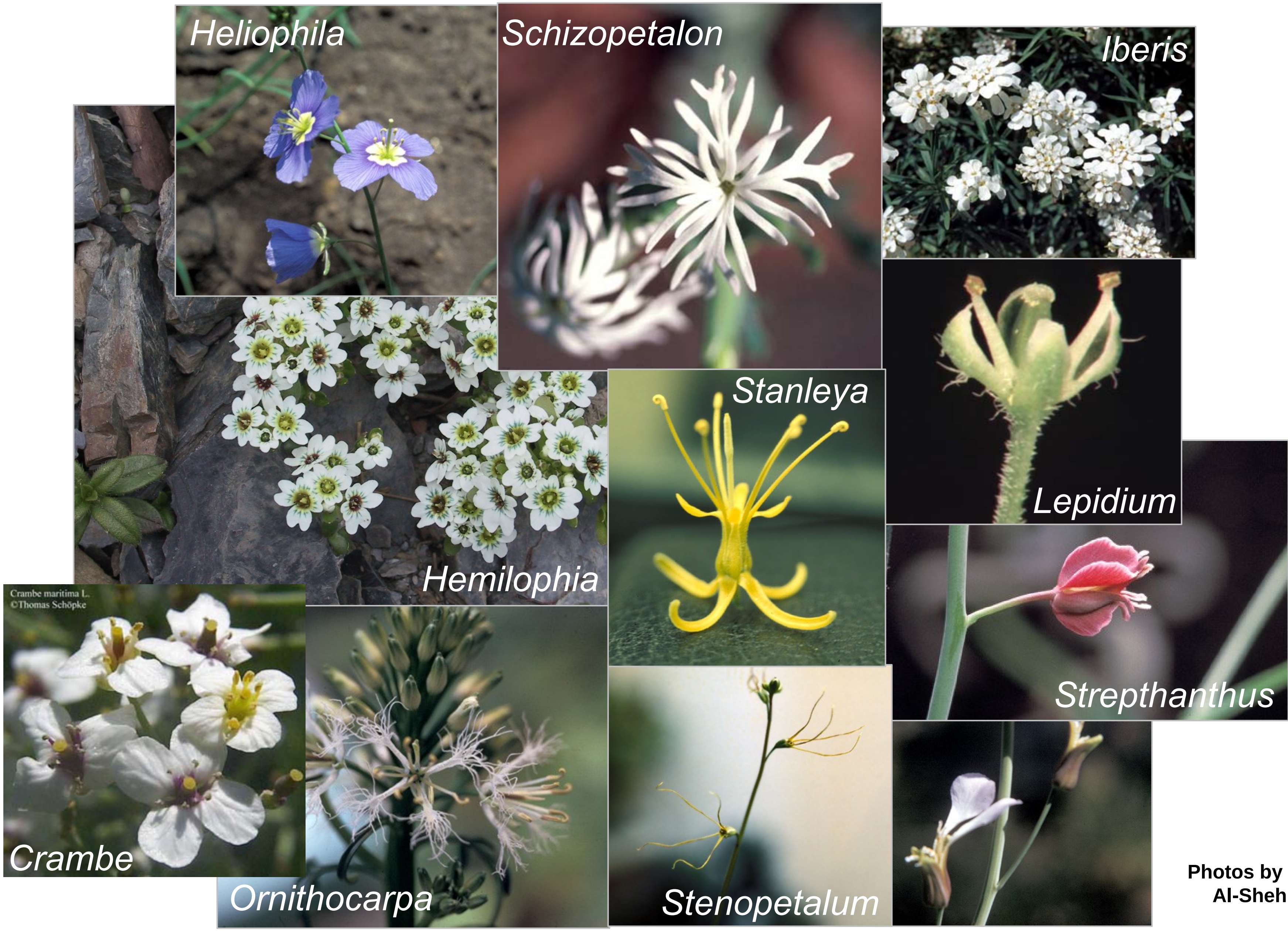
Brassicaeae – An introduction to family-wide Biodiversity: workshop

