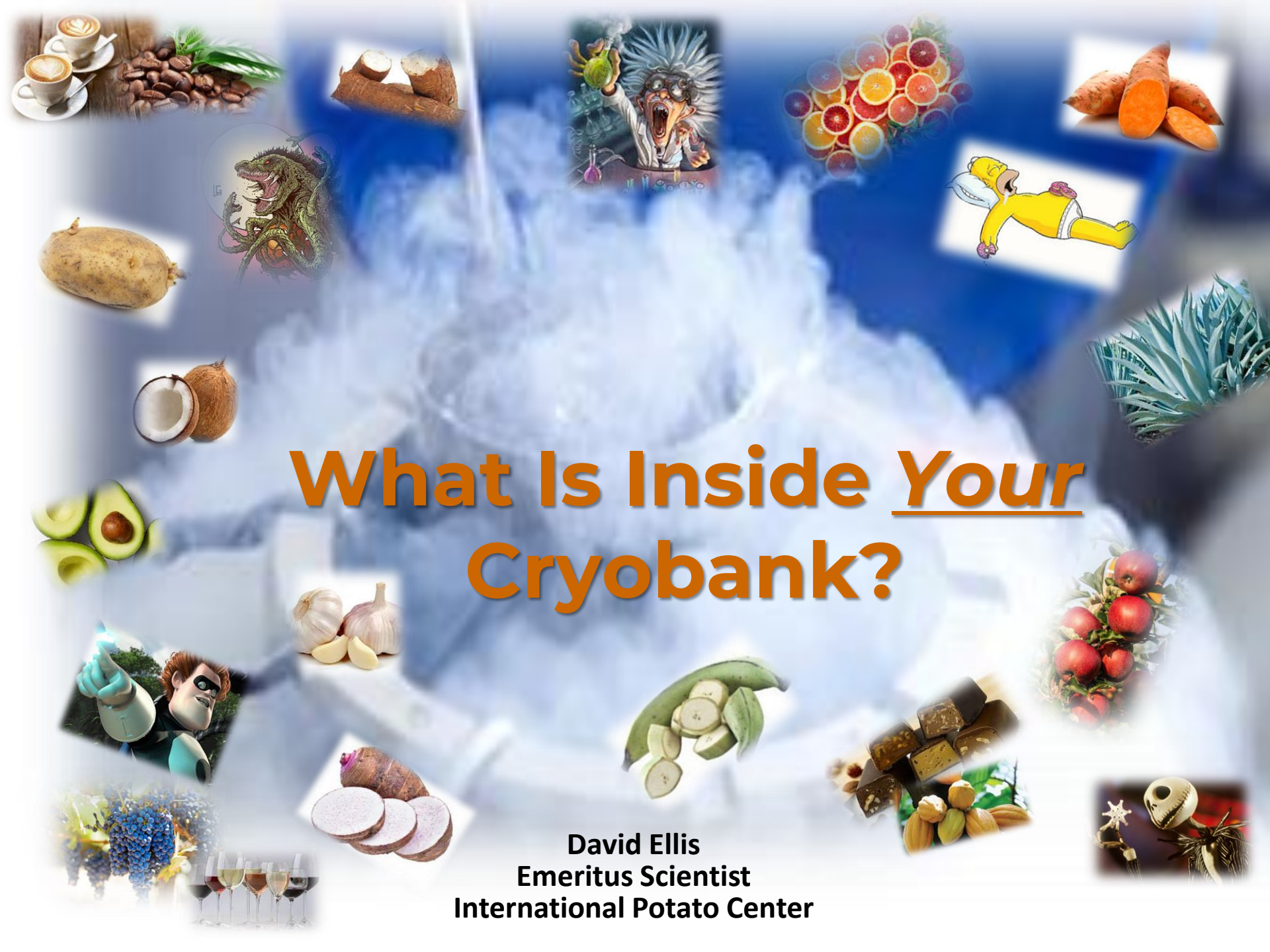


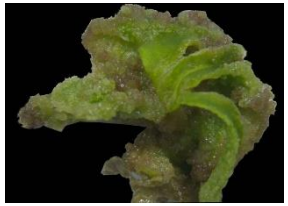
What Is Inside *Your* Cryobank?

