# The process and lessons of exchanging and managing *in-vitro* elite germplasm to combat CBSD and CMD in Eastern and Southern Africa

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## Abstract

Varieties with resistance to both cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) can reverse food and income security threats affecting the rural poor in Eastern and Southern Africa. The International Institute of Tropical Agriculture is leading a partnership of five national (Malawi, Mozambique, Kenya, Tanzania and Uganda) cassava breeding programs to exchange the most elite germplasm resistant to both CMD and CBSD. This poster documents the process and the key learning lessons. Twenty to 25 stem cuttings of 31 clones comprising of 25 elite clones (5 per country), two standard checks (Kibandameno from Kenya and Albert from Tanzania), and four national checks (Kiroba and Mkombozi from Tanzania, Mbundumali from Malawi, and Tomo from Mozambique) were cleaned and indexed for cassava viruses at both the Natural Resources Institute in the United Kingdom and Kenya Plant Health Inspectorate Services, in Kenya. About 75 *in-vitro* plantlets per clone were sent to Genetic Technologies International Limited, a private tissue culture lab in Kenya, and micro-propagated to ≥1500 plantlets. Formal procedures of material transfer

between countries including agreements, import permission and phytosanitary certification were all ensured for germplasm exchange. At least 300 plantlets of each elite and standard check clones were sent to all partner countries, while the national checks were only sent to their respective countries of origin. In each country, the *in-vitro* plantlets were acclimatized under screen house conditions and transplanted for field multiplication as a basis for multi-site testing. Except for Tomo, a susceptible clone, all the clones were cleaned of the viruses. However, there was varied response to the cleaning process between clones, e.g. FN-19NL, NASE1 and Kibandameno responded slowly. Also, clones responded differently to micro-propagation protocols at GTIL, e.g. Pwani, Tajirika, NASE1, TME204 and Okhumelela responded slowly. Materials are currently being bulked at low disease pressure field sites in preparation for planting at 5-8 evaluation sites per country. The process of cleaning, tissue culture mass propagation, exchange and local hardening off/bulking has been successful for the majority of target varieties. Two key lessons derived from the process are that adequate preparations of infrastructure and trained personnel are required to manage the task, and that a small proportion of varieties are recalcitrant to tissue culture propagation.

**Key words:** exchange, in-vitro, germplasm, CBSD and CMD

## Introduction

Cassava (*Manihot esculenta* Crantz) is the most important food staple in Africa, ranking number one root crop and followed by yam and sweetpotato (FAO, 2011). With over 140 MT of annual root production (FAO, 2013), cassava is the major source of carbohydrates in the diet of millions of people in sub-Saharan Africa (SSA) and is grown as a famine reserve crop owing to its tolerance to harsh environmental conditions (Nassar and Ortiz, 2007). Moreover, the crop has an enormous potential to graduate from a mere famine reserve crop to an important economic driver within the agriculture sector in different SSA countries where entrepreneurs have started to exploit its industrial business potentials to produce high quality flour, starch, beverages and animal feeds.

However, cassava production is direly threatened by the two viral diseases: Cassava Brown Steak Disease (CBSD) and Cassava Mosaic Disease (CMD) (Legg et al., 2011; Alicai et al., 2007). The diseases are both or singularly the current principal biotic factors affecting cassava production in East and Southern Africa (Legg et al., 2011; Alicai et al., 2007). CBSD is localized in this sub-region with high risks of spreading to the western Africa sub-region if not contained (Legg et al., 2011). Also it has been reported (Mbanzibwa et al., 2010; Mohammed et al., 2012; Legg et al., 2011; Ogwok et al., 2015) that different virus species and strains occur in different agro-ecologies and generate different interactions with the host which complicates the evaluation and selection processes for resistance. CBSD has been characterized to be caused by two species of viruses, each with different isolates, and occupying distinct agro-ecologies (Mbanzibwa et al., 2010; Ogwok et al., 2015, Legg et al., 2015, Ogwok et al., 2016). Also documented is varied response of genotypes across species and isolates (Mohammed et al., 2012; Legg et al., 2015). This further justifies the exchange of elite germplasm between countries to validate their resistance to the virus types in different countries.

Collaborative efforts with different national cassava breeding programs have identified CMD/CBSD resistant or tolerant germplasm. However, these have been evaluated under (narrow) different environmental and virus species and strains (Legg et al., 2011). The exchange of germplasm between countries enhances germplasm diversity available to partner countries that not only can be evaluated and released as new varieties but also can be used for adaptability breeding to combat CBSD and CMD in different countries.