SPP 1529
ADAPTOMICS

Basic rules of seed storage and gene banks

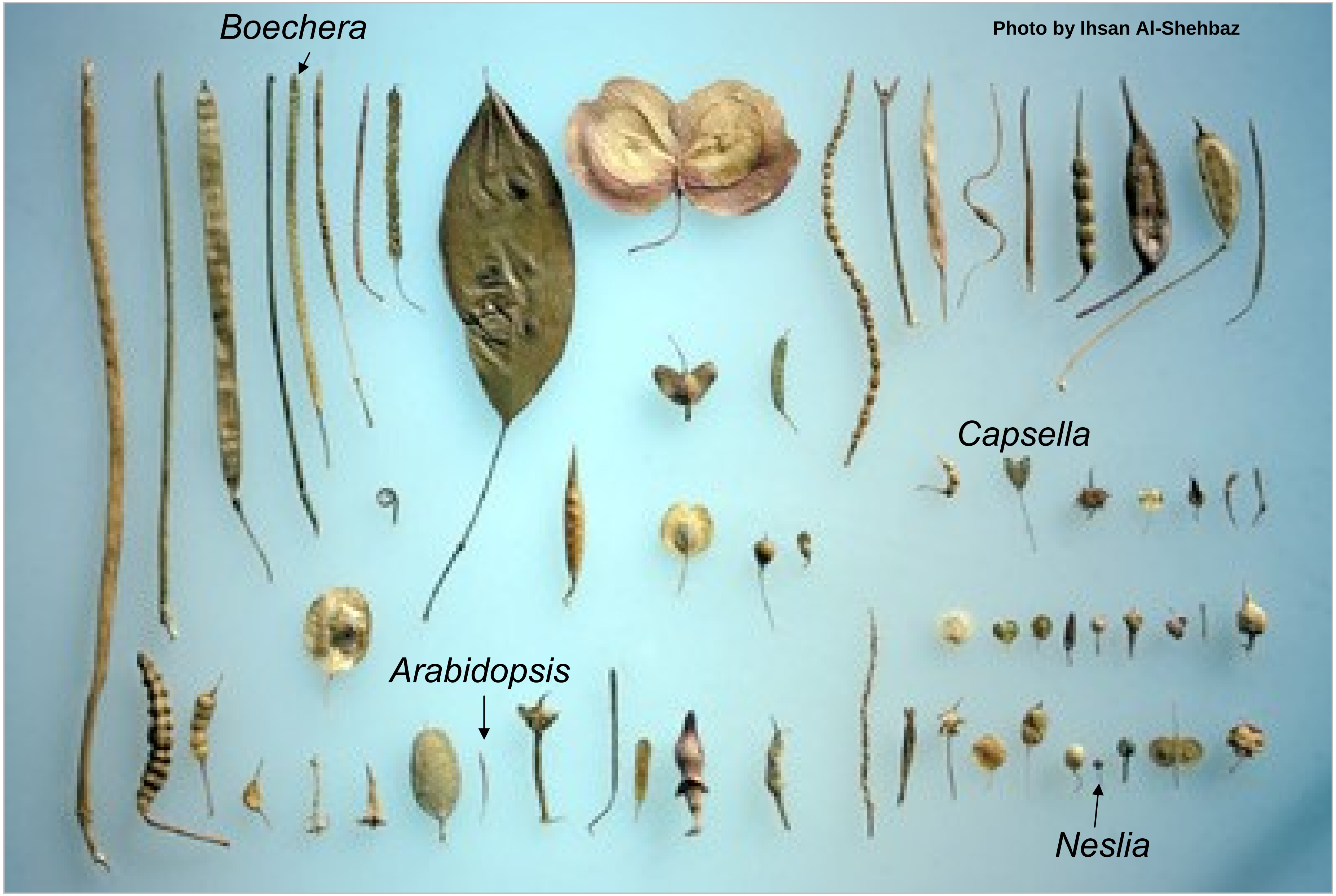
Klaus Mummenhoff, Osnabrück

Brassicaceae



Photos by Ihsan Al-Shehbaz

Fruit Diversity in Brassicaceae



Seed drying and difficult seeds

Seed storage is the preferred method for 90% of the six million accessions conserved in ex situ collections worldwide because it is practical and economical.

Inherently difficult seeds

- recalcitrant seeds
- orthodox but dormant
- orthodox but short-lived

Handling and storage difficulties

- immature orthodox seeds dried too rapidly
- orthodox seeds insufficiently dried prior to storage and/or stored under poor conditions

less longevity, low viability

- Seeds that have been damaged by insects

Objectives

That you know about and understand:

- the key factors affecting the extent and rate of drying
- different drying methods

Reasons to dry orthodox seed

- Prolong storage
- Tolerate sub-zero temperature
- Prevent germination
- Reduce damage by insects, mites and microbes

Orthodox seeds: Seeds that can be *dried to low moisture* content and *stored at low temperatures* which *increase seed longevity*.

Recalcitrant seeds: Seeds that loose viability when dried or stored at low temperatures

Reasons to dry orthodox seed

- **Prolong storage - seed moisture content**

Reducing **seed moisture level** (drying) slows down physiological ageing processes and so increases seed longevity

The most important factor for seed storage is the <u>r</u>elative <u>h</u>umidity (RH) of the air that will generate a defined <u>s</u>eed <u>m</u>oisture <u>c</u>ontent (smc)

% mc = fresh weight of seed – (dry weight of seed / fresh weight of seed) X 100
Seed Testing Association [ISTA] 2005

Smc > 30%	non dormant seeds may germinate
Smc 18-30%	deterioration by microorganism
Smc 18-20%	respiration, in poor ventilation the generated heat can kill seeds
Smc 8-9%	little or no insect and fungal activity
Smc < 3-4%	immune to insect and fungi attack, but this ultra-dry storage may cause seeds deteriorate faster than those maintained at a slightly higher mc
SMC 5%	optimal for Brassicacae

Reasons to dry orthodox seed

- **Prolong storage seed - seed moisture content**

Rough rule of thumb: *For each 1% decrease in smc, the storage life of the seed doubles*

Reasons to dry orthodox seed

- **Prolong storage seed - temperature**

The second most important factor for storage is temperature; **high temperature will cause deterioration**