David Ellis
Emeritus Scientist
International Potato Center

Evolution of Cryobanking with Plants

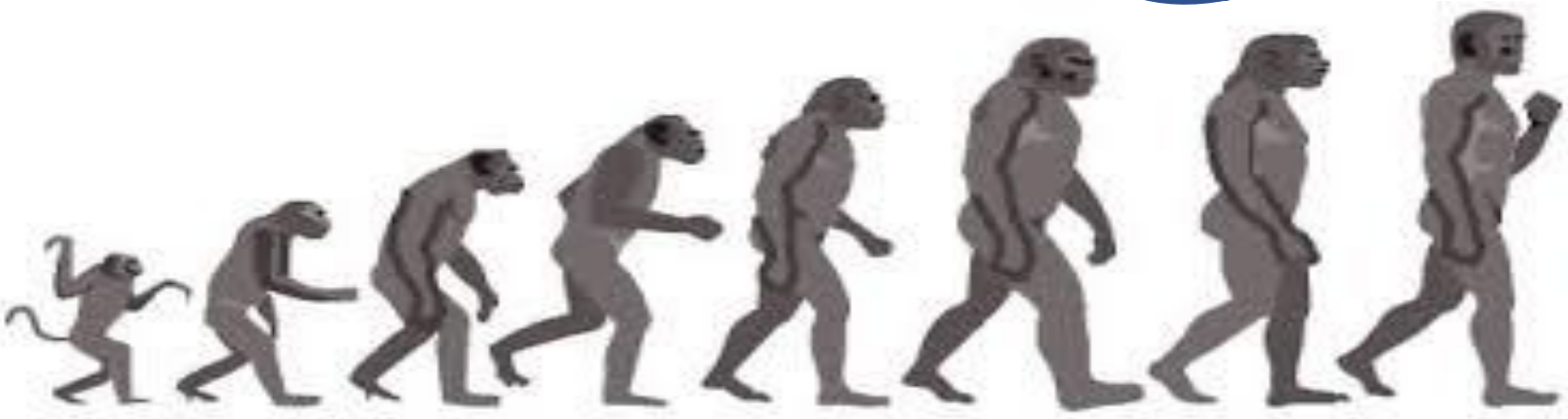
Pre 1980's
ANYTHING surviving is good!

1980-90's
Want something leaf-like and future generations will be able to regenerate



1990-2000's
Want something that *looks* meristematic

2010 - today
Must have a normal looking plant with roots that can survive ex vitro



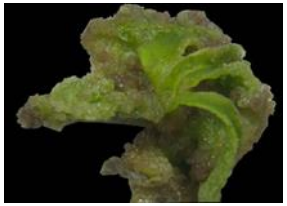
Today's plant cryobanks

- Will future generations know what is in the cryobank?
- Vials with varied purposes and numbers
- Quality standards continue to evolve
- Embrace superior quality standards
  - Admit what we have
- When was the last time you did housekeeping & cleaning of your cryobank?

Plant Cryopreservation Today

- **No uniform standards or guidelines**
- **Genebank managers are averse to throwing anything away**
  - ***"It may be of use in the future"***
  - ***"How do I know this diversity is securely conserved"***
  - ***"Knowledge will improve, and future generations will be able to regenerate it"***

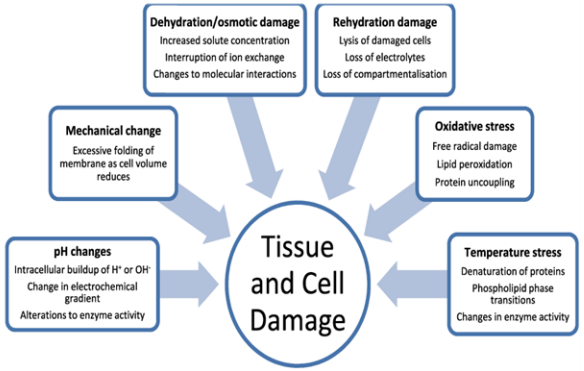
- **What will our great-grandchildren find in our cryobanks?**
  - **At CIP, we threw away 50+% of the potato and 90% of the sweetpotato cryo collections in 2013-2014**
  - **Non-regenerable, contaminated, dead**



