

Diagnostics, Phytosanitation, Phytosanitary Regulations and Germplasm Transfer

An overview

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Some terms

Pest = Pathogens, insects, mites, nematodes, etc.

Any species, strain, biotype injurious to plants and plant products

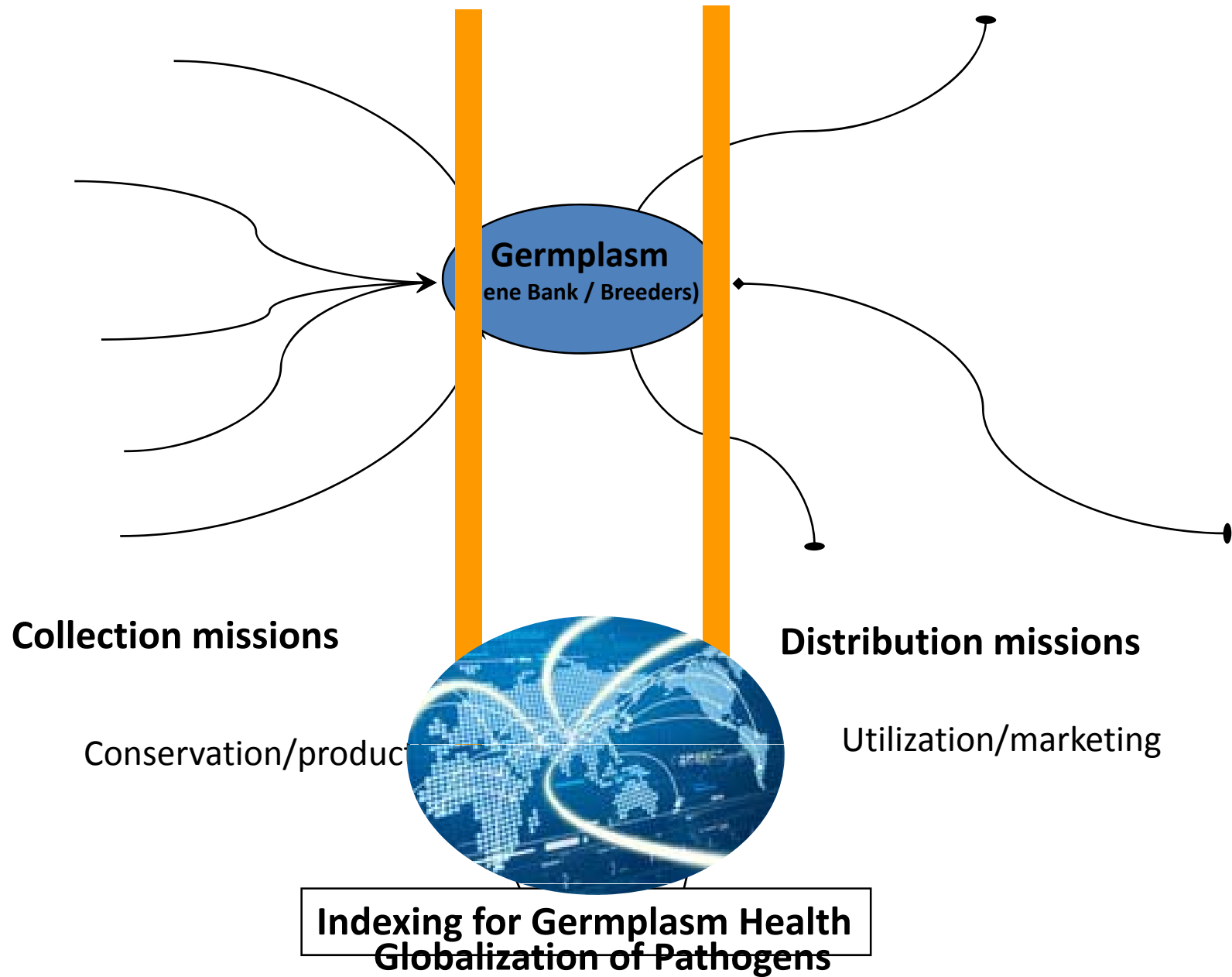
Virus negative: plants tested negative to a particular virus

CBSD-free: Free of all virus species presently known to cause CBS

Virus clean or clean germplasm: Seldom used in official documents

Diagnosis: Identification of the cause of the problem

Diagnostic tools: To ascertain presence, absence or quantify pests



Germplasm exchange: risks

- Movement of germplasm involves a risk of accidentally introducing quarantine pests.

- Germplasm sometimes are contaminated with harmful pests.

- Pathogens harmful to the same species
(e.g. cassava to cassava)

- Can also carry pathogens that do not harm the carrier, but harmful to other species
(e.g. cassava carrying spores of maize pathogen)
(spores, insect eggs, etc)