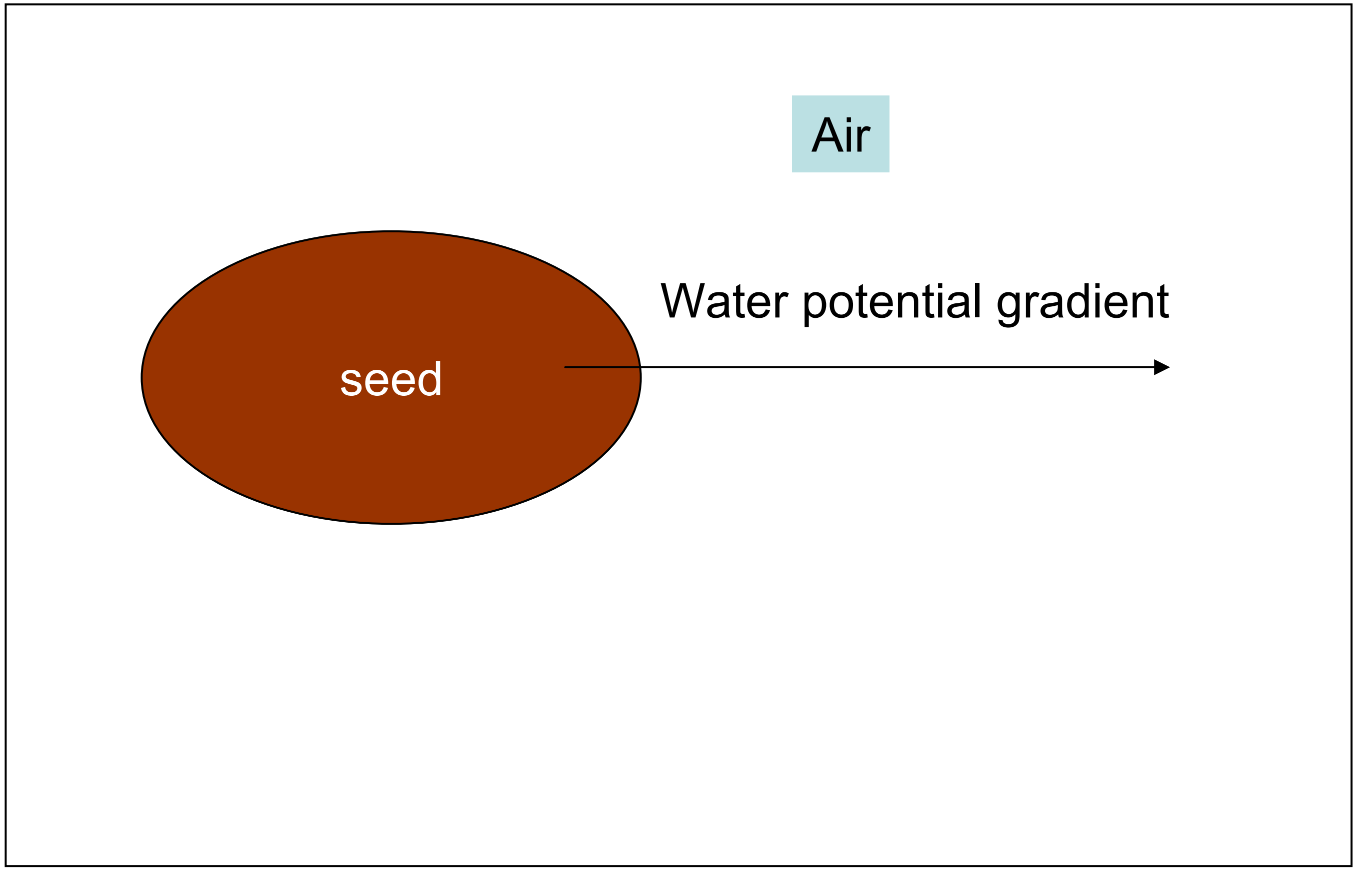
RH is temperature-dependent. Sealed seed storage containers are therefore important to prevent seed re-wetting

Rough rule of thumb: *For each 5.6°C decrease in seed storage T, the storage life of the seed doubles*

At smc < 14% no ice crystals; thus storage of dried seeds at subzero T in a dry atmosphere further improves longevity

Reasons to dry orthodox seed

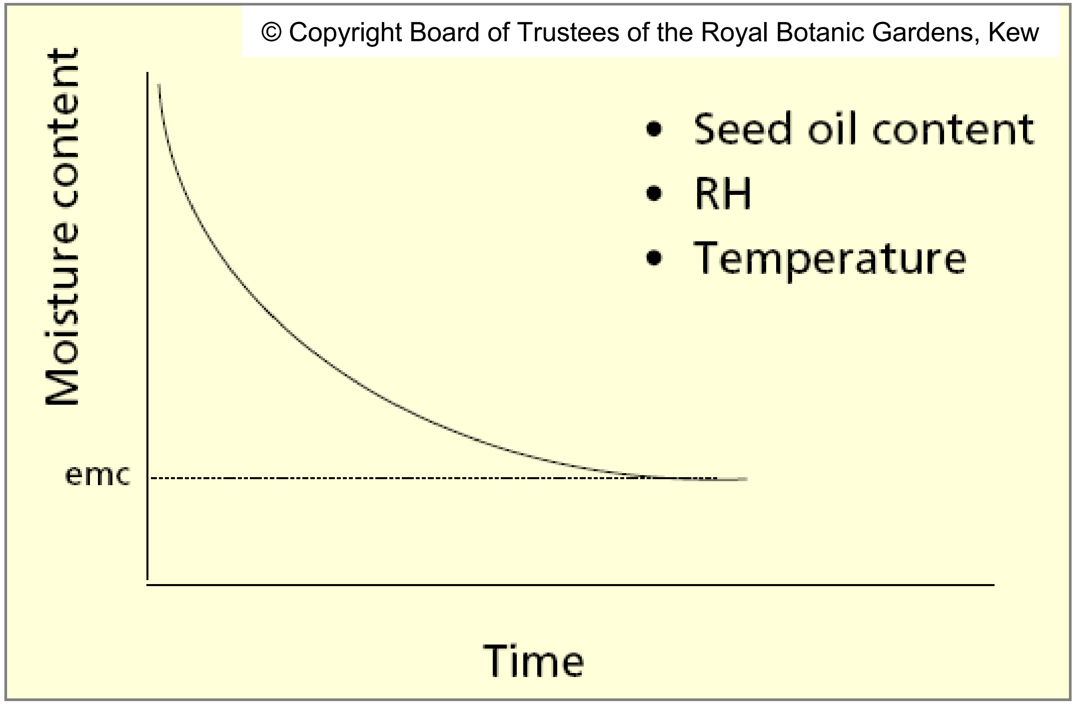
How seeds dry



Wet seed in air with low RH

Seed drying procedures

What affects the **extent** of seed drying?



Emc - equilibrium moisture content

How far can you dry down seeds?