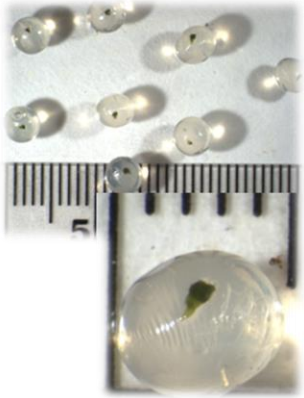
- **I fear, and know from past personal experiences, most plant cryobanks are storing subpar material**

Categories of material in plant cryobanks

1. **Fragments of research or archived material**
   - Leftovers from protocol development and research
   - Vials remaining from research efforts
   - Should <u>not</u> be confused with cryobanking or cryopreservation of PGR collections
   - Low # accessions, not operational
   - Material cryopreserved by different methods
   - Cryobank lacks uniformity/predictability
   - Data in database is minimal if present at all
   - Objective = publication, research, protocol development



Categories of material in plant cryobanks



2. Remnants from initial cryobanking

- Tied to short-term funding
- Small number of accessions (<20 accessions)
- Often limited # vials
- Material cryopreserved by different methods
- Cryobank lacks uniformity/predictability
- Data in database is minimal if present at all
- Objective = initiate cryobanking, show ability to attract funding, publication

Categories of material in plant cryobanks

3. Operational cryobanking

- Cryobanking larger PGR collections (>100 accession)
- Focus on future use and preservation of diversity
- Written standards of excellence in place and followed
- Well defined written operational protocols
- Uniformity, predictability for future generations
- Every vial documented in database
  - Method, whole plant viability, person responsible, date
- Permanent printed labeling – nothing handwritten

Cryobanking = legacy for the future

- **Want to leave 100% predictability for future genebank managers**
  - **Known regeneration results with every vial**
  - **Protocols for cryo and viability assessment documented**

- **Make things as easy as possible for future genebank managers**
  - **High quality, predictable material**
  - **Time and resources will always be precious and limiting**

- **Identity verified, phytosanitary clean**

Your Programs are Critically Important

- **Increasing need for secure long-term protection of diversity**
  - **Back-up of collections crucial**
  - **Growing uncertainty and limiting resources**

- **Five of the ten most important crops for humans are vegetatively propagated**
  - **Potato, cassava, sweetpotato, yam, and banana**

- **All PGR collections that cannot be stored long-term as orthodox seed at risk**
  - **Food crops**
  - **Horticultural species**
  - **Medicinal species**
  - **Trees**

Points to Consider in Plant Cryo

- **Cryo reported with >40 different crops**
  - **Why then are there only a handful of crops with 100+ accessions in cryo?**
  - **The focus has been to publish on few genotypes, not entire collections**

  Difference between developing a vaccine with some efficacy vs vaccinating an entire population

- **Critical need for secure long-term conservation of PGR collections**
  - **Globally, everyone needs the capacity to secure their collections**
  - **Not everyone needs to actively do in vitro and cryopreservation**

- **A wide range of plant tissues can be cryopreserved - pollen, seeds, shoot tips, dormant buds, cell suspensions, embryonic cultures, somatic and zygotic embryos and callus**
  - **One size does not fit all - no one method works for everything**
- **Most cryo methods require *in vitro* (exceptions pollen, seed, dormant buds)**
  - **Opportunity and curse – in vitro can be the limiting factor**
- **Genotyped and disease-free starting material is critical**

Cryo Feasibility Study 2017

Australian Government
Australian Centre for International Agricultural Research

Federal Ministry for Economic Cooperation and Development

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra
Swiss Agency for Development and Cooperation SDC

Bioversity International

CGIAR

CIP International Potato Center

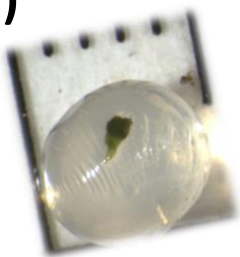
CROP TRUST

Acker, J.P., Adkins, S., Alves, A., Horna, D. and Toll, J. (2017). Feasibility study for a safety back-up cryopreservation facility. Independent expert report: July 2017.Rome (Italy): Bioversity International. 100p.