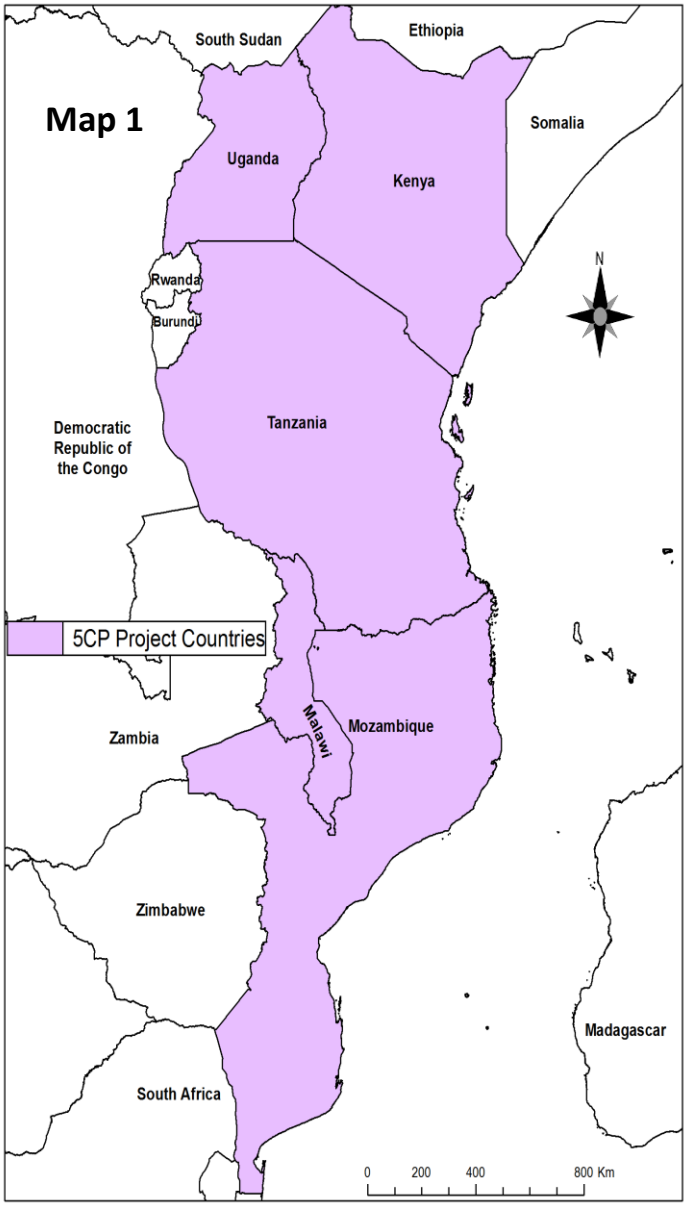
Past experiences of CMD pandemic management underline the significance of host resistance and joint action among partners hence the need for sharing of elite germplasm among the CBSD affected countries. Indexed tissue culture (TC) plants are the only recognised standard way by quarantine regulations/regulators for exchanging advanced germplasm between countries or breeders (Lebot, 2009; Mwangangi et al., 2014; FAO…; Ntaruhunga and Legg, 2007). Other approaches like open quarantine which was used in the past during the CMD crisis to quickly exchange promising germplasm, faced criticism from virologist on the possible risks of introducing disease/pests in new areas (Ref). Also, sharing of less riskier botanical seeds has been used but requires more time and funds at national levels to develop promising varieties (Ref). The exchange and use of indexed TC plants also takes care of the long standing critism, by virologists, of unfair comparisons of breeding materials that are not clean since and possibility at different inoculum levels (Dr. Legg, personal communication).

Breeding for dual resistance is currently being pursued as the only cost effective and sustainable way to tame the devastating effects of the viral diseases in the sub-region. Whereas meaningful resistance has been found for CMD, limited success is documented for CBSD resistance (Ref). And the desired goal of the breeding efforts is stable dual resistant genotypes for the two viral diseases.

The overall goal of the process was to combat the cassava viral diseases, CMD and CBSD through deployment of resistant and clean stock diverse germplasm. The immediate goal of the process was to exchange elite germplasm between countries most affected by the CMD and CBSD for adaptability breeding.

