RUFORUM Working Document Series (ISSN 1607-9345), 2018, No. 17 (1): 739-746.
Available from *http://repository.ruforum.org*

Research Application Summary

Trends in cassava brown streak disease diagnostics for certification of healthy cassava planting materials and future perspectives: A review

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Abstract

Cassava (*Manihot esculenta* Crantz) is important for food security and livelihoods of more than 500 million people in sub-Saharan Africa particularly for poor subsistence farmers. However, its productivity is hampered by cassava brown streak disease (CBSD), the most devastating viral disease of cassava in East and Central Africa. Cassava brown streak disease (CBSD) often leads to necrosis of storage roots making them unfit for consumption and unsuitable for industrial processing. The disease has been reported to be caused by two species of ipomoviruses; Cassava brown streak virus (CBSV) and Ugandan Cassava brown streak virus (UCBSV) which belong to the Potyviridae family. Cassava brown streak disease is reported to be transmitted by whitefly vectors and disseminated over long distances through farmers' exchange of infected planting materials and across the borders through trade. This poses great risk to international and domestic exchange of cassava germplasm. Many cassava producing countries in Africa including Kenya have inadequate mechanism for adoption of improved varieties as well as lack cassava seed certification systems. Virus indexing is very key to ensure that only the plantlets that are disease-free are deployed in the field for further multiplication. The rapidly increasing spread of CBSD to new cassava-growing regions in Africa and the cases of re-emergence of CBSD is a real threat to food security. Recent studies have revealed high sequence diversity of CBSV virus strains. This variability makes diagnostics difficult and there is therefore a need for development of more robust and broad spectrum diagnostic tools for rapid detection of the CBSV virus strains in order to inform implementation of effective control measures. These tools would also be utilized for accurate detection of the viruses to improve certification of cassava planting material in greenhouse and field multiplication. This review provides insights into the trends in cassava brown streak disease diagnostics for certification of healthy cassava planting materials as well as future perspectives in strengthening cassava seed certification. Future research needs and novel genomic approaches will provide basis for improving the existing molecular diagnostic tools or development of new ones suitable for detection of newly discovered virus strains. This will also offer potential to overcome some of the drawback of the current control strategies of the disease.

Key words: Cassava brown streak disease, genomic approaches, seed certification, sequence diversity, virus detection

Résumé

Le manioc (*Manihot esculenta* Crantz) est important pour la sécurité alimentaire et les moyens de subsistance de plus de 500 millions de personnes en Afrique subsaharienne, en particulier pour les pauvres petits agriculteurs. Cependant, sa productivité est entravée par la maladie des stries brunes du manioc (MSBM), la maladie virale la plus dévastatrice du manioc en Afrique orientale et centrale. La maladie des stries brunes du manioc (SBM) entraîne souvent la nécrose des racines de stockage, les rendant impropres à la consommation et impropres à la transformation industrielle. Il a été signalé que la maladie était causée par deux espèces d'ipomovirus; Virus de la striure brune du manioc (VSBM) et Virus ougandais de la striure brune du manioc (VUSBM) qui appartiennent à la famille des Potyviridae. On signale que la maladie des stries brunes du manioc est transmise par les vecteurs de l'aleurode et disséminée sur de longues distances grâce à l'échange d'agriculteurs de matériel végétal infecté et à travers les frontières par le biais du commerce. Cela représente un grand risque pour les échanges internationaux et nationaux de matériel génétique de manioc. De nombreux pays africains producteurs de manioc, dont le Kenya, ne disposent pas de mécanismes adéquats pour l'adoption de variétés améliorées et manquent de systèmes de certification des semences de manioc. L'indexation des virus est essentielle pour garantir que seules les plantules exemptes de maladies sont déployées sur le terrain pour une multiplication ultérieure. La propagation rapidement croissante du MSBM dans les nouvelles régions productrices de manioc en Afrique et les cas de réémergence du MSBM constituent une menace réelle pour la sécurité alimentaire. Des études récentes ont révélé une grande diversité de séquences de souches de virus SBM. Cette variabilité rend le diagnostic difficile et il est donc nécessaire de développer des outils de diagnostic plus robustes et à large spectre pour la détection rapide des souches du virus SBM afin de guider la mise en œuvre de mesures de contrôle efficaces. Ces outils seraient également utilisés pour une détection précise des virus afin d'améliorer la certification du matériel de plantation de manioc en multiplication en serre et au champ. Cette revue donne un aperçu des tendances dans les diagnostics de la maladie des stries brunes du manioc pour la certification des matériels de plantation de manioc sains ainsi que des perspectives futures pour renforcer la certification des semences de manioc. Les futurs besoins de recherche et les nouvelles approches génomiques fourniront une base pour améliorer les outils de diagnostic moléculaire existants ou en développer de nouveaux adaptés à la détection de souches virales nouvellement découvertes. Cela permettra également de surmonter certains des inconvénients des stratégies actuelles de lutte contre la maladie.

