

The Cassava Diagnostics Project (CDP)

A review of 10 years of research



2008–2018

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Compiled by
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The Cassava Diagnostics Project (CDP): A review of 10 years of research 2008–2018

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DEDICATION

We dedicate this Monograph to the memory of Shubi Katagira, who faithfully served as the project accountant at TARI-Mikocheni for many years.

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ABOUT THE DONORS

Bill & Melinda Gates Foundation (BMGF)

The Bill & Melinda Gates Foundation (BMGF) is a private organization founded by Bill and Melinda Gates that aims to enhance healthcare and reduce extreme poverty across the globe. One of BMGF's areas of focus is agricultural development based around the key principles of fostering breakthrough discoveries in scientific research, strengthening the tools available to farmers across the world, and providing better data systems for agriculture.

UK Department for International Development (DFID)

The Department for International Development (DFID) is a United Kingdom government department responsible for administering overseas aid. The goal of the department is "to promote sustainable development and eliminate world poverty". It supports research and programs around the globe aimed at ending extreme poverty.

ABOUT THE PARTNERS

Tanzania Agricultural Research Institute (TARI)–Mikocheni, Tanzania

The Tanzania Agricultural Research Institute (TARI)–Mikocheni is a public agricultural research institute under the Ministry of Agriculture in Tanzania. Among its mandates is conducting and promoting agricultural biotechnology research for socio-economic development in the country.

Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya

The Jomo Kenyatta University of Agriculture and Technology (JKUAT) is an educational institute in Nairobi specializing in agricultural research and innovation. JKUAT aims to provide accessible, quality training to its students and focuses on collaborating with other educational institutions to contribute to the social and economic development of Kenya.

Department of Agricultural Research Services (DARS), Malawi

The Department of Agricultural Research Services (DARS) was created by the Malawi Government in 1938 with the aim of developing agricultural technologies. Today its mission is to address challenges to Malawi's agricultural productivity by conducting research into cutting-edge technologies and promoting specialist services across Malawi.

Instituto de Investigação Agrária de Moçambique (IIAM), Mozambique

The Instituto de Investigação Agrária de Moçambique (IIAM) focuses on strategic research and dissemination of technologies. Created in 2004, IIAM brings together several areas of agrarian research sectors. Its mission is to generate knowledge and technological solutions for the sustainable development of agribusiness and food and nutritional security in Mozambique.

Rwanda Agriculture Board (RAB), Rwanda

The Rwanda Agriculture Board (RAB) is an autonomous body formed to improve food security and livelihoods in Rwanda by championing the development of the agricultural sector. RAB looks to implement national agriculture and animal husbandry policies through research and innovation around sustainable crop and natural resource management.

National Crops Resources Research Institute (NaCRRI), Uganda

The National Crops Resources Research Institute (NaCRRI) in Uganda is a public agricultural research institute under the policy guidance and co-ordination of the National Agricultural Research Organisation (NARO). This institute's mandate is to address the challenges that affect the country's staple crops, with the goal of improving the health and wealth of the country's population.

Zambia Agriculture Research Institute (ZARI)

The Zambia Agriculture Research Institute (ZARI) is Zambia's largest agricultural research institution. ZARI's mandate is to develop and adapt crop, soil and plant protection technologies in order to provide the country's farmers with a high-quality, appropriate and cost-effective agricultural services.

North Carolina State University (NCSU), Raleigh, North Carolina, USA

North Carolina State University is a pre-eminent research institution that was founded on a specialization in agriculture and engineering but has now expanded across disciplines. Its agricultural research services aim to improve productivity, profitability and sustainability through its developments in knowledge and technology.

Rutgers University (RU), New Jersey, USA

Rutgers University in New Jersey is committed to delivering excellent teaching, conducting ground-breaking research, and supporting local, county, and state citizens. Its school of environment and biological sciences focuses on research that addresses real-world problems around food security and sustainability.

COUNTRIES TARGETED FOR CDP IMPLEMENTATION



INTRODUCTION

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Cassava virus research and sustainable disease management in East and Southern Africa

About 60% of the world's arable land is in Africa (FARA, 2014). The overall African population engaged in agriculture is estimated at 530 million and is expected to exceed 580 million by 2020 (NEPAD, 2013). The proportion of the population in Africa reliant on agriculture is 48% and in East Africa this figure is almost 70%. Agriculture is the predominant source of employment and livelihood and a way of life for most African citizens. This sector provides employment for 50% of the labor force – 47% of these workers are women and the majority of farmers are subsistence farmers. However, the agricultural sector provides only an average of 15% of GDP to the continent (ADB, 2016). The increasingly low contribution of agriculture to the economy is a sign of low productivity and limited value addition.

