LSOP Title	Parasitic weed seed germination assay
LSOP No.	LSOP22
Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *UQ -waste* *OGTR*
Risk Assessments	
Approved by	*Milos Tanurdzic*
Date Approved	11/10/2021
Date Effective	18/06/2021
Next Review Date	18/06/2026
Contact for Assistance	plantsuccess@uq.edu.au

1.0 Scope

This LSOP covers use of the O. minor seeds for seed germination assays.
This does not cover the use of all parasitic weeds.

2.0 Definitions

min – minutes

mL – milli Litre

3.0 Materials and Equipment

1. 70% ethanol
2. Bleach
3. Tween20
4. Pipette and Pipette Tips
5. Tubes
6. Tissues
7. Alfoil
8. Centrifuge

9. Laminar flow cabinet
10. 96 well plates
11. filter-sterilised 2.5 mM hepes buffer (305 mg/500 mL; can be filter-sterilised directly into Eppendorf tubes)

4.0 Prescribed Actions

Preparation

1. Wear gloves and buttoned-up lab coat. Clear and clean a large section of lab bench. Put on UV lamp in laminar flow cabinet.
2. Prepare fresh 50% bleach (6.25%) + 2 drops of Tween20 (from a P200 tip) per 50 mL. Gently mix by inversion.
3. Lay down moist tissues. Moisten outside of tubes with a little ethanol

   NB: ethanol removes some static electricity as it evaporates.
4. Carefully open the seeds over the wet tissues and tap about 50 uL of seeds into a new Eppendorf tube.

   NB: Don't do this in the laminar flow cabinet because it may blow out some seeds.

Sterilising (similar to *Arabidopsis*)

1. Add 1 mL of 70% ethanol. Use a mini centrifuge to spin down the seeds (as much as possible).
2. Pipette off the ethanol onto the wet tissues. Click tip off onto the tissues too. The tissues will trap the seeds. Place tissues inside a glove and into yellow bin.
3. Add 1 mL of the bleach solution and treat for 10 min. Mix by inverting the tube every 2 min.
4. Turn on the laminar flow cabinet and wipe it down with ethanol. Now work in the cabinet.
5. Spin down seeds, pipette off bleach and discard onto a tissue, also click tip off onto tissue.

6. Add sterile water, mix by inversion, spin down and pipette off water. Repeat this rinse step 3 times.

7. Add 1 mL filter sterilised hepes buffer. Wrap the tube in foil and store in the dark at ~21°C for 7 days

Dispensing

1. Seeds will fall to the bottom of the tube, so mix them and quickly suck up 50 uL with a pipette.
2. Dispense them into a sterile 96 well plate. Due to variation, include up to 10 reps.
3. Add hormones and control solutions and incubate a further 4 days in the dark.
4. Score the percent germination for each well and average it. Good luck!

5.0 Appendix A

Orobanche minor (clover broomrape) seeds can be obtained from the UWA team or Jane Prider (jane.prider@sa.gov.au). O. minor originates from the Mediterranean and is naturalised in southern parts of Australia. Although it is not considered a weed, we do not want to spread it! O. ramosa is also available from Jane Prider, but is a noxious weed (in southern states), so don't use it. Native broomrape (O. cernua var. australiana) occurs in Australia, but we don't know of anyone who has tested it.

Precautions

Do not allow dry seeds to fall on benches or floor. The seeds are extremely small and may stick to people's clothing and shoes, which may unwittingly be taken outside into the gardens. Wet seeds tend to stick to plastic tubes and tips, so all discarded seeds and plastic tips and tubes should be carefully wrapped up and placed in yellow bins.