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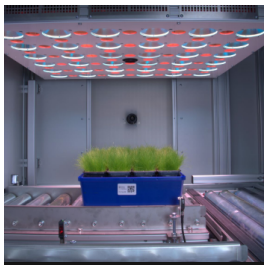
Plant material preparation and salt imposition for PlantScreen analysis



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Protocol status: Working
We use this protocol and it's working

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Abstract

The following protocol on plant material preparation and salt imposition for PlantScreen analysis will cover:

- Agar preparation for seeds
- Sowing germination trays
- Sowing PlantScreen trays
- PlantScreen preparation
- Salt stress imposition
- PlantScreen protocol

Attachments

DOCX

bkwun.docx

24KB

Guidelines

Note: During the PlantScreen protocol, it is important to stay commited to a consistant time of day, as variation in time may have a profound effect on the result.

Materials

Reagents

- Agar
- ddH_20
- Plantscreen trays (P trays with 20 pots)
- Germination trays (G trays with 24 pots)
- Soil
- NaCl
- Seeds

Equipment

- Growth chamber
- 200 ml beaker
- 1.5 ml eppendorph tubes
- 2 L bottles

Troubleshooting

Safety warnings

See SDS (Safety Data Sheet) for safety warnings and hazards.

Adding 0.1% agar

1 Weigh out 0.2 g of agar.

2 Put agar into a 200 mL beaker.

3 Add 200 mL of normal water.

4 Put in microwave to dissolve for 00:03:00 .

5 Leave agar to cool.

6 Cover beaker with foil or cling film.

7 Add 100 µL to each mini tube.

8 Add 0.1% agar to eppendorfs, vortex well.

8.1 Place in fridge at 4 °C for 72:00:00 .

9 Check the eppendorf tubes the next day. Vortex again to ensure the seeds are dispersed in solution.

Sowing germination trays

10 Pot sieved soil for germination trays (G Trays with 24 pots).

10.1 Add 2.5 L of water to saturate the soil and cover with transparent lid.

11 Remove excess water from all trays and leave to drip for one day before sowing.

12 Prepare traylist for Plantscreen and ID stickers for pots.

13 Cut the end of a pipette tip. Use a pipette and the wide tip to sow seeds.

Note

Seperate seed by pipetting in a line.

14 Sow the germination trays according to the Master GTray List.

Note

¬30 seeds per pot per accession.

15 Put trays in the growth chamber with conditions set at:

	Temperature (°C)	Time
	22	12 (day)
	20	12 (night)

150 µmoles/m^2/s (12 panels with 25% white LED illumination + 20% far-red LEDs).
60% humidity.

16 Remove lids after 3-5 days, earlier if tray lids are too humid.

Sowing PlantScreen trays

17 Pot sieved soil for Plantscreen trays (P Trays with 20 pots), while weighing each pot to have ± 60 g of soil.

Note

Tare one empty pot and then weigh each pot + soil.

17.1 Add 2.5 L of water to saturate the soil and cover with transparent lid.

18 Remove excess water from all trays and leave to drip for one day before transplanting.

19 Let plants grow for 1 week, then perform transplantation.

Note

Choose similar sized seedlings.

20 Transplant 2-3 seedlings per pot by taking seedling with soil around it.

21 Cover with transparent lids for 3-5 days.

Note

Do not cover completely. Leave an open area to reduce humidity formation.

22 Put trays in the growth chamber with conditions set at:

	Temperature (°C)	Time
	22	12 (day)
	20	12 (night)

150 µmoles/m^2/s (12 panels with 25% white LED illumination + 20% far-red LEDs).
60% humidity.

Arrange trays to be in batches of 6 on the shelves for maximum light incidence on plants (no shadow).

23 Remove extra seedlings (thin-out) after 1 week.

PlantScreen preparation

24 Register trays in PlantScreen by using the import function in the PlantScreen Registration program.

25 Add the reference weight of all the trays using the Plant Watering program.

26 Water trays in PlantScreen to the specified percentage of field capacity.

27 Let plants grow until they reach the 10-leaf rosette stage (approx. 21-23 days old) to apply salt stress.

28 Water trays in PlantScreen to the specified percentage of field capacity the day before salt imposition.

29 Prepare weigh boats of NaCl in 2L bottles for salt imposition using distilled water (dH_2O).

29.1 Prepare bottles of dH_2O for control plants.

30 Remove all Australian pots to ease the process of salt imposition the next day.

Salt stress imposition

31 [M] 250 millimolar (mM) NaCl (29.22 g) in 2 L of water for 01:00:00 bottom-watering using black trays and small pots only.

PlantScreen protocol

32 RGB, FC (Lightcurve) and IR, takes about 1 hour and 20 mins. 01:20:00 1h 20m

> Note
>
> Make sure to stay committed to this timing for future scannings, because time of day can have a profound effect.

33 Turn off all lights.

34 Dark adaptation delay for 00:15:00 .

35 FC measurement with Light curve protocol.

36 Turn IR light on 100%, others off.

37 RGB and IR measurement.

38 Turn on the 3 lights at 22%.