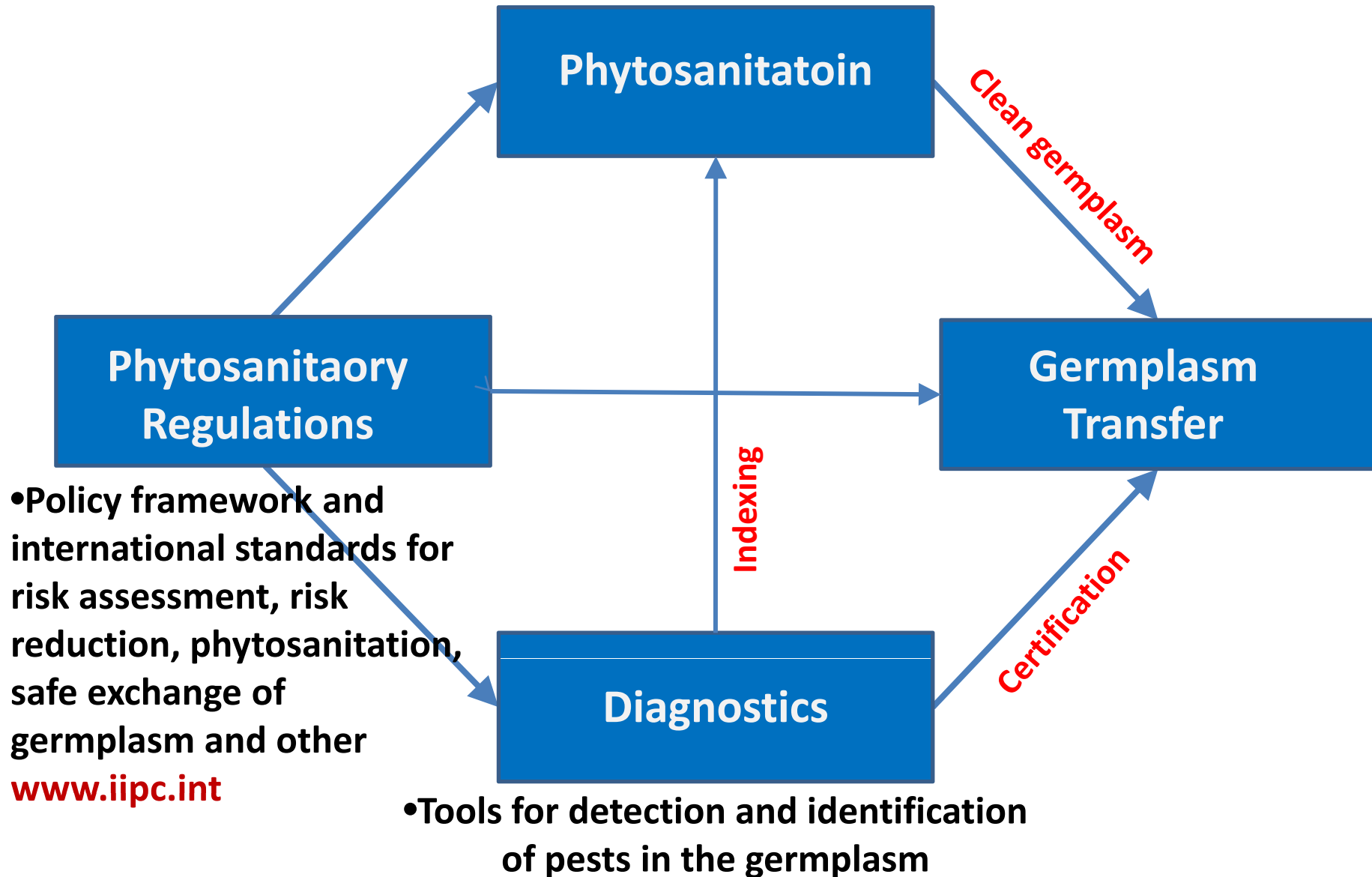


Clean germplasm is prerequisite for safety of conserved germplasm and for the safe distribution of germplasm

Interception of non-indigenous pests at US ports

- 725,000 pest (non-indigenous insects, mites, molluscs, nematodes, plant pathogens and weeds) interceptions recorded in US Port-interception network from 1984 to 2000.
- 62% of intercepted pests were associated with baggage,
- 30% were associated with cargo and
- **7% were associated with plant propagative material.**
 - 50,750 in 17 years (ca. 3,000 interceptions per year)

- Measures to remove pests infested/infected plants
- Elimination of pests from infected/infested tissues (therapy)



International regulations and technical guidelines

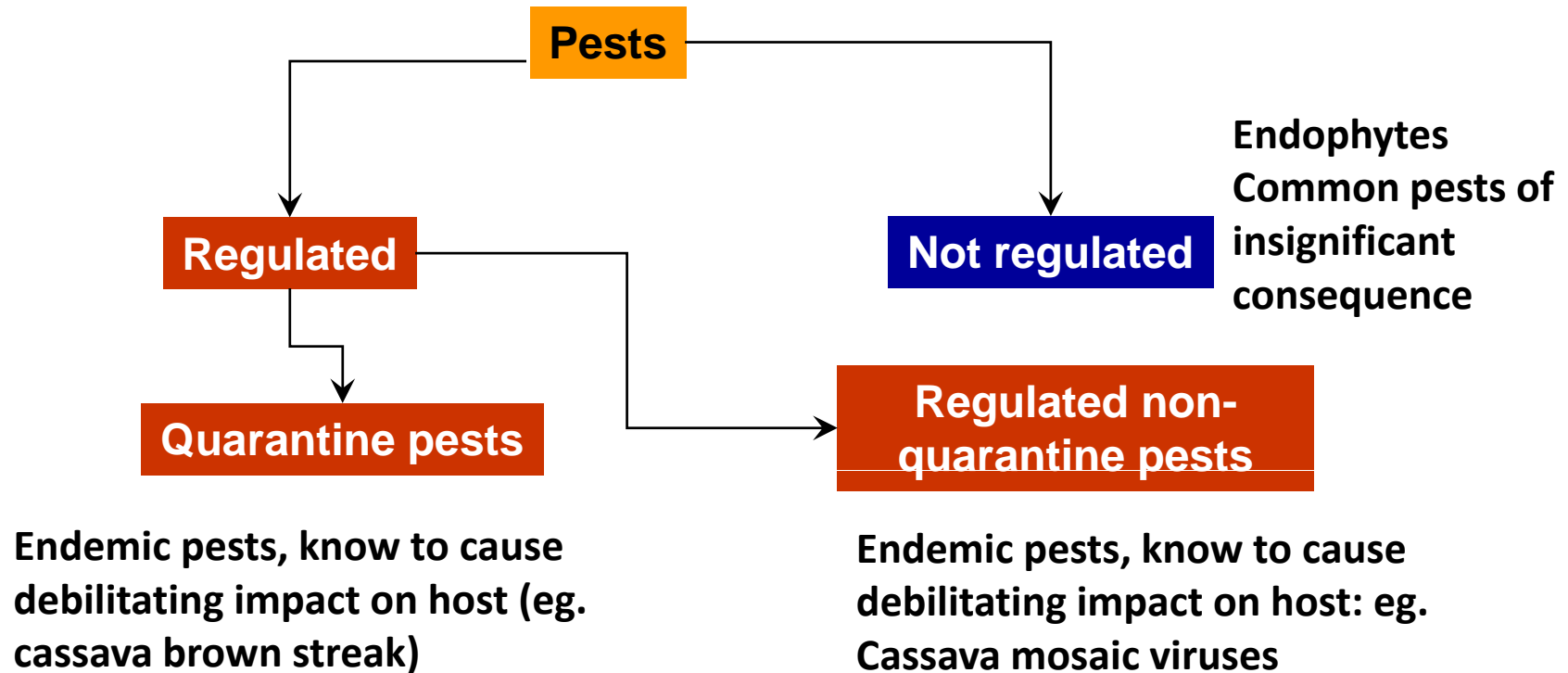
International Standards for Phytosanitary Measures (ISPMs) of FAO-IPPC



ISPM 1 to 36
www.ippc.int

Challenges for cassava conservation

- Pest matters, irrespective of disease causing potential



NPPO provides the list of quarantine pests and non-quarantine pests

Major cassava pests in Africa (regulated non-quarantine pests)

Indigenous pests

- Cassava mosaic viruses
- Whiteflies, fungal diseases, root scales etc

Pests of regional importance

- *Cassava brown streak virus*
- *East African cassava mosaic virus* – Uganda (EACMV-UG)