## Target countries

Five countries including Malawi, Mozambique, Kenya, Tanzania and Uganda agreed to exchange their best bet five cassava clones for adaptability breeding aimed at combating the two major cassava viral diseases, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). The countries represent areas most devastated by the two diseases and form the eastern and southern sub-region.




## Target cassava germplasm

The targeted germplasm (Table 1) included: a) five elite cassava clones chosen by each of the target countries, and b) two standard check clones i.e. Kibandameno from Kenya and Albert from Tanzania. The elite clones were selected for exchange based on their high tolerance/resistance levels to both CMD and CBSD, root yields and dry matter content. The standard checks Kibandameno and Albert, are known for their susceptibility to CMD and CBSD respectively.

## Partner institutions

IITA led the initiative through its Eastern African Hub, in Dar es Salaam Tanzania. The five countries were represented by their respective agricultural research organisations or departments, namely: National Agricultural Research Organization (NARO) in Uganda, Kenya Agricultural and Livestock Research Organisation (KALRO) in Kenya, Department of Research and Development, (DRD) in Tanzania, Instituto de Investigação Agrária de Moçambique (Agrarian Research Institute of Mozambique - IIAM) in Mozambique, and Department for Agricultural Research Services (DARS) in Malawi. Other partner institutions included Natural Resources Research Institute (NRI) in the United Kingdom, Kenya Plant Health Inspectorate Services (KEPHIS) in Kenya, and Genetic Technologies International Limited (GTIL) in Kenya.

## The process

It was a five step process as shown in Figure 1 and discussed below:

1. **Selection and submission of 'dirty' elite and check clones:** Between August and December 2012 each country obtained and submitted to the Natural Resource Institute (NRI) United Kingdom and Kenyan Plant Health Inspectorate Services (KEPHIS) Kenya between 20 and 25 stem cuttings from asymptomatic plants of each of the best five clones (Table 1) for virus cleaning. To avoid intra-clonal variation, stem cuttings were obtained from one plant. The initiative considered the two laboratories as backups of each other in the role of virus cleaning and indexing.

2. **Virus cleaning and indexing:** Upon arrival at the two laboratories, the stem cuttings were sprouted and grown in pots under quarantine for about 3 months while observing the plants for virus symptoms. At NRI, the asymptomatic plants were separated from symptomatic ones, and leaf samples taken for further virus diagnostics using PCR procedures (Maruthi et al., 2002; Aloyce et al., 2013) for CMVs and Real-time Reverse Transcription Polymerase Chain Reaction (Real-time RT PCR) procedures (Adams, et al., 2013; Abarshi et al., 2012) for CBSVs. The virus negative plants of each clone were initiated into tissue culture (TC) and micro propagated to raise over 50 plants. For clones with only virus positive plants, meristems were used to initiate TC plants which were incubated under thermotherapy conditions and left to grow for 8 – 10 weeks before reintroducing them in the quarantine glasshouse and diagnosed to confirm the absence of the viruses. At KEPHIS, meristem tips of asymptomatic plants were initiated into TC and later diagnosed for viruses. The negative plants were micro propagated to raise over 50 plants while the positive ones were incubated in the thermotherapy chamber at 38$^{o}$C for 21 days before picking the meristems for TC initiation. Upon establishment, the plants were tested again for viruses, and the cycle was repeated for the positive plants until negative plants were obtained and micro propagated to over 50 plants.

3. **Micro-propagation and distribution:** From NRI, approximately 75 TC plants per clone were sent to Genetic Technologies International Limited (GTIL) a private TC laboratory in Nairobi Kenya and micro propagated (photo 1A) to more than 1500 plantlets, enough to provide at least 300 plantlets per clone per country. Using cylindrical or cuboid plastic

transparent containers, GTIL initiated in the media a maximum 75 plantlets per container, labelled, allowed 2 weeks to develop roots and shoots, sealed, packaged and sent to the partner countries. It is this step that marked the exchange of germplasm, since each country submitted 5 clones but was about to receive 20 more clones on top of the 5 clones. To avoid spillage and damage during shipment, the media was made more solid. The containers were placed in bigger boxes and surrounded with shock absorbing materials. The boxes were also marked with an arrow up direction and labelled fragile. The plants were sent by road to Uganda and Tanzania or as cargo with Kenya airways to Malawi and Mozambique. Kenya plants were hardened at GTIL. To ease quarantine authority clearance at entry points, all the shipments were accompanied by copies of the import permit and phytosanitary certificate from KEPHIS. At the first point of entry, the consignments were cleared for entry by plant protection officials of respective countries and received by the national cassava research team or project team representatives.

