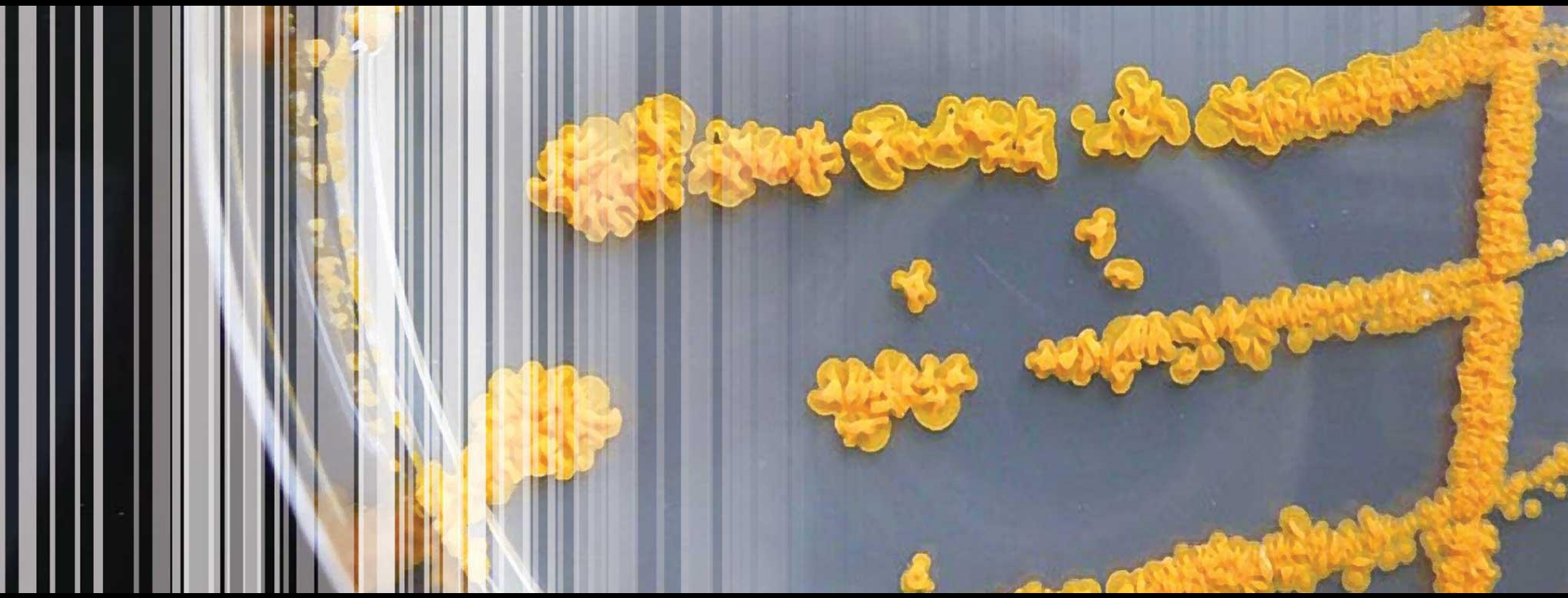

Handling and preservation of plant seeds for patent purposes

Peter N Green, NCIMB

Presentation content

1. Why should a national bacterial culture collection offer plant seed deposit and preservation facilities?

2. What are the differences between accessioning plant seed and other biological materials?

3. The preservation process and viability testing

4. Difficulties and solutions associated with viability testing and banking

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Why should a national culture collection offer seed deposit and preservation facilities ?

- There is a demand; particularly for patent purposes.
- National specialist centres of excellence (such as Kew at Lakehurst Place in the UK) offer seed gene banking facilities, but not for patent purposes
- NCIMB is one of only two global major national collections which offer such a service
- Seed are no different from other living biological materials – there is a need (arguably greater) for their long term preservation!
- Microbial culture collections already have some of the required expertise and equipment.

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What are the differences between accessioning plant seed & other biological materials for patent purposes?

- Whether bacterium, fungus, alga or other living biota, one of the prime acceptance criteria for patent purposes is the demonstration of **viability.**
- Numbers of propagules in the deposit - is it an issue?
- How do we measure or assess viability in plant seeds and hence acceptance of a deposit for patent purposes?
- Any regulatory problems?

