In vitro conservation

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Cassava Germplasm: Collection Process and Action Plan for South, East and Central Africa

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In vitro conservation

Maintenance of explants / propaguls in sterile (aseptic, pathogen-free environment) and favorable artificial conditions via successive subcultures allowing material renewal and germplasm multiplication

Mainly for vegetatively propagated crops:

Species that produce recalcitrant seeds (or Hardly produce seeds)

Heterozygosis ⟹ desired traits not always maintained

Germplasm multiplication, storage of more than 1000 species

In vitro conservation

International Standards edited by Commission on PGRFA (FAO/Bioversity)

- Standards for acquisition and initial handling
- Standards for behavior testing and vigor and viability assessment
- Standards for hydrated storage of recalcitrant seeds
- Standards for *in vitro* culture and slow growth storage
- Standards for cryopreservation
- Standards for documentation
- Standards for distribution and exchange
- Standards for security and safety duplication

In vitro conservation

International Standards edited by Commission on PGRFA (FAO/Bioversity)

- Standards for acquisition and initial handling
- Standards for behavior testing and vigor and viability assessment
- Standards for hydrated storage of recalcitrant seeds
- **Standards for *in vitro* culture and slow growth storage**

- Identification of optimal storage conditions for *in vitro* conservation according to the species

  - Whole plantlet / shoot, or organs for species where these are naturally formed

    - Regular monitoring system (quality and possible contamination)

- Standards for cryopreservation
- Standards for documentation
- Standards for distribution and exchange
- Standards for security and safety duplication

In vitro slow growth medium term storage

Optimal subculture duration / interval through growth reduction

Growth conditions regulation:

Physical growth limitation
- Low temperature
- Low light/restricted photoperiod
- Minimal containment
- Minimal O2
- Osmotic (water) stress

Chemical growth limitation
- Growth regulator retardation
- Growth inhibitors

Minimal nutrition
- Low macro nutrient levels
- Low micro nutrients levels

Efficient use of resources, labor and risk of contamination

In vitro conservation

Facilities requirements for adapted *in vitro* conditions / crop or specie:

- ✓ Control environment, mainly temperature and light (Hr 40–50%)
- ✓ Light intensity between 50 to 200 µmol m^{-2} s^{-1}
- ✓ Avoid external contamination (from field material, operator)
- ✓ Controlled Air flow (AC, positive pression)
- ✓ Continuous energy supply (Back-up generators)
- ✓ Adequate shelving (local made can do) + Safety Duplication place
- ✓ Sterilization equipment (autoclave) and Post Flask management facilities
- ✓ Documentation (inventory and monitoring system like IITA OGD or Grin Global)

In vitro conservation

Cassava in vitro slow growth medium term storage

Culture conditions

	CIAT Multiplication (4E medium) + Conservation (NP medium)			**IITA** Cassava culture medium		
	Tº	**Photoperiod**	**Light intensity** µmol m^{-2} s^{-1}	**Tº**	**Photoperiod**	**Light intensity** µmol m^{-2} s^{-1}
Pre-culture	**27-28 ºC**	12 h	18.5	**25-27 ºC**	12 h	38
Conservation	**24 º C**	12 h	18.5	**19-20 ºC**	12 h	43

In vitro conservation

Advantages:

- Pathogen cleaning (indexing), away from biotic and abiotic pressions (60 % of loss in Uganda due to P&D)
- Rapid multiplication purposes, dissemination and active collections
- Allow easier germplasm exchange (continuously available)
- Useful tool for more research (genetic transformation, big scale micropropagation, biotechnology studies, ...)

In vitro conservation

Advantages (Continued):

- Alternative and complementary to field collection ("safe duplication" of field)
- Safer and more secure in a longer perspective than field bank (safer and longer)
- More cost effective than field bank (labor, space and costs reduction)
- Gain of space (2-3 ha in the field *vs.* half a conservation room)

Security requirements:

Purity: safe from contaminating organisms.

Accuracy: correct identity.

Stability: Conformity (trueness-to-type).

***In vitro conservation* « better » than field bank**

IITA Cassava Germplasm Flow

Cassava FieldBank
3499 acc.

Post flask process

Cryobanking

In vitro introduction / Regeneration

Virus indexing

In vitro GeneBank

Subculture (Separated unit)

Repatriations

Transfers

NIGERIA

BENIN Rep.

Distribution

Safety Duplication (Black box)

In vitro conservation

Cassava *in vitro* conservation

Around 47 countries hold cassava genebanks

Only 12 have *in vitro* cassava genebank (many combine pathogen cleaning with rapid multiplication and gene bank conservation)

Largest in CIAT, EMBRAPA, Argentina and IITA

Very few *in vitro* cassava genebanks in Africa (Ng and Ng, 2002)

Contrast: Good system but very rare!?!?!?

In vitro conservation

Limitations:

- Still labor intensive
- Contamination and mixture risk (loss of accessions, operator error…)
- Tissue aging
- Possibility of genetic instability (higher risk of somaclonal variation than in seed / sexual propagation)

Not as common as on plant regenerated from single cells, callus or adventitious buds

Continuous improvement of the system…

In vitro conservation

Cryobank: Conservation at ultra-low temperature (-196^{o}C) using cryogenic techniques

Long term and safe conservation

Cost effective conservation

Avoid *in vitro* disadvantages and limitations

BUT... to be part of a global genebank strategy with precise targets, QMS, sustainability and priorities (Less requested accessions, clean and unique material, high importance accessions, presence in the *in vitro* genebank???)

Thanks for your attention !!!

IITA Genetic Resources Center (GRC): http://www.iita.org/genetic-resources-center

Crop Genebank Knowledge Base (CGKB): http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=390&Itemid=557&lang=english

System-wide Genetic Resources Programme (SGRP): http://www.sgrp.cgiar.org/?q=node/432

Genebank standards for PGRFA: http://www.fao.org/docrep/meeting/027/mf804e.pdf