The Science Agenda for Agriculture in Africa (FARA, 2014) is an African-owned and African-led process that emphasizes the application of science and technology for agriculture to double agricultural productivity by 2025 and connect science to the end-users. Africa has the potential to increase the value of its annual output from about \$280 billion (in the late 2000s) to around \$800 billion by 2030 (McKinsey Global Institute, MGI 2010). There is a consensus within Africa that such a vital sector as agriculture needs to be transformed using the catalytic power of science and technology.

In this monograph, using cassava (*Manihot esculenta* Crantz) as a model crop, we demonstrate how the application of science and technology can play a key role in transforming agriculture in Africa and how this can benefit smallholder farmers.

Cassava is an important source of food in developing countries after maize, rice and wheat and is grown mainly by subsistence and small-scale farmers. This root crop is a source food for an estimated 800 million people in Asia and in the tropics – about 300 million people in sub-Saharan Africa (SSA) depend on cassava as their source of daily calories. It is a staple food throughout Africa – that is, an item of good nutritional value that is consumed as the major part of the daily diet of a large part of a country's population – as is the case in the seven countries that participated in the Cassava Diagnostics Project (CDP).

Cassava is well adapted to poor soils, and its economically desirable attributes include drought tolerance and the ability to store the roots for lengthy periods in the ground, allowing flexible

harvesting over long periods. Thus, cassava is considered a major food security crop in many developing countries. Additionally, cassava is a valuable source of starch with potential industrial uses, such as in animal feed, biofuel, paper, textiles and food processing applications. Overall, healthy cassava can contribute nutritionally and economically to the countries where it is grown.

However, the benefits of this crop are seldom felt in the East and Southern African countries where cassava is grown. Among the many challenges farmers face are plant diseases that significantly reduce crop yields, often caused by viral pathogens. Viral diseases are difficult to prevent, and once established there are few ways to counter their impact on yield. As a result, development and deployment of resistant crop varieties remains the most effective manner to combat the evolving threats of plant viral diseases. Underpinning such efforts is the need for robust diagnostic capacities to identify the species and strains of viral pathogens infecting crop plants and their associated vectors, as well as their related wild species, and to understand their distribution within a given geographical region.

To achieve this aim, in-depth studies of the pathogens using state-of-the-art molecular tools can detect evolutionary or environmental changes and inform the management of viral diseases so as to maximize crop yields for farmers. Another important factor in combating plant diseases is to understand the means by which crops become infected and how the diseases are spread at local level – from farm to farm, village to village – and further afield to district and regional levels.

An integrated approach is required to translate the knowledge gained in the laboratory into solutions that can be incorporated into the farmers' practices so that they adopt them readily. Diagnostic capacities must be simple, cost-effective and robust enough to be used in resource-constrained countries like those in SSA.

In order to achieve this aim, a formal phytosanitary framework is required to (1) develop disease-free cassava plants by plant breeders, (2) make disease-free planting materials available to farmers, (3) formulate an education program so that the farmers recognize cassava viral disease symptoms on their crops and (4) train extension workers involved in outreach programs so that they can provide assistance to farmers. To enable the project to meet these aims, funding was received from the Bill & Melinda Gates Foundation (BMGF) and the UK Department for International Development.

Cassava viral diseases

The main viral pathogens constraining healthy cassava production in East and Southern Africa are cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). CMD is widespread in SSA, whereas CBSD has been reported from East African countries and around the Great Lakes region.

Both CMD and CBSD have seriously reduced yields across the continent, often forcing farmers to abandon their fields. In 2005 for example, CMD alone caused crop losses of 4 million t/year in Tanzania, Uganda, Rwanda and Burundi. It is estimated that US\$2–3 billion is lost annually due to CMD in SSA (Scholthof et al., 2011). These viral diseases have caused severe food shortages among resource-poor farmers in Africa, threatening food security across the continent.

Symptoms of CMD in cassava vary from mild mosaic to severe symptoms of leaf curl, leaf distortion, yellowing and plant stunting – depending on the infected cultivar, virus species/strain (mixed infections are common) and climatic conditions. The major symptom types in CBSD include yellowing

of older leaves along the major veins, round or elongated brown streak-like lesions on the stems and, most destructively, production of brown, corky, necrotic lesions within the storage roots.

Root and leaf symptoms of CBSD are highly variable and depend on host genotype, virus species and environmental conditions. Co-infection with more than one virus species or strain is a common feature in the etiology of CBSD, often leading to increased symptom severity.