Mots-clés: maladie des stries brunes du manioc, approches génomiques, certification des semences, diversité des séquences, détection des virus

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Introduction

Cassava (*Manihot esculenta* Crantz) is a major staple root crop in the world and offers a great potential for food security. It has long been used as a famine reserve and food security crop produced mainly through smallholder subsistence farming systems. Low input use, rudimentary technology, high post-harvest losses and minimal processing characterize these farmers. The crop is a great source of income for many poor households. About 90%

of cassava roots produced is used as food. The roots are a rich source of carbohydrates of which starch constitute 31% of fresh weight. Cassava can be grown under a wide range of Agro-Ecological Zones and is a drought tolerant and water-use-efficient crop, able to grow in marginal environments with erratic rainfall, poor soils and under low intensity management, and has a flexible harvest period (Burns *et al*., 2010; FAO, 2013). The potential of cassava production and utilization has been highly impacted by both biotic and abiotic constraints. Diseases caused by viruses especially cassava brown streak disease and cassava mosaic disease are the most damaging biotic stress in cassava production. In East and central Africa, yield losses of more than >$1 billion every year have been reported (Legg *et al*., 2006; Legg *et al*., 2011; Patil *et al*., 2015). Cassava brown streak disease (CBSD) is transmitted by the whitefly vector (*Bemisia tabaci*) and are disseminated through use of infected planting materials. Use of infected planting materials is the common source of virus inoculum and dissemination of viral diseases in farmers' fields. There is therefore need to strengthen cassava seed certification schemes to curb the CBSD problem to improve the livelihoods of millions of resource-poor farmers depending on this crop.

Existing cassava seed systems in East Africa. Clean seed systems is critical for the effective management of cassava brown streak disease. However, access to quality seed by farmers requires proper understanding of a functioning seed system. In most East African countries where cassava brown streak disease is a major problem, cassava seed certification systems are largely informal. This is characterized by farmers exchanging planting materials or recycling own cassava cuttings every year. In such a system, the dissemination of viral diseases is common especially for vegetatively propagated crops. Farmers plant and saturate their fields with a mixture of local cassava varieties which are low-yielding and highly susceptible to diseases. This makes adoption of new/improved varieties a challenge. The mixture of different varieties with different adaptation potential to environmental conditions including disease pressure makes it difficult for the farmers to meet the quality standards required by the processors who are usually interested in a certain variety and not a mixture of varieties. The practice of exchanging planting material by farmers further leads to spread of the disease. Therefore, there is a need to devise a mechanism for adoption of improved cassava varieties through establishing a sustainable seed multiplication and distribution system The multiplication sites need to be established in areas where there is low disease pressure and materials must be virus indexed using sensitive diagnostic tools to reduce the effects of disease in cassava production (McQueen *et al.,* 2015).

A formal seed system is a well constructed system that involves chain of activities leading to certified seed (Louwaas, 2012). Intensive awareness on importance of certified planting materials is important for sustainable implementation. This will create enabling environment and attract private investment and development of seed business as well as sustainable system to supply adequate quality, disease-free cassava planting materials. Once certified materials have been distributed, farmers must be trained thoroughly on the identification of disease symptoms to enable sufficient rouging to reduce spread of CBSD in fields (McQuaid *et al.,* 2015; Legg *et al.,* 2017). Ensuring freedom of diseases through virus indexing in planting materials is central to any voluntary or mandatory certification scheme for production of healthy cassava planting material.