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Number of seeds in a deposit

- There are no absolute hard and fast rules just guidelines.
- The International Board for Plant Genetic Resources (IBPGR) in their “Handbook of Seed Technology for Genebanks” recommend that a minimum of 2500 seeds be accepted for a normal banking exercise.
- However patent deposits are not “normal.”

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Number of seeds in a deposit

- In many cases, where a new or novel cultivar is involved in a cross-breeding experiment, there may only be one plant when the initial patent is filed with only a few hundred seeds available at the time of deposit. Thus patents **must,** in our view, be treated differently in terms of minimum number of propagules for acceptance.

- Deposits can always be topped or replenished under rule 6.2 and Article 4 of the Budapest Treaty

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Number of seeds in a deposit

- For the reasons stated NCIMB **encourages** depositors to deposit at least 2500 seeds to cater for viability testing, loss during preservation and the furnishing of samples, but have a **minimum acceptance** limit set around 250.

Regulatory criteria

- Restrictions on seed imports are less than those for living plants or plants in soil

- Nevertheless for some species there are controls to be observed both within counties and across various geographical regions in order to prevent the spread of non-indigenous plant pests.

- In the UK we follow the guidelines for phtosanitary certificates or plant passports as directed in The Plant Health (Great Britain) Order 1993 et al.

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The preservation process

- **NCIMB accept only “orthodox” seeds**
- Plant seeds can be stored either chilled at a controlled relative humidity or frozen.
- Many of the large gene banks use the former method.
- However at NCIMB, for a number of reasons, we store seed frozen at minus 20^{0}C.
- However before freezing, in order to minimise tissue damage, seeds firstly have to be dried to a residual water content of around 5-6%

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Orthodox and recalcitrant seeds

- **Recalcitrant seeds** (sometimes known as unorthodox seeds) are seeds that do not survive drying and freezing during ex-situ conservation. Moreover, these seeds cannot resist the effects of drying or temperatures less than 10° C; thus, they cannot be stored for long periods like *Orthodox seeds* because they can lose their viability. Plants that produce recalcitrant seeds include avocado, mango, lychee, some horticultural trees, and several plants used in traditional medicine.

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Seed drying

- AT NCIMB seeds are dried in a specially adapted incubator which functions at a relative humidity such that seeds will be dried in a matter of days to the desired residual moisture content.

- Seed water content can be estimated either by destructive or non-destructive methods.

- NCIMB estimates moisture content with the use of instrumentation designed to measure water activity (Aw) which is a measure of free unbound water.

Viability – how is it defined and assessed in plant seeds?

- **Viability** is assessed by **germination** testing and we accept deposits only where germination alone has been **unequivocally demonstrated.**

- Thus we only score seeds which have visible emergence of either **plumule** and/or **radicle** as **positive for viability**

- What of other tests for viability?

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“Alternative” tests for viability

- Most alternatives to germination involve detection of embryo enzymes and merely indicate a potential to germinate and hence **potential viability**

- Commonly accepted test such as the tetrazolium test also can be difficult to interpret and requires a highly skilled eye

- Remember the result of a viability test may have to withstand legal interrogation in the event of a patent being challenged!

