### Research Application Summary

## Advances in cassava research in management of Cassava Brown Streak Virus Disease in Uganda

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### **Abstract**

Cassava is an important food crop in Africa where it is affected by two main virus diseases, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). CMD occurs in all the cassava-growing countries on the continent and it has been much researched on. CBSD occurs mainly along the East African coastal areas, and although the disease was first reported in 1936, it started receiving more research attention only after 2004 following its re-emergency as a devastating disease in the East African region. Recognising the gravity of the threat, a proactive programme was initiated at Makerere during 2005 under the BIOEARN Programme to bridge the knowledge gaps in order to combat the disease and stabilise production of this important food crop. Activities focused on identification and diversity of CBSV, developing diagnostic tools for the virus, using conventional and genetic transformation methods for developing CBSD-resistant varieties, and developing an array of integrated pest and crop management options suited for small-holder agriculture. Tissue culture applications were also sought in the areas of rapid multiplication of new varieties, low cost multiplication protocols, virus elimination and regeneration protocols for genetic engineering. Recent surveys in Uganda indicate re-emergency of CBSD with worrying food security and economic consequences. An important feature of CBSD in Uganda is that incidence is highest and severity greatest in CMD-resistant varieties that are being promoted for the management of the CMD pandemic. In view of the present importance of CBSD in Uganda, it is timely to develop a strategy to manage this devastating disease of cassava. This paper highlights the major advances in research towards management of CBSD in Uganda and in the East African region.

Key words: BIOERN, Brown streak, Cassava, East Africa, Uganda

Résumé

Le manioc est une culture vivrière importante en Afrique où elle est affectée par deux maladies virales principales, la maladie

de la mosaïque du manioc (CMD) et la maladie des stries brunes (CBSD). Le CMD apparait dans tous les pays du continent qui produise le manioc. Beaucoup de recherches ont déjà été effectuée sur ce phénomène. Le CBSD apparait principalement le long des zones côtières de l'Afrique de l'Est, et bien que la maladie a été signalée pour la première en 1936, il a commencé à recevoir une attention de recherche seulement après 2004 suite à sa réapparition urgence comme une maladie dévastatrice dans la région de l'Afrique de l'Est. Reconnaissant la gravité de la menace, un programme proactif a été initié au Makerere en 2005 au titre du Programme BIOEARN pour combler les lacunes dans les connaissances afin de lutter contre la maladie et de stabiliser la production de cette importante culture vivrière. Les activités ont porté sur l'identification et la diversité des CBSV, le développement d'outils de diagnostic pour le virus, en utilisant des méthodes conventionnelles de transformation génétique pour développer les variétés résistantes au CBSD, et développe une gamme de lutte antiparasitaire intégrée et les options de gestion des cultures adaptée aux petites exploitations agricoles. La culture de tissus a également été recherchée dans les domaines de la multiplication rapide de nouvelles variétés à faible coût protocoles de multiplication, l'élimination du virus et des protocoles de régénération pour le génie génétique. Des études récentes en Ouganda indiquent résurgence de CBSD avec des conséquences inquiétantes sur la sécurité alimentaire et économique. Une caractéristique importante de CBSD en Ouganda est que l'incidence est la plus élevée et la plus grande sévérité dans les variétés résistantes CMD, qui sont promues pour la gestion de la pandémie de CMD. Compte tenu de l'importance actuelle des CBSD en Ouganda, il est opportun d'élaborer une stratégie pour gérer cette maladie dévastatrice du manioc. Ce document souligne les avancées majeures dans la recherche en matière de gestion des CBSD en Ouganda et dans la région de l'Afrique de l'Est.

Mots clés: BIOERN, strie brunes, manioc, Afrique orientale ou Afrique de l'Est, l'Ouganda

Cassava is one of the most important food staple crops for

**Background** 

more than 200 million people in East and Central Africa. Human population growth rates in this sub-region continue to be one of the highest in the world, and consequently there is an urgent need to match this growth with concomitant increases in food production, using one of the most robust crops in terms of resilience to climate variability The crop was prioritised by the

New Partnership for African Development (NEPAD) as one of the crops to combat poverty and food and nutrition insecurity in Africa, and is a high priority commodity in the Uganda national research and development agenda.