4. ***In-vitro* conservation:** The clean plants at KEPHIS served as a back-up for micro propagation activities at GTIL, and 5 to 10 plants per clone were later sent to the countries as *in-vitro* back-ups. The backups were meant to serve both immediate need of replacing plantlets of any clone lost during the acclimatization process and long term need of clean stocks for pre-basic seed production systems of identified varieties per country. Apart from Uganda and Kenya, early indications are that countries lost majority of the backup plants for all or majority of the clones. Erratic power supplies faced by majority of NARS tissue culture facilities were reported as the main cause of plant losses through contaminations.

5. **Acclimatization and field multiplication:** In the countries, the TC plantlets were checked for contamination, physical damage and registered. The plantlets were allowed two to seven days at room temperatures, in TC laboratory growth rooms or normal rooms, to recover from transit stress. The plantlets were then carefully separated from the media and introduced into individual small cups filled with vermiculite or forest soil sand mixture, and placed in nutrient enriched water baths. Each potted plant was covered with a transparent polythene bag to create micro humid conditions. In Tanzania, the potted plants covered with polythene bags were further placed in a bigger humidity chamber. After one week, the polythene bags were cut open at one end, and after two weeks, fully opened (photo 1B). The tender plants were sprayed with fungicide and insecticide while irrigating with nutrient enriched water. After a month the plants were transplanted into bigger polythene bag containers (also used in potting tree and flower seedlings) with sterilized soil. By the end of the month the plants were ready for transplanting into the field for multiplication. Fields located in areas with very low CBSD and CMD pressure were identified and used for multiplication (Photo 1C). In drought prone sites (in Kenya, Tanzania and Mozambique) irrigation infrastructure were ensured. In Malawi, the cold/winter period (June – October 2014) after acclimatization forced further delay of transplanting the plants to the multiplication field to avoid death due frost. In Uganda, field multiplication was rain fed and no fertilizer application was made. To minimize disease infection fields were isolated from any old cassava crop and rogued of any virus symptomatic plants till maturity.

## Capacity preparedness for Acclimatization

Both human and infrastructural capacity preparedness is critical and was undertaken to successfully receive and manage big numbers of TC plantlets of the germplasm. IITA in collaboration with Uganda's cassava research program (also a regional Centre of Excellency for Cassava Research) at National Crops Resources Research Institute (NaCRRI), conducted a two weeks practical training on post-flask management. Each country (except Tanzania which sent four technicians) was represented by two senior technicians who formed the core team to receive and manage the TC plants of germplasm in their respective countries. Also, two staff from KEPHIS and GTIL each participated in the training. The trainees were equipped with both theoretical and practical knowledge and skills for preparing and handling TC plants for acclimatization and multiplication purposes. The key infrastructure required were the TC laboratory for *in-vitro* conservation of clean stocks, insect proof screen houses for acclimatization work. Whereas no improvements were made on the TC laboratories, screen houses were repaired in Malawi and Mozambique, and new ones constructed in Kenya (for KEPHIS) and Tanzania.

## Quality control mechanisms

To ensure genotype purity as well as virus clean status before the exchange of germplasm, the following quality control activities were under taken. At laboratory level during cleaning, micro-propagation, and acclimatization, labelling structures that allow traceability were observed by all partners. Additionally, IITA in collaboration with Mikocheni Agricultural Research Institute (MARI) and Biosciences Eastern and Central Africa (BecA) Hub, conducted independent virus diagnostics to verify the clean status, and fingerprinting to verify the genotype purity, respectively.

## Node stem cuttings multiplication technique to increase hardened TC plants

To mitigate the challenges of plant deaths during post-flask management and ensure that all the clones have a sufficient number of hardened plantlets for field multiplication, the few surviving plants were further multiplied using two or three node stem cuttings both in the screen houses (for hardened plants between 2 and 3 months old and ready for transfer to the field) and in the multiplication fields (for plants 4 to 6 months after establishment). In both cases, the mother stock sprouted with multiple shoots in addition to three or more plantlets or plants generated from stem cuttings. Through this technique, the countries that suffered early plant deaths during acclimitization were able to increase the number of plants of the targeted clones. The technique helped to save time and costs of acquiring and acclimating new sets of TC plants from the TC laboratory (GTIL) by the affected countries. It also ensured that there were sufficient stem cuttings for at least 20 elite clones to establish regional trials by the end of 2015 in all countries. This technique can be helpful in multiplication of hardened virus indexed cassava plants under insect-proof screen houses for pre-basic seed production under cassava seed systems. (Bernado et al., 2002; Wambua et al., ---; IITA, 2014)