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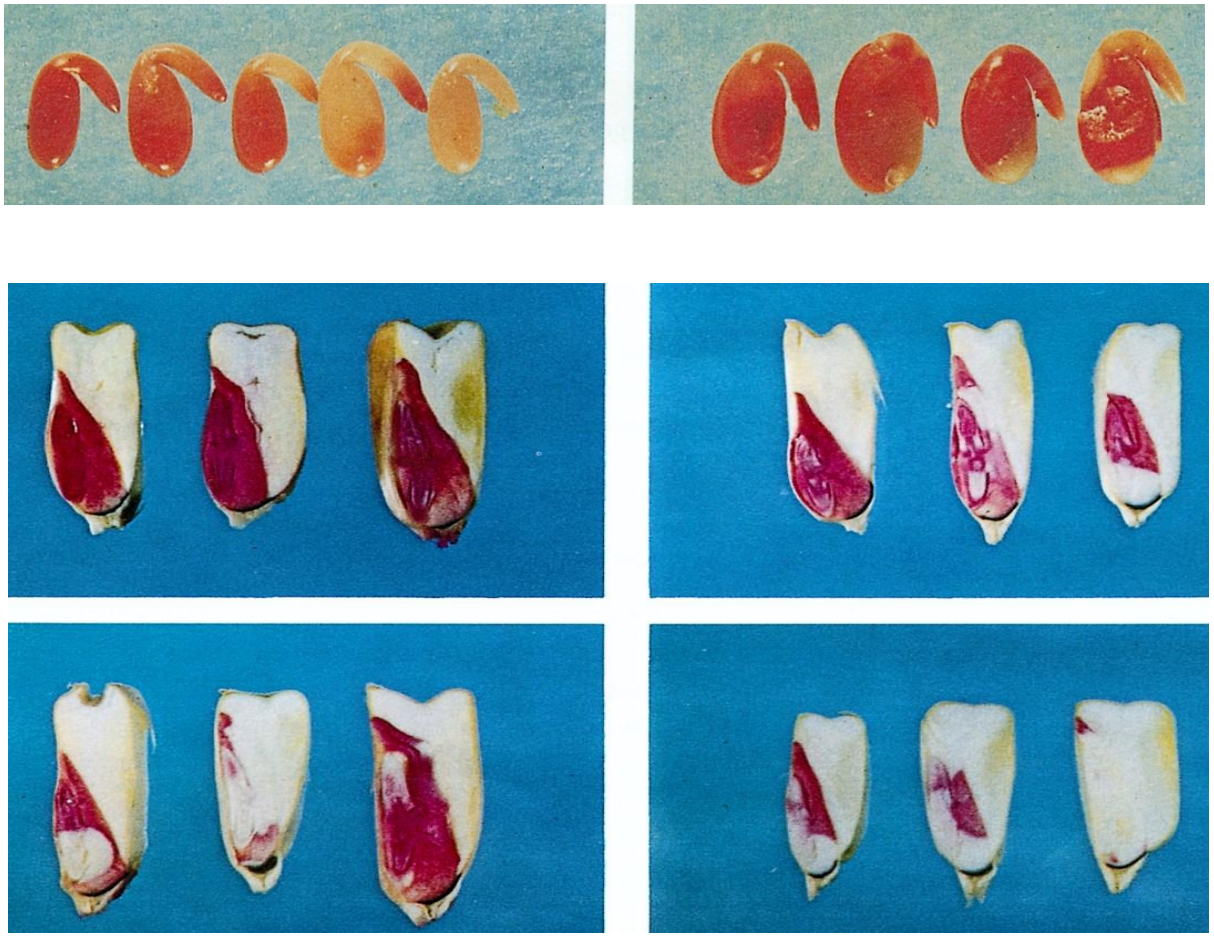
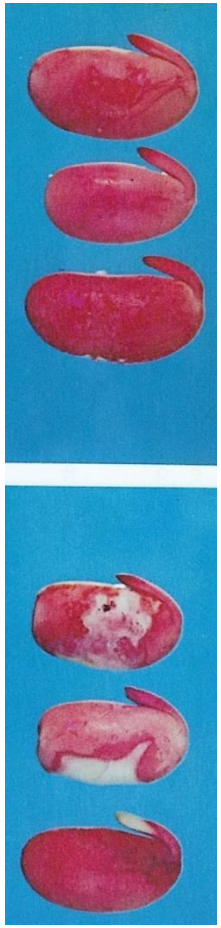
Tetrazolium test – which seeds are likely to germinate ???





Flow diagram of the accession process

Viability test (10-25 seeds)

↓

Positive viability (effective date of deposit)

↓

Seed drying (to desired residual moisture content)

↓

Freezing at minus 20^0C

↓

Percentage germination (post drying c.100 seeds)

↓

Banking and documentation

Germination protocols

- Germination protocols can be extremely varied and are often species specific (IBPGR, ISTA)
- Some seeds will germinate optimally only in light, some in dark, some in either.
- Some seeds will germinate at a constant temperature others require alternating temperature and/or light /dark cycles
- Some seeds require additional pre-treatment regimes
- Surprisingly many of the seeds NCIMB handle are relatively easy.

