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Collecting viable seeds: Seed biology and storage



**BILL & MELINDA
GATES foundation**

Bart Panis

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The Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT) is part of CGIAR, a global research partnership for a food-secure future.

Question related to wild banana seeds

- What is the conservation status of wild bananas
- Where do we need to collect banana seeds ?
- How can we improve germination frequency of *Musa* seed?
- Shows *Musa* seed recalcitrant/ Intermediate/orthodox storage behaviour?
- Is seed behaviour species / batch dependent?
- How to yield and process *Musa* seed so it is in optimal condition to be stored
- How many seeds need to be stored to store the population's diversity



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Overcoming barriers to the conservation and use of banana seed germplasm

Simon Kallow
5th April 2022
PhD Public defence

Alliance



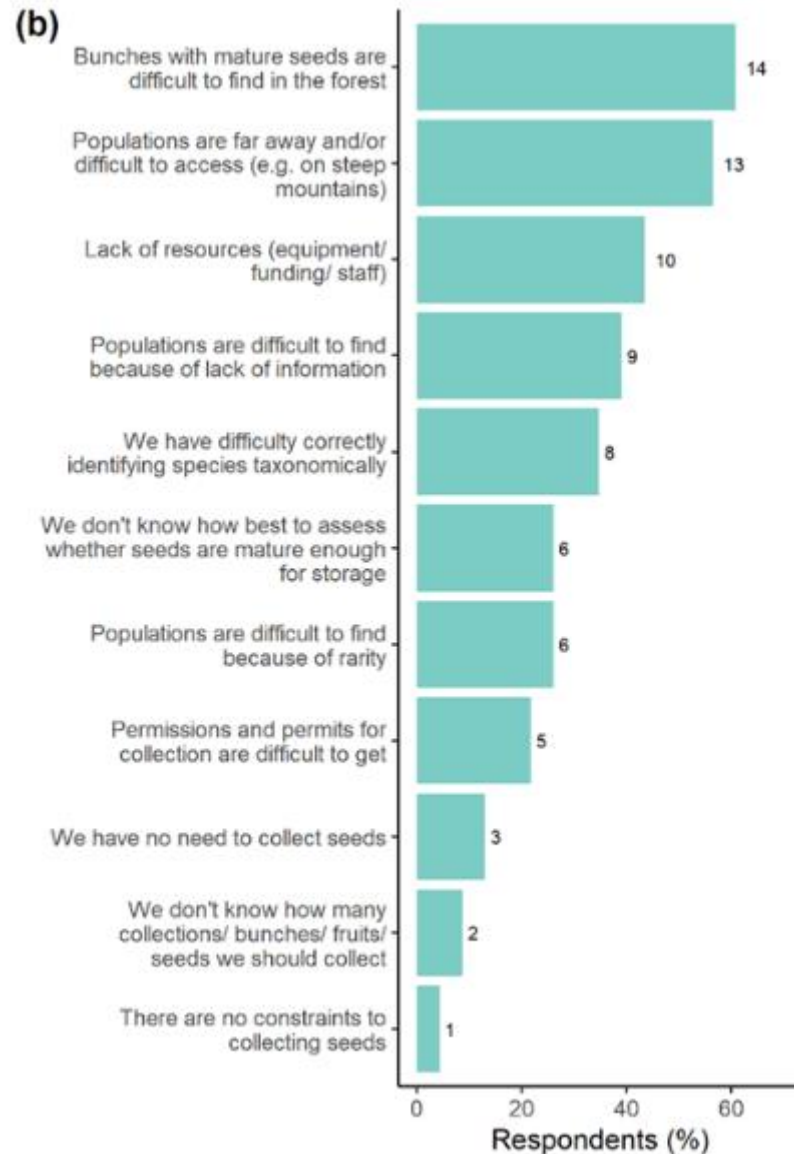
**Botanic Garden
Meise**



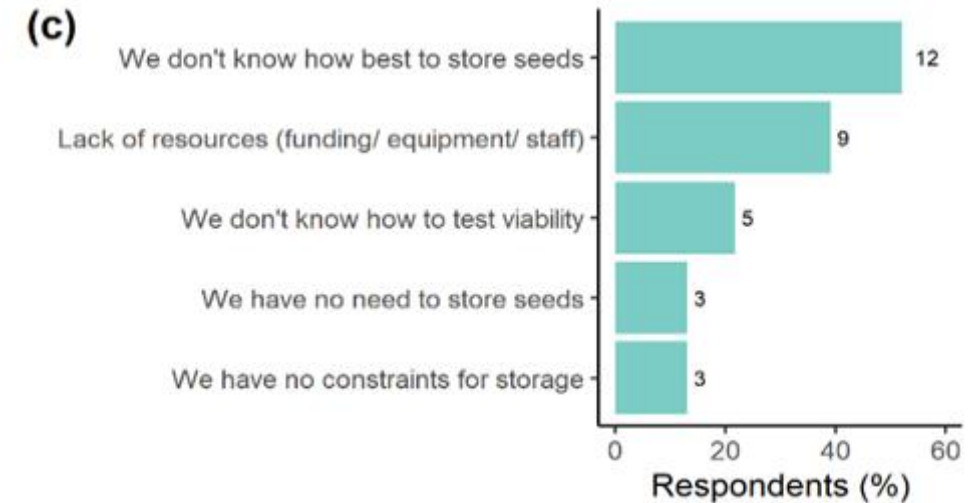
MILLENNIUM
SEED BANK
PARTNERSHIP
Kew



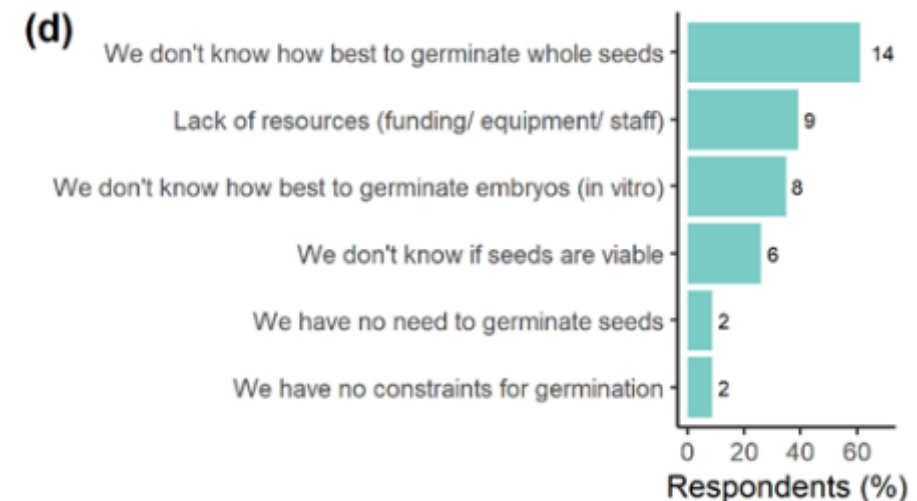
Constraints for collecting



Constraints for storage



Constraints for germination



Conservation status of wild bananas (13 out of 22 institutes responded)