Cassava mosaic disease is caused by cassava mosaic begomoviruses (CMBs) (family *Geminiviridae*; genus *Begomovirus*). The virus is spread by the whitefly *Bemisia tabaci* Gennadius (Hemiptera) (Dubern, 1994) and also through movement of CMB-infected planting material (Harrison, 1987). In SSA, there are eight recorded cassava-infecting CMBs (Brown et al., 2015) including: *African cassava mosaic virus* (ACMV), *Cassava mosaic Madagascar virus* (CMMGV), *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Cameroon virus* (EACMCV), *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Kenya virus* (EACMKV), *East African cassava mosaic Zanzibar virus* (EACMZV) and *South African cassava mosaic virus* (SACMV).

Early studies on the distribution of CMBs in SSA indicated that ACMV was widespread in most cassava-growing areas in west, central, eastern and southern Africa, with the exception of the coastal areas of Kenya, Tanzania and Mozambique. Indeed, ACMV is considered one of the top 10 pathogens affecting cassava.

In contrast, EACMV was believed to be restricted to coastal Kenya, Tanzania, Mozambique, Zanzibar and Madagascar, and some areas of Malawi (Harrison et al., 1991, 1996). However, subsequent studies showed that EACMV also occurred inland in western Kenya and western Tanzania (Gibson et al., 1996; Legg et al., 1999; Were et al., 2003, 2004). In West Africa (Cameroon, Guinea, Ivory Coast, Nigeria, Senegal and Togo), EACMCV is the predominant species (Fondong et al., 2000; Ogbé et al., 2003, 2006; Okao-Okuja et al., 2004).

These viruses are transmitted by the polyphagous cryptic whitefly complex *B. tabaci*, mechanically transmitted through grafting and sap inoculation of herbaceous plant species, and are propagated through infected stem cuttings.

Whitefly

About the organism

Whiteflies *Bemisia tabaci* (Gennadius) (*Hemiptera: Aleyrodidae*) are phloem (sap) feeders. They cause direct damage in some hosts by extracting large quantities of sap. *Bemisia tabaci* is a vector of plant begomoviruses of the *Geminiviridae*. These whitefly-transmitted viruses (WTV) are among the most destructive plant viruses and early virus infection can result in total crop loss.

Although the problems caused by *B. tabaci*, both as pest and vector, have been recognized for more than 100 years, serious damage had been limited to a handful of crops in particular geographic areas. This scenario has changed over the past two decades. The known WTVs have extended their geographic range and other WTVs are emerging in new crops and geographic zones around the world. Whitefly infestations have become severe in both traditional and non-traditional food and industrial crops throughout the tropics.

Role in CMD and CBSD

A key feature of the geographical areas severely affected by CMD and CBSD is the presence of high whitefly populations on cassava plants. The persistent mechanism of transmission of CMBs by *B. tabaci* (viruses can be retained up to 9 days) facilitates long-distance movement of virus populations (up to 38 km in a year) and has important consequences for the pattern of virus spread. Examples of the steady progression of these diseases can be found in published literature (for example, Otim-Nape et al., 1997, 2000; Legg, 1999; Pita et al., 2001). These studies demonstrate changes in the viruses as well as documenting their route from northern Uganda moving southwards at approximately 20 km/year and reaching Kenya. In both countries, severe to total crop failure was experienced.

With these historical events in mind, one aim of the CDP project was to gain a better understanding of the whitefly and other insects that might contribute to the viruses causing CMD and CBSD. To this end, the collection of whitefly and other insects found on the plants became part of the surveys for plant material sampling to assess these insects. Although not all insects collected were studied comprehensively, they were identified to group or family level. The findings of the whitefly and insect assessments are detailed under the individual country chapters. The work carried out by individual partners also shows that whitefly may not be solely responsible for the spread of the viruses causing CMD and CBSD and that the dissemination of infected plant cuttings may play an important role in this problem.

Making disease-free cassava plants available to farmers

This has been a major objective for all the partner countries. Efforts to achieve this objective are described in the individual chapters of this document. It is widely acknowledged that, in addition to interacting with plant breeders, it is essential that farmers trust the material they are acquiring from the breeders. A formal certification system is thus vital to the success of the endeavor to manage CMD and CBSD. Tanzania has in place the Tanzania Official Seed Certification Institute.