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Common germination formats

- **Between** moist **Paper** (**BP**)
- On **Top** of moist **Paper** (**TP**)
- **Water Agar** (**WA**)
- **Soil/Compost** (**S/C**)

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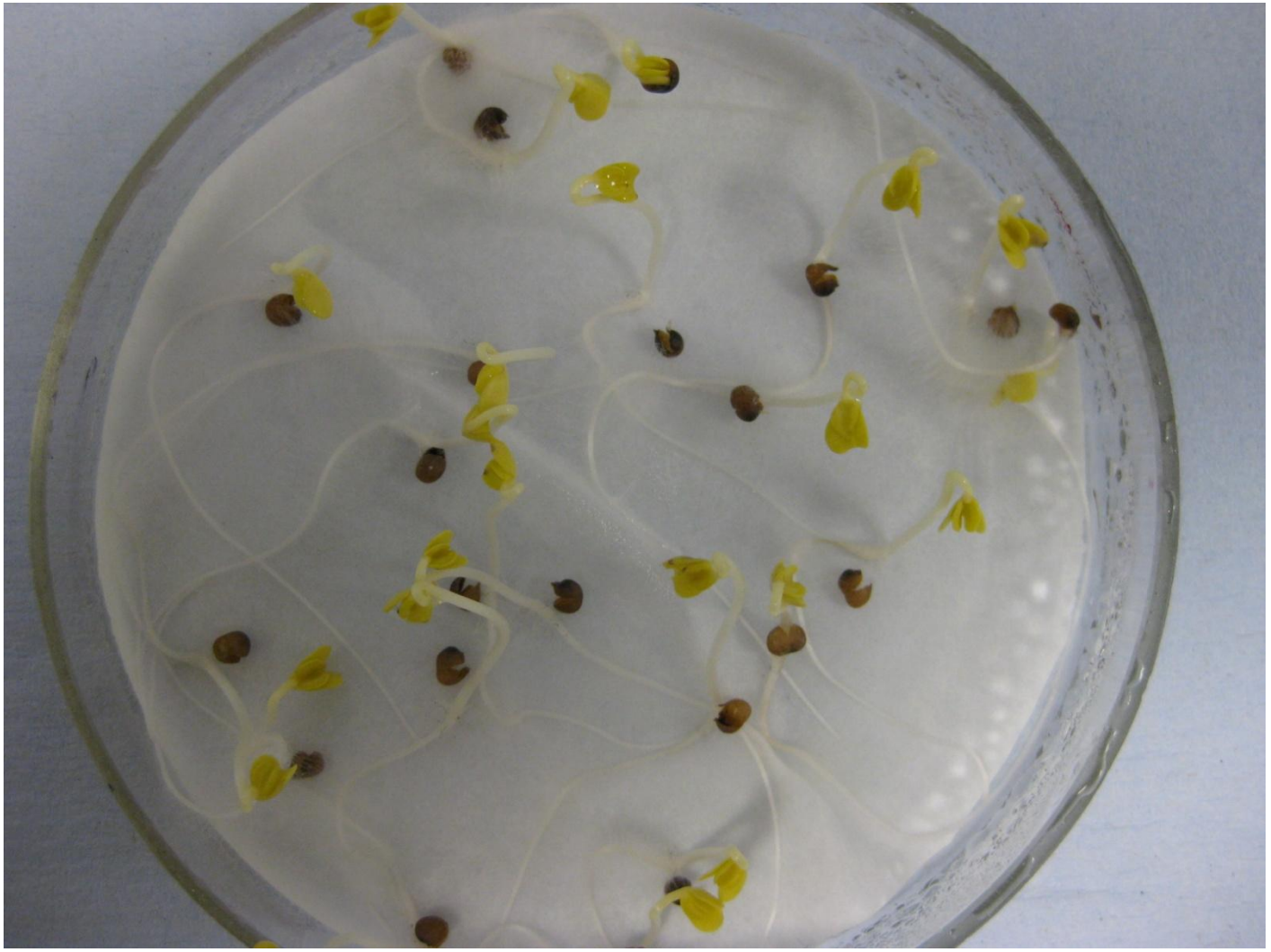
Germination on top of moist paper (TP)