https://cgspace.cgiar.org/handle/10568/91009

"A major global initiative is urgently needed to accelerate the development and implementation of cryo cryopreservation"

- **Commissioned to investigate the feasibility and need of a safety back-up facility for cryopreserved collections of vegetatively propagated and recalcitrant seed crops**
- **<u>No Svalbard Seed Vault equivalent</u> for vegetatively propagated or recalcitrant seed crops**
- **<u>Genetic resources collections</u> conserved in field or *in vitro* genebanks (at-risk, not long-term)**



Conclusions from Cryo Feasibility Study

- Cryopreservation = best long-term conservation option for clonal and recalcitraint seed crops collections
  - (+) Lower running costs, increased longevity, greater genetic stability
  - (-) High initial costs, skill and technical challenges

- Cryopreservation has huge benefits for long-term secure back-up
  - In vitro – costly, difficult transport, need to continually replenish
  - Infrastructure needs modest (5K accessions) but should double in 10yrs

- Need to accelerate the development and implementation of cryopreservation to safeguard clonal and recalcitrant seed crop collections
  - ~100,000 Annex 1 accessions currently at risk in field and *in vitro* genebanks

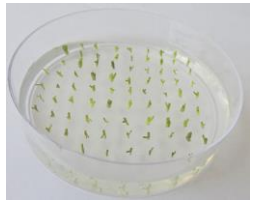
- CGIAR ideally positioned for proposal development and seek donor sponsorship

At what Cost?

- **Cryopreservation of potato at CIP**

Cryo team

- 16 technicians trained in cryo
- >550 potato and ~130 sweetpotato accessions into cryo per year

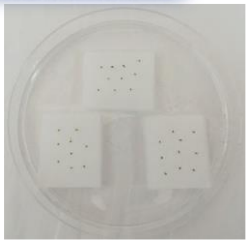


Challenge

- 4,747 potato accessions maintained *in vitro*
- $80/yr to maintain each *in vitro* potato accession (~$380K/yr)

Cost of cryopreservation

- ~$400 to put a potato accession into cryo (based on 500 accessions/yr)
- Once in cryo – annual cost per accession ~$7/yr
- Savings in 6 years – and continues for >century



Success

- Five yrs ago 15% of the *in vitro* potato collection in cryo
- Today 90%+ of *in vitro* potato collection cryopreserved and ready for back-up (4,374 potato accessions)*

*Data as of 1 May 2023

Response is a Vision for the Future

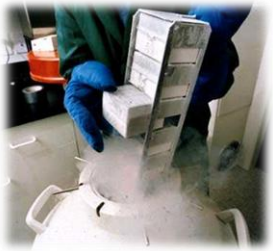
Global Plant Cryopreservation Initiative

- **Focus on recalcitrant and clonal crop collections in the developing world**
- **Regional centers of excellence/capacity (hubs)**
- **Capacity building**
  - **know-how, awareness, support**
- **Safety cryo back-up**
- **Global plant cryo network**

Focus is collections in developing world, but we need support from the developed world = all of you!

The Vision Needs to be for Centuries

- **The focus with cryobanking needs to ensure we leave usable genetic resources for our grandchildren**



- **Requires collective and unified effort**
  - **Ensure highest quality material in cryobanks**
  - **Guidelines for monitoring viability over time**
  - **Operational cryopreservation protocols for genetic resources collections**
  - **Global long-term cryopreservation safety back-up**

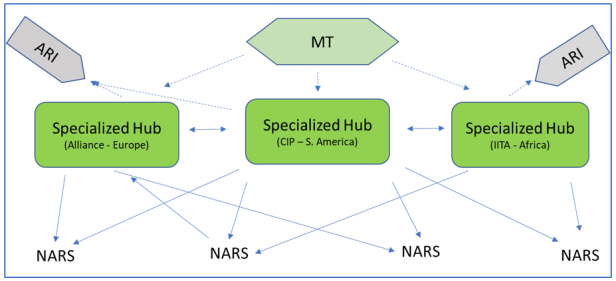
- **Quality Management System for plant cryobanks are critical**