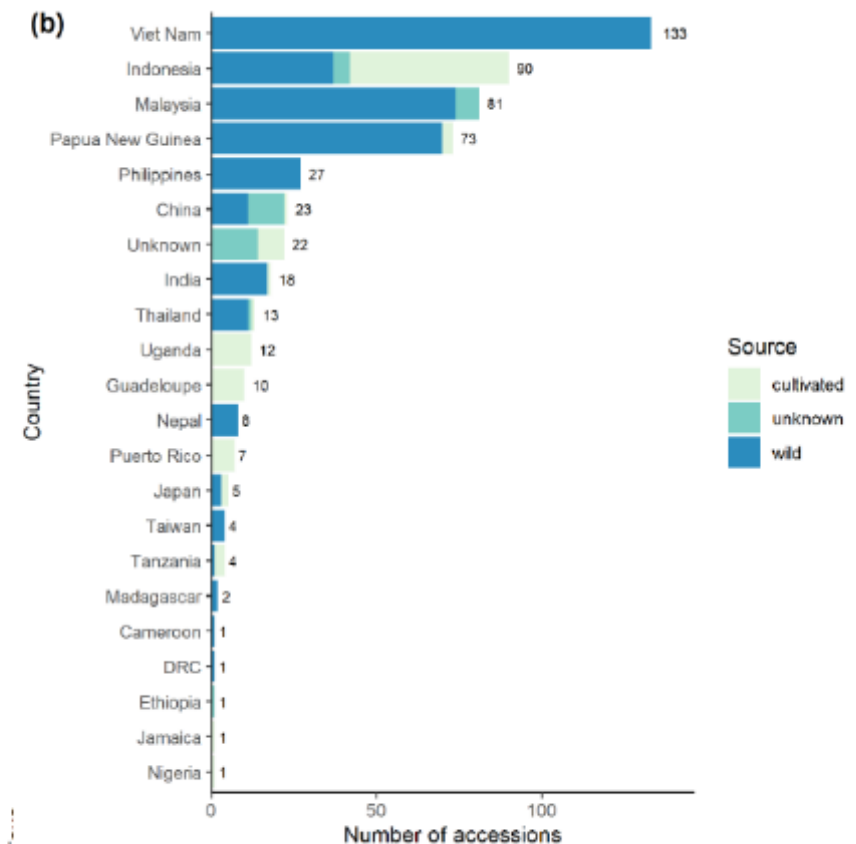
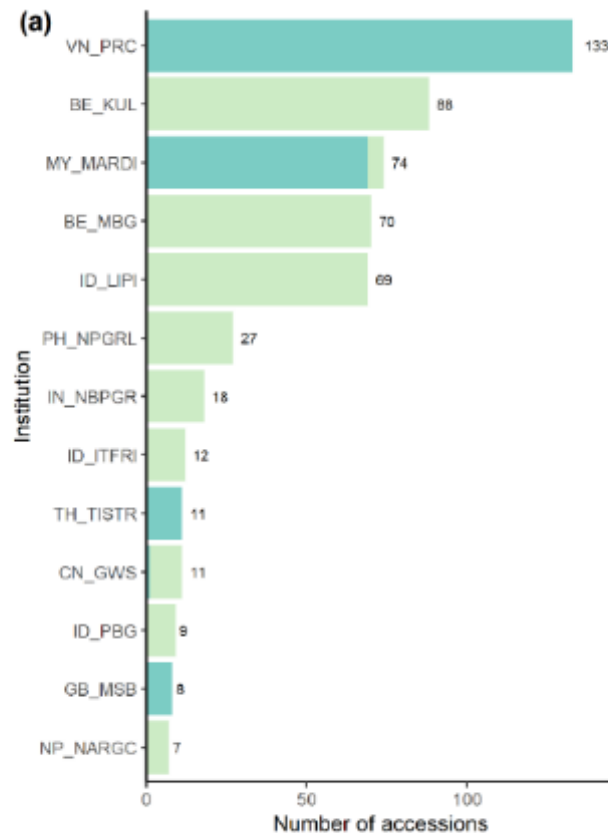
13
institutions

10
countries

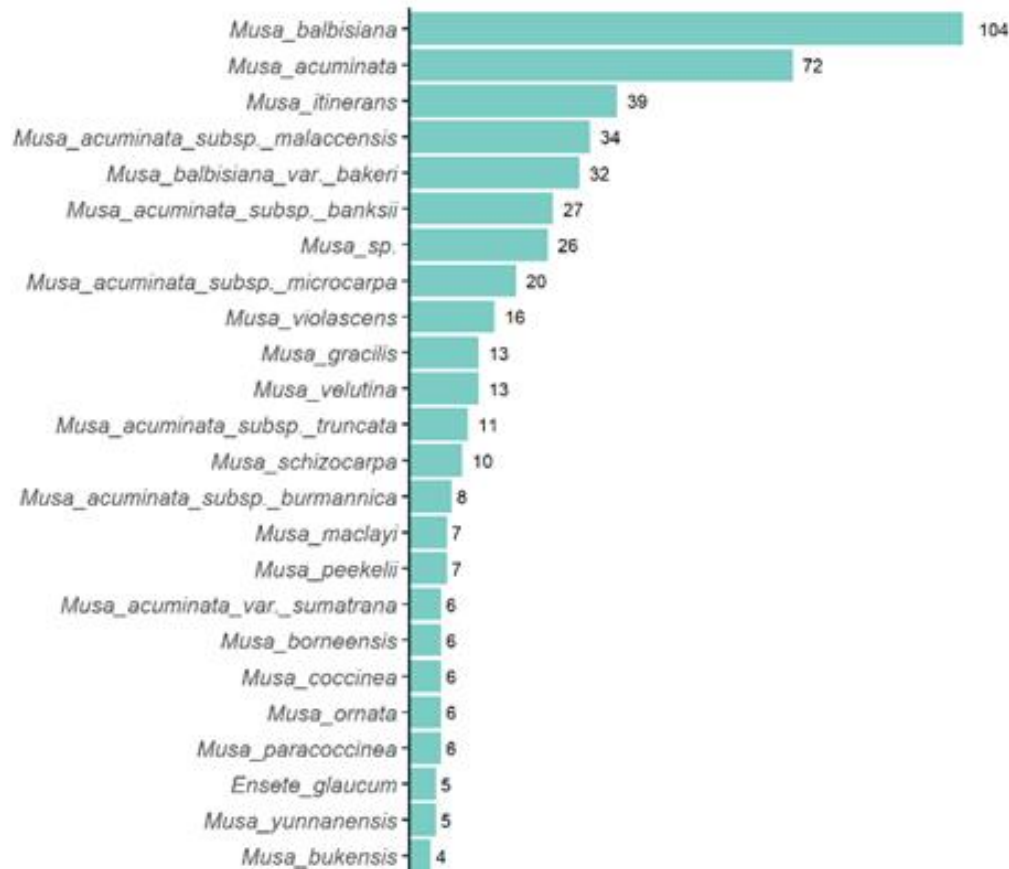
537
accessions

430,000
seeds

56 taxa



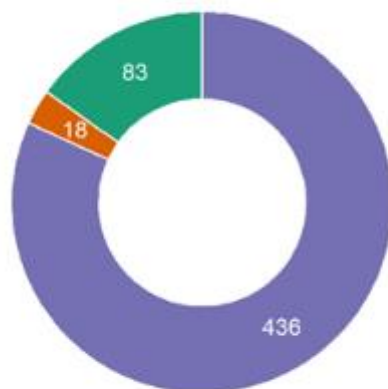
50 Musa taxa, five Ensete, and one Musella



55 % available, 27% not available, 1 % available only domestically, 17% distribution policy not known.

How is wild banana seed stored ?

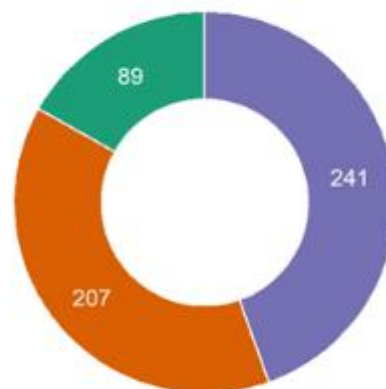
(a)



MC (%)

- <10
- >=10
- unknown

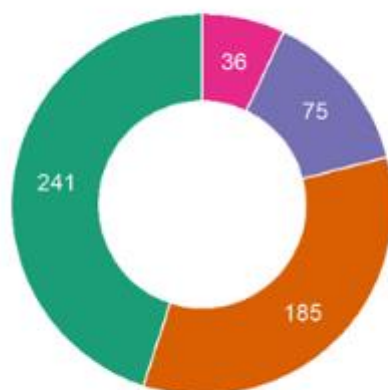
(b)



eRH (%)

- <=15
- >15
- unknown

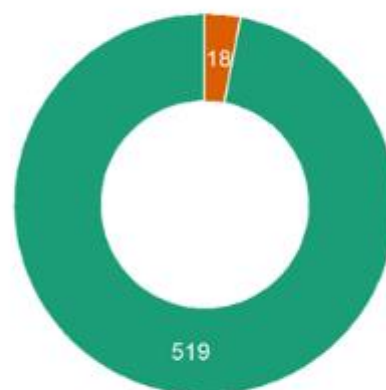
(c)



Temperature (°C)

- <=0
- >0 <=5
- >5
- unknown

(d)



Stored cryogenically (%)

- no
- yes

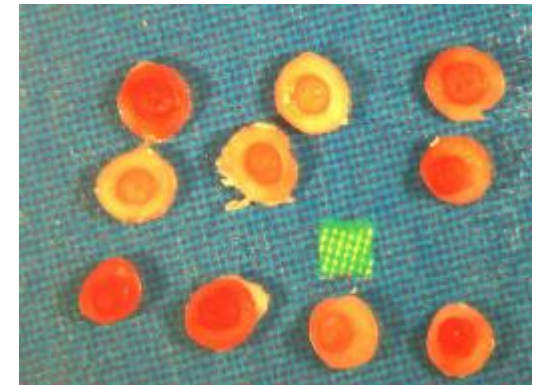


Wat is the viability of the seeds?

