Cassava Diagnostics Network for Africa (CDNA)

1. Introduction

The working group on the establishment of Virus, Bacterial and Whitefly Diagnostics, subsequently renamed as ‘Cassava Diagnostics Network for Africa (CDNA)’ was co-chaired by Lava Kumar (IITA, Nigeria) and Stephan Winter (DSMZ, Germany).

- Hamza Azali (Université des Comores, Union of Comoros)
- Hélène Delatte (CIRAD, France)
- Wilmer Cuellar (CIAT, Colombia)
- Maruthi Gowda (NRI, UK)
- Zinga Innocent (LBSAD, Central African Republic)
- Ralf Koebnik (IRD, France)
- Samuel Offei (WACCI, Ghana)
- Joseph Onyeka (NRCRI, Nigeria)
- Isabelle Robene (CIRAD, France)
- Fidèle Tiendrébéogo (INERA, Burkina Faso)

2. The group discussion focused around the following topics:

- What types of diagnostic tools are available?
  Types of procedures and tools; actors in tech development and application; relative merits of various methods; and expertise in national programs; major constraints for adoption/application of diagnostics
- Who are using the diagnostics?
  Main applications of diagnostics; organizations involved technology development; key stakeholders adopting diagnostics
- What are the major challenges to diagnostics application and adoption in Africa?
  Critical gaps in current diagnostics; development of new tools (for established and emerging threats); validation of promising methods and harmonization of procedures (ring test); and proficiency testing of labs and expertise
- How to strengthen cassava diagnostics capacity in Africa?
  On-line indent of tools, expertise and capacities; directions to improve linkages between cassava R&D community in Africa; identification/development of reference labs in Africa; and establishment of cassava diagnostic network

3. What types of diagnostics tools are available?

A range of qualitative and quantitative diagnostics for cassava pests and diseases in use are listed in the Table 1. PCR and RT-PCR-based assays are the most popular and widely used. Real time RT-PCR assays are gaining popularity for the detection of CBSVs in particular. RT-LAMP developed as a field-based assay for CBSVs, but not widely used. ELISA for cassava
viruses is not among the routine choice of methods, but the technique seems to hold great promise for the detection of CBSVs. Deep sequencing of small RNA and computational assembly (next generation sequencing) for discovery of new viruses was applied for the detection of viruses associated with frog skin disease in Colombia.

Diagnostics are frequently used for the detection of viruses causing mosaic and brown streak diseases in Africa; viruses associated with mosaic and frog skin disease in Latin America; Candidatus phytoplasmas and Xanthomonas axonopodis pv. manihotis (the causal agent of cassava bacterial blight) in Asia and Latin America; and identification of whitefly (Bemisia tabaci) biotypes across all the continents.

Diagnostics using generic and specific PCR methods have also been used but less frequently for the characterization of anthracnose disease caused by Colletotrichum gloeosporioides, and several cassava pests such as mealybugs (Phenacoccus manihoti), root scales (Stictococcus spp.), spiraling whitefly (Aleurodicus dispersus), green mite (Mononychellus tanajoa) and endosymbionts in cassava whitefly.

Table 1. Frequently used tools in the diagnosis of major cassava pests and pathogens

<table>
<thead>
<tr>
<th>Pest/Pathogen</th>
<th>Tool</th>
<th>Comments</th>
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<tbody>
<tr>
<td><strong>Cassava mosaic begomoviruses</strong></td>
<td>ELISA</td>
<td>TAS-ELISA using a mix of mono- and polyclonal antibodies; used for differentiating ACMV and EACMV-complex in 1990s. ELISA usage is less frequent or nil mainly due to less sensitivity, dearth of antibodies and lack of resolution to distinguish EACMVs.</td>
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<tr>
<td></td>
<td>PCR / real</td>
<td>Several primers and PCR procedures have been developed by different laboratories for the specific detection of begomovirus species, or any the species using generic primers in uniplex and multiplex format. Most of the multiplex assays (some procedures combine detection of begomoviruses and ipomoviruses) have targeted the detection of ACMV, EACMV-like viruses and EACMV-UG. Both qualitative and quantitative assays have been established for all the cassava begomoviruses occurring in Africa and south Asia.</td>
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<tr>
<td></td>
<td>time PCR</td>
<td></td>
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<tr>
<td><strong>Cassava brown streak viruses</strong></td>
<td>ELISA</td>
<td>TAS-ELISA has been developed for both CBSV and UCBSV and its usage demonstrated successfully; however this assay is less frequently used due to doubts over sensitivity, however, there is no evidence to compare relative sensitivity of ELISA vs other diagnostics. Some efforts are on-going to establish peptide-based antibodies for CBSV and UCBSV and development of LFD-type kits.</td>
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<tr>
<td></td>
<td>RT-PCR and</td>
<td>Several primer pairs and RT-PCR procedures have been</td>
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Real time RT-PCR developed by different laboratories for the specific detection of the two impomovirus species in uniplex and multiplex format. Relative merits of various assays are not known. Wide variation in detection sensitivity and specificity has been reported with most of the procedures. Lately, real time RT-PCR is gaining prominence for CBSVs detection.

RT-LAMP RT-LAMP assays were developed for the detection of CBSV and UCBSV. Only one procedure is available.

Viruses infecting cassava in Latin America

RT-PCR protocols have been established for the detection of several RNA viruses cassava common mosaic; cassava torado-like virus; cassava polero-like virus, cassava frog skin-associated virus and several RNA viruses identified in cassava in Latin America

Whitefly biotyping

PCR and sequencing Mitochondrial CoI gene amplification and sequencing is the routine method of choice for the identification and classification of biotyping cassava whiteflies. Biotype specific primers are also available.

