Equilibrating seeds to specific moisture levels

Technical Information Sheet 09

Seed banks may need to equilibrate or maintain seeds at a specific moisture level, for example for comparative longevity studies (see Technical Information Sheet 01), testing desiccation tolerance, seed/fruit ripening or producing seed sorption isotherms.

This information sheet presents a protocol for preparing lithium chloride (LiCl) solutions and explains how to use the solutions to equilibrate or maintain seeds at specific moisture levels and to produce seed sorption isotherms.

Equilibrating seeds

LiCl solutions produce environments of various relative humidities (RH) in which to equilibrate seeds.

- To equilibrate seeds to a specific moisture level, make up a LiCl solution to generate the desired RH (see Box 1).
- Add the prepared LiCl solution to suitable, labelled containers (to at least 20% of total volume). The Millennium Seed Bank (MSB) uses 200ml of LiCl solution in 1L storage jars (Fig. 1)
- Place the seeds in an open receptacle, supported above the LiCl solution (e.g. use a plastic support and mesh).
- Seal the container and allow seeds to reach equilibrium. This will take two weeks or more, depending on seed size/structure.
- To determine when the equilibrium point has been reached, either monitor the loss/gain in weight of seeds until there is no further change, or measure seed equilibrium relative humidity using a hygrometer (see Technical Information Sheet 05).

Figure 1: Air-tight jar with lithium chloride solution, showing plastic mesh support to hold seeds above solution.

Box 1: Preparing lithium chloride solutions

- Add the specified weight of lithium chloride to 200ml deionised water (Table 1). Use a magnetic stirrer to mix.
- Once prepared, allow the solutions to equilibrate in sealed containers for at least 24 hours, ideally at a constant temperature of 20°C ± 0.5°C.
- This will produce enough LiCl solution to equilibrate a maximum of 100g of seed. If you are working with smaller amounts of seed, you may reduce the amount of LiCl and volume of water.
- LiCl is an expensive chemical. The solutions may be re-used indefinitely, provided they are uncontaminated. Store as a concentrated salt in clearly labelled containers at 5°C.

Health & safety

- LiCl mixed with water generates heat (exothermic reaction). Cover to avoid water loss.
- LiCl salts are irritants. Wear lab. coat, gloves and safety glasses.
- If solution contaminates skin, wash with copious quantities of water.
- If solution comes into contact with mouth or eyes, seek immediate medical assistance.

Table 1: LiCl weights

Weight of LiCl (g)	RH (%) generated at 20°C
174	11
147	15
128	20
104	30
88	40
74	50
60	60
50	70
34	80
26	85
20	90
8	95

Isotherms

A seed isotherm represents the relationship between equilibrium moisture content (emc) and equilibrium relative humidity (eRH) at a given temperature (Fig. 2).

Seed isotherms show what the emc will be at a specific RH level, i.e. the moisture level of the seeds under prevailing conditions. They can be used to help manage the seed drying process, by reducing the need to carry out destructive determinations of moisture content (gravimetric).

The relationship between emc and eRH for a particular seed lot will vary depending on:

- Seed composition - at a given eRH, oily seeds have lower emc than non-oily seeds. This is because the dry matter in starchy seeds attracts and holds water molecules but the oil in oily seeds does not.

  For example, the emc of *Brassica napus* (~40% oil content) at 70% eRH and 25°C is ~9.0% while that of wheat (~2% oil content) under the same conditions is ~14%.
- Whether seeds are gaining moisture (absorption) or losing moisture (desorption) - at a given eRH, seeds that are drying will have a higher emc than if they were absorbing moisture, due to the different physical processes involved in absorption/desorption.
- Temperature - at a given eRH, the warmer it is, the lower the emc, because water is more able to vapourise and diffuse out of the seed at higher temperatures.

Isotherms have a typical reverse-sigmoid shape (Fig. 2). The inflections or kinks in the isotherm curve define three 'water-binding regions' that relate to physiological activity:

Region I: little physiological activity of any sort; seeds are in a relatively inert state.

Region II: seed ageing processes can occur; the higher the eRH, the greater the damage.

Region III: normal metabolic processes may occur (although germination will not be initiated at less than 100% eRH). If oxygen is present, damage from ageing processes can be repaired.

Drying according to seed bank recommendations limits the physiological activities which cause seed ageing and allows seeds to be stored at sub-zero temperatures without freezing damage.