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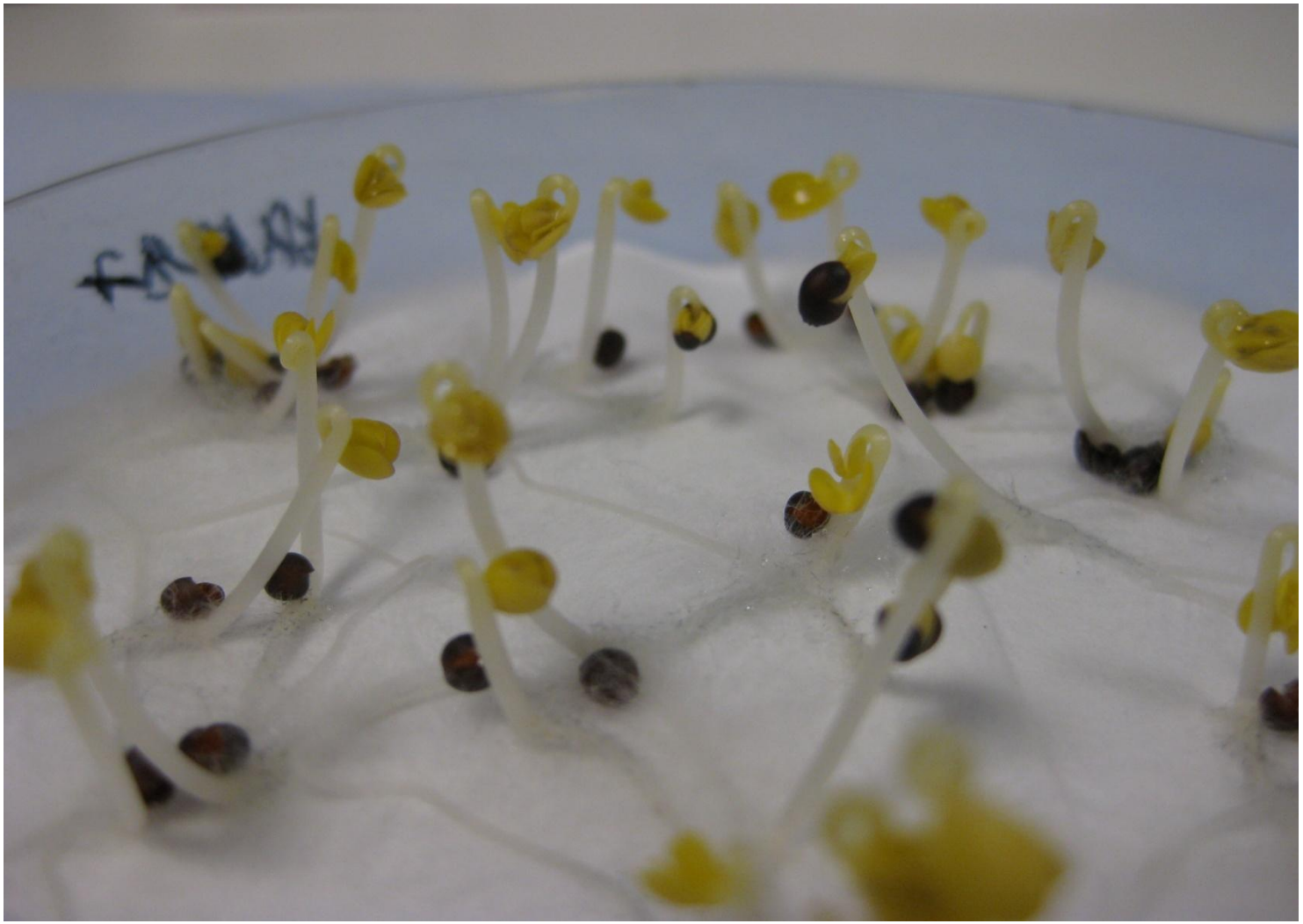