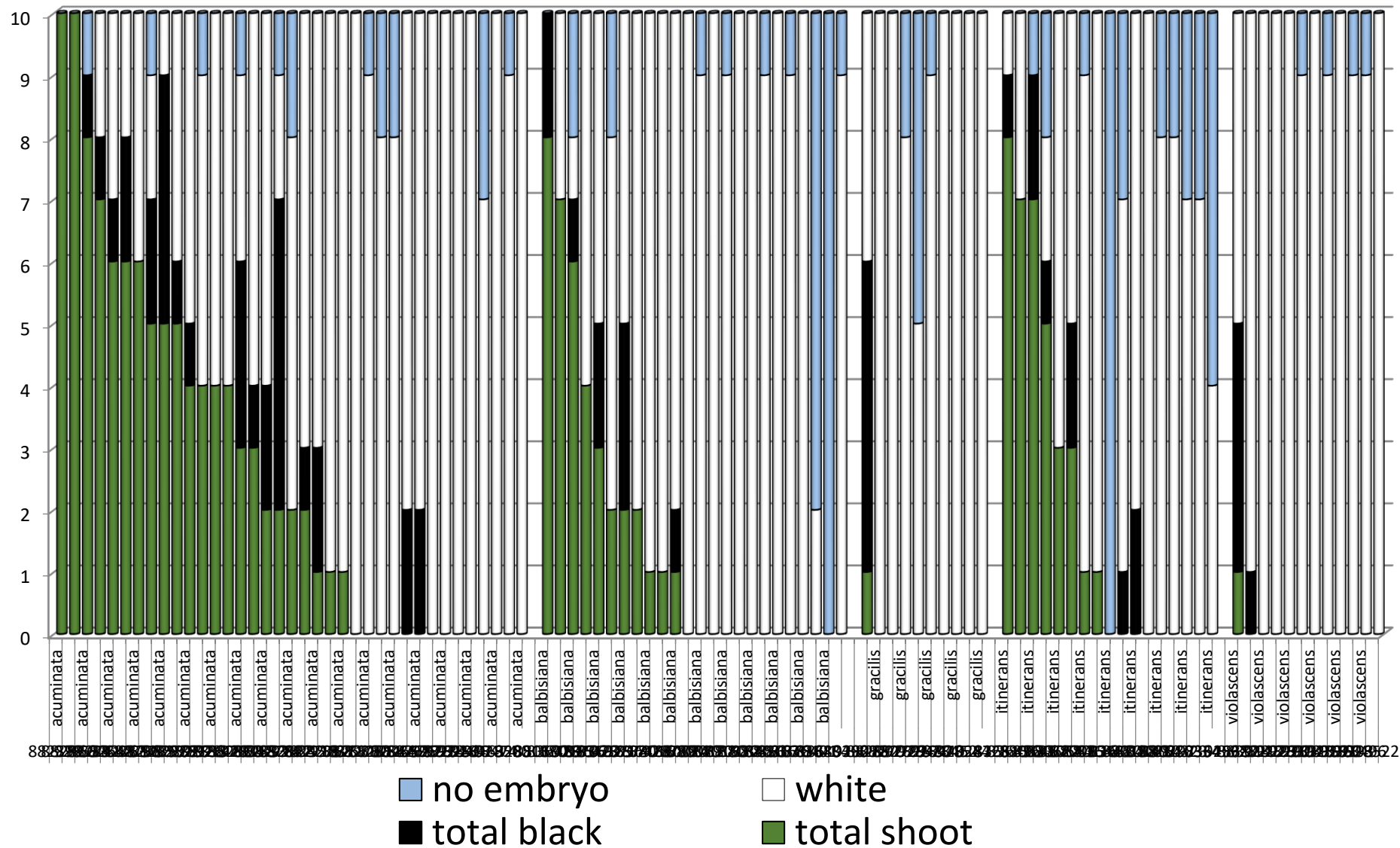
- Median viability was 25 %
- For more than half of accessions (52 %) had not been viability assessed
- 71 accessions (13 % of total) no viability

Problem: how viability was tested

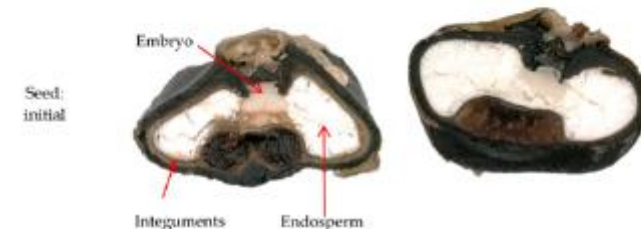
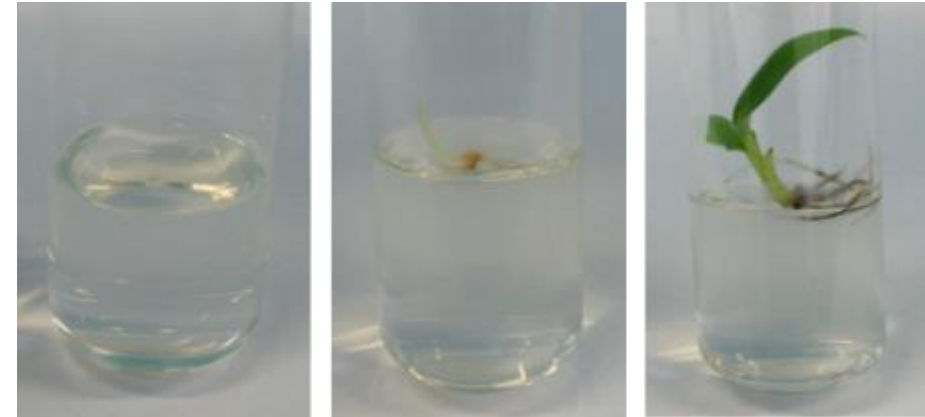
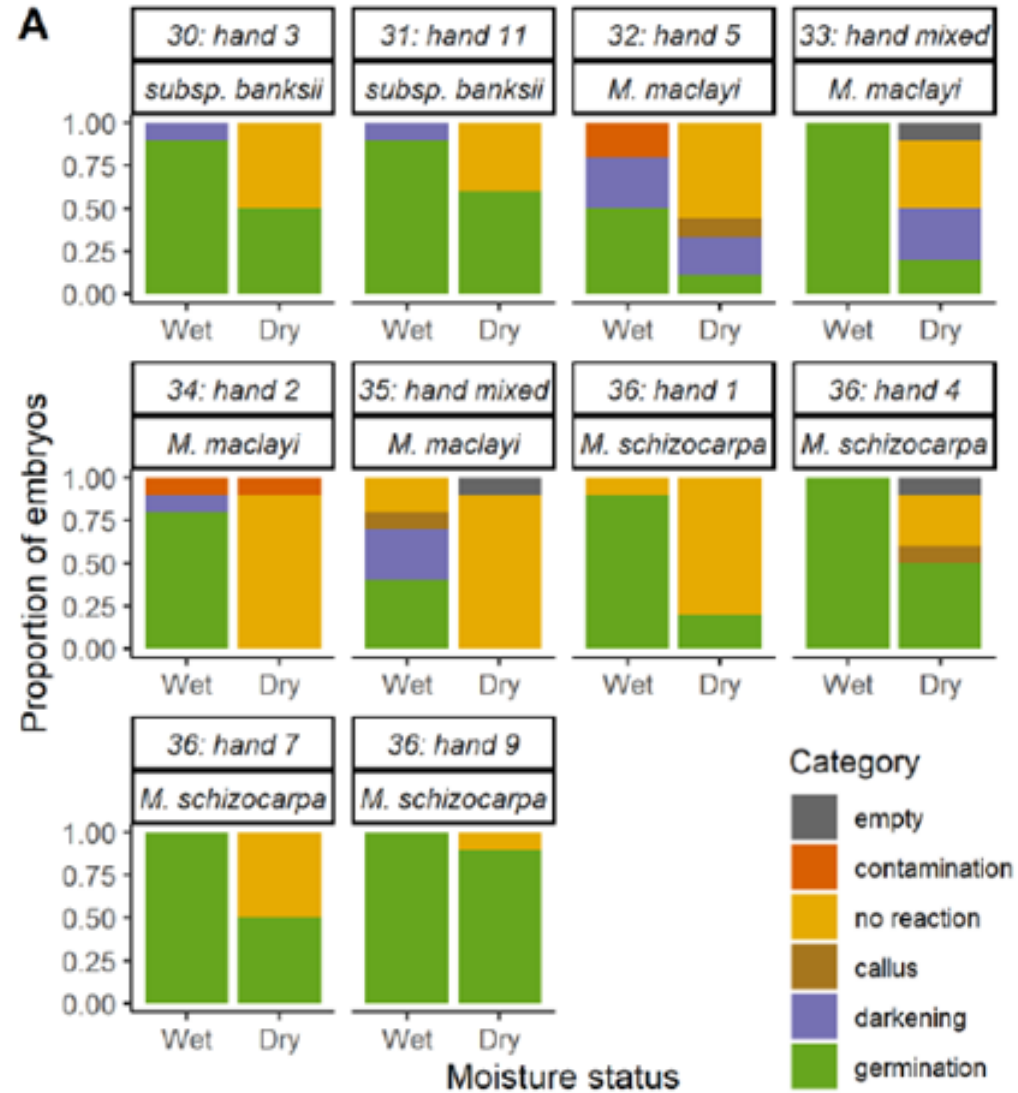
- Embryo rescue; 29 % (31 % viability)
- Whole seed germination; 18% (47 % viability)
- TTC (1%, 93% viability)