With minimal guidelines, cryo provides the only secure long-term cost-effective safety back-up of clonal and recalcitrant seed collections

The Global Plant Cryopreservation Initiative

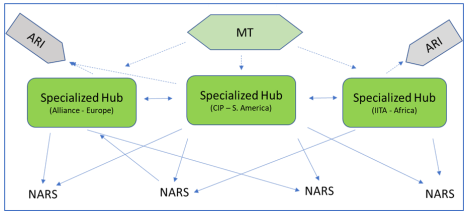
- **Current model based on three Cryopreservation Centers of Excellence = "*Cryopreservation Hubs*"**
- **Specialized hubs based on CG centers**
  1) **Existing infrastructure**
  2) **Expertise, ongoing cryopreservation programs,**
  3) **Regional locality**
  4) **Access to partners in the developing world**

- **<u>European Hub</u> - Alliance-Bioversity - *in trust* banana collection**
- **<u>Americans Hub</u> – CIP- *in trust* potato and sweetpotato collections**
- **<u>African Hub</u> - IITA – *in trust* cassava, yam, and banana collections**



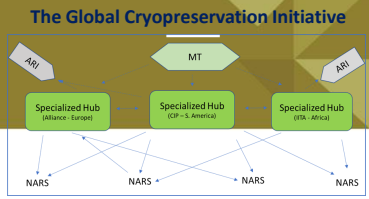
Role of Hubs

- **Hubs will :**
  - **Provide expertise for development of methodologies**
  - **Move research results into operational protocols**
  - **Capacity building activities**
  - **Services for operational cryopreservation**
  - **Cryo safety back-up**
  - **Coordinate development and maintenance of database and network**



- **Structure will be dynamic and will evolve**
  - **Hubs could be added, modified or changed based on crop, need and donor preference**

Ten initial target Crops



- **Seven Annex 1 crops**
  - **Banana, cassava, potato, aroids, coconut, sweetpotato, yam**
- **Three non-Annex 1 crops**
  - **Ulluco, coffee, cacao**
- **Seven clonal crops**
  - **Banana, cassava, potato, aroids, sweetpotato, yam, ulluco**
- **Three recalcitrant seed crops**
  - **Coffee, cacao, and coconut**
- **Transfer of materials through the Standard Material Transfer Agreement (SMTA) of the ITPRGFA**
- **Focus on collections, <u>not</u> research unless protocol development is needed**
  - **Build on success of potato and banana**

Network for Integrated Global Plant Cryo

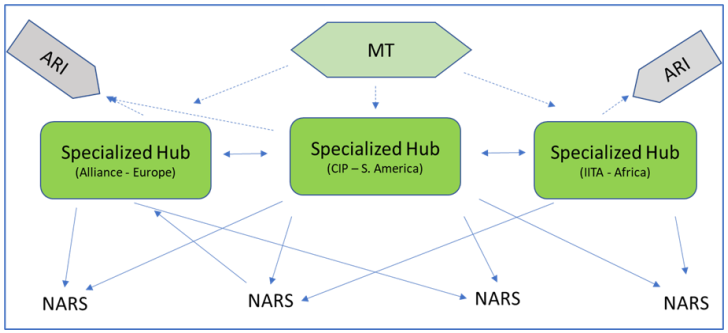
- **Specialized Hubs = foundation for a cryopreservation network for global clonal and recalcitrant crop genetic resources collections**
  - **Plant Cryo Community of Practice**

- **Comprehensive – include NARS and other interested parties (NGOs, academia, industry groups, donors)**
  - **Groups such as the ECPGR Cryo WG will be essential partners**

- **Database of clonal and recalcitrant seed collections**
- **Directed workshops to maintain contact and to monitor status of collections**
- **Forum for sharing experiences, concerns, information, ideas**
  - **Critical during COVID**
- **Provide capacity and coordinate movement of phytosanitary clean materials**

If not now, when? A Global Cryo Initiative!

- **Proposal + summary drafted**
- **Steering committee = CGIAR Clonal Community of Practice + Global Crop Diversity Trust**
- **Government of Belgium offered funding for a cryo back-up facility**
- **Fundraising campaign launched and initial project funded (Darwin Initiative)**



Thank you

For doing what you do for future generations!