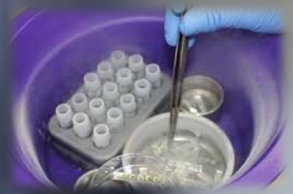


When is it *Good* to be Left Out in the Cold?

The Global Plant Cryopreservation Initiative

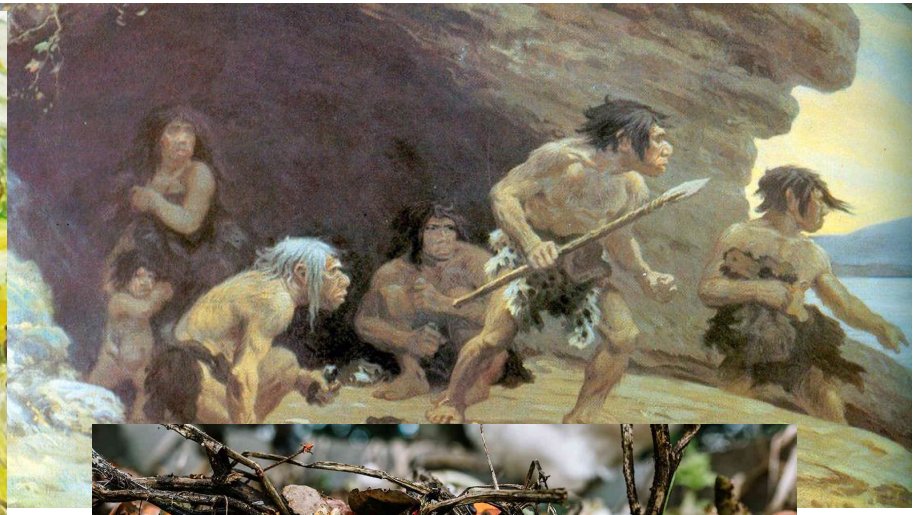



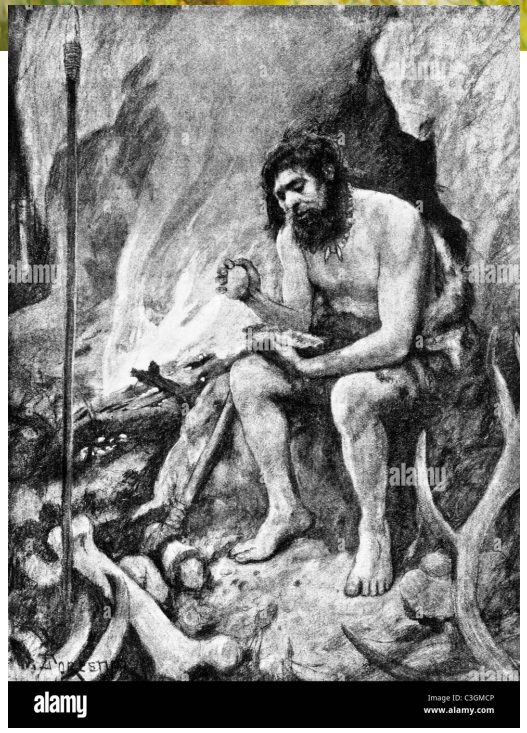


GROW Webinar
24 August 2023

David Ellis
Emeritus Scientist
International Potato Center

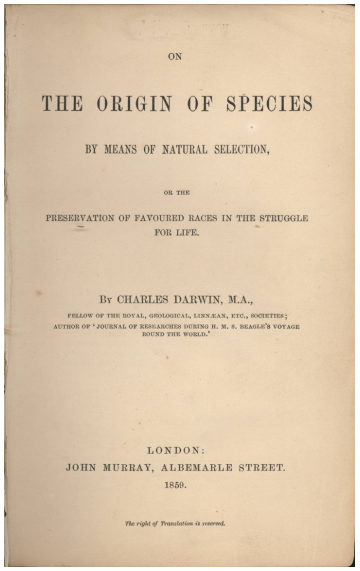
Imagine the world 15,000 years ago





On the Origin of Species (Darwin 1859)

- Most students know *On the Origin of Species* as a treatise for natural selection
- Alternative title *Preservation of Favoured Races in the Struggle for Life*
- Darwin recognized the power of natural selection, but also the power and place of artificial selection



ON

THE ORIGIN OF SPECIES

BY MEANS OF NATURAL SELECTION,

OR THE

PRESERVATION OF FAVOURED RACES IN THE STRUGGLE FOR LIFE.