Seed drying procedures

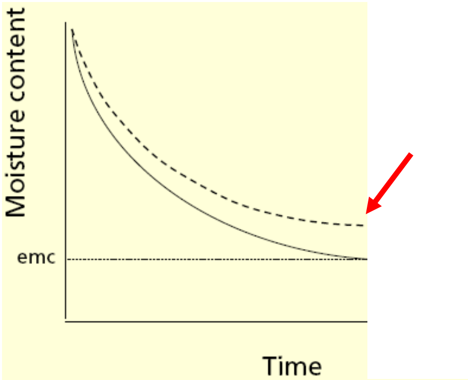
What affects the **extent** of seed drying?

emc (%) of oily and non-oily seeds dried under different conditions

FAO/IPGRI Standards	15% RH 15°C	15% RH 20°C	10%RH 15°C	10%RH 25°C
Triticum aestivum (2% oil)	6.1	5.9	5.0	4.6
Brassica napus (42% oil)	3.8	3.6	3.0	2.8

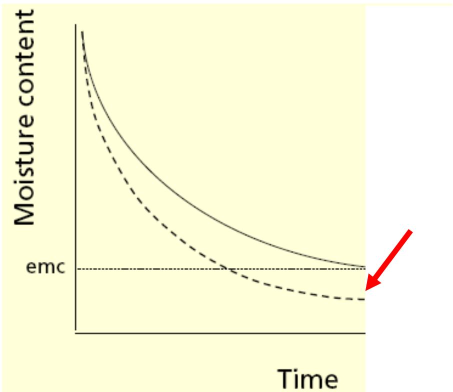
(IPGRI) International Plant Genetic Resources Institute
(FAO) Food and Agriculture Organization of the United Nations

Seed drying procedures



What if seeds are dried at a higher RH ?

Seeds dry more slowly and reach a higher emc

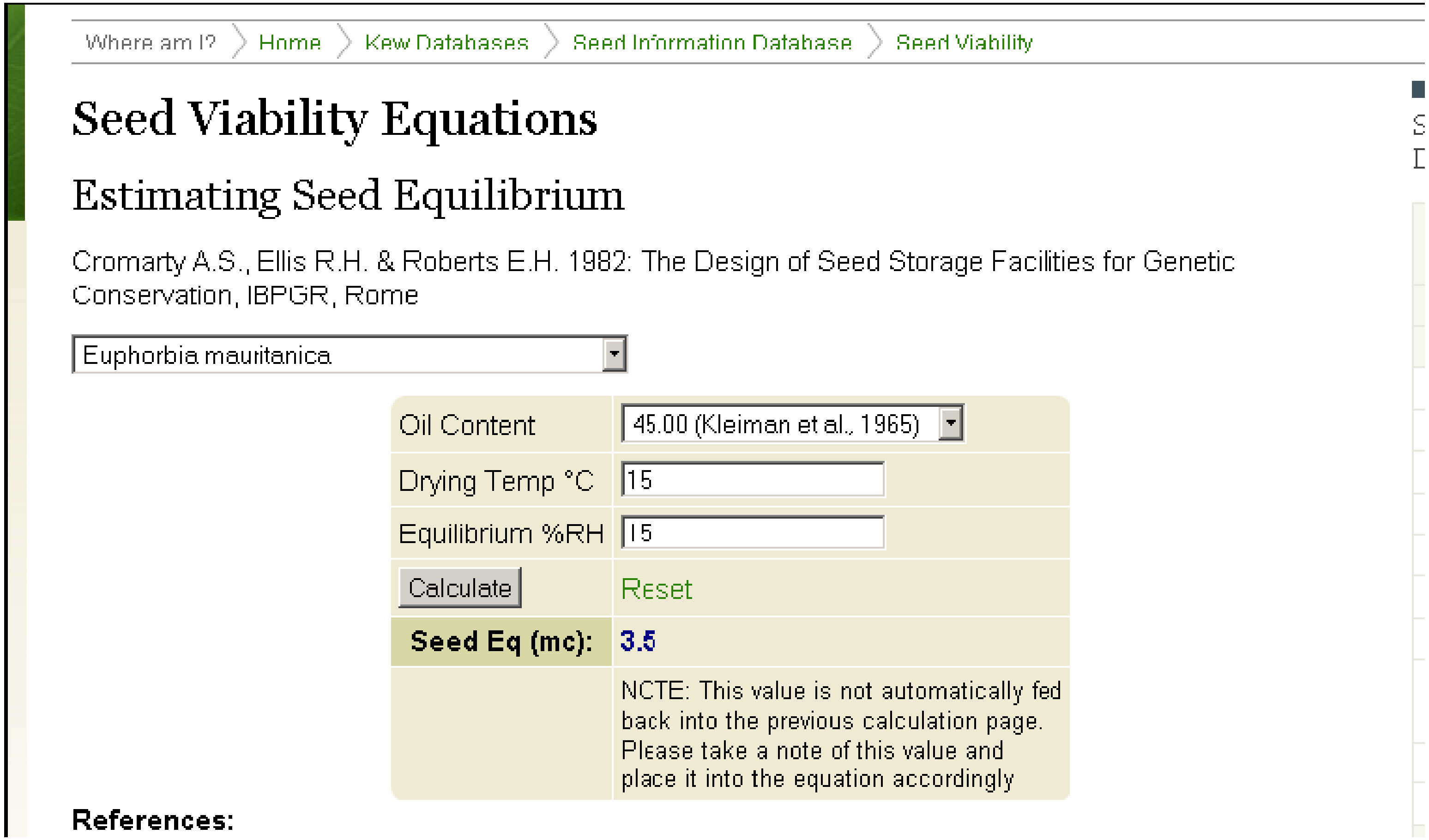


What if seeds are dried at a higher temperature ?

Seeds dry more quickly and reach a lower emc

Seed drying procedures

Estimating equilibrium moisture content (emc)



Kew's Seed Information Database (SID) includes a simple conversion tool to estimate emc if seed oil content and drying conditions (temperature and RH%) are known.

http://data.kew.org/sid/viability/index.html

Seed drying procedures

What affects drying **rate**?