Current methods applied for diagnosis of viruses causing cassava brown streak disease. Plant diseases caused by viruses are among the major constraints of any plant health and seed system. Accurate identification and characterization of the causal agents of these diseases especially in the ever increasing cases of emerging diseases is fundamental for front line diagnosis and supports

implementation of pest management strategies. In the case of detection of viruses causing cassava brown streak disease, virus-indexing is key in supporting cassava seed certification systems as well as for quarantine regulation to prevent entry and spread of the disease during international cassava germplasm exchange (Legg *et al*., 2011; McQuaid *et al*., 2017). Virus indexing using highly sensitive diagnostic techniques before the materials undergo further multiplication is vital to ensure that all cassava planting materials are free from CBSVs before dissemination (Arbashi *et al*., 2010; Mwangangi *et al*., 2014). Several methods have been in the past and/or are presently applied in diagnosis of cassava brown streak disease. In this review, such methods are discussed, highlighting the opportunities as well as the challenges of such methods, with focus on the future perspectives to support cassava clean seed system.

Symptomatology: diagnosis based on symptoms. The differentiation of plant viruses using a range of symptoms has been used since ancient times (Mathews, 1980). The method is commonly used as a first step towards identification of many plant viruses. It is also used in cases where rouging of diseased plants is applied as a control measure. This is also widely applied in cassava especially during farmer awareness on management of cassava cassava brown streak disease. Rouging of diseased cassava plants and replacement with uninfected cassava materials has been practiced in many countries in East Africa and reported to significantly lower the spread of cassava brown streak disease (Legg *et al*., 2017). This however requires intensive training of farmers on identification of disease symptoms as expressed on cassava leaves and stems. Symptoms of cassava brown streak disease on leaves are normally associated with the minor veins of leaves, including a feathery necrosis along the veins, as well as blotchy yellow chlorosis (Maruthi *et al*., 2005). On stems, symptoms of brown lesions are usually observed under the bark of the stem with mature stems showing the streaks most strongly especially under the bark of leave scars. Stem symptoms appear more difficult to recognize and are not a consistent feature of the disease except in highly sensitive varieties.

The CBSD often leads to necrosis of storage roots affecting palatability and marketability (Hillocks *et al*., 2002; Alicai *et al*., 2007). Diagnosis of CBSD by the use of symptoms on roots is not practically applicable to farmers because the crop has to be in the field for at least eight months and farmers can only observe the symptoms during harvest. In addition, root necrosis may not be apparent until 12 or more months after planting in tolerant cultivars. As reported by Nichol *et al*. (1950), CBSD symptoms vary in terms of severity and expression which also depends on the cultivar, the viral strain, the age of crop when the crop was infected as well as environmental conditions. This makes diagnosis of CBSD using symptoms difficult. Alicai *et al*. (2007) reported that mosaic symptoms of CBSD are less conspicuous and farmers are often unaware of the problem until the roots are harvested. Some varieties also express symptoms on roots but not on the leaves. Such difficulties in using symptoms would lead to further spread of the disease through movement of infected cassava cuttings to areas which CBSD has previously not been present (Tomlinson *et al*., 2018). High sensitive diagnostic methods are therefore paramount to supplement the visual inspection of symptoms since many CBSD infected plants may remain symptomless leading to further spread of the disease (Arbashi *et al*., 2010).

Serological methods. Serological techniques are widely used in detection of various plant viruses. The technique includes use of Enzyme Linked Immuno-sorbent (ELISA) (Clark and Adams, 1977). ELISA is simple to perform, sensitive if there is availability of virus-specific monoclonal antibodies, and easily used in large-scale analysis. However, these methods are not as specific and sensitive compared to nucleic acid based assays due to chances of getting false positives where cross-reaction of viruses occur. The cross-reaction is due to high homology between different potyviruses. Presences of many shared epitopes makes it difficult to distinguish between two closely related strains/species (CIP, 2007). ELISA kits using monoclonal antibodies for detection of CBSVs have been developed and are available (winter *et al*., 2010). However, ELISA requires fresh leaves and may not be sensitive enough to detect viruses causing CBSD in latent infections. Therefore, there is need for more sensitive methods for detection of viruses in screening germplasm and for certification of healthy and quality cassava planting materials.