MSB: 5 Species

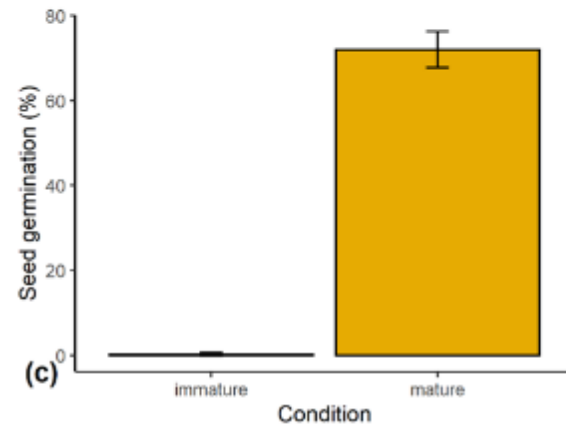
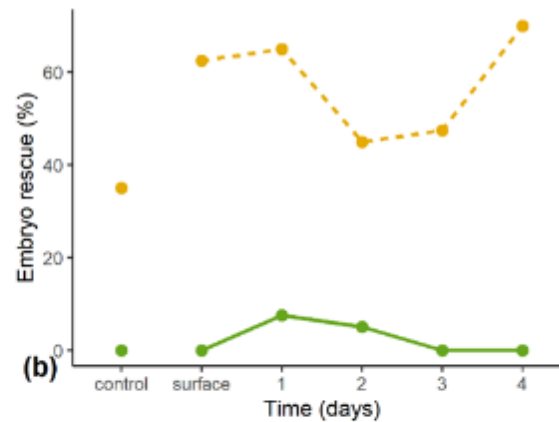
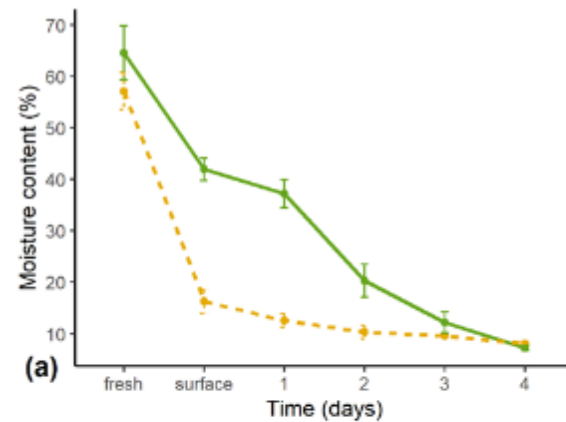


Problem! Many collected seed batches do not germinate / do not survive drying



The effect of maturity on viability and drying

- Executed on two bunches *M. balbisiana* of the same accession but with different ripeness of the field collection in Los Banos



