R. F. W. 1947. Breeding cassava for virus resistance. *East African Agricultural Journal* 15: 154-160.

Olsen, K. M. and Schaal, B. A. 1999. Evidence on the origin of cassava: phylogeography of *Manihot esculenta. Proceedings of the National Academy of Sciences* 96 (10): 5586-5591.

Olsen, M. K. 2004. SNPs, SSRs and inferences on cassava's origin. *Plant Molecular Biology* 56 (4): 517-526.

Otim-Nape, G. W., Thresh, J. M., Bua, A., Baguma, Y. and Shaw, M. W. 1998. Temporal spread of cassava mosaic virus disease in a range of cassava cultivars in different agro-ecological regions of Uganda. *Annals of Applied Biology* 133 (3): 415-430.

Taylor, N. J., Halsey, M., Eliana, G. S. and Fauquet, C. M. 2012. Cassava for Africa. *GM Crops and Food* 3 (2): 93-103.

Wasswa, P., Alicai, T. and Mukasa, S. B. 2010. Optimization of in vitro techniques for cassava brown streak virus elimination from infected cassava clones. *African Crop Science Journal* 18 (4) 235 - 241.

Winter, S., Koerbler, M., Stein, B., Pietruszka, A., Paape, M. and Butgereitt, A. 2010. Analysis of cassava streak viruses reveals the presence of distinct virus species causing cassava brown streak disease in East Africa. *Journal of General Virology* 91 (5): 1365-1372.