However, cassava production in the region and in Uganda is restricted by a diverse set of constraints. The most economically important are the two virus diseases: cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). Both have been recognised in the region since 1930s, but have become increasingly damaging in recent years. CMD is caused by viruses of the family Geminiviridae: genus Begomovirus, referred to collectively as cassava mosaic geminiviruses (CMGs). Nine species are currently recognised, of which eight have been reported from Africa. CMD occurs wherever cassava is grown in Africa, from Senegal in the north-west to Mozambique in the south-east, as well as on the off-shore islands of Madagascar, Mauritius, Seychelles, Zanzibar and Cape Verde. The biology of the CMGs has been the subject of much study since the early 1990s. Key areas of interest included: molecular characterisation, vector transmission, field and regional-level epidemiology, resistance breeding and management.

The re-emergency of CBSD after 2002 led to even more devastating consequences. Our recent studies show that CBSD is caused by at least 2 distinct ipomoviruses. The most prevalent virus in the coastal and lowland areas of east Africa retained the name CBSV while the second virus was named UCBSV. These CBSD associated viruses are gaining in severity, threatening food and livelihood securities for millions of farmers and cassava consumers in region. CBSD causes a dry necrotic rot in the storage roots, leading either to decimating yields and/or significant reductions in quality. Current estimates show that CBSD causes economic losses in excess of \$100 million annually.

Recognising the gravity of the threat, a proactive programme was initiated at Makerere during 2005 under the BIOEARN Programme to bridge the knowledge gaps in order to combat the disease and stabilise production of this important food crop. Activities focused on identification and diversity of CBSV, developing diagnostic tools for the virus, using conventional and genetic transformation methods for developing high-yielding, CBSD-resistant varieties, and developing an array of integrated

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pest and crop management options suited for small-holder agriculture. Tissue culture applications were also sought in the areas of rapid multiplication of new varieties, low cost multiplication protocols, virus elimination and regeneration protocols for genetic engineering.

# Current Status of CBSD in Uganda

Cassava brown streak disease (CBSD) was first described in Tanzania during the 1930s (Storey, 1936). Unlike Cassava mosaic disease (CMD) which is widely distributed wherever cassava is grown in Africa, CBSD was restricted mostly along the East African coastal cassava-growing regions and hence considered for a long time as a low altitude (< 1000 masl) problem. Nicholas (1950) reported that the disease was endemic in all East African coastal cassava growing areas from the north-east border of Kenya to southern Tanzania and Zanzibar (Thresh and Mbwana, 1998) and that was widespread at lower altitudes in Malawi.

In Uganda, CBSD was first reported in 1945 on materials imported from the then East African Cassava Breeding Station at Amani in Tanzania. The materials were imported to control the CMD epidemic of the 1930's and 1940's (Storey 1936; Jennings 1957; Jameson 1964). The affected crops at Bukalasa were destroyed and the intensive eradication programmes fully controlled the disease. The problem was not noticed until 1994 when symptoms typical of CBSD were observed in a field near Entebbe on the northern shores of Lake Victoria. A decade later symptoms of CBSD were again observed on cassava in some locations in central Uganda; this time in relatively higher incidences, albeit localised, on some of the popular CMDresistant varieties being grown countywide by farmers. This caused serious concern as cassava production in the country had just been restored through use of CMD-resistant varieties following devastation by the severe CMD epidemic in the 1990s. The emergency of CBSD in the high altitude areas led to proposing the possibility of having a new strain(s) of the CBSV or due a general replacement of tolerant varieties with more susceptible ones following deployment of varieties in the fight against CMD. There was therefore need to establish the prevalence of the disease in the country and the reason for increased severity and/or spread in order to institute corresponding mitigation strategies.