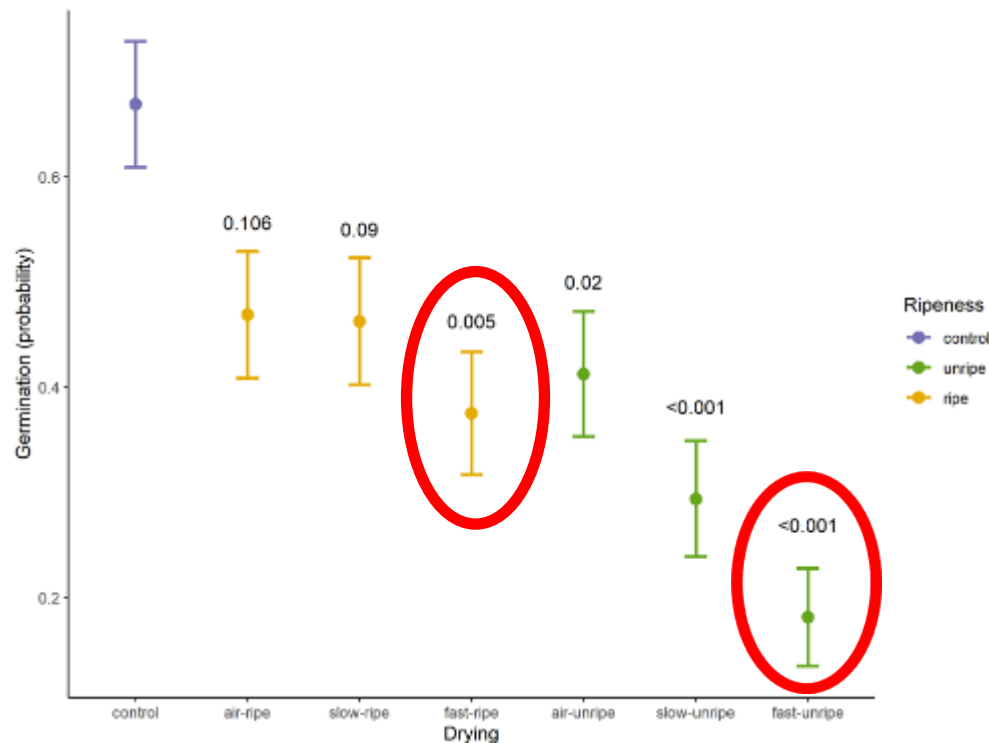
Maturity

- immature
- mature



The effect of fruit ripeness and drying rate on seed germination

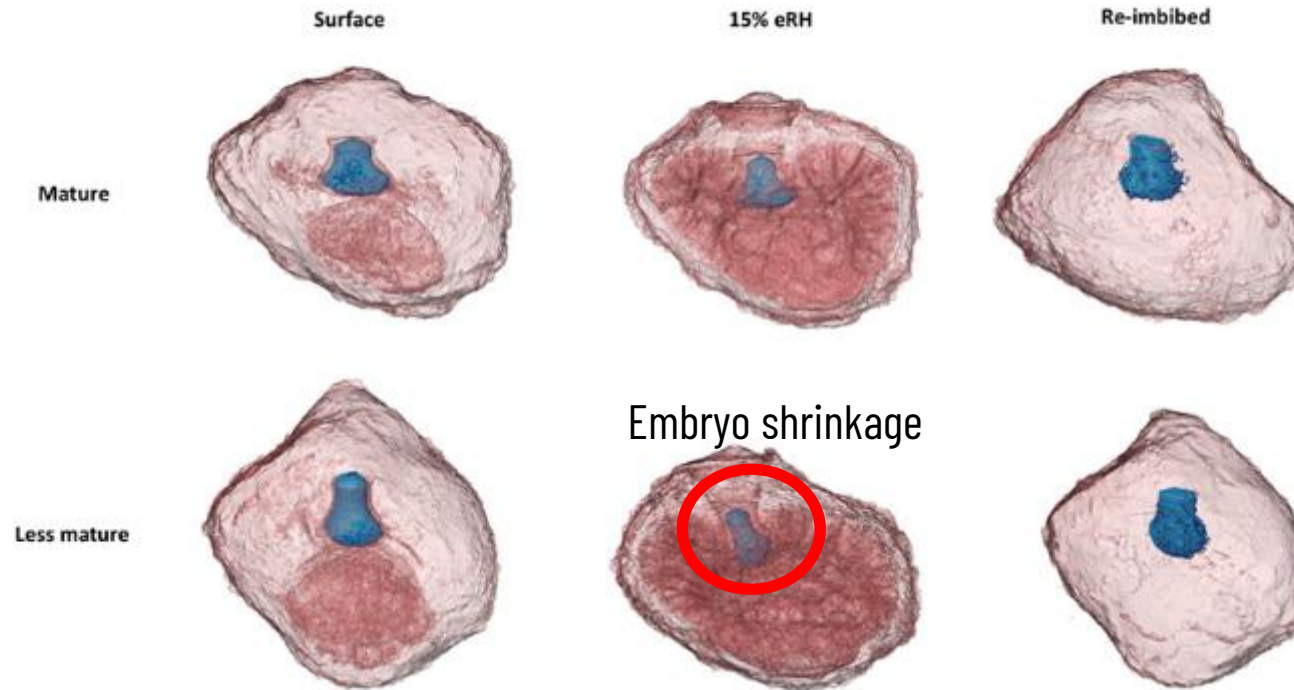
- Executed on a bunch of *M. balbisiana* with a ripe and a non-ripe part collected from a home garden in Viet Nam during a collecting mission



Fast drying problematic !

The effect of drying and imbibition on seed morphology

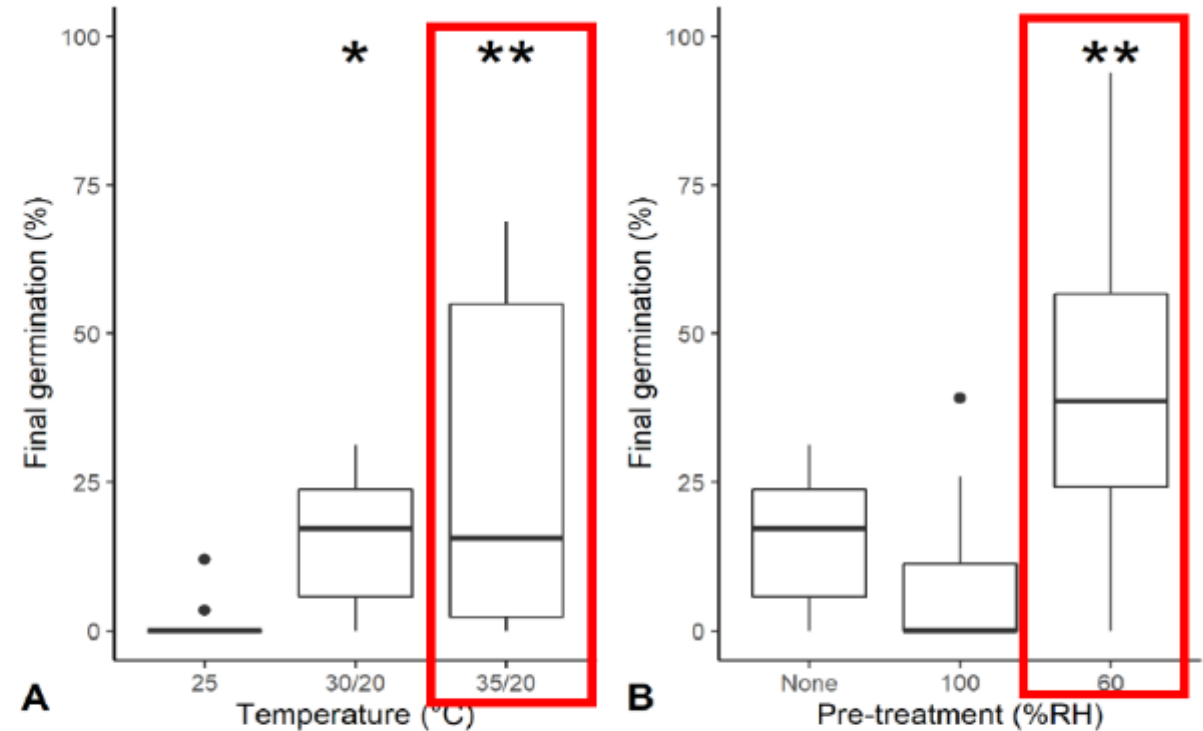
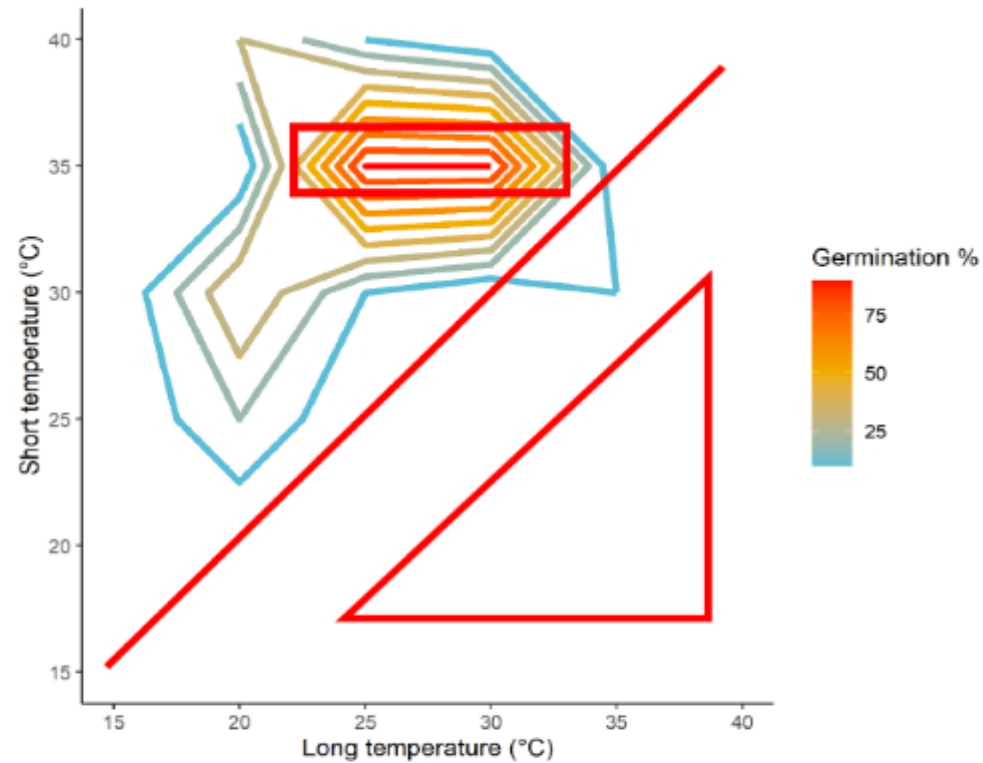
- Executed on two bunches *M. balbisiana* of the same accession but with different ripeness from field collection in Guadeloupe (CIRAD)