Exotic pests

- Green spider mite (*Mononychellus tanajoa*)
- Cassava mealy bug, (*Phenacoccus manihoti*)
- Cassava Bacterial Blight (*Xanthomonas axonopodis*)
- Indian cassava mosaic virus

Quarantine pests of cassava in Africa

Pests not present in the continent

1. Cassava common mosaic virus (??)
2. Cassava green mosaic virus
3. Cassava vein virus
4. Cassava X virus
5. Frog Skin Disease (complex etiology)
6. Cassava antholysis (phytoplasma-like)
7. Cassava witches broom (Phytoplasma)
8. Super Elongation (*Sphaceloma manihoticola*)

Risk of pest spread through planting materials

Pest	Stems	Sterile tissue culture	seed	Sterile certified tissue culture plants*
Bacteria	+	-	-	-
Fungi	+	-	-	-
Virus	+	+	- (?)	-
<i>Insects & other</i>	+	-	-	-

Stems: carriers of all pests

Tissue culture plants: Only viruses (some endophytic bacteria)

Virus elimination requires combination of tissue culture and therapy techniques (cryotherapy, etc) and selection of plants following virus indexing.

The viruses of cassava in Africa

African cassava mosaic virus (ACMV)
Indian cassava mosaic virus

East African cassava mosaic virus (EACMV)
South African cassava mosaic virus (SACMV)
East African cassava mosaic Cameroon virus
East African cassava mosaic Malawi virus
East African cassava mosaic Zanzibar virus
East African cassava mosaic Kenya virus
EACMV-Uganda (recombinant virus)
Cassava mosaic Madagascar virus

Cassava brown streak virus (Ipomovirus)
Cassava brown streak Uganda virus

*Cassava Ivorian bacilliform virus**
*Cassava Kumi virus**
*Cassava 'Q' virus**
Cassava common mosaic virus (Potexvirus)*



Challenges to cassava conservation

What goes in stays

- Gene banks conserves cassava
- Cassava conserves pests, particularly viruses

- Cassava is seriously affected by accumulation of pathogens in the propagation material.
 - High risk of pathogen spread through stems
 - Constraint to field gene banks
High risk of infection & re-infection; cause germplasm decline and genetic erosion
 - Restrictions on exchanging stems
 - International exchanges require in vitro plants

Challenges to cassava conservation: Virus threat to micropropagation

Tissue culture cleanses
pests and pathogens, but
not viruses.


- *In vitro* conservation is emerging as mainstay for conserving clonal crops.
- Effectiveness of in vitro therapy procedures depends on the **genotype, host susceptibility, infection status, virus strains, type of therapy procedure and practice**
- Procedures capable of eradicating cassava viruses are time consuming.
- Requires expensive capacity and facilities, and round the clock access to water and power.

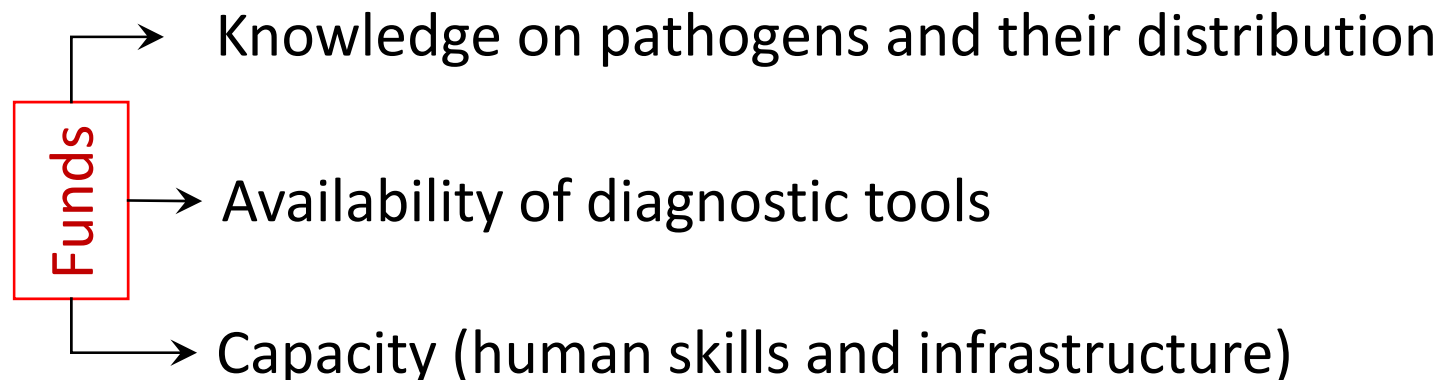
Challenges to cassava conservation: Virus diagnostics

High diversity is a bane to accurate diagnosis of viruses

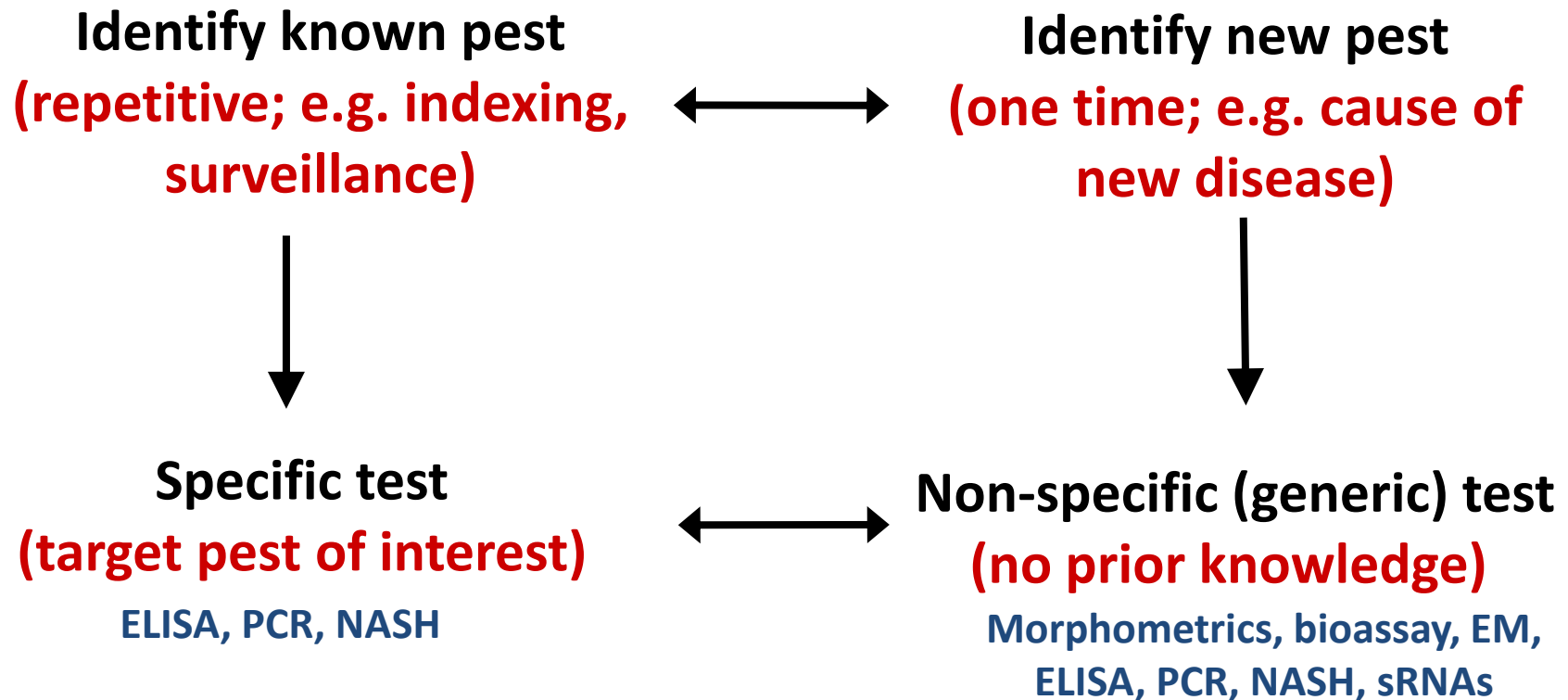
- ELISA, and PCR-based tests (conventional and RT-PCR) are used for the detection of cassava viruses. Recently even siRNA based methods are used for diagnosis.
- High diversity poses challenges to the virus detection.
- Generic (**broad-specific**) diagnostics are better for virus detection for quarantine monitoring, and virus indexing.