Subsequent surveys indicated that the disease spread to 25 districts within the country. In the districts of Mukono, Wakiso and Luwero, CBSD occurs at a high incidence and reached

epidemic proportions averaging 60%. An important feature of CBSD in Uganda is that incidence is highest and severity greatest in CMD-resistant varieties that are being promoted for the management of the CMD pandemic. This is most prominent for the two varieties TME 14 and TME 204 that have proved to be highly popular with farmers, and have therefore spread very rapidly within and between farming communities. In fact, contrasting levels of susceptibility of cassava genotypes to CBSV and CMGs have been observed. Genotypes that are highly tolerant or resistant to CMGs were observed to be highly susceptible to CBSD and vice versa. CBSD therefore poses a new threat to the livelihoods of millions of Ugandans who had recovered from the devastating effects of the CMD. This disease threat translates into food insecurity and loss of incomes for cassava farmers in the affected areas.

Epidemiology of CBSD

Recent surveys in Uganda indicate re-emergency of CBSD with worrying food security and economic consequences. An important feature of CBSD in Uganda is that incidence is highest and severity greatest in CMD-resistant varieties that are being promoted for the management of the CMD pandemic. This is most prominent for the two varieties TME 14 and TME 204 that have proved to be highly popular with farmers, and have therefore spread very rapidly within and between farming communities. Importantly, CBSD symptoms can also now be seen in diverse local cultivars, although in this case symptoms appear to be less severe. It also seems likely this 'new' spread of the virus causing CBSD is being enhanced by the superabundance of the whitefly vector, Bemisia tabaci, a phenomenon that was also associated with the CMD pandemic. This suggests that all parts of Uganda already affected by the CMD pandemic with concomitant super-abundant whitefly populations are vulnerable to CBSD spread. In view of the present importance of CBSD in Uganda, it is timely to develop a strategy to manage this devastating disease of cassava.

In several countries, however, where CBSD is prevalent, it is apparent that much use of CBSD infected planting material is an effective means of perpetuating and disseminating the disease (Jennings, 1957). Also Storey (1936) demonstrated that CDSD was graft-transmissible and that, cuttings from affected plants invariably gave rise to plants showing CBSD symptoms. Mechanical transmission by use of sap is also possible but this is easier from cassava to a number of herbaceous hosts than between cassava plants (Lister, 1959).

There is evidence, however, of natural spread between plants as clones introduced from West Africa or other areas that are free from CBSD have become infected when grown at sites in Mozambique, Malawi, Kenya and Tanzania (Bock, 1994). Plants raised from seed introduced from West Africa have also become infected at these sites. Storey (1936) speculated that this natural spread was most likely to be mediated by the whitefly (*Bemisia species*). Although, however, until recent there were only a few transmission experiments that were conducted, transmission of CBSD was unsuccessful with the whitefly Bemisia tabaci and with six species of aphids in an experiment conducted in Kenya (Lennon *et al.*, 1986).

Also from experiments conducted by Hillocks et al. (2001) in Tanzania with diseased and disease free planting stocks of a range of local CBSD susceptible varieties and locations, it was revealed that the peak of the disease corresponded with the period of peak whitefly numbers. All this circumstantial evidence seems to suggest that B. tabaci and B. afer may indeed be the vectors, but that conditions used so far in transmission studies have not been appropriate to demonstrate this (Maruthi et al., 2005). In some transmission experiments conducted in Tanzania, transmissions were achieved using B. tabaci, but symptoms appeared in only one test plant on each of two repeated experiments. More recent transmission studies show that like CMGs, CBSV is transmitted by B tabaci and via the stem cuttings that are often used for replanting without any quality check. Recent cage transmission experiments and field surveys show that transmission of CBSV is sporadic However, rate of transmission was low (maximum 22%) even when using high whitefly numbers of up to 120 per target plant. Spread of CBSD in the field coincided with increases in whitefly numbers; further supporting the evidence that B. tabaci is a vector of CBSV. CBSV is sporadically spread by B. tabaci at a seemingly low rate and given the incidence and prevalence level of CBSV in low and high altitude areas, suggests planting material as key vehicle in the spread of the virus.