X-ray computer tomography (CT) scanning and image analysis methods

Germination

- Executed with *M. acuminata* subsp. *malaccensis*



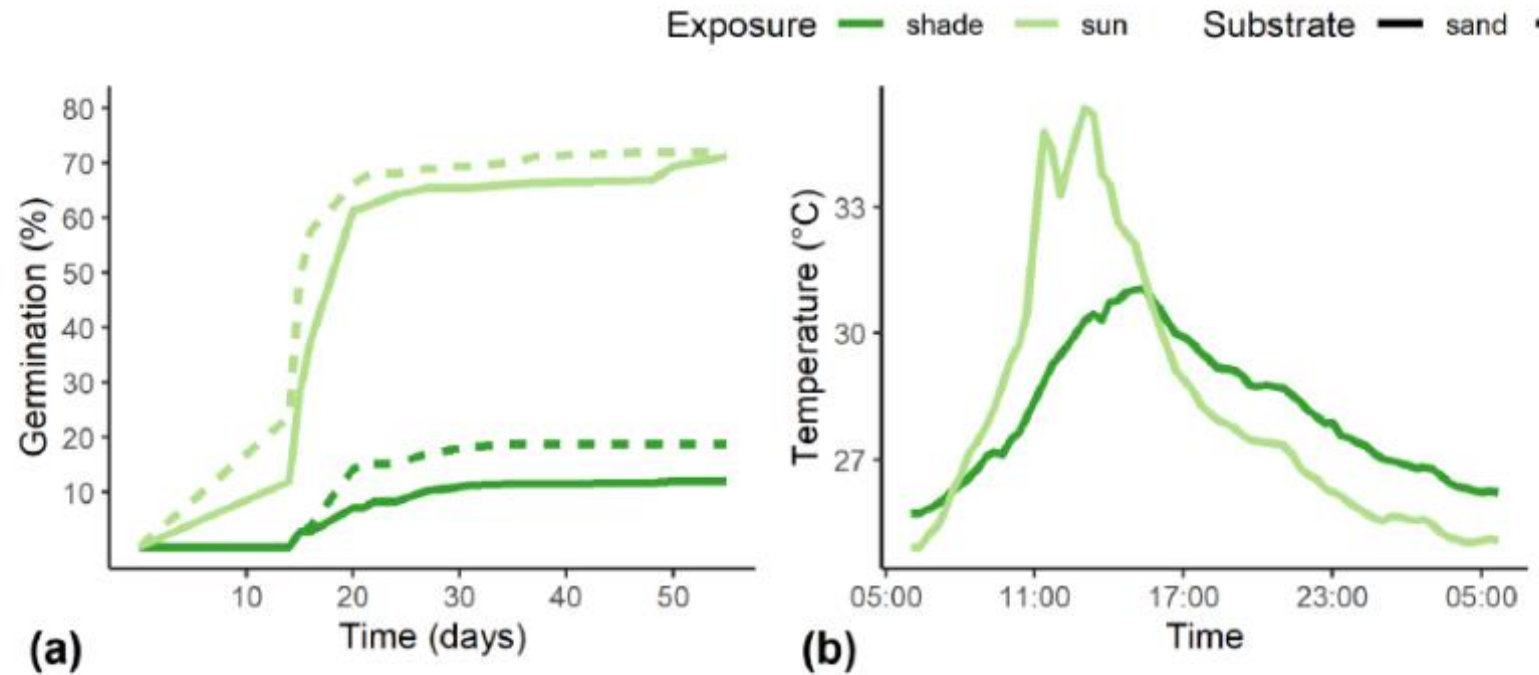
Seeds need alternating temperatures

Optimal maximum temperature is 35 °C

Minimum temperature is 20-30°C

Pre-treatment in constant temperature increases germination

Optimal temperature range for germination mimics forest gaps but also seed burial dept



In vitro germination experiment

- Method of Simon Kallow

Phase 1

- $T = 25^{\circ}\text{C}$
- RH: 60-70%
- 1-3 months



Phase 2

- $T = 20^{\circ}\text{C}$
- On moist sand
- 7 days



Phase 3

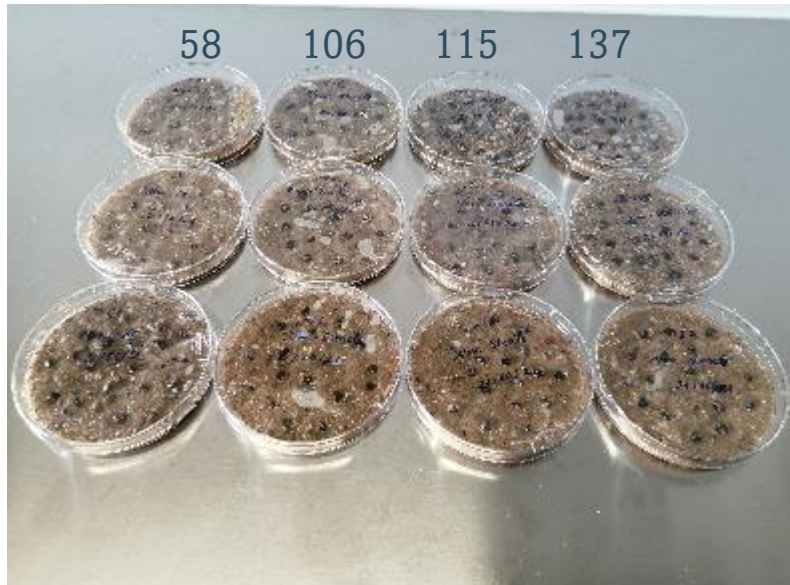
- 8 hours: $T = 35^{\circ}\text{C}$ (+light) $\leftrightarrow$ 16 hours: 20°C (dark)
- On moist sand
- 2 months



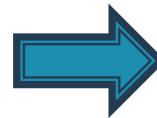
G1

G2

In vitro germination experiment



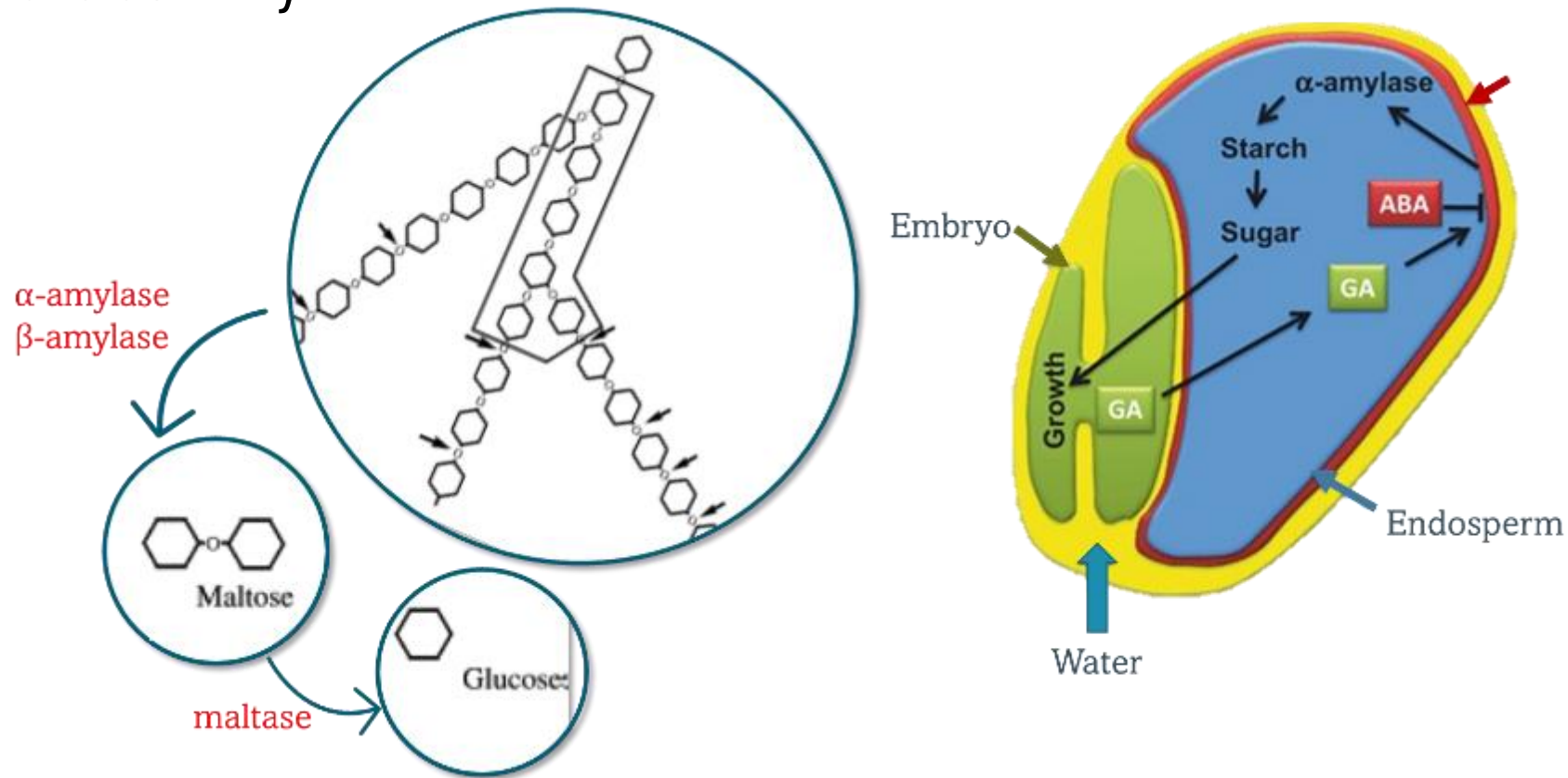
Embryo rescue of 24
non-germinating
seeds per batch



Still viable embryos present in the seeds,
not triggered to germinate

Amylase activity test

- Reason for low germination despite the embryos are alive according ER test? Lack of amylase activity?