Interaction with agricultural extension workers and farmers

From the start of the project in all partner countries, trust was built between project teams, government agricultural extension departments, local government administration and farmers. Their permission was sought in surveying their fields, and it was made clear to them that their cooperation was valued and essential to combat the disease problems they face. Farmers whose land was surveyed were taught how to recognize plant disease, and plots were designed and cultivated with their preferred cassava varieties as well as disease-free varieties in order to demonstrate the benefits of using clean planting material. Agricultural shows, printed material, radio and television broadcasts and other educational events were used to inform farmers about accessing clean planting material. These endeavors are described in detail within the individual chapters of this document.

In addition to direct interaction with farmers, CDP worked closely with extension staff. These staff can usually communicate with farmers in their dialect and offer practical assistance. As with farmers, it is essential that extension staff can recognize cassava diseases. Project teams were therefore active in providing training and information to this sector of the workforce – their work is detailed within the individual chapters of this document.

Disease diagnostic protocols

The overall aim of the project was to manage cassava virus diseases and their associated vectors sustainably using standardized and harmonized protocols. Among the protocols developed by CDP and shared among the network countries and beyond included that for disease surveillance (Sseruwagi et al., 2017). The parameters standardized and harmonized included a disease severity scoring scale (1–5), assessment of CMD and CBSD incidences, sample collection and determination of whitefly abundances in the cassava fields. The second protocol was the establishment of cassava demonstration plots (Sseruwagi et al., 2014), with standards developed for their design and size. Finally, laboratory disease diagnostic protocols were developed, standardized and harmonized, including sample storage, processing, primer design, PCR and results analysis. These protocols were shared through training, joint surveys and technical backstopping visits by project scientists. All CDP countries used the same protocols in their respective countries. In addition, a number of PCR primers for detection of various *cassava brown streak virus* and CMB species/strains were developed and shared widely for routine use in indexing cassava planting materials before materials were provided to farmers. All the protocols listed above were also shared with scientists in the WAVE (West African Virus Epidemiology) project for use in West African countries.

Origin and organization of the CDP

The goal of the CDP was formally stated and discussed at the Project Inception Meeting in December 2008. Using laboratory-based evidence that the capacity to diagnose disease is essential for effective and sustainable control of CMD and CBSD, the project aimed to enhance capacity to diagnose, characterize, monitor and sustainably manage viruses affecting cassava productivity. Partners within the CDP project were Tanzania, Kenya, Uganda, Rwanda, Mozambique, Malawi and Zambia. The general goal of the project was to improve cassava production in the region. Key gaps were highlighted by the partners. All partner countries lacked reliable information concerning cassava disease problems such as correct identification of diseases and hotspots, baseline data, surveillance, disease mapping for distribution, and diagnostics linked to resistance breeding. These criteria were considered vital to assist the National Agricultural Research Systems (NARS) in predicting disease spread and formulating control options.

In order to achieve this goal, CDP developed an effective diagnostic tool – that was simple, cost-effective and robust – to enhance the capacity of NARS in modern molecular diagnostics. An overview of the project budget and the disbursement procedures to participating countries was presented.

The key budget lines explained were Personnel and Administration – each partner country was headed by one scientist and a research assistant covered by the country's budget, but it was stressed that as all partners were government employees at their respective NARS, no fringe benefits would be issued because this would be the contribution of each country's government to the project. Regional and local travel would follow the partner country's government rates.

Equipment allocation guidelines were such that all countries had the same funds for similar types of equipment. The equipment was either purchased centrally by the Principal Investigator (PI) and shipped to respective partners, or each country purchased the material themselves depending on their procurement procedures.

A small budget was set for contract services, mainly the sequencing of isolates from partner countries by other advanced countries with sequencing facilities, where these are not present in partner countries.

There were no sub-grants or consultancies as there was no budget for such expenditure, but for all participating countries, 10% of direct costs could be charged by the institution to support administrative issues rendered to the project.

Provision was made for four MSc students, from CDP partner countries, to be supported by the project starting in year two. Partner scientists attending annual rotational workshops and meetings were also supported financially by the project. The organizing countries were supported by the PI office in using the allocated budgets. Funds were disbursed annually following proper accounting of previous disbursement at the agreed reporting date as per the project contract.

Common challenges for all partners included lack of information sharing, lack of staff training and retention of key project staff rather than them leaving for other employment with attractive packages within the region/partner country. Partners were reassured that attractive top-ups equivalent to other programs within the region would be provided to country team leaders to support them.

The procurement of key equipment and consumables was addressed because some partner countries had difficult procedures. A solution was to have central procurement points where the supplier could be directed by the PI office to deliver the consignment to the respective countries – this would take into account special cases where countries might require formal supporting letters for equipment provided for researchers by a donor.

