

Seed storage/plating/germination assay

Seed Storage:

- For general storage seeds should be placed in small (approx 2" x 3") glassine bags, and labelled with the genotype, date of harvest and your name. Seeds should be stored at constant temperature of 24C to allow after-ripening to occur.

Seed plating/germination:

- All media should autoclaved and contain either 0.7 % or 1 % (w/v) agarose (Park Scientific) for plates to be used horizontally or vertically respectively.
- Use of different media:
 - Germination of seeds for growth of plants: Media contains ½ strength MS salts; it should be pH 6.2 prior to autoclaving, adjusted using 1M KOH. The pH will then drop to 5.8 after autoclaving.
 - When assaying after-ripening (ie. Dormancy status of seed) use water agarose (0.7% w/v) only.
 - Hormones should be added via a sterile filter after the media has been autoclaved and allowed to cool to 'hand-hot' (50-55 °C).
- Seed sterilisation: Seeds are washed in an eppendorf tube in 1ml 5% (v/v) Jeyes Parazone for 5 minutes, then twice in sterile distilled water. When analysing freshly harvested seed it is useful to include a tiny amount of Triton X-100 (eg. tip of a yellow tip dipped into Triton then into 20 ml of sterile water or Parazone) in the solution to avoid the seeds sticking to each other and the tip end.
- Sterile seeds should be plated in sterile water with sterile Ultipette tips.
- Where mesh is used as a solid support for seeds, it should be 100 µM and sterile (by double wrapping in aluminium foil and autoclaving).
- The plates are sealed using micropore tape (3M).
- Normally square plates (10-15 cm) are used.
- Where seeds are to be moist chilled (stratified), the seeds should be plated onto the media, the plates sealed with micropore tape, and wrapped in aluminium foil. The plates should then be incubated at 4 °C for 3 days before transfer to constant light.
- Plates should be placed in constant at 22 °C, 120-145 µmol m⁻² s⁻¹, 4 x Osram L58W/535 bulbs per shelf. **Germination is normally scored after 7 days (but see below).**
- Seedlings from ½ MS plates should be transferred to soil after 10-14 days in constant light (when the first true leaves have formed). Do not use seedlings grown on water-agarose for transfer, as they establish badly.

Germination Index assay:

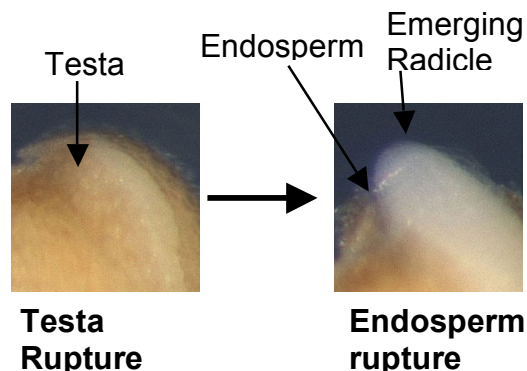
To provide an indication of rapidity of germination, the germination Index (GI) can be used:

1. Germination is scored as testa and endosperm rupture (TR, ER) (see pictures for examples). The germinated seeds are counted once, at the same time of day every 24 hours for 7 days. ER should be assessed for GI scores.

2. The germination index is based on work from Walker-Simmons 1988 (Enhancement of ABA responsiveness in wheat embryos by high temperature. *Plant, Cell and Environment* **11**, 769-775).

$$GI = (7 \times n_1 + 6 \times n_2 + 5 \times n_3 + 4 \times n_4 + 3 \times n_5 + 2 \times n_6 + 1 \times n_7) / (\text{total days} \times \text{total number of grains})$$

where n_1, n_2, \dots, n_7 are the number of grains (or embryos) that germinated on the first, second, and subsequent days until the 7th day, respectively; 7, 6, 1 are the weights given to the number germinated on the first, second and subsequent days, respectively. The maximum GI is 1.0.



Chemicals/Equipment

- **PGP-Type Agarose**
Park Scientific Ltd., Order No. 1039.
- **MS**
Murashige & Skoog Basal Salt Mixture (MS).
Sigma, M5524
1xMS = 4.3 g/l, ½ MS = 2.15 g/l.
- **Utipette tips**
Barky Instruments (www.barkyinstruments.com), Order No. SCP-100
(once bags of tips have been opened, keep in 70 % ethanol and rinse through with water before use).
- **Mesh**
Lockertex / Clarcor Ltd., Fabric type: NY/MO/100/32/980.
Tel: 019250644371; <http://www.clarcoruk.com/lockertex/index.htm>)