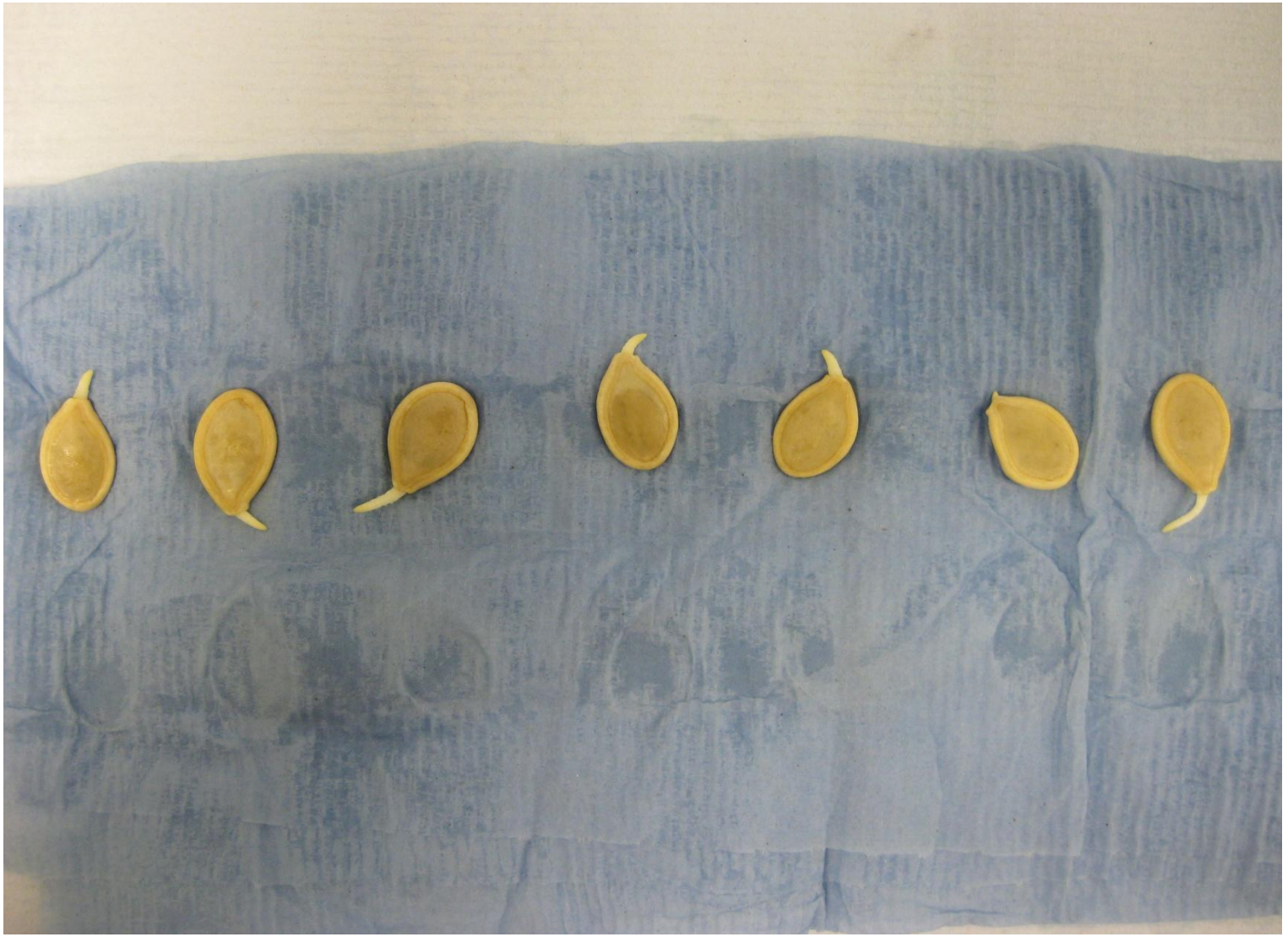
Germination on top of moist paper (TP)