Pest detection: requirements

- Symptomatic stage: Confirmation of pathogen
 - Asymptomatic stage: Confirmation of infection & pathogen
 - Undetectable stage
 - Germplasm (dormant)
 - Low-titers of pathogens
- 
- Diagnostics**

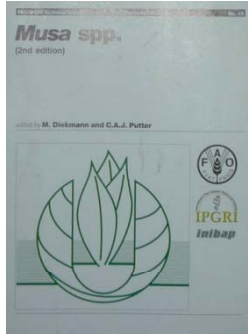


Choice of methods depends on the need

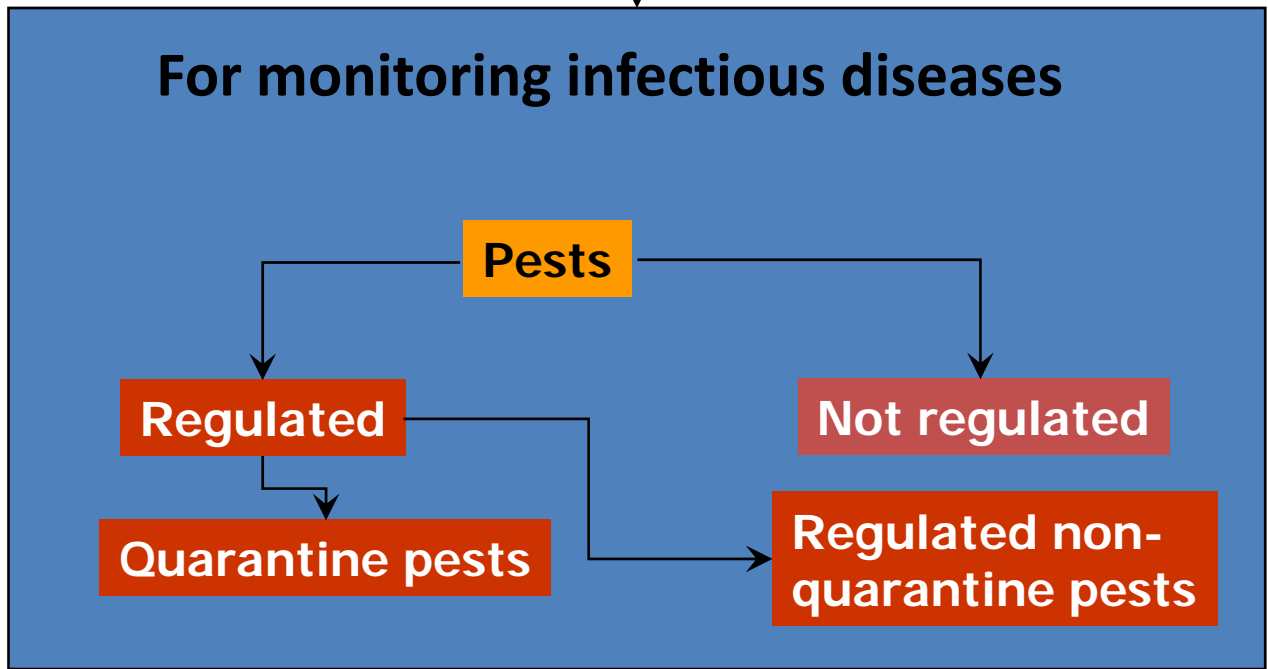


- PCR-based assays are commonly used for cassava indexing
- Small RNA-based approach is gaining popularity as a useful broad-specific tools

Guidelines for use of diagnostics



International Plant Protection Convention (IPPC)
WTO-SPS guidelines
Regional and Country-specific guidelines