Practical Tips

- Monitor seed weight to determine equilibrium. Once seeds reach a constant weight they are at equilibrium. Seeds in high RH environments will equilibrate quickest.

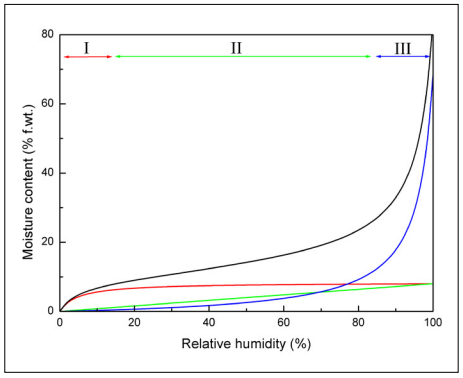

Figure 2: Typical moisture sorption isotherm (black) showing the relative proportions of strong (red), weak (green), and loosely (blue) bound water in regions I, II and III.

- There is a risk of fungal growth at RHs above 85%. Determine emc before seeds go mouldy.
- Work quickly when weighing seeds for emc determination, especially when dealing with small seeds which rapidly gain or lose moisture. Place the equilibration chamber close to the balance and work with one sample at a time.
- Monitor the RH of the LiCl salt solutions with a hygrometer. To help maintain a stable environment, open the container only when necessary.
- Solutions generating RHs below ambient levels (circa 50%) will gradually take up moisture and will need to have additional LiCl added to maintain the desired concentration.
- Solutions generating RHs above ambient levels will gradually dry out, requiring the addition of pure deionised water to maintain concentration.

Acknowledgements

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Further reading

ISTA (2007). International Rules for Seed Testing. International Seed Testing Association, Bassersdorf, Switzerland.

Sun, W.Q. (2002). Methods for the study of water relations under desiccation stress, pp. 47-91. In: M. Black and H.W. Pritchard, Desiccation and Survival in Plants: drying without dying. Commonwealth Agricultural Bureaux International, Wallingford, UK.

Box 2: Producing seed sorption isotherms

- Practically, it is easier to produce an absorption isotherm. Small samples of dried seeds are allowed to absorb moisture at a range of RH levels. Once equilibrium is reached, emc is determined.
- Prepare a range of LiCl solutions (see Box 1) in suitably-sized air-tight containers. Fill each container with solution, to at least 20% of the total volume, and label with concentration and RH level.
- Place a small sample (about 5g is sufficient) of fully dried seeds above each LiCl solution (use a plastic support).
- To ensure enough points in Region I of the isotherm, equilibrate a sample of seeds with 5% RH silica gel.
- Allow the seeds to reach equilibrium. This will take 14 days for seeds of up to 2mm diameter, or longer depending on seed size structure.
- Determine seed eRH using a hygrometer (see Technical Information Sheet 05), unless the seed sample is too small to adequately fill the sample chamber.
- Determine seed moisture content of each seed sample (ISTA, 2007) and plot emc against eRH.

Equipment specifications*

Description	Model/Product	Supplier
Electronic top pan balance, 4 or 5 decimal place	A&D Instruments 131-096 GR series analytical balance 210/42g, 0.01mg read w/auto cal: GR-202-EC	Jencons PLS https://uk.vwr.com
Air-tight plastic or glass containers	Bormioli Rocco Fido jars can only be purchased in bulk via https://www.scientificlabs.co.uk (but not in their online catalogue) If using alternatives test vessels for integrity	Fisher Scientific Ltd. www.fishersci.co.uk
Perforated plastic support/mesh on which to place seed material above LiCl solution	e.g. sections of plastic drain pipe with flat circular platforms	Locally available
Lab-based hygrometer	HC2-AW-USB-SW sensor with USB interface, connected to laptop/PC running HW4-E software. Range: 0 to 100% RH, -40 to 85 °C.	Rotronic Instruments (UK) Ltd. www.rotronic.com
Fan-assisted and ventilated oven	Moisture Extraction Oven with Digital Controls	Genlab www.genlab.co.uk

*Please note that the above equipment is used by the Millennium Seed Bank and has been chosen carefully using our many years' experience. The list of suppliers is for guidance only and does not represent an endorsement by the Royal Botanic Gardens, Kew. The manufacturer's instructions must be followed when using any of the equipment referred to in this Information Sheet.