Germination between paper (BP)



Germination on top of moist paper (TP)

Germination between paper (BP)

Germination in water agar (WA)

Difficulties and solutions associated with banking and viability testing

These can be many and varied but include the following:

- Hard seededness
- Water sensitivity
- Imbibition injury
- **Fungal overgrowth**
- **Dormancy**

Fungal or bacterial overgrowth

- Many seeds, and in particular some of the grain crops such as barley (Hordeum vulgare), can be overgrown by fungal contaminants which are part of the natural seed flora and these can inhibit or lyse the seed embryo as a result of enzyme activity or competition for O_2
- This effect can be partly countered by the use of washing, fungicides such as copper (Cheshunt compound), sterilants such as hypochlorite or proxide, silver nitrate, mercuric chloride, fungal and/or bacterial antibiotics, and/or in combination with removal of seed covering structures

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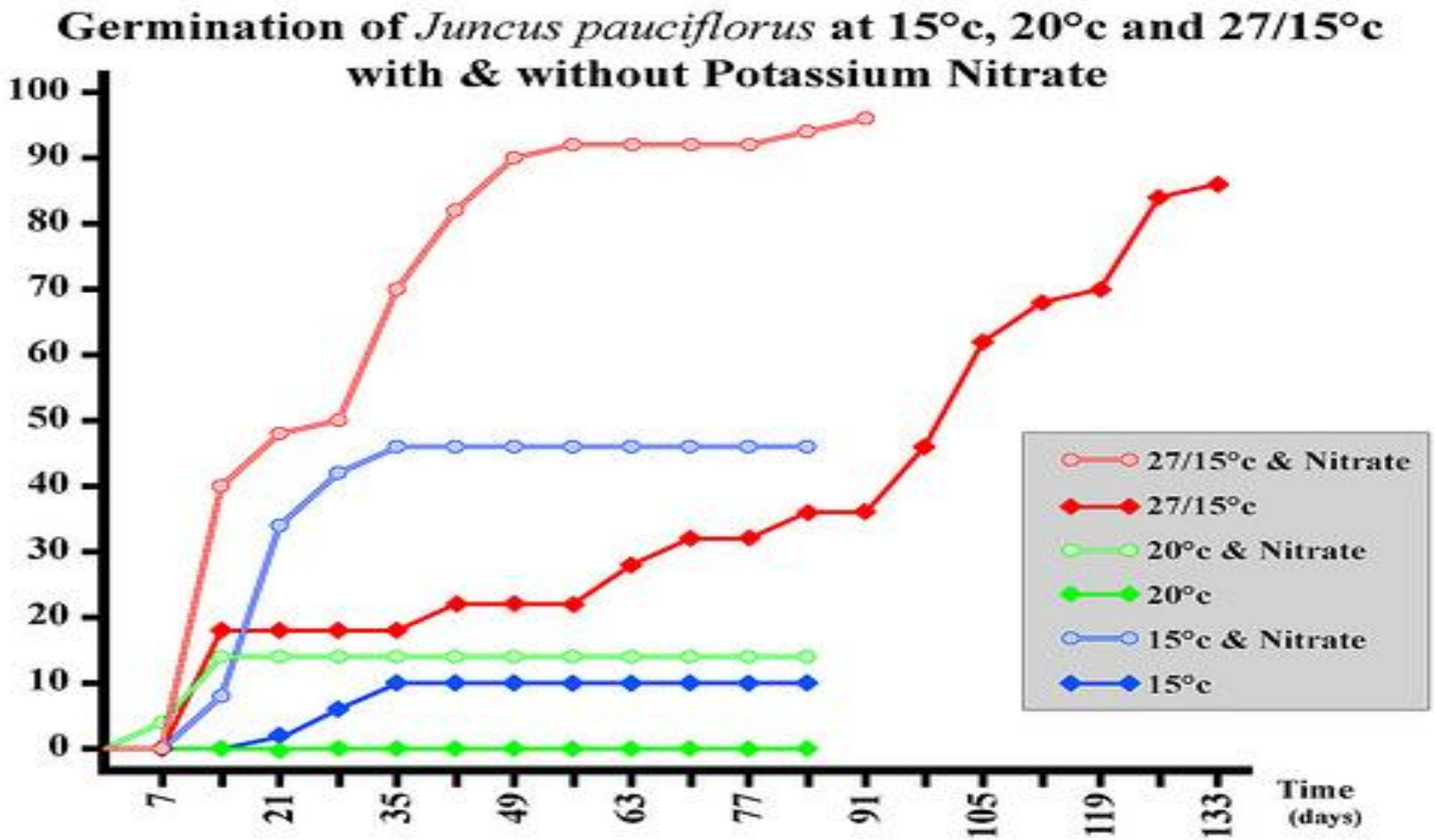
Pictorial example of fungal overgrowth

Treatments to remove dormancy

- Pre-soaking
- Pre-chilling (not freezing)
- Freeze/thaw cycling
- Removal or puncture/scarification of testa
- Alternation of germination tempertures/lighting
- Application of chemical agents (nitrates, nitrites, hypochlorite, azides, cyanide, cytokinins, gibberellins etc.)

Examples of treatments to break dormancy and/or improve germination



What is “satisfactory germination”

- IBPGR & other experts suggest that an **85%** or above % germination is satisfactory in terms of the likliehood of survival for that seed in storage for the 30 years required for a patent deposit.