Amylase activity test

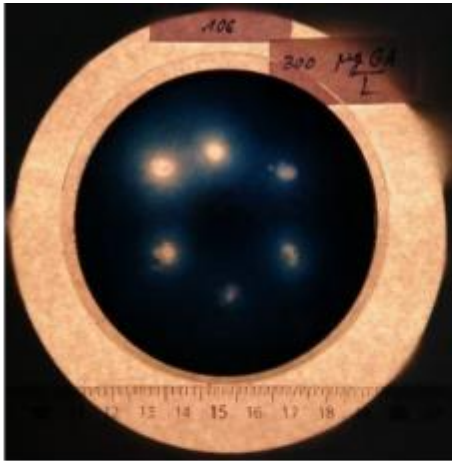


Amylase activity test



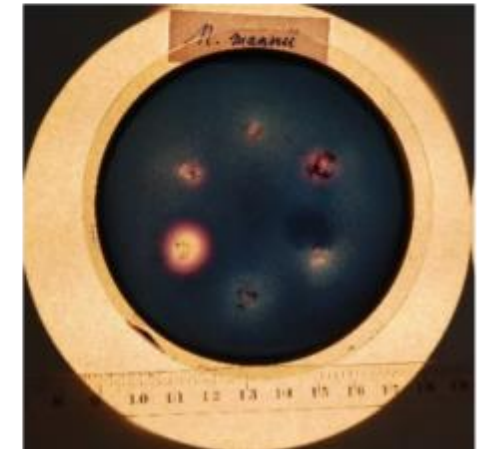
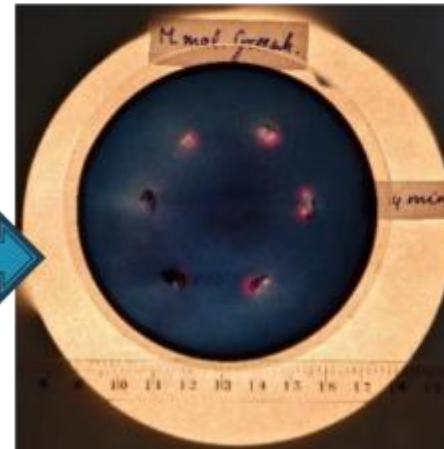
Amylase activity test

Also cooked seeds show these spots:
Interfering compounds??



Spots = not immediately visible!

Fresh *Musa acuminata* ssp. *malaccensis* and *M. mannii* do show immediately pink spots



Chipping experiment



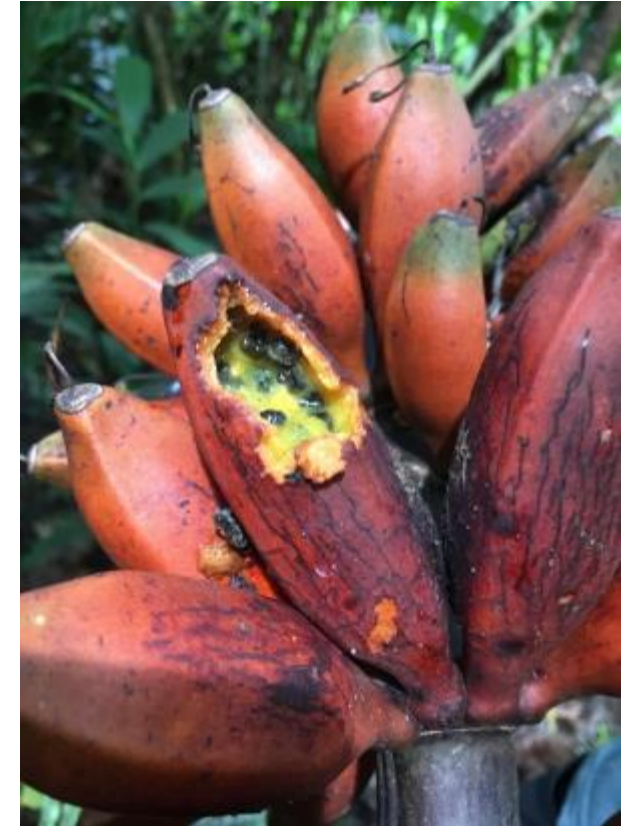
Contamination!

1/4 chipped seed
and
2/4 unplugged seeds
germinate without
alternating temperatures

Non contaminated seeds can germinate when sugar is provided
(problem with breaking down starch)