Prior to 2004, CBSD had never been recorded at high incidence above 1000 masl., and was primarily known as a disease of the lowland cassava-growing areas of East Africa, including the shores of Lake Malawi. However, from late 2004 onwards it became apparent that CBSD was becoming more and more widespread in parts of south central Uganda (Alicai *et al.*, 2007). IITA and Uganda's National Agricultural Research Organisation

conducted a survey of cassava pests and diseases in January 2007 which incorporated an assessment of CBSD leaf symptoms for all of the 493 fields sampled through 26 of the most important cassava-growing districts in the country. CBSD was recorded from 40 of the fields, distributed virtually throughout the country, but with greatest incidence in south, central areas, corresponding to the area in which cultivation of TME and TME-like varieties is most widespread. The national cassava program work indicated that more than 30 districts are attacked (Alicai *et al.*, 2007).

## **Economic Importance** of CBSD

Cassava is a major staple food and income generating crop in Uganda. It is the most important root crop and accounts for 11% of all crops grown in the country and 3,833,485 households (74.8%) of all households in Uganda are directly involved in Agriculture (Uganda National Household Survey, 2005). Uganda is ranked tenth among the world's leading cassava producers and in Africa it is ranked sixth with approximately 5.4 million tonnes of fresh storage roots (tubers) produced annually (FAO 2002). Cassava is particularly popular because of the ease to cultivate it, low input requirement, and tolerance to low rainfall and poor soils and easily propagated through stem cuttings.

The use of stem cuttings, as a means of propagating cassava exposes the crop to the risk of virus accumulation. Vegetative propagation tends to result in perpetuation and build up of virus infection to high levels in the field if the propagation material is not indexed and may act as source of inoculum. The foliar symptoms of CBSD are less conspicuous and farmers are often unaware of the problem until the crop is harvested and symptoms on the storage tubers become evident. Such root tubers are rendered unfit for human consumption. This severely limits utilisation of cassava, as the disease affects the most economic part of the plant. Therefore, CBSD is a more important cause of food insecurity than was previously believed because the root damage does not become apparent until the crop is harvested. The July 2003 issue of 'CgiarNews' reported that losses due to CBSD were over US\$ 100 million in the first few months of the outbreak.

Records from Mukono district agricultural office indicate that there are 87,000 acres of land under cassava cultivation. Of this acreage, 30% is affected by the disease and since an acre of cassava fetches 1 million Uganda Shillings, the 30% infected fields are costing Mukono district alone 26 billion in lost revenue.

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This translates into 460 billion shillings for the 20 affected districts if the level of damage and acreage are averaged across the affected districts. CBSD has continued to spread with increasing economic losses. This put more pressure on the National Cassava Programme of NARO to constantly breed and rapidly develop new varieties that are resistant to CBSD.

## Molecular Detection, and Diversity studies

Until 2008, there was a paucity of sequence information for CBSV. Using RT-PCR technique, the complete genomes of two genetically distinct isolates (MLB3 and KOR6) were cloned and sequenced. Also the 3' proximal region of 16 isolates was sequenced. Additional sequences were retrieved from validated sequence databases and analysed using modern bioinformatics tools. The identity of complete genomes of MLB3 and KOR6 isolates obtained in this study were compared with other nine complete genomes that were from other laboratories ranged between 69.0-99.3% nt and 73.6-99.4% aa identities, respectively. At CP level, comparison between all isolates showed identities that varied from 70.1 to 99.8% and 75.6 to 99.7% at nt and aa levels, respectively. A neighbour joining tree for non-recombinant CP sequences revealed two phylogenetic groups that were supported by high bootstrap values (100%), suggesting distinct species. For one group the old name CBSV (Isolate KOR6) has been retained whereas for the second group the name Ugandan cassava brown streak virus (UCBSV; Isolate MLB3) was proposed. The CBSV group constitutes isolates that are predominately found in the lowlands (<1000 masl) while the UCBSV isolates are more commonly found in the high altitude (>1000 masl) areas of East Africa.