## Key achievements

Two main acheivements were accomplished under the intiative. First, up to 30 clones (25 elite, 2 standard checks and 4 national check) were successfully virus cleaned and indexed, and only 27 clones exchanged among target countries. The other four were returned to respective mother countries as national checks. The exchanged germplasm represents the first-of-kind program to share in such large numbers of virus indexed *in-vitro* elite germplasm between countries as a joint action to combat CBSD and CMD. Additionally, the germplasm presents a unique opportunity to identify premier varieties with high tolerance to both CBSD and CMD in addition to being used as parents to generate more good progenies with high virus resistance background. The clean stocks are a big asset to initiation of production and access of clean prebasic seed for respective varieties.

The second achievement is a strong partnership built between breeders and virologist in national and international intitutions (Photo 1D) that ensured successful exchange of elite germplasm. It represents a joint action to reverse the devastating effects of the two deadly viral diseases on cassava. The partnership also allowed cross learning between partners at all levels of the process. It has also presented a unique opportunity for breeders and virologists to work together to combat CBSD and CMD through elite resistant germplasm and clean seed.

## Learning experiences

- There were varied responses of clones to virus cleaning and indexing process. While majority of the clones cleaned easily of the viruses others like Tomo, F19-NL and Kibandameno were hard to clean, contributing to delayed timelines of the process. Tomo (not listed), a check clone from Mozambique never cleaned of the viruses at KEPHIS despite repeated efforts, and the reasons for this behaviour are unclear. However, the specificity of genotype response to the cleaning and indexing protocols has been reported (Ref). Future efforts should be made to clean this clone to confirm the observed response under this initiative.
- There were varied response of clones to micro-propagation protocols at GTIL. While majority responded positively to the protocols others such as Pwani, Nase1, Tajirika, and Okhumelela were recalcitrant and called for optimization of media for micro propagation. The recalcitrant clones also caused delays and held behind the timelines for delivery of the plants to countries. Recalcitrant behaviour among clones has been reported in cassava (Acedo and Labana, 2008; Bernado et al., 2002; Abd Alla et al., 2013). There is need to further investigate and establish the optimal protocol that is suitable for micro-propagation of most of the genotypes.
- Extreme temperatures affected adversely the acclimatizing plants. For example extreme cold night temperatures in Nairobi either slowed growth or caused death of the acclimatizing plants in Kenya. On the other hand, hot screen house conditions scotched the plants at Kibaha and Maruku in Tanzania to death. These experiences suggest that temperature conditions are important in selecting a station where acclimatization of cassava plants should be conducted, specifically, shaded cool or fresh environments with no extreme temperatures are recommended (FAO, 2010). The extreme hot conditions

were overcome by introducing a shade net inside the screen house, but can also be overlaid on top of the white insect proof net.

- Bulk packaging of the TC plantlets (75 per container) at GTIL was cost effective but presented a number of challenges. First, the plantlets were exposed to contamination during shipment due to ineffective sealing of large containers. Second, the plantlets were vulnerable to damage during removal for hardening. The roots of the plantlets grow and intertwine into a network, making is most difficult to separate the plants without root or plantlet damage during removal. Third, for some clones the plantlets in the middle of the container tended to be slow in root development resulting into plantlets with no or underdeveloped roots that have high chances of early death during acclimatization. Single test tube TC plant packaging is the commonly used method and is associated with limited contamination and damages during removal (Ref). However, test tubes can be expensive and bulky.
- Requirement of a skilled and committed personnel to carefully and constantly monitor the acclimatizing plants, keenly observing progress of almost every plant, was critical. Therefore training and supervision of personnel is key. Assigning the task to one committed personnel readily available during the two months of acclimatizing plants will yield success.
- We experienced variation across countries in capacities for *in-vitro* conservation of elite germplasm. To date most of the countries, except Uganda (Centre of Excellency for Cassava Research) and Kenya (kept at KEPHIS), have lost at least half of the *in-vitro* back-up plants due to infrastructure inefficiencies such as power failures. For example in year one of plantlets' delivery, Tanzania lost more than half of the materials due to erratic power supplies at MARI where the tissue culture facilities are located. Malawi and Mozambique have since experienced similar challenges.