By CHARLES DARWIN, M.A.,

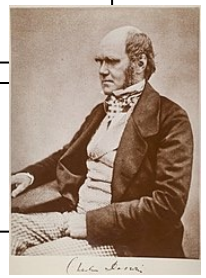
FELLOW OF THE ROYAL, GEOLOGICAL, LINNEAN, ETC., SOCIETIES;
AUTHOR OF 'JOURNAL OF RESEARCHES DURING H. M. S. BEAGLE'S VOYAGE ROUND THE WORLD.'

LONDON:
JOHN MURRAY, ALBEMARLE STREET.
1859.

The right of Translation is reserved.

Slow though the process of selection may be, if feeble man can do much by his powers of artificial selection, I can see no limit to the amount of change, to the beauty and infinite complexity of the coadaptations between all organic beings, one with another and with their physical conditions of life, which may be effected in the long course of time by nature's power of selection *(page 54)*

Thus the appearance of new forms and the disappearance of old forms, both natural and artificial, are bound together *(page 148)*



Take Home Message!

- **Genetic diversity is critical for both natural and artificial selection**
- **And … the genetic diversity in plants is disappearing at an alarming rate**
  - **2.3 plant species disappear every year***
  - **Current extinction rate of plants is 500 times greater than natural or background extinction rates ***

St. Helena Olive
(Nesiota elliptica)
Extinct 2004

Franklin Tree
(*Franklinia alatamaha*)
Extinct in the wild since 1803

*Humhreys et al. 2019. Nature Ecology & Evolution https://doi.org/10.1038/s41559-019-0906-2

Importance of Are Genetic Resources

- **Green Revolution – Norin 10**
  - **Germplasm from 3 countries**
  - **Impacted 25% of the global population**

- ***Oryza nivara* – wild rice species**
  - **Source of grassy stunt virus resistance in tropical rice for past 36 years**

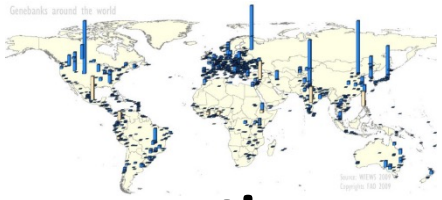
- **C88 LB^{r} potato**
  - **Pedigree of at least 17 accessions**
  - **Benefit to poor farmers = $192 million/year**

- **Wheat variety 'VEERY'**
  - **3,170 crosses from 51 accessions from 26 countries**

Ex situ conservation of plant diversity

- **Plant diversity is mostly stored as botanical seed in genebanks**

Crop Diversity Conserved in Genebanks



- Genebanks are incredible places to visit
- Wealth of diversity that will feed the world for generations
  - Teach the children

- Dedicated – Soviet Union Bureau of Applied Botany (The Vavilov Institute) during siege of Leningrad
  - 115 expeditions, 64 countries, 380,000 samples, 20 yrs
  - Siege lasted 872 days – 1,500,000 died

- Impossible to assess future economic value of one accession versus another

- Vulnerable
  - Afghanistan - war
  - Philippines – natural disaster (typhoon)

Svalbard Global Seed Vault

- FAO standard for plant genetic resources collections = accessions backed up distant from the primary repository
- Svalbard Global Seed Vault – an incredible asset and success!
  - Over 1.25M seed samples from 231 countries*
  - 1,161 genera, 6,120 species*
- However …. half of the PGR collections of 10 most important crops to global human livelihood **cannot** be stored in Svalbard
  - Not the potatoes you had for dinner
  - Nor the cassava or sweetpotato sustaining smallholder farmers in Africa
  - Nor the coffee seed for your cappuchino nor cacao in the chocolate you gave to loved one

- Focus = genetic resources collections which <u>cannot</u> be safeguarded in Svalbard

* https://seedvault.nordgen.org/ - 21 August 2023

Why Can't They be Stored in Svalbard?

- Svalbard has a single storage condition -20°C
- Only seed which can be dried down to <10% MC can survive in long-term storage at -20°C

- Exceptions are:
  - Clonal crops propagated vegetatively to maintain their unique genetic attributes – these crops are not maintained by seed
  - Plants which produce seed that cannot tolerate drying down = recalcitrant seed – this seed dies when dried down
  - Plants which produce seed which can tolerate drying down but the seed is short-lived (<20 yrs) at -20°C

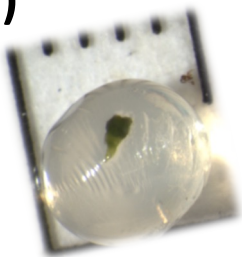
- Potato, cassava, banana, taro, yam, coffee, cacao, palm, citrus, apples, grapes, avocado, coconut, garlic, ulluco, sweetpotato + many more!