Interestingly, the CBSV and UCBSV genomes contained a Maf/HAM1-like sequence (HAM1h) (678 nt; 226 aa) recombined between the replicase (RNA polymerase) and CP domains in the 3'-proximal part of the genome, which is known to be highly conserved in the family *Potyviridae* (Mbanzibwa *et al.*, 2009b). The HAM1 is wide spread in cellular organisms such as plants, fish and animals and man. Homology of HAM1 of these two viruses with cellular Maf/HAM1 pyrophosphatases suggests that it may intercept noncanonical nucleoside triphosphates to reduce mutagenesis of viral RNA. However, its function in the biology of the virus has not been fully elucidated.

Using the accumulated sequence data for the two viruses, an RT-PCR based diagnostic tool was developed. The tool can be used for simultaneous detection of single and mixed infections

of CBSV and UCBSV. Employing this diagnostic tool, it was established that the two viruses co-infect cassava in East Africa and co-infection were as high as 67-100 % in some places around the Lake Victoria.

In general, these studies contribute to the understanding of the genetic diversity and structure of CBSD associated viruses in East Africa and general evolution of viruses in the family *Potyviridae*. Genetic diversity studies have have clearly demonstrated the occurrence of two distinct viruses that are associated with CBSD, information that was hitherto not been apparent. The information generated will also benefit as well as guiding CBSD resistance breeding work.

Cassava Tissue Culture and Transformation Research

Vigorous cassava tissue culture research was initiated at Makerere University Plant tissue Culture Laboratory at Kabanyolo and National Crops Resources Research Institute (NaCRRI) during 2005 and 2008, respectively. Key research areas focused on rapid multiplication of new varieties, low cost multiplication protocols, virus elimination and regeneration protocols for genetic engineering. Successful micro-propagation depends on the kind of culture to be initiated, the purpose of the proposed culture and the plant species to be used. Cassava micro-propagation uses nodal cultures, where stem pieces carrying single or multiple nodes are utilised. Rapid in vitro multiplication protocols have been optimised for a number of existing varieties (Wasswa et al., 2011). However, given the fact that often there are salient differences in in vitro variety responses, there is need to continuously optimise protocols for rapid multiplication. Currently, were are optimising in vitro protocols for the new, high yielding and CBSD tolerant varieties namely NAM 130, 72-TME 14 and NASE 14 (MH96/4271).

We have been able to optimise in vitro techniques for CBSV elimination from infected Ugandan farmer preferred cassava cultivars. The best plantlet growth in terms of height was observed on MS medium supplemented with 0.5 mg l-1 BAP and 0.1 mg l-1 2,4-D. Highest CBSV elimination efficiency of 40%, with 49% plantlet survival was observed at 36 °C for 8 hours darkness and 40 °C for 16 hours light. These results indicate that in vitro techniques can greatly enhance CBSV elimination and, thus, provide a means of CBSD management through dissemination and conservation of popular but CBSD susceptible cultivars. Because cassava is mostly grwn by subsistence and resource poor farmers, in vitro protocols ought

to be cost effective in order to make the cost of tissue culture derived planting material affordable to the resource poor farmers.

Somatic embryogenesis is useful for regeneration and genetic transformation of cassava because it produces the target tissue required for gene (DNA) insertion. However, the initiation of somatic embryos (SE) in cassava is reported to be facilitated by the presence of auxins, reduced forms of nitrogen and carbon, given the appropriate explant, culture media and environmental conditions are in place (Taylor et al., 2004). Generation of embryogenic callus is a key step in genetic engineering of many crop species, including cassava. Protocols for generation of friable embryogenic callus (FEC) have been lacking for Ugandan cassava genotypes, thereby delaying their genetic engineering for agronomic and other desirable traits. Work at NaCRRI and in collaboration with DDPSC led to to determining conditions suitable for production and regeneration of FEC in the Ugandan cassava genotypes; Aladu and Ebwanateraka, and control cultivar 60444. Immature leaf lobe explants were established on Murashige and Skoog (MS) based media for initiation of organised embryogenic callus (OES). To produce FEC, resulting OES were established on Gresshoff and Doy based callus induction media with varying levels of sucrose, maltose, tyrosine, tryptophan, naphthalene acetic acid (NAA) under light and dark conditions. Subsequently, FEC was subcultured to MS-based embryo maturation and embryo regeneration media. The amino acid tyrosine favoured production of FEC in Aladu and Ebwanatereka, but not in 60444, while 20 g/L of sucrose trigged production of FEC in Aladu and 60444, but 40 g/L of sucrose was superior for Ebwanatereka. Media supplemented with 1 ml/L naphthalene acetic acid NAA facilitated embryo regeneration in Ebwanatereka and 60444, while Aladu responded better to 5 ml/L NAA. Light, tyrosine and sucrose were essential for FEC production in Uganda cultivars while NAA was required for regeneration of somatic embryos.