• Proximity to emc

• Seed size and structure
Large seeds dry more slowly than small ones

• Depth of seeds in bags
Many small seeds deep in bag – moisture migration equivalent to that in large seed

• Air speed/ventilation
High air speed: Water potential gradient between seed and air MAXIMISED

• Temperature
Increased temp. → decreased RH → increased water potential gradient → increased rate of diffusion
Be careful with too high T; heat reduces viability: best is ca. 15°C

Seed drying procedures

Step 1: Predict moisture content
Oven drying method: (destructive) 103-130°C

Moisture meters (non-destructive): measures electrical resistance of seed material

Step 2: Prepare seeds for drying
Place (not too many) seeds in porous bags; cotton, paper

Step 3: Dry the seeds
(i) Initial drying to reduce mc to safe levels to avoid damage to sensitive seeds
(ii) Final drying to mc recommended for final coservation in gene banks

(i) Initial drying
- Passive drying in a room with good ventilation and air circulation
- Active drying under forced ventilation

Seed drying procedures

Step 3: Dry the seeds
(ii) Final drying

- **Dry room or drying chamber** at 10-**15**% RH and 10 - 25°C (FAO / IPGRI Genebank Standards)
  The Millennium Seed Bank (MSB) dries seeds at 15% RH, 15°C.

- **Desiccant - Silica gel** / charcoal / dried rice

- Sun / shade drying

- **Saturated salts / Lithium Chloride solutions**

- Air conditioned room / vehicle

- Incubator drier

Seed drying procedures

Dry room or drying chamber/Cabinet

Drying rooms should be fitted with an airlock to minimise moisture entering from outside.

The room should be sized on the basis of peak annual demand.

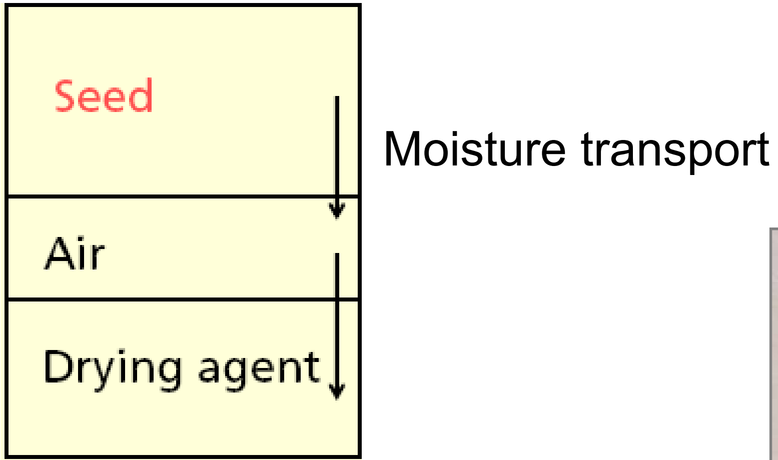
A typical dry room, set at 10-15% RH at 10-25°C, and with the air circulating at about 6 changes per hour, will dry seeds of any species and at any mc in about 30 days.


Drying cabinet


Royal Botanic Gardens, Kew

Seed drying procedures

Desiccant - Silica gel / charcoal / dried rice

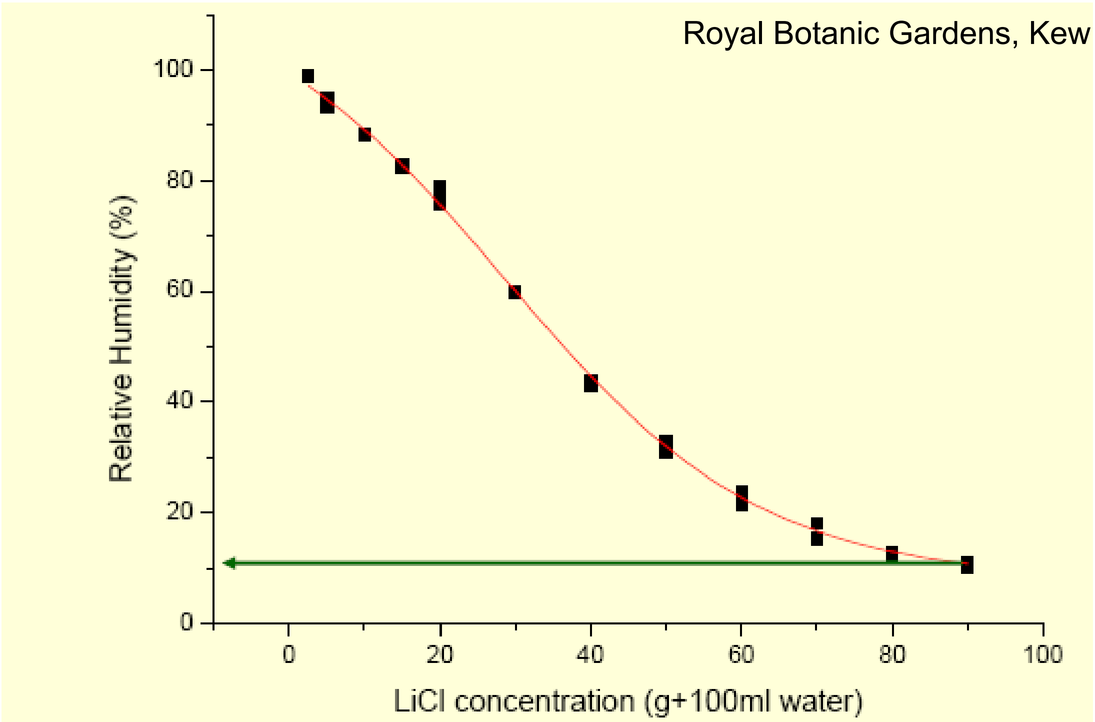


Drying time (~one month) depends on the initial moisture content of the seeds, the amount of seeds, the type of seeds and the dryness of the silica gel.

Silica gel-to-seed ratios 3:1

Seed drying procedures

Saturated salts
Lithium Chloride: at 20°C produces a RH of about 12%
Calcium Chloride: at 25°C produces a RH of about 30%



Air-tight jar with lithium chloride solution, showing plastic mesh support to hold seeds above solution

Only plastic coated metal components should be used as salts may be very corrosive.