Candidatus phytoplasma (cassava witches broom)

PCR and sequencing Universal primers targeted to P6 and P7 genes are routinely used for amplification of phytoplasma, and specific identification is based on RFLP or sequencing.

Cassava bacterial blight (Xanthomonas axonopodis pv. Manihotis)

PCR and sequencing Universal primers to 16S RNA is being used for generic level detection. Specific primers are also available.

4. Who are using the diagnostics?

Diagnostic applications mainly targeted towards (i) characterization of pathogens and pests; (ii) understanding population diversity; (iii) virus indexing of causal agents in field samples; (iv) phenotyping for virus resistance in breeding and transgenic programs; and (v) production of virus-free planting material.

Some of the key organizations involved in the development of cassava diagnostics technologies are listed in the Table 2. Several other universities and national programs are also involved in the development of technologies either alone or in collaboration with organizations listed in the Table 2.

Many more organizations are involved in application of diagnostics. At least one laboratory with adequate basic facilities (nucleic acid extraction, thermal cyclers and gel electrophoresis) for PCR-based diagnostics is available in almost all the countries.
Sophisticated facilities for advanced diagnostic applications are rather limited and available in few labs in sub-Saharan Africa.

### Table 2. List of some key organizations involved in the development of diagnostic tools for cassava pests and pathogens

<table>
<thead>
<tr>
<th>Organization</th>
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<tbody>
<tr>
<td>CIAT (Colombia &amp; Vietnam)</td>
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<tr>
<td>CIRAD (France)</td>
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<td>CTCRI (India)</td>
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<td>DDPSC (USA)</td>
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<td>DSMZ (Germany)</td>
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<td>ETH (Switzerland)</td>
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<td>FERA (UK)</td>
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<tr>
<td>IITA (Nigeria, Kenya &amp; Tanzania)</td>
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<tr>
<td>IRD (France)</td>
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<td>ILRI-BecA (Kenya)</td>
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<td>MARI (Tanzania)</td>
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<tr>
<td>NACCRI (Uganda)</td>
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<tr>
<td>NRI (UK)</td>
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<td>University of Witwatersrand (South Africa)</td>
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5. **What are the major challenges?**

**Key objectives:**
- Strengthen diagnostics capacity of the national and regional programs/cassava community in Africa
- Enhance utilization of diagnostics in surveillance, clean planting material production and research

**Status:**
- Diagnostic tests for priority pathogens available (e.g. CBSVs, CMGVs, frog skin-associated viruses, CBB and generic tools)
- Differential capacities for diagnosis at national, regional and international level

**Approach:** Discussed current capacities, who is doing what and needs

**Diagnostics gaps:**
- Technologies, tools (lab and field)

**Application gaps:**
- Expertise (skill), capacity (infrastructure) and priority uses

**Standards:**
- Protocols, reference labs and controls

**Networking:**
- Who are working, where are they and how best to connect?

**Diagnostic methods:**
- Several methods available for CMGVs and CBSVs.
- SOPs (harmonized methods) are not available.
- Validation of available methods to identify best-bet methods/protocols.
- An open call will be made to nominate protocols
- A steering group to scrutinize submissions and shortlist promising methods for organizing a ring test in various labs
  [to check on ELISA/PCR and improve it (generic test for Gemini presence/CBSV/Xanthomonas cassava), S Winter (agrees to give free ELISA sero material and positive controls) FOR GEMINI/ (M. Gowda/ I. Robene / L. Kumar?)—> group to work on standard procedure for PCR].
- Open to all labs to participate and share reagents methods / ring testing

**Need for new pathogen tests**
- Frogskin disease (W. Cuellar, CIAT)
- CBB+ *Xanthomonas cassava* (BETTER SIMPLE TEST, need to be develop)  
  IRD/CIRAD
- Whitefly species (and subgroupings) differentiation?, on COI/RFLP (to be developed on SSA1, 2, 3, 4 and hitchhiking whiteflies species like IO, MED and ALSO B afer): IITA/NRI/CIRAD
- Phytoplasma (witches broom) testing in Asia (W. Cuellar, CIAT)
- Any other???

**Reference labs:**
Expert labs for technical backstopping at national, regional and international level
La Réunion (CIRAD)/ NRI/Burkina/RCA/INRAPE (Comores)/IITA/DSMZ/WAKI-Legon/ others...
  - To share applications, expertise, reference standards and capacity development (advocacy, funds, group testing...)=> fill a form

**Capacity development**
- Network: to share experiences and knowledge
- Workshop for various groups to work together to test protocols
- Training: Three regional diagnostic trainings (West-Central, East-South, & IO)
- FUND RAISING? (S Winter: German national funding opportunity for organizing specific diagnostic workshops; I Robene: E prpv fundings for organizing such a similar workshop)
- Develop a dossier/inventory on available diagnostics, diagnostic capacity and who is who
- Capacity assessment survey

**Steering group:** To drive the action points
Samuel Offei, Lava Kumar (coordinate), Maruthi Gowda, Isabelle Robène (organization), Stephen Winter, Wilmer Cuellar, Fidèle Tiendrebeogo, Hélène Delatte, Valerie Verdier
Send email around to the whole group to share experience, protocols, and communicate developments

Questions?
- How can diagnostic team helps the user needs?
- What about the use of digital diagnostics?
- How about field-based diagnostics?
- Where is the timeline/prioritization?
- Quick diagnostic kits (research required)
- Training different levels of users (most technical to least technical; training the trainees)
- Communication to the non-scientists (NGOs, journalists, policy makers)
- Harmonization should include sampling
- Preservation of isolates
- Validation of tests against broad range of strains
- Criteria for nominating protocols for ring test
- Maps, database, links to database
- Service labs