## Conclusion

This process is the first of its kind ever known to successfully facilitate exchange the largest number of clean virus indexed elite germplasm to combat CBSD and CMD. However, the success comes with adequate preparations of infrastructure and trained personnel to manage the different sub-processes in the frame work of an effective partnership. Also, a small proportion of varieties that are recalcitrant to tissue culture propagation can slow the process and hence need for more time. The clean stocks of all exchanged clones will offer partner countries unique opportunity to test and confirm dual resistance or susceptibility taking into account the different species of CBSVs and CMVs in the region. Also clean stocks for the seed systems. The success of this process demonstrates the potential in quick solutions to biotic challenges through a joint action of germplasm exchange.

## Acknowledgement

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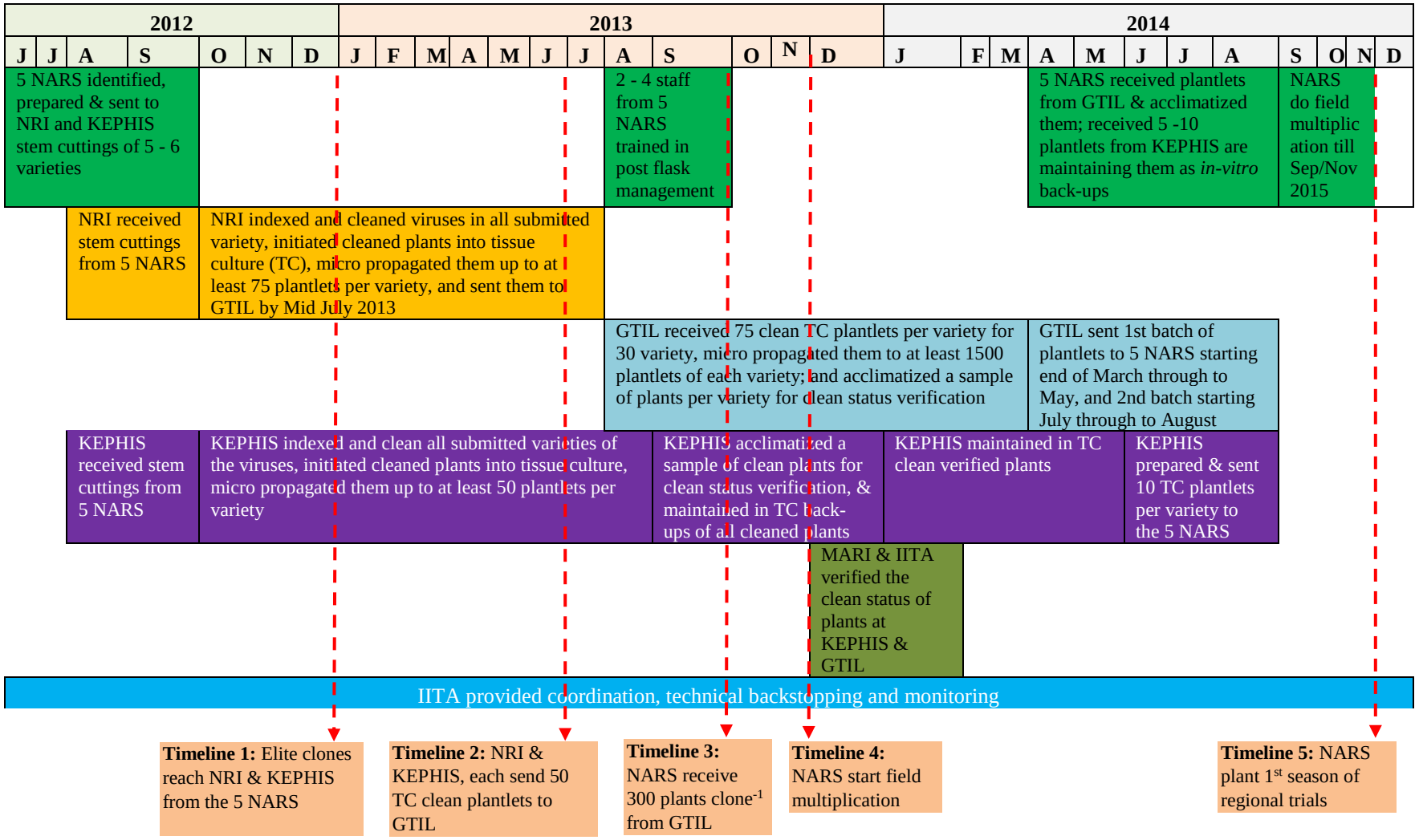
2012
2013
2014
J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
5 NARS identified, prepared & sent to NRI and KEPHIS stem cuttings of 5 - 6 varieties
2 - 4 staff from 5 NARS trained in post flask management
5 NARS received plantlets from GTIL & acclimatized them; received 5 -10 plantlets from KEPHIS are maintaining them as in-vitro back-ups
NARS do field multiplication till Sep/Nov 2015
NRI received stem cuttings from 5 NARS
NRI indexed and cleaned viruses in all submitted variety, initiated cleaned plants into tissue culture (TC), micro propagated them up to at least 75 plantlets per variety, and sent them to GTIL by Mid July 2013
GTIL received 75 clean TC plantlets per variety for 30 variety, micro propagated them to at least 1500 plantlets of each variety; and acclimatized a sample of plants per variety for clean status verification
GTIL sent 1st batch of plantlets to 5 NARS starting end of March through to May, and 2nd batch starting July through to August
KEPHIS received stem cuttings from 5 NARS
KEPHIS indexed and clean all submitted varieties of the viruses, initiated cleaned plants into tissue culture, micro propagated them up to at least 50 plantlets per variety
KEPHIS acclimatized a sample of clean plants for clean status verification, & maintained in TC back-ups of all cleaned plants
KEPHIS maintained in TC clean verified plants
KEPHIS prepared & sent 10 TC plantlets per variety to the 5 NARS
MARI & IITA verified the clean status of plants at KEPHIS & GTIL
IITA provided coordination, technical backstopping and monitoring
Timeline 1: Elite clones reach NRI & KEPHIS from the 5 NARS
Timeline 2: NRI & KEPHIS, each send 50 TC clean plantlets to GTIL
Timeline 3: NARS receive 300 plants clone-1 from GTIL
Timeline 4: NARS start field multiplication
Timeline 5: NARS plant 1st season of regional trials