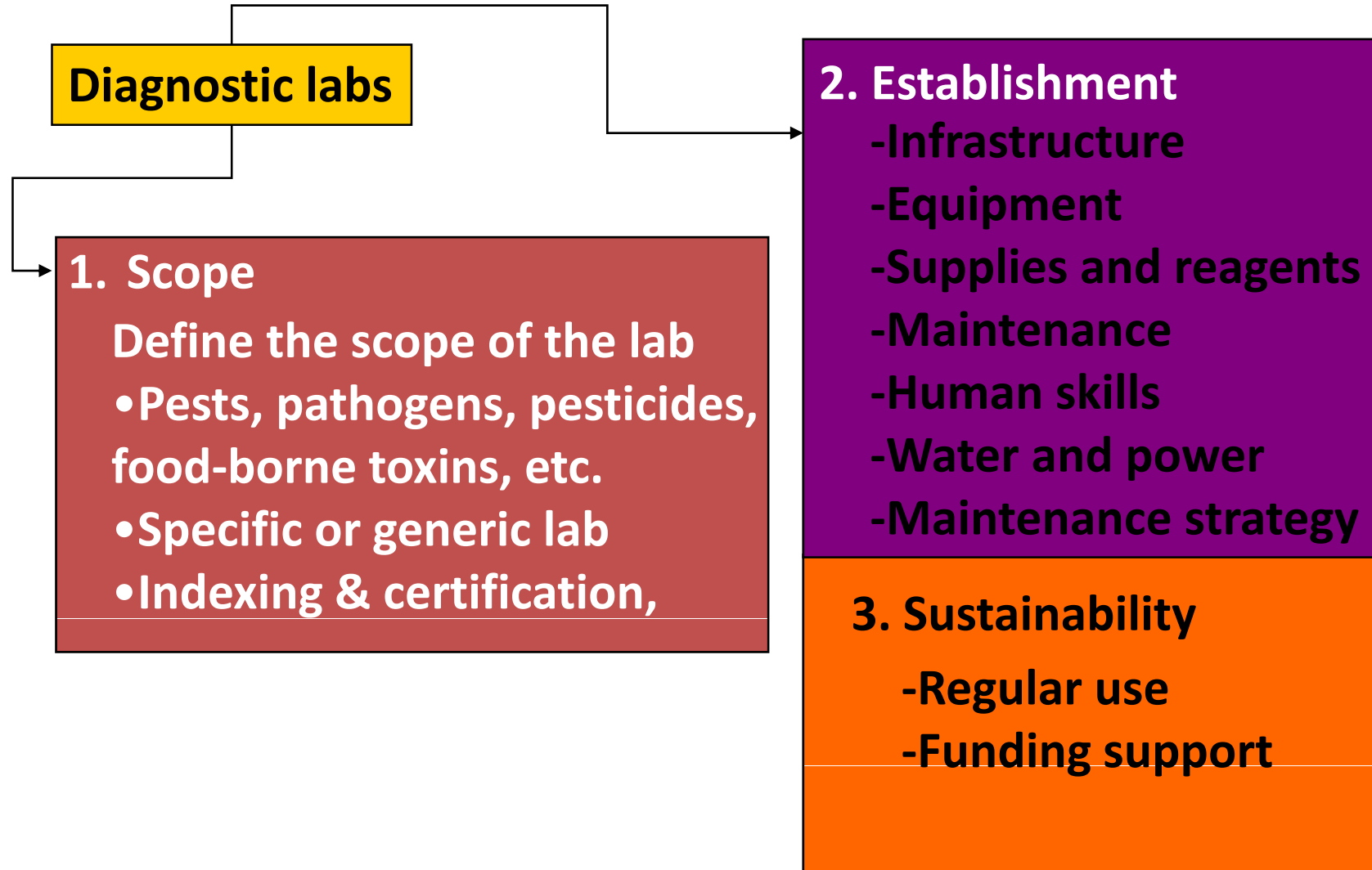
Product quality certification

- Planting material
- Product quality (inputs as well as plant products)

Plenty of options for cassava pest diagnostics

- Diagnostics is a very well established field, but not saturated
- Tremendous progress over last 3 years
- Low-tech to very high-tech tools
- Simple cost-effective tools to most expensive tools
- Availability is not an issue, application and practice matters.

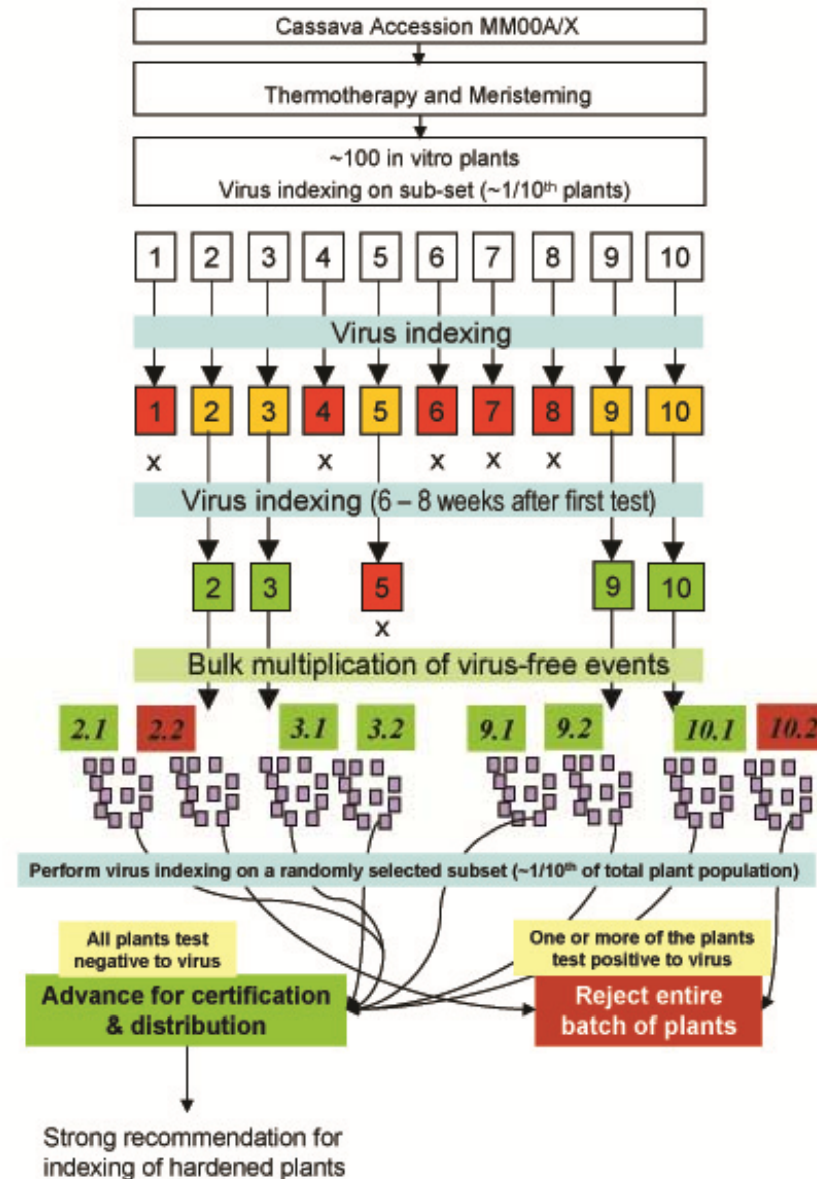
Sustainability of diagnostics



Cassava Phytosanitation

Scheme for in vitro production of virus-free cassava clones

	Variety	Total plants tested	Number that tested negative to CBSV
1	MM06/0011	20 ^{TC}	15
2	MM06/0024	20 ^{TC}	10
3	MM06/0138	20 ^{TC}	3
4	MM06/0131	20 ^{TC}	3
5	MM06/0019	20 ^{TC}	9
6	MM06/0079	20 ^{TC}	11
7	MM06/0013	20 ^{TC}	1
8	MM06/0045	20 ^{TC}	20
9	MM06/0012	20 ^{TC}	20
10	MM06/0023B	20 ^{TC}	8
11	MM06/0139	20 ^{TC}	2
12	MM06/0124	20 ^{TC}	4
13	MM06/0112	20 ^{TC}	3
14	MM06/0076	20 ^{TC}	6