Progress has also been registered in developing artificial microRNAs and cassava brown streak disease resistance in *N. benthamiana*, as an alternative method to the hairpin (RNAi) mediated resistance to the CBSD. About 100 TME 204 cassava transgenic lines expressing siRNA derived from CBSV and UCBSV coat protein each individually in collaboration with the DPSC and NaCRRI have been developed. Otherwise, 2 coat

protein sequences from UCBSV and CBSV for the TME204 cassava product, resistant to both viruses have been fused. Challenging the plants with CBSV and UCBSV so far has showed good resistance to both. Other genomic regions, like the 3' UTR, and do not seem to yield much siRNA, and therefore not a good target for either viruses.

## Management of CBSD

The intensive monoculture of crop plants today invites the epidemic spread of many diseases and pests, among the most important of which are viruses. Rapid vegetative propagation and a flourishing movement of planting material have made matters worse. There are at present no practical treatments to cure virus-infected plants once they are set out in the field. The production and distribution of virus-free propagating material has proved highly successful in controlling virus diseases in many crops, and can be of wider application in management of cassava viral diseases, including CBSD. The strategy of control through virus-free stock aims to dilute the amount of virus infection by the supply of large quantities of healthy planting material. Inevitably, this material will sooner or later become infected and is regarded as expendable; to be replaced as soon as noticeable deterioration occurs. The key lies in maintaining the foundation mother stock isolated, or otherwise fully protected against re-infection, so that only the progeny propagated from it are exposed to the hazards of ordinary cultivation. Phytosanitary programmes require (i) proper diagnosis of CBSD, (ii) reliable and affordable virus indexing methods, (ii) appropriate techniques for virus elimination that can be employed whether no healthy material is found for farmer preferred cultivars, and (iv) establishment of virus indexed foundation stocks for distribution to farmers.

The benefits of selecting of CBSD-free stems when replanting were clearly demonstrated and recommended (Storey, 1936; Hillocks *et al.*, 2001) although advocating a phytosanitation programme to farmers has some drawbacks including the need for major educational and training input For experimental purposes, planting material free of CMD were obtained from infected plants using heat treatment (Kaiser and Teember, 1989). A combination of meristem tip culture and heat treatment together with appropriate virus indexing techniques can effectively result into cassava planting material free of CBSD (Wasswa *et al.*, 2011). Farmers can also been introduced to the control of CBSD through phytosanitation e.g. it may be worth while roguing the infected individual. Roguing was used

in Uganda and effectively worked to rid the country of CBSD when it had been introduced in planting material from Tanzania (Jameson, 1964). The method was also successful in Tanzania when producing symptomless breeding stocks from populations that were originally showing symptoms of CBSD (Mtunda *et al.*, 1998).

Also, a key facet of CBSD management, given its apparently restricted distribution, will be the prevention of movement between countries and region through the implementation of strict quarantine procedures. It is therefore critical that movements of germplasm in vegetative form should be strictly controlled (through meristem tip culture and thermotherapy) and that virus indexing laboratories that test tissue culture material prior to export are fully equipped for CBSV diagnosis.