Impact

The CDP had a significant impact both direct and indirect in all seven project network countries and beyond following its implementation. Over the ten years (2008–2018) that the CDP team worked together they sustained the network and performed its research goal of addressing cassava problems using harmonized strategies. Some of the key visible results of CDP are described below.

Both the human and infrastructure disease diagnostic capacity in the project countries was strengthened through training and establishment of laboratory facilities. Researchers in the project countries can now go to the field, collect disease and pest samples for analysis in the laboratories, and use the laboratory results to inform pest and disease management strategies.

University students and other research scientists in the project countries who might have left and gone abroad to conduct research work are now using the laboratory and greenhouse infrastructure established by the CDP project to conduct their research – a noticeable benefit for the project and their country.

Organizations such as the International Institute of Tropical Agriculture, research institutes, local government, government departments and NGOs involved in cassava improvement in the project countries are using information generated by the project. A good example is Tanzania in which local government in all the major cassava-growing areas received and used disease prevalence maps

generated by CDP. These maps contributed to decisions on where to multiply, screen and deploy cassava varieties, as well as strengthen phytosanitary regulations.

The visibility of the research institutes hosting the CDP increased, and resulted in these institutions attracting more funding through new collaborative projects and grants. These new collaborations have led to joint projects, publications and exchange visits, as well as the acquisition of new laboratory infrastructure. The CDP inspired many young research scientists in Africa to write grant-winning proposals for initiatives such as the Program for Emerging Agricultural Research Leaders (PEARL) funded by the Bill & Melinda Gates Foundation.

For smallholders within the CDP partner countries, the impact of the project has been positive. Those farmers who received virus-indexed clean planting cassava material through the project recorded yield increases from 5 to up to 40 t/ha. This has resulted in increased income and household food security.

Further afield, CDP supported the initiation of the WAVE project which addresses similar cassava virus diseases and their associated vectors in West Africa using a CDP model.

Challenges

From a science point of view, although *B. tabaci* was found to transmit CBSD, the transmission percentage was low and thus involvement of other agents in CBSD transmission cannot be definitively excluded.

Running regional projects such as the CDP include dealing with the human, administrative and logistical elements. The diversity of people, countries, government procedures, institutional arrangements and capacities influence project implementation. Practical challenges include slow procurement processes due to long tendering procedures and expensive laboratory equipment and supplies – often due to involvement of brokers as well as burdensome customs regulations and laws in some countries causing delays in delivery of laboratory supplies. Furthermore, biosafety regulations are not in place in some countries and there is low level of awareness among the public on application of biotechnology for crop improvement.

Conclusions

A number of stakeholders benefited from the implementation of the CDP – there is now a strong connection with the end-users of the science and technology. Farmers who used to get less than 5 t/ha of cassava before the project intervention are now getting 20–45 t/ha after receiving virus-free improved cassava material from the project. This has significantly contributed to improved household food security and income. The CDP also contributed significantly to enhanced human and infrastructure in the project countries through the acquisition of laboratory equipment and screenhouses and training of young scientists. This, in turn, contributed to the emerging of bioscience capacity in Africa – leading to the realization of the vision of seeing bioscience playing a key role in agricultural transformation in Africa.

The success of the CDP greatly depended on teamwork and notable attributes displayed by the team. These include: dedication and commitment, self-motivation, a high level of cooperation

among the project teams, and a high degree of transparency especially from the project leadership. Additionally, effective communication, consistent hard work and a high level of government support in all the project countries was instrumental.

Recommendations for future work

The Cassava Diagnostics Project is a good model to use for other staple crops. Researchers could benefit from our experience on a number of areas: How to start a large project? How best to find partners for collaboration? Could calls for proposals be a useful source for finding potential partners or seeing who else is addressing similar areas of research?

The project has demonstrated the value of cooperation between plant pathologists and plant breeders in a range of areas such as:

- Having the scientific capability to investigate complex scientific as well as practical problems and to provide training programs
- Advising on, or assisting with, setting up the required infrastructure for breeders to carry out their work efficiently
- Understanding the challenges faced by breeders and proposing solutions that fit readily with their usual operations
- Providing information to government bodies to highlight the potential benefits of this cooperation
- Fostering trust and respect in all areas of the project – scientific, technical and administrative. This is of paramount importance among partners in a project such as CDP
- Fostering trust and respect between scientists and farmers, i.e. treating farmers as partners in the endeavor
- Taking responsibility to share knowledge – scientific, technical as well as administrative know-how
- Using a common platform to which all partners have access – so that information is easily available
- Practicing a philosophy of cooperation: *If you wish to receive, you must be prepared to give.*

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