**Table 1.** Elite germplasm cleaned, multiplied and exchanged among 5 countries in Eastern and Southern Africa during 2012/2014.

| Variety name | Country of Origin | Fresh Root Yield (t/ha) | DM content (%) | Reaction to diseases | | Release status |
|---|---|---|---|---|---|---|
| | | | | CMD resistance | CBSD resistance | |
| KBH2002/066 | Tanzania | 34.1 | 28.0 | Moderate | Moderate | Released |
| Pwani | Tanzania | 50.8 | 29.2 | Moderate | Moderate | In pipeline |
| Mkumba | Tanzania | 23.3 | 27 | Weak | Moderate | In pipeline |
| KBH2006/026 | Tanzania | 30.0 | 29.0 | Moderate | Moderate | Released |
| Kizimbani | Tanzania | 28.6 | 28.0 | Moderate | Moderate | Released |
| Kiroba* | Tanzania | 20.0 | 32.0 | Weak | Moderate | Released |
| Mkombozi* | Tanzania | >20 | ~30 | Strong | Weak | Released |
| Albert** | Tanzania | fair | good | Strong | Susceptible | Not released |
| Sangoja | Malawi | 35 | 33 | Moderate | Moderate | Released |
| Sauti | Malawi | 30 | 34 | Moderate | Moderate | Released |
| Yizaso | Malawi | 35 | 33 | Moderate | Moderate | Released |
| Kalawe | Malawi | 28 | 36 | Moderate | Moderate | Released |
| CH05/203 | Malawi | 33 | 34 | Moderate | Moderate | In pipeline |
| Mbundumali* | Malawi | 25 | 37 | Susceptible | Susceptible | Recommended |
| Coliacanana | Mozambique | 20.0 | 33.0 | Weak | Moderate | Released |
| N'ziva | Mozambique | 22.0 | 35.1 | Weak | Moderate | Released |
| Okhumelela | Mozambique | 20.0 | 32.8 | Moderate | Moderate | Released |
| Orera | Mozambique | 23.0 | 32.0 | Weak | Moderate | Released |
| Eyope | Mozambique | 25.0 | 32.0 | Moderate | Moderate | Released |
| LMI/2008/363 | Kenya | 69 | 27 | Moderate | Moderate | In pipeline |
| F19-NL | Kenya | 39.4 | 25 | Moderate | Moderate | In pipeline |
| Tajirika | Kenya | 61 | 25.7 | Moderate | Moderate | Released |
| Shibe | Kenya | 68 | 26 | Moderate | Moderate | Released |
| F10-30-R2 | Kenya | 58 | 40 | Moderate | Moderate | Adv. yield trial |