Another attempt for CBSD management is the use of resistant varieties. Selection for resistance to CBSD began at Amani in northern Tanzania as far as 1937, where resistance was transferred from cassava related species, such as, Manihot glaziovii Muell-Arg, M. dichotoma Ule, M. catingae Ule and 'tree' cassava, believed to be a natural hybrid between M. esculenta Crantz and M. glaziovii Muell-Arg (Nichols, 1947; Jennings, 1957). Also, a few cassava cultivars, such as, cv. Macaxiera Aipin are also resistant to CBSD. Other two shrublike species M. saxicola and M. melanobasis are also highly resident to CBSD but their roots contain high concentration of hydrocyanic acid. Also conventional breeding for resistance to CBSD requires too much time, with each generation taking not less than 3 years and a series of backcrosses are needed to remove the undesirable characteristics, such as, tree like characteristics and high cyanide level, while retaining resistance to CBSD (Jennings, 1957). Nonetheless, varieties have been identified in southern Tanzania and Malawi which are either resistant to infection by CBSV or express very mild symptoms which do not have an effect on yield. No information is currently available on mechanisms of resistance, and there has as yet been no major effort to use conventional breeding approaches to develop CBSV-resistant germplasm. However, preliminary screening by NARO raises hope of identifying resistant varieties. The only hope lies in the fact that some few varieties such as NASE 3 and two pre-release varieties; 2961 and 0067 commonly called "Akena" seem to be tolerant or escape the disease. Therefore with more research a suitable resistant cultivar may be developed.

Therefore, the relative merits of any approach compared to others need to be evaluated. It seems that resistant and tolerant varieties together with field sanitation and use of disease free planting material may all have an important role to play in managing CBSD, but considerable research remains to be done on the conditions under which each is most appropriate and on how best to combine them into an integrated strategy.

# **Seed Multiplication and Delivery Systems**

Accessibility to improved cassava planting material still remains a problem in most African countries. This is mainly attributed to the absence of an effective distribution system of improved crops, technical expertise, and funding (Roy-Macauley, 2002). Lack of well organised seed distribution system for vegetatively propagated crops in East Africa has contributed, partly, to the slow rate of dissemination of new improved varieties. New varieties are traditionally disseminated through farmer to farmer variety exchange and limited sale of cuttings in market at the onset of rains. Besides the slow rate of dissemination of new varieties, lack of well organised seed distribution systems also results in spread and intensification of viral diseases in vegetatively propagated plants.

Multiplication and deployment of improved varieties will necessitate development high throughput seed delivery systems achieved through a participatory approach involving key stakeholders in a public-private partnership. This is also need to create increased awareness and training in a bid to accelerate uptake and promotion. Among the prerequisites for establishment of a well organised seed system are: i) availability of improved seed (foundation seed, basic seed), (ii) protocols for rapid multiplication of disease free planting materials, (iii) protocols for cleaning planting materials, (iv) well trained personnel on rapid multiplication and their maintenance, and (v) perhaps most important, there must be a well defined and institutionalised private/public partnership that is self sustaining to maintain primary, and secondary nurseries and deliver the seed to the farmers.

For optimum harnessing of the tissue culture in production of quality planting materials, there is need to develop agronomic packages for screenhouse/nursery handling of the plantlets. This will involve consideration for hardening and weaning of cassava plantlets. This is the most delicate step constraining mass propagation of clean cassava, because of the high mortality of the plantlets that is registered during this stage of transfer to

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soil. Beyond weaning, procedures for delivery of hardened small micropropagated plants to nurseries and their establishment requirement need to be evaluated. This will include developing low cost alternative substitutes, developing field level diagnostic tools and devising environment friendly and suitable packaging materials.

## **Capacity Building**

With support from development partners and government, some physical infrastructure and human capacity have been strengthened and in some cases put in place in Uganda. These include new human capacity to develop new varieties, tissue culture and virus indexing laboratories, and sreenhouse both at Makerere and NaCRRI. Most of the research activities at Makerere have been carried out as graduate research topics at MS and PhD levels. To this effect, several MSc and PhD students have been trained in various disciplines of cassava research including molecular biology, virology, genetic transformation, and tissue culture and seed systems.

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