- **Why isn't there a Svalbard equivalent for these crops?**

Cryo Feasibility Study 2017

"A major global initiative is urgently needed to accelerate the development and implementation of plant 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)**



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

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

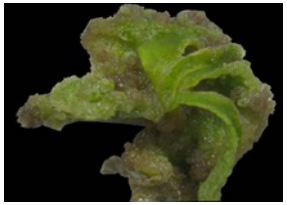
- **Need to <u>accelerate the development and implementation of cryopreservation</u> to safeguard clonal and recalcitrant seed crop collections**
  - **~100,000 Annex 1 accessions currently at risk in field and *in vitro* genebanks**

- **<u>CGIAR ideally positioned</u> for proposal development and seek donor sponsorship**

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"***

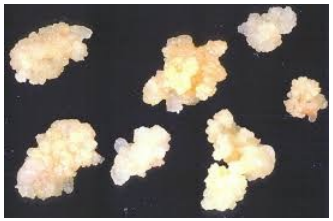
- **What will our great-grandchildren find in our cryobanks?**
  - **At CIP, we threw away 50+% of the potato and 90% of the sweetpotato cryo collections in 2013-2014**
  - **Non-regenerable, contaminated, dead**



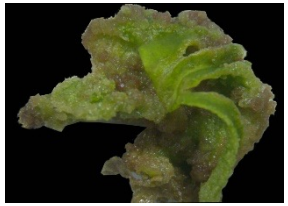
- **I fear, and know from past personal experiences, most plant cryobanks are storing subpar material**

Evolution of Cryobanking with Plants

Pre 1980's
ANYTHING surviving is good!

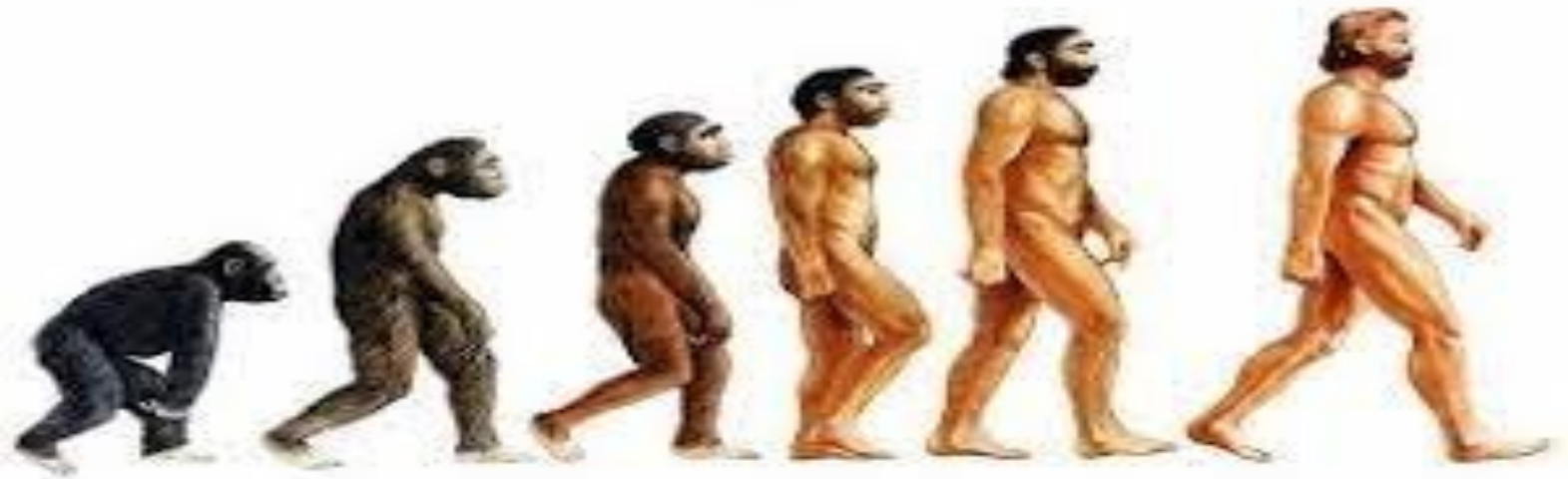


1980-90's
Future generations will be able to regenerate anything that *looks* leaf-like