| | | | | | | |
|---|---|---|---|---|---|---|
| Kibandameno** | Kenya | 26.1 | 40 | Susceptible | Susceptible | Not released |
| TZ130 | Uganda | - | - | Strong | Moderate | Released |
| NASE14 | Uganda | 31.2 | 35.0 | Strong | Moderate | Released |
| NASE18 | Uganda | 38.6 | 35.5 | Strong | Moderate | Released |
| NASE1 | Uganda | 14.9 | 32.5 | Strong | Moderate | Released |
| NASE3 | Uganda | <10 | 30.0 | Moderate | Moderate | Released |

* National susceptible checks returned only to countries of origin; ** are standard susceptible checks distributed with the elite clones

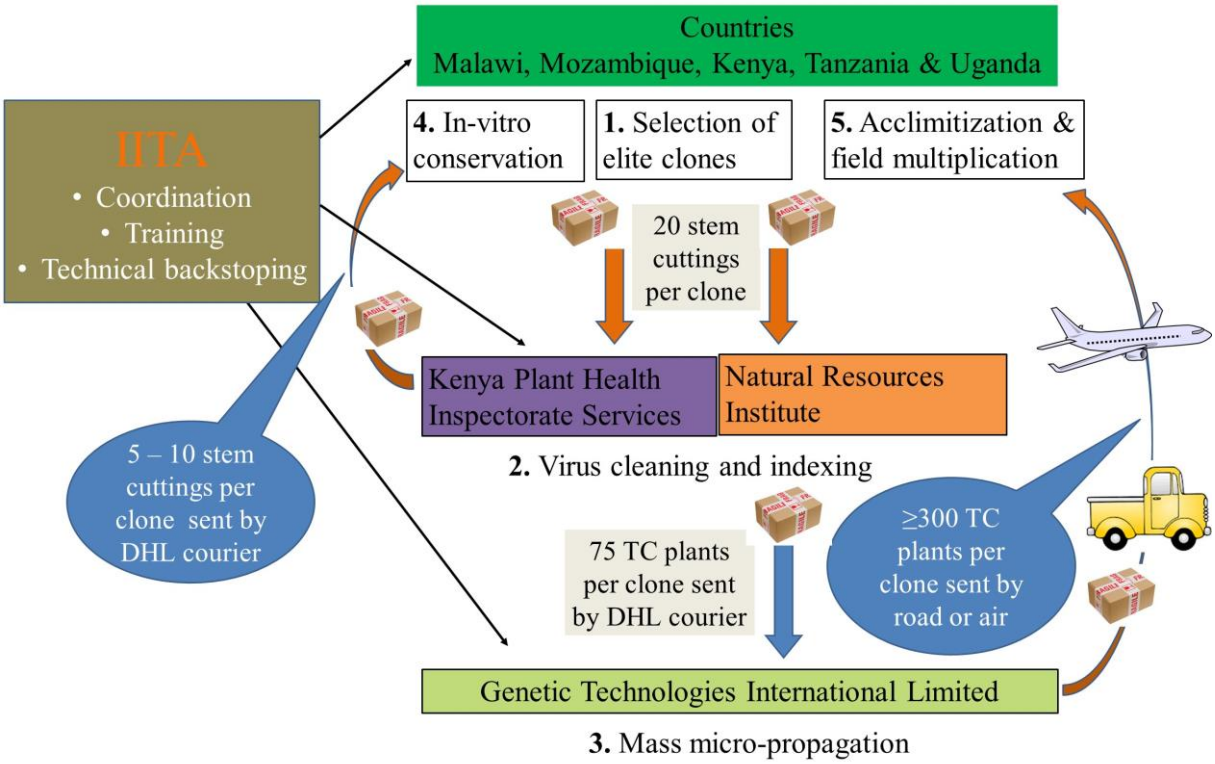


**Figure 1:** Schematic flow of the cleaning, multiplication and exchange process of the germplasm





**Photo 1. A**, micro propagation at GTIL; **B**, acclimitization at Kibaha; **C,** field multiplication of EG at Kandiyani Malawi; and **D,** partners of breeders and virologists in field in Malawi.