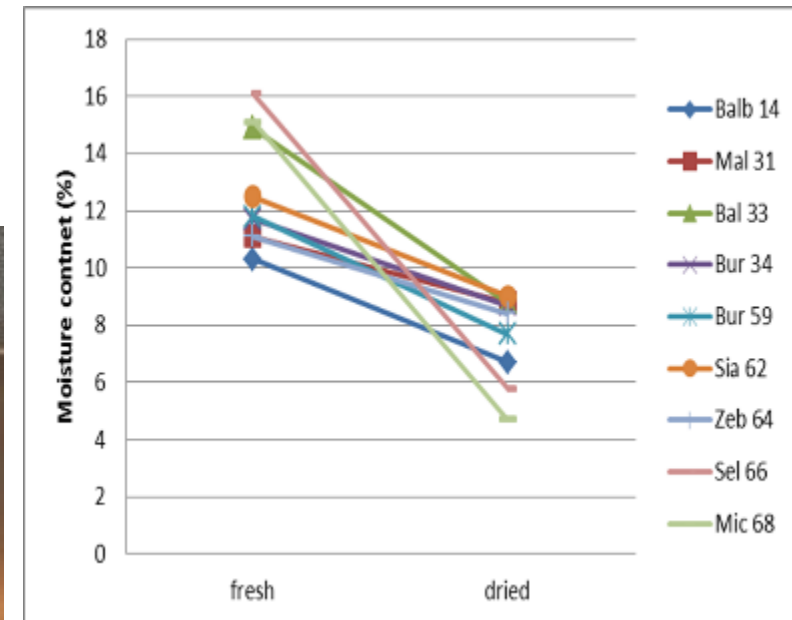
Conclusion

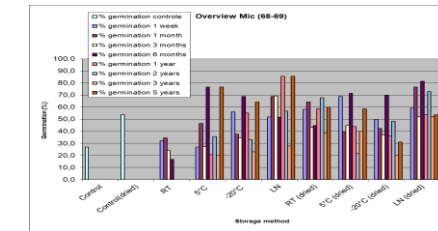
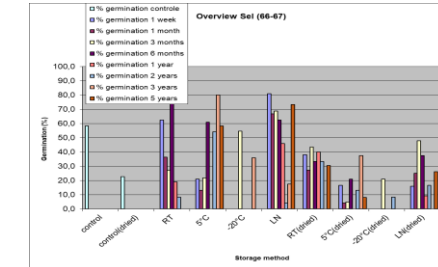
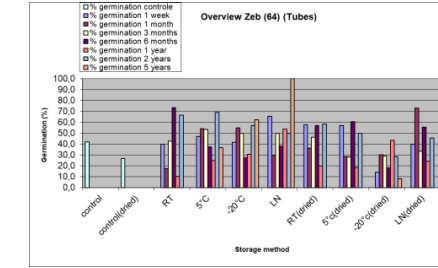
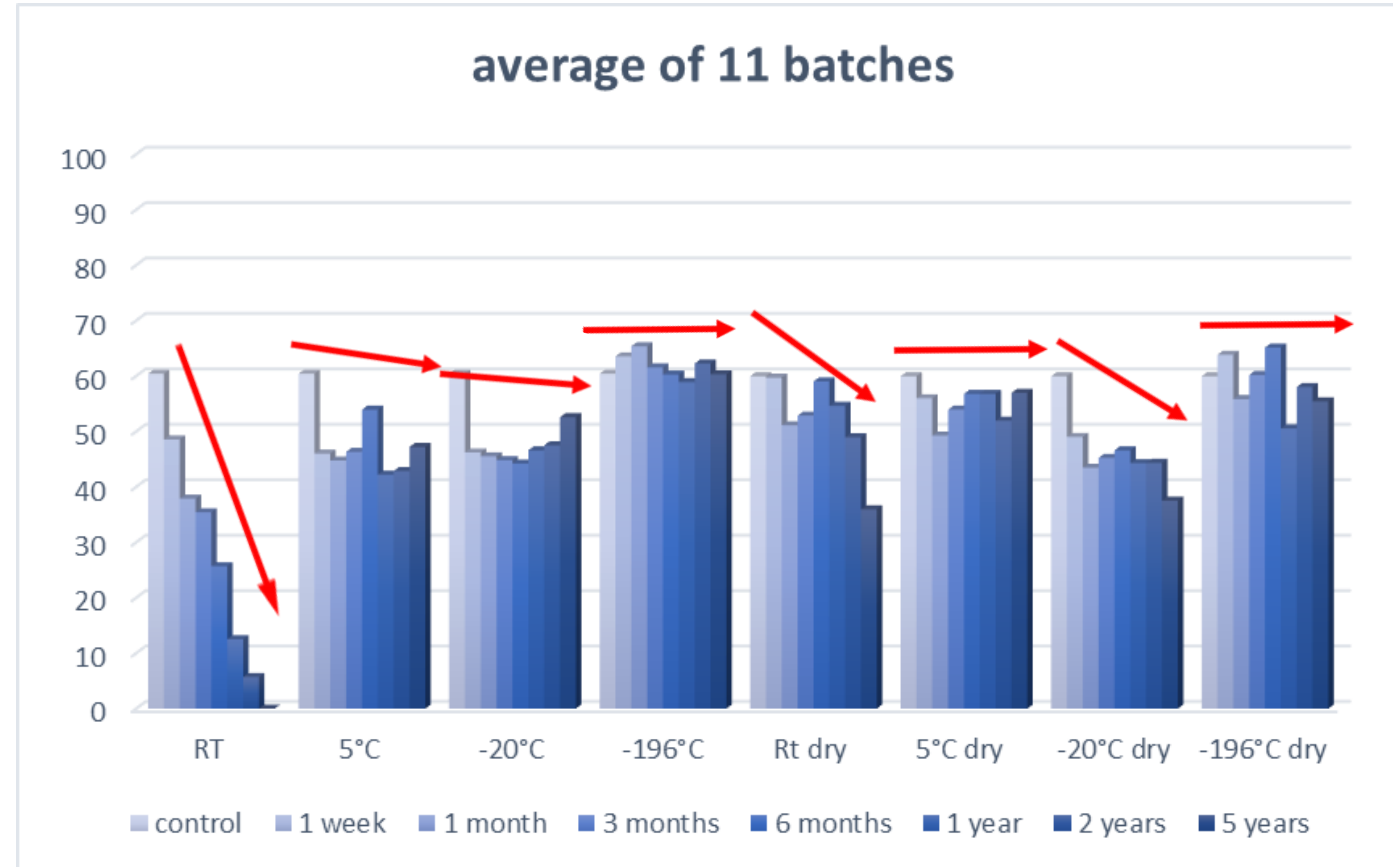
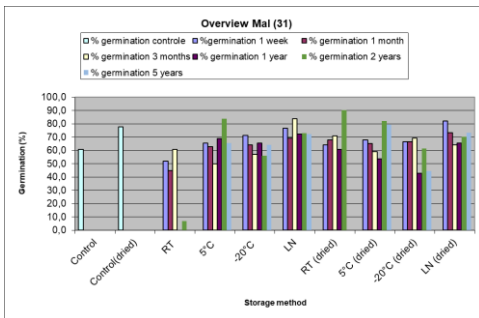
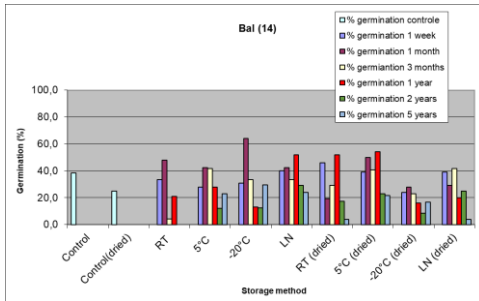
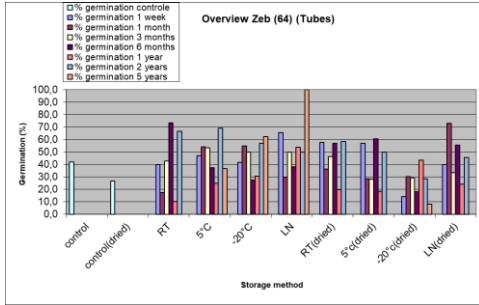
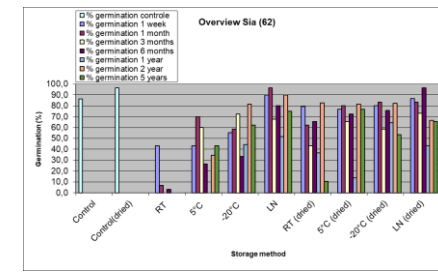
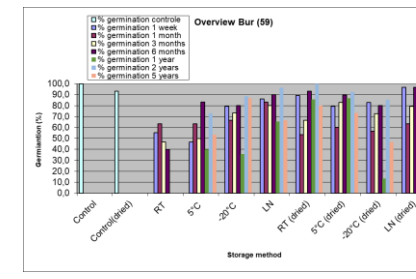
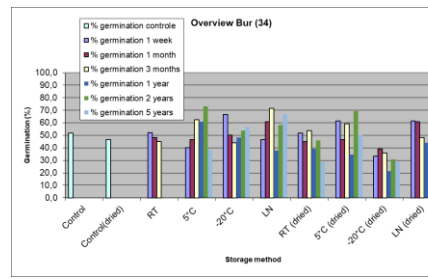
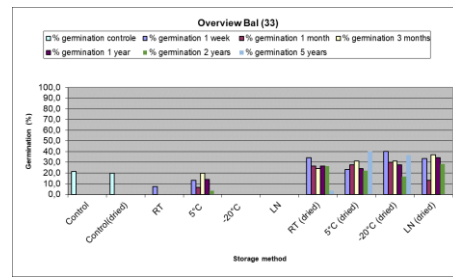
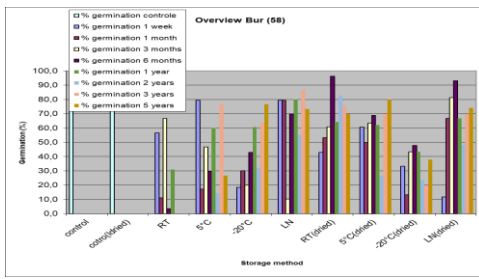
- Seed maturity is extremely important for (i) good germination and (ii) good storability.
- Problem: when collecting in the forest; seldom the right maturity phase is found. Is after-ripening a possibility?
- Drying rate is also important to keep viability... not too fast
- Germination problem is still not fully solved



Storage of seed batches , continuation of previous years

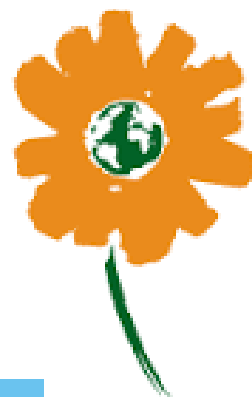
- Different seed lots (focus on *M. accuminata* and *M. balbisiana*)
- Dried and non-dried seed
- Storage at **room temperature, 5°C (Fridge), -20°C (freezer)** and -196°C (Liquid nitrogen) for 1 week, 1 month, 3 months, 6 months, 1 year, 2 years, 5 years, 10 years, 20 years, 50 years.
- Embryo rescue after storage





Best : Liquid nitrogen storage

Alliance



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THE BELGIAN
DEVELOPMENT COOPERATION **.be**



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AUSTRALIA
CREATE CHANGE





Thanks!