Seed drying procedures

Key points:

• Seeds die rapidly under ambient conditions

• Drying can increase seed longevity by 1000 times

• RH, temperature & oil content influence the *extent* of drying

• Temperature, seed size / structure, seed bed depth & ventilation influence the *rate* of drying

• Dry-room conditions of 15% RH, 15°C are recommended

Seed drying procedures

Seed storage concept of the Leubner group (RHU, London), optimised for *Lepidium* and *Arabidopsis*

The best long term storage conditions to preserve the ***physiological state of a seed*** *is to dry it down to ca. 5% smc*

Seed storage of fresh (can be dormant) or after-ripened seeds (dormancy release)

Seed after-ripening: Storing the seeds in open tubes/porous bags above a saturated $Ca(NO_3)_2$ solution. Above this solution 51-54%RH is generated at 25ºC and will cause an optimal smc of ca. 10% for fast after-ripening.

The time depends on ecotype and is weeks to months.

Seed drying procedures

Seed storage concept of the Leubner group (RHU, London), optimised for *Lepidium* and *Arabidopsis*

1. **Initial drying:** Passive drying in a room with good ventilation and air circulation (1 week); avoid re-wetting.

2. **Final drying:** Storing the seeds in open tubes/porous bags above a saturated (!) LiCl solution. Above this solution 15% RH is generated and at 5-15 ºC and a smc of ca. 5% is caused within ca. 1 week.

3. **Long-term seed storage that preserves the physiological state** (**either after-ripened or fresh**) **is best at -20 ºC.** Seed storage at -20 ºC requires sealed tubes /bags to prevent moisture uptake

Seed drying procedures

Reasons, why seeds do not germinate:

Dormancy: is considered the failure of **viable seeds** to germinate, even under environmental factors that are otherwise favourable for its germination

Quiescent seeds: no germination at all, usually due to low smc; they are alive but lack some environmental factor (water) for germination

Low viability/longevity: depends on seed maturity and post-harvest conditions

Fully ripened seeds with high initial viability retain their viability longer than immature seeds

Inadequate storage conditions also play a main role in the low seed viability

Seed viability testing

2,3,5-<u>T</u>riphenyl<u>t</u>etrazolium <u>c</u>hloride (TTC)

TTC belongs to the group of Tetrazolium salts and is metabolically reduced in plant cells to a water-insoluble red-coloured Formazan.

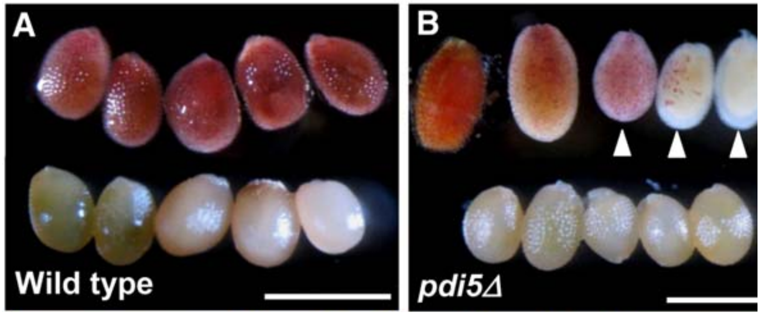
This reaction can be used to determine the viability of cells.

Not only single cells, but also the viability of whole seeds can be tested with TTC

Embryos from ungerminated seeds are treated with TTC: if they turn red, they are classified as vital and potentially viable, if not, they are most likely dead and non-viable.

TTC test is destructive!

Seed viability testing



(**A**) and **(**B**)** Tetrazolium-based embryo viability test. Bar = 500 µm.
Top row: wild-type embryos show a uniform red color (viable seeds).
Bottom row: wild-type embryos boiled for 30 min are light pink (negative control).

The Plant Cell 20, 2008

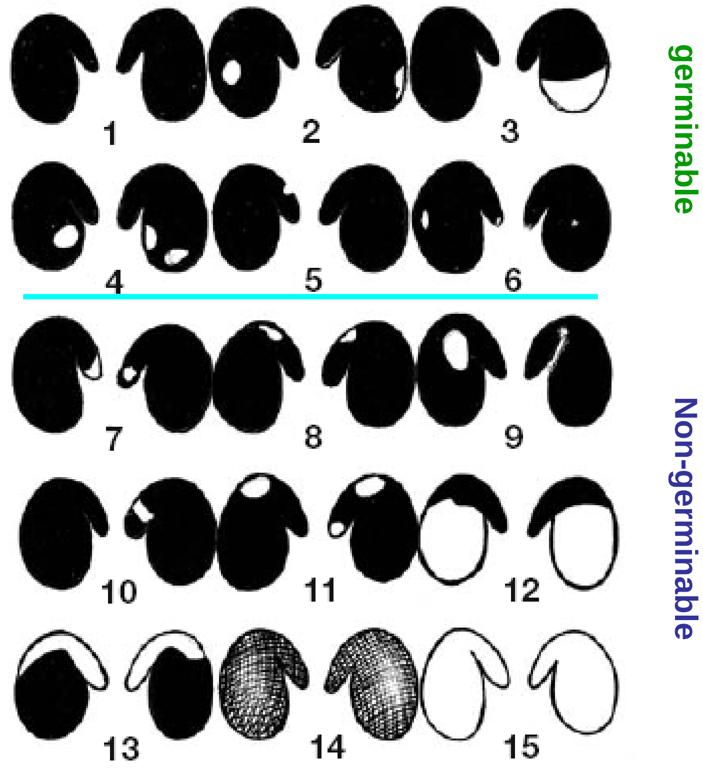


Figure 5.9. Staining pattern after tetrazolium test in dicot seeds. Illustrations depict both sides of seeds. Numbers 1–6 are germinable seeds and numbers 7–15 are nongerminable seeds (adapted from AOSA, 2005).