IPCC guidelines for cassava germplasm exchange

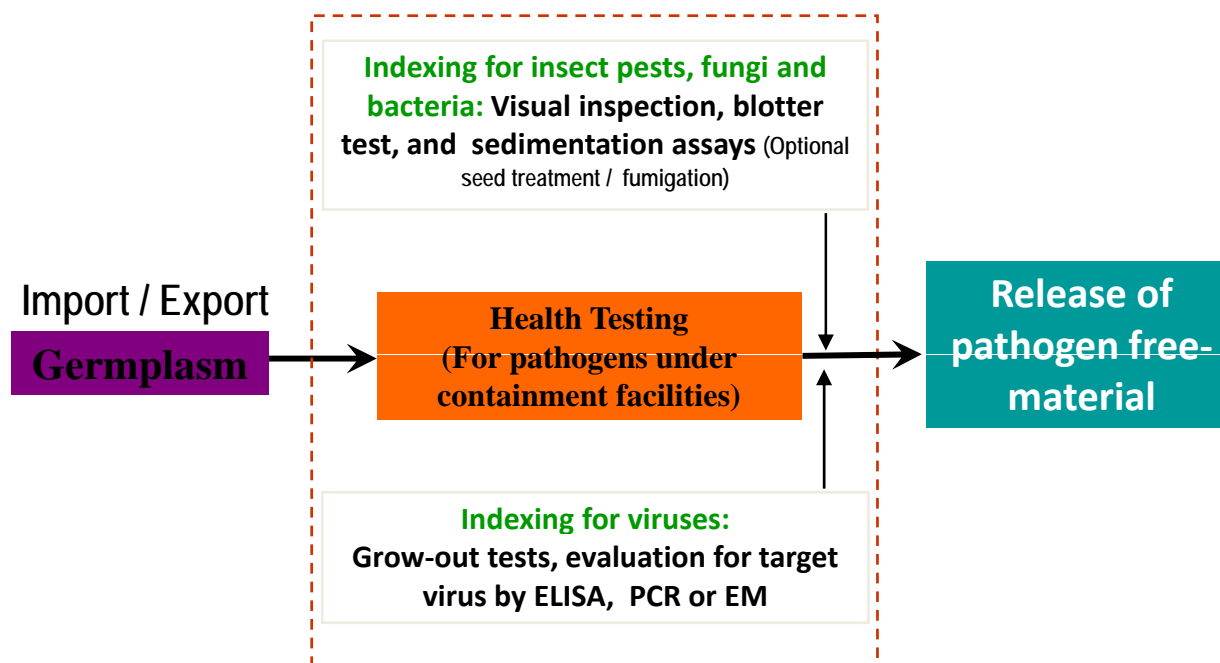
Vegetative (in vitro) material:

- Import permit and phytosanitary certificate
- Declaration certifying the absence of virus, bacteria, and nematodes (particular those quarantine/regulated pests **(based on country pest lists)**)
- Compulsory treatment of consignment before shipment into Africa.
- Exchange only virus tested, virus-free materials.
- Cassava seed: Seed sanitation and in vitro plants derived from meristems of regenerated plants

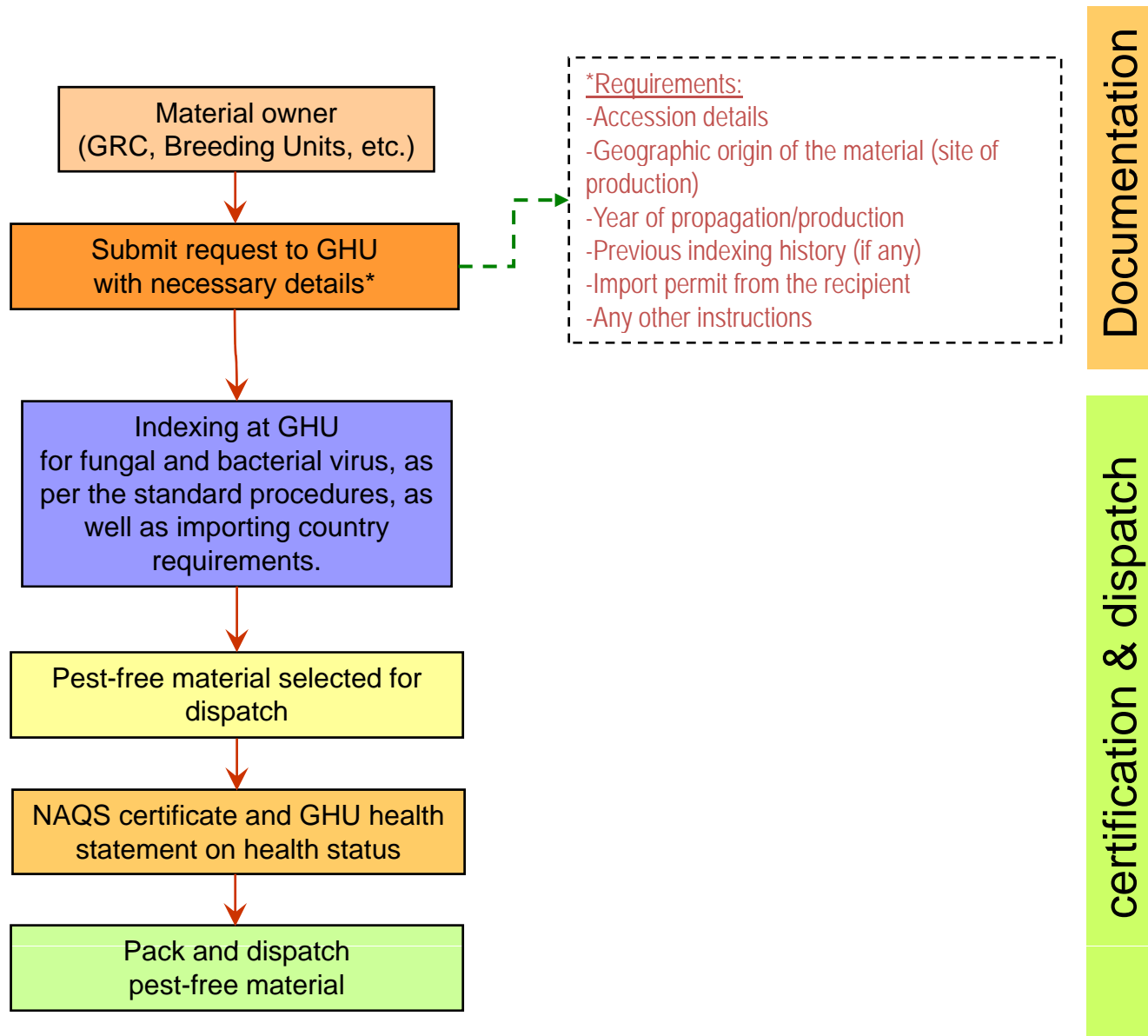
Country specific guidelines can differ from IPPC and IAPSC guidelines