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

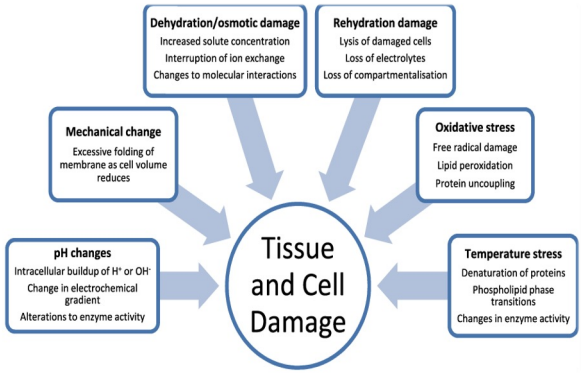
- Will future generations know what is in the cryobank?
- Vials with varied purposes and numbers
- Quality standards continue to evolve
- Embrace superior quality standards
  - Admit what we have
  - Garbage in = garbage out
- When was the last time you did housekeeping & cleaning of your cryobank?

3 Categories of Material in Plant Cryobanks

1. **Fragments of research or archived material**
   - Leftovers from protocol development and research
   - Not cryobanking of PGR collections

2. **Remnants from initial cryobanking**
   - Tied to short-term funding
   - Objective = cryobanking but falls short

- **Both categories leave cryobanks with:**
   - Low # of accessions (<20), limited # vials, not operational
   - Material cryopreserved by different methods lacking uniformity/predictability
   - Data in database is minimal if present at all
   - Objective = initiate cryopreservation, show ability to attract funding, publication
   - Neither category is adequate for long-term conservation of PGR collections



Categories of material in plant cryobanks

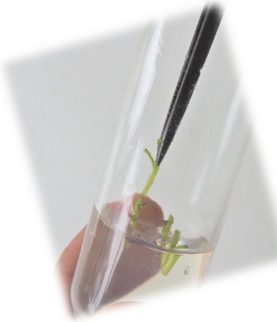
3. Operational cryobanking

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

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

- Critical need for secure long-term conservation of PGR collections
  - Globally there is the need to secure all PGR collections



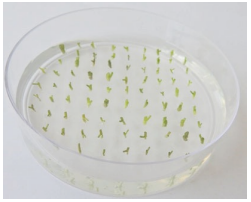
- A wide range of plant tissues can be cryopreserved - pollen, seeds, shoot tips, dormant buds, cell suspensions, embryonic cultures, somatic and zygotic embryos and callus
  - One size does not fit all - no one method works for everything
- Most cryo methods require *in vitro* (exceptions pollen, seed,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

- Genotyped and disease-free starting material is critical
- Not everyone needs to actively do in vitro and cryopreservation
  - Requires a long-term secure funding commitment

At What Cost?

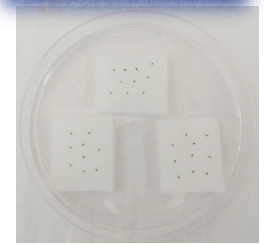
"Cryopreservation is too expensive"

- Infrastructure is needed as is a secure liquid nitrogen supply
- Not everyone should and can do cryopreservation
- But all managers should understand cryobanking
- Centralized teams to support cryo work and maintain safety back-ups



- Cryo *is* the <u>only</u> secure long-term back-up for these crops
- Compared to in vitro, the investment is paid off in 6 yrs

"How do you know cryopreserved material can live for centuries?"



- Nothing lives forever
- Plant cryopreservation since early 1970's
- Easy to mess up, but when done right no indication of routine losses of viability to date
- Theoretical half-live of cryopreserved lettuce seed >500 years*

*Walters et al. 2004. Cryobiology. https://doi.org/10.1016/j.cryobiol.2004.01.007

Cryobanking = legacy for the future

- **Want to leave 99+% 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**

Cryo 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

A Vision for the Future

Global Plant Cryopreservation Initiative

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

The Vision Needs to be for Centuries

- We need 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

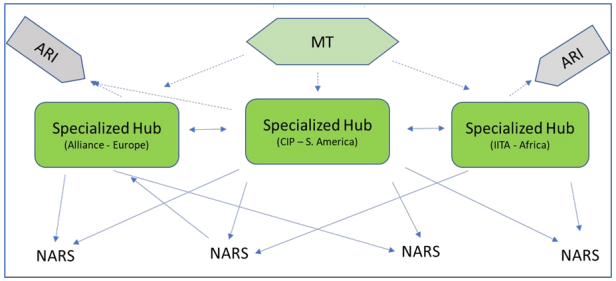
- Quality Management System for plant cryobanks are critical