Seed viability testing

Non-viable or deteriorated seeds leak more solutes than viable or vigorous seeds when placed in water due to aged, damaged or non-functional cellular membranes and cellular rupture

Seed leakage: Amino acids, proteins, sugar, phenolics, ethanol, ions

Measuring seed leakage and thus viability

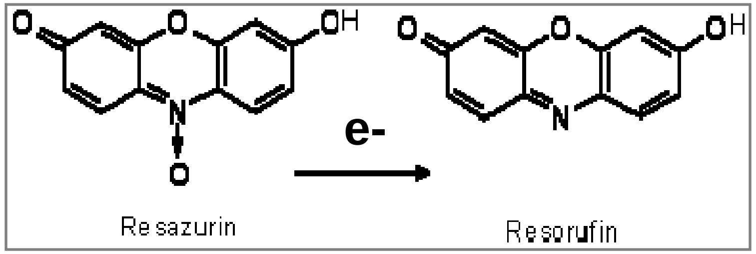
Electrical conductivity of seed leachate, light absorbance at 260nm, sinapine or amino acid leakage test

Either tests are destructive or methods are not simple and quick and require pretreatment and often many seeds

Seed viability testing

Single seed viability test using Resazurin (Min & Kang 2011)

Resazurin* is a redox adjuvant, non-toxic, water soluble dye, that is reduced by e$^-$ transfer reactions associated with respiration to an easily measured water-soluble flourescent product *Resorufin



Resazurin (blue) is reduced to *Resorufin* (pink) and *Dihydroresorufin (*colourless)

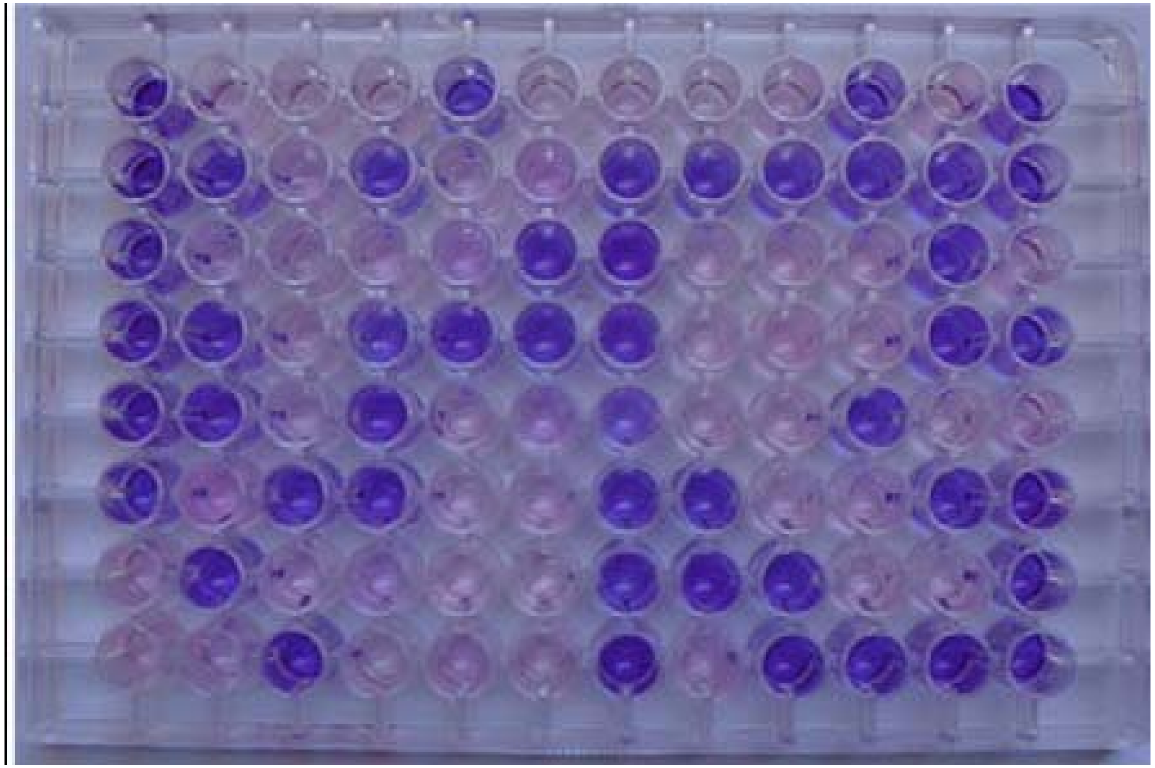
Test: Yeast, and samples of fresh and differentially aged *Brassicaceae* seeds

Respiration of yeast is activated by the solute leaked from aged seeds (**ethanol**) and then the blue colour of *Resazurin* reduced to *Resorufin* and *Dihydroresorufin* to be pink and colourless.

Min & Tan, 2011. A simple, quick, and nondestructive method for Brassicaceae seed viabilioty measurement with single seed bas using Resazurin