Reverse –Transcriptase polymerase chain reaction (RT-PCR). Application of molecular diagnostics in disease detection provides a more reliable rapid alternative to traditional biological assay and serological techniques with greater sensitivity and specificity (Mumford *et al*., 2000). Polymerase chain reaction (PCR) is the most widely used molecular diagnostic technique due to its fast and easy to use protocol. Variants of PCR like real-time polymerase chain reaction has been widely applied for detection of main plant pathogens including fungi (Bohm *et al*., 1999), bacteria (Schaad *et al*., 1999), viruses (Mumford *et al*., 2000), and viroids and phytoplasma (Bianco *et al*., 2004). The real-time technology has also been developed for the specific identification of virus vectors (Walsh *et al*., 2005). Real-time PCR based methods provides greater sensitivity, specificity and also reduces risk of cross contamination since post-amplification processing is not required. Cassava diagnostics using RT-PCR has greatly accelerated identification of viruses associated with CBSD (Monger *et al*., 2001). Recent advancement in cassava diagnostic techniques has been reported and the case in point is the simultaneous detection of CBSV and UCBSV as reported by Mbazibwa *et al*. (2011b) and Abarshi *et al*. (2012). Multiplex PCR, which was reported to be reliable, requires less time and labour and has been utilized in simultaneous detection of cassava viruses. With the increased whole genome sequencing and availability of complete genome sequences for cassava brown streak viruses, this will largely lead to the design of even more primers for the detection of possible new virus strains.

Reverse –Transcriptase Loop-mediated isothermal amplification (RT-LAMP). Fast, accurate and simple and sensitive describes the criteria for a diagnostic tool for use in rapid detection of diseases and pests. Notomi *et al*. (2000) described a novel nucleic acid amplification of DNA under isothermal conditions. LAMP uses 4primers to amplify 6 different regions of the target gene thus enhancing its specificity. Tomlinson *et al*. (2012) developed a simple RT-LAMP for detection of cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) which demonstrated greater specificity and sensitivity than ELISA and RT-PCR based methods. The most significant advantage of LAMP is the ability to amplify specific sequences of DNA under isothermal conditions between 60-65 C. LAMP can be carried out with a simple system and can be monitored in real time. This makes LAMP much easier to adapt in diagnostic laboratories with little resources. The method has been used and adopted in the diagnosis of many plant pathogens and pests (Tomlinson and Boonham, 2008), including viruses (Tomlinson *et al*., 2012; Almasi *et al*., 2013). For on-field detection during certification of cassava planting material, there is need for

adoption of such a technique that is quick, simple, affordable and accurate. Application and use of lyophilized laboratory reagents with no nucleic acid extraction step would be useful for certification to assure cassava planting materials are disease-free.

Sequencing. Next generation sequencing technique has been reported as a powerful tool for identification and discovery of new viruses in disease complexes. Recent studies using next generation sequencing (Ndunguru *et al*., 2015) revealed diversity in CBSV strains which calls for rapid advancement of the current diagnostic techniques. The technology is also used to investigate possibility of new viruses. There is also need to apply next generation sequencing to determine and annotate the complete transcriptomes for whitefly and other vectors phloem colonizing cassava in order to infer the possible genes involved in virus transmission. This could possibly unravel better understanding about the virus-vector interactions and could be used for vector-control strategies.

Conclusion

Future perspective requires a diagnostic tool that can be used for field studies. This will enhance quick and rapid detection of viruses and especially to support inspections during certification of cassava planting materials since in some cases, infected materials may not have noticeable symptoms.

Acknowledgement

This work was supported by the Regional Universities Forum for Capacity Building in Agriculture. We thank Kenya Plant Health Inspectorate Service and the University of Nairobi for their support. This paper is a contributon to the 2018 Sixth African Higher Education Week and RUFORUM Biennial Conference.

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