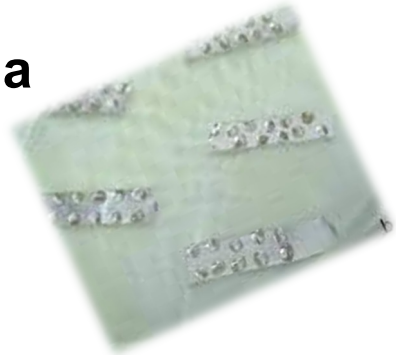
With minimal guidelines, cryo provides the only secure long-term cost-effective safety back-up of clonal and recalcitrant seed collections

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**

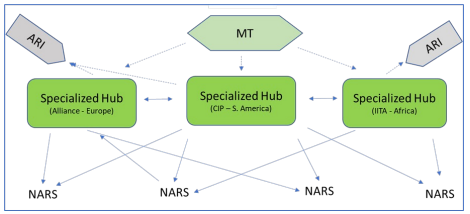
- **<u>European Hub</u> - Alliance-Bioversity - *in trust* banana collection**
- **<u>Americans Hub</u> – CIP- *in trust* potato and sweetpotato collections**
- **<u>African Hub</u> - IITA – *in trust* cassava, yam, and banana collections**





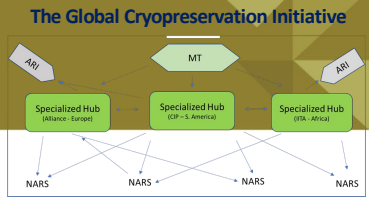
Role of Hubs

- **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**

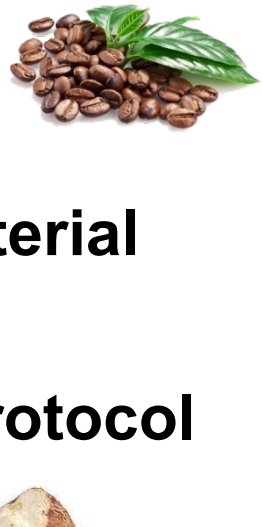


- **Structure will be dynamic and will evolve**
  - **Hubs could be added, modified or changed based on crop, need and donor preference**

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**
- **Materials available 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

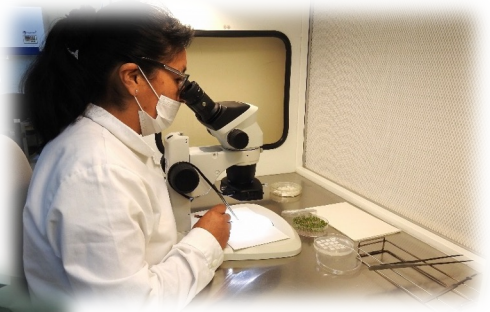
- Specialized Hubs = foundation for a cryopreservation network for global clonal and recalcitrant crop genetic resources collections
  - Plant Cryo Community of Practice

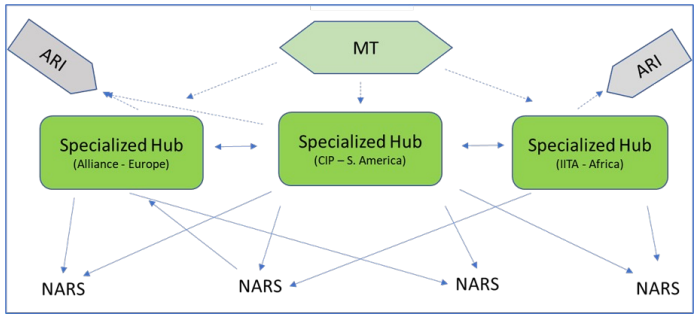
- Comprehensive – include NARS and other interested parties (NGOs, academia, industry groups, donors)
  - Essential partners will include groups such as the ECPGR Cryo WG and the Latin American Plant Cryo Network

- 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
- Fundraising campaign launched
- Initial pilot project funded (Darwin Initiative)
- Plant cryopreservation network for Latin America is being developed
- CIP building a new state-of-the-art cryobank
- Capacity building programs ongoing





CGIAR

CROP TRUST

Thank you

For doing what you do for future generations!

A poll will pop up on your screen for 1 minute after the presentation, please indicate if you are interested in staying informed as the Global Plant Cryopreservation Initiative is launched