Seed viability testing

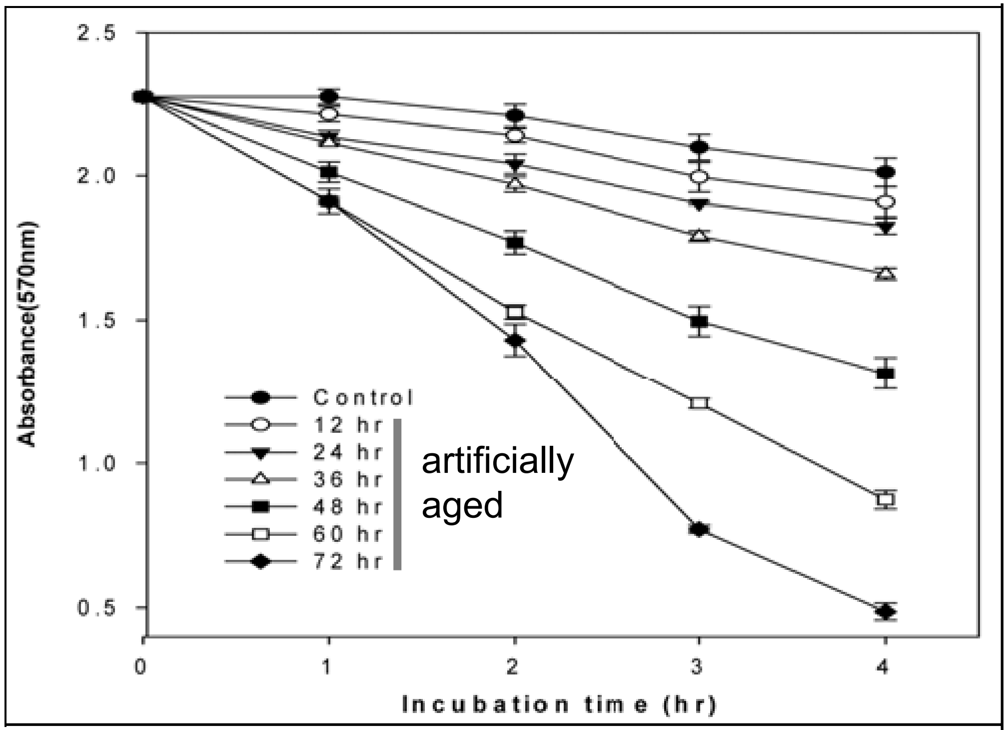
Single seed viability test using Resazurin (Min & Kang 2011)



Resazurin-test for 48 normal seeds and 48 dead seeds from the artificially aged (0-72h, 45°C) *Raphanus* seeds

Seed viability testing

Single seed viability test using Resazurin (Min & Kang 2011)

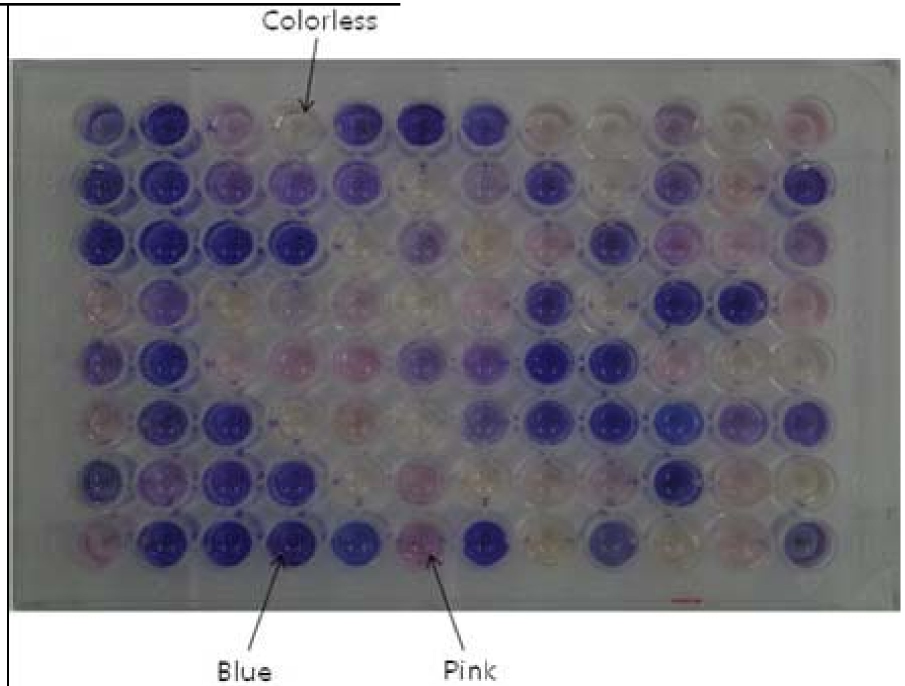


Time course absorbance change of artificially aged (0-72h, 45°C) *Raphanus* seeds soaked in Resazurin / yeast solution at 570 nm

Seed viability testing

Taxon	seed sample	Storage at 4°C (year)
Raphanus sativus	J	18
	R	7
Brassica oleracea	D	7
var. *Capitata,* cabbage	T	6
	H	7
var. *botrytis* Broccoli	G	19

As a result, the average of germination percentage of the blue, pink and colorless fractions of six naturally aged seed lots were 96%, 89% and 7%, respectively.



The three color fractions of the Resazurin for the naturally aged (7-18 years, 4°C) radish seeds

Seed viability testing

Single seed viability test using Resazurin (Min & Kang 2011)

The relationships between germination percentage, MGT (mean germination time) and the color fractions of the **Resazurin** test of artificially aged *Raphanus* seeds

Ageing time (hr)	Germination (%)				Absorbance (570 nm)	Color (%)			MGT (day)
	Total germination (%)	Normal	Abnormal	Dead		Blue	Pink	Colorless	
0	100	99	1	0	2.011	100	0	0	1.1
12	99	99	0	1	1.909	95	5	0	1.2
24	79	73	6	21	1.825	68	26	6	1.8
36	77	69	8	23	1.659	50	21	29	2.3
48	25	22	3	75	1.337	25	19	56	2.8
60	15	15	0	85	0.873	0	8	92	3.7
72	0	0	0	100	0.487	0	3	97	-

Seed drying procedures

Seed storage concept Gomez-Campo (Brassicaceae Germplasm Collection, Madrid)

Ultra-dry storage
Long term storage between 1 and 3% smc and between +5°C and -5°C in sealed glass tubes on silica gel

Rejuvenation cycles after 25-50 years

Warning: other authors stress that ultra-dried seeds may deteriorate faster than those maintained at a slightly higher smc

Seed drying procedures

Probert, R.J. (2003). Seed viability under ambient conditions and the importance of drying, pp.337-365. *In*: R.D Smith, J.D Dickie, S.H. Linington, H.W Pritchard & R.J Probert (eds) *Seed conservation: turning science into practise.* Royal Botanic Gardens, Kew, UK.

http://www.kew.org/msbp/scitech/publications/SCTSIP_digital_book/pdfs/Chapter_19.pdf

Groot, PC, de Groot L. 2008. Seed quality in genetic resources conservation. CGN, Wageningen University