- Ideally a germination in excess of 95% is ideal but some seeds have much poorer “viabilities.”

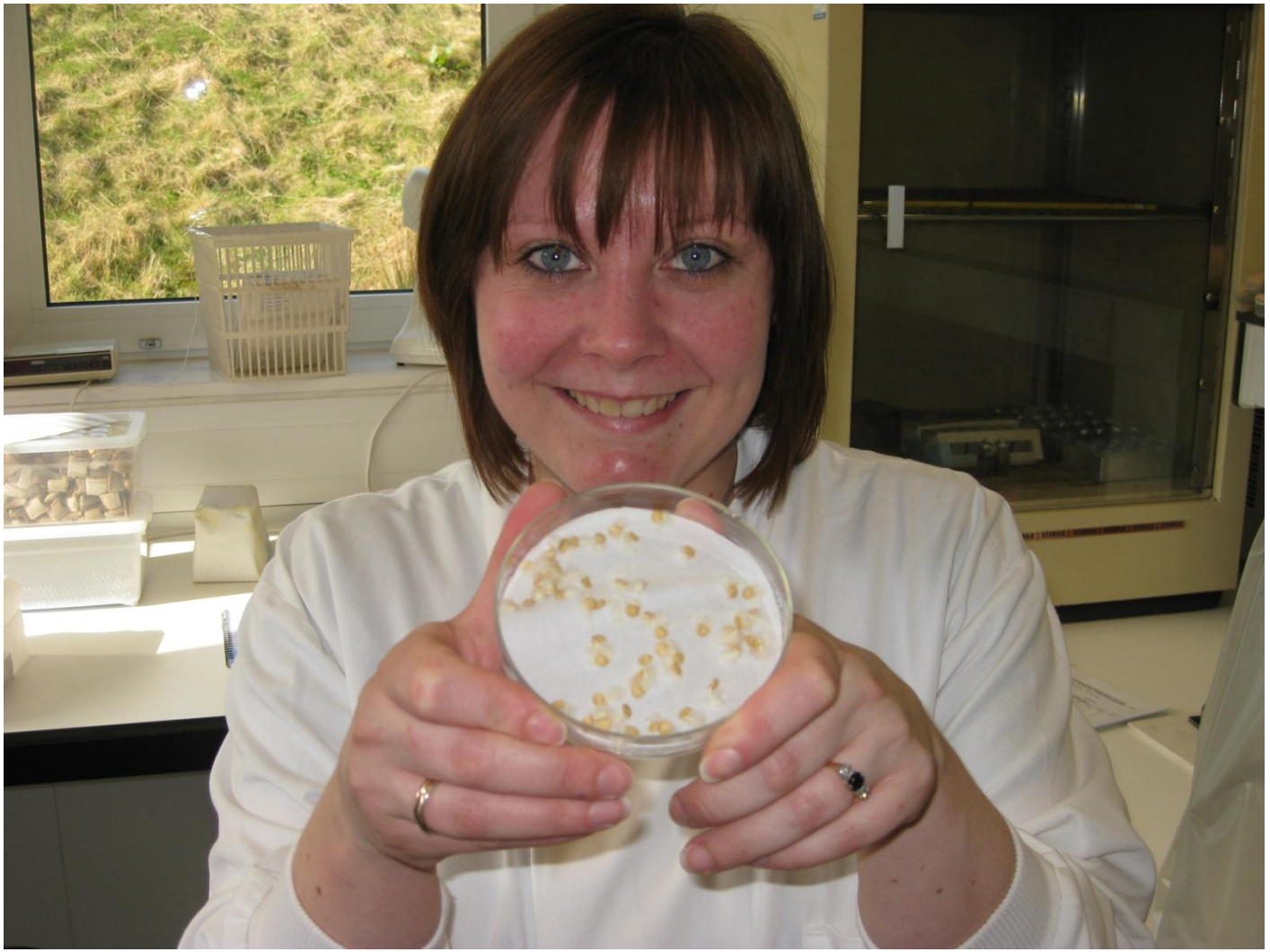
- Remember with a patent deposit if only 1 seed per hundred germinates that is sufficient for the deposit to be accepted although the chances of such a deposit still remaining “viable” after 30 years is low

Popularity of seeds versus bacteria

You be the judge

Curator's given me another bug to accession !

A seed deposit – that's more like it!



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Obrigado!