IITA Germplasm Health Unit

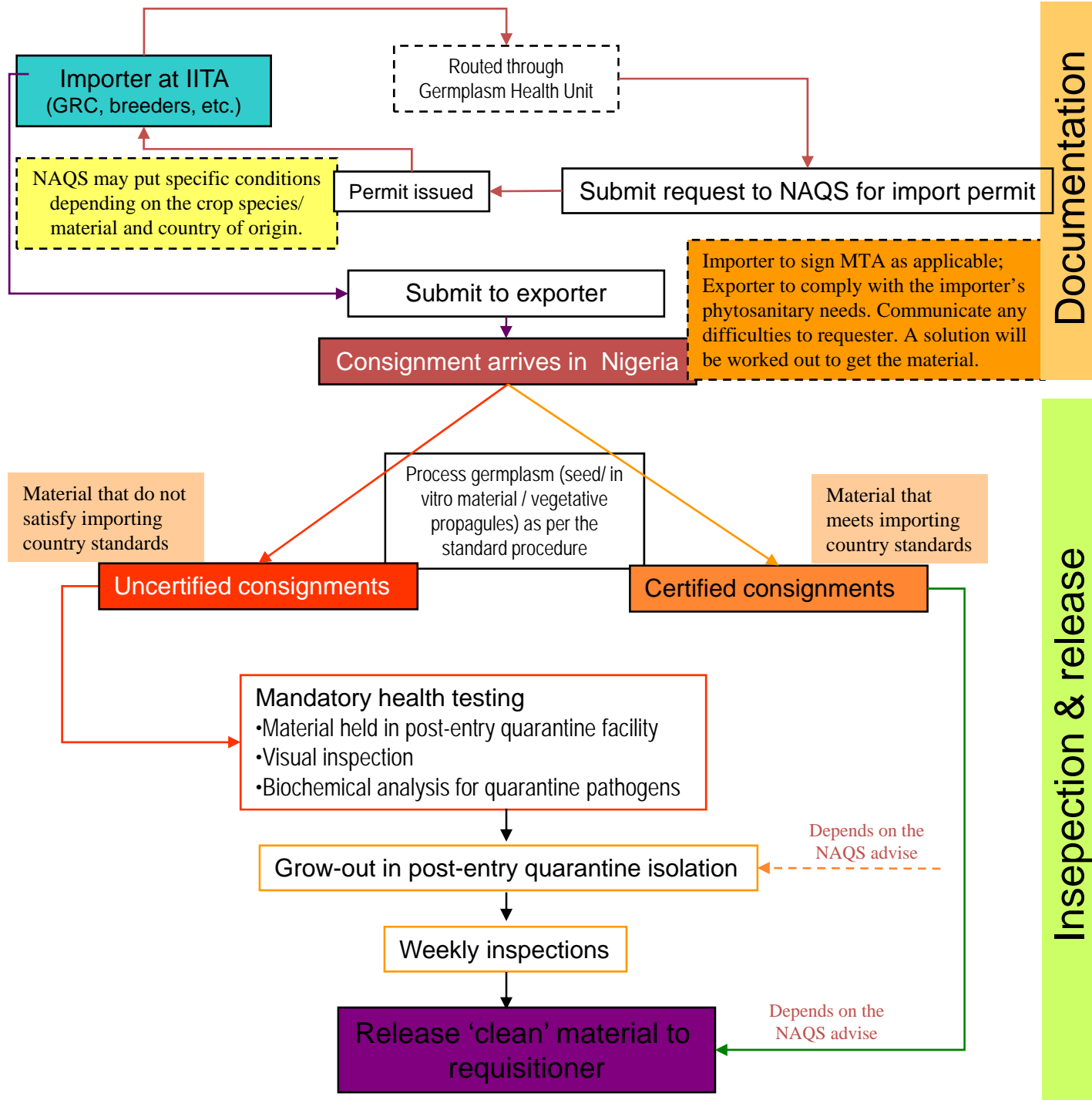
- Adjunct to NPPO
- Prevent the spread of pests and pathogens through germplasm exchange
- Produce healthy planting material & safe conservation and exchange procedures
- Ensure compliance with national and international regulations



Requirements for germplasm export

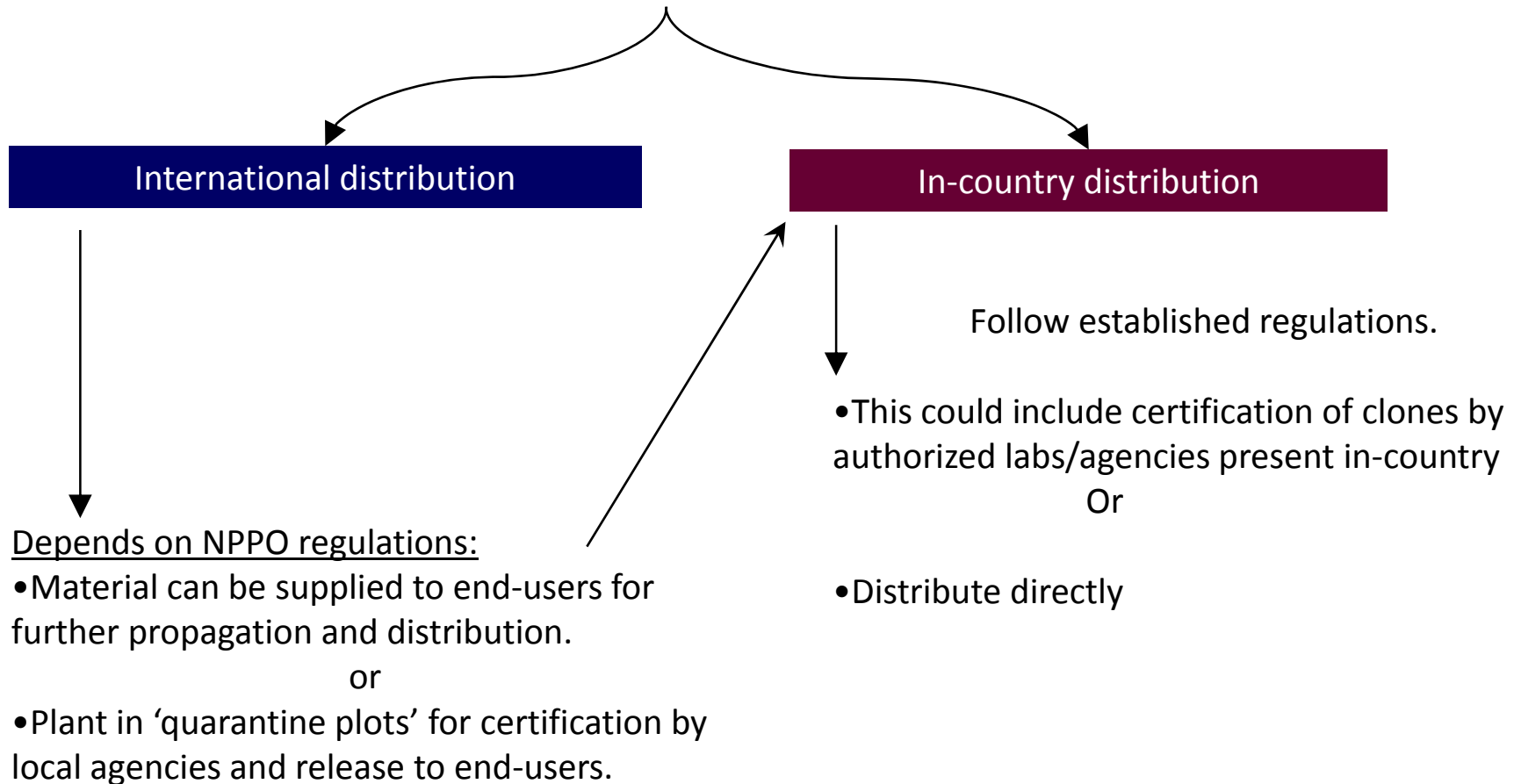


Procedure for importing planting material



Distribution of imported material

Certified Clones that meets the importer NPPO guidelines are released



Germplasm exchange with a country **with** regulations

Regulator:

- **Create awareness about the policy and standards**
- **Assess the producer constraints and find solutions (policy/technical)**

Producer / client

- **Strictly comply with the regulatory guidelines**
- **Discuss any constraints with the regulators**

Germplasm exchange with a country **without** regulations

Regulator:

- Follow the IPPC standards
- Identify gaps and establish a feasible model

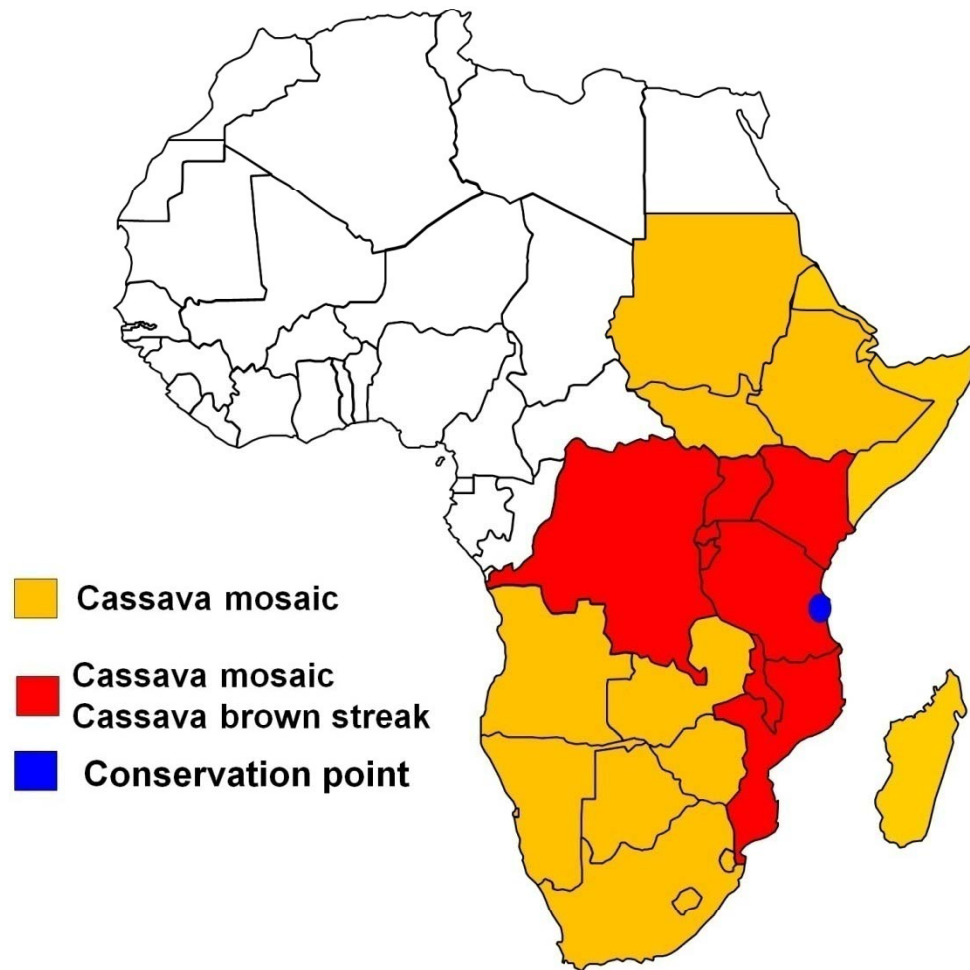
Producer/client

- Adopt international standards
- Work with regulators to establish guidelines

Other issues

- Phytosanitary permits are issued based on the NPPO analysis of risk, priority, end use, etc.
 - Permit to import **prohibited (quarantine) plant material** for research purpose
 - Permit to import plant material for research purpose
 - Permit to import plant material for processing and industrial use (not meant for propagation)
 - **Most stringent regulations apply for the material imported or exported for 'propagation purpose'.**
- Retrospective indexing

Risk of transferring material



- Within Africa, biggest risk is CBSD, which is already present in Tanzania
- Requires a perfect phytosanitary system for re-distribution

Endnote

- **Risk assessment, analysis and phytosanitary system should be kept in place prior to commissioning collection missions for conservation of germplasm in a central facility (perform in consultation with NPPO)**
- **Create awareness about the advantages (benefits) of the process, facility and phytosanitary procedures in place, to the participatory countries.**
- **Establish a virus indexing, certification and documentation system (traceability), and obtain accreditation from TPPC.**
- **Establish a safe measures to harden virus-free plants for exchange with countries with inadequate capacity to handle TC plants.**