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Marchantia spores production in Microboxes

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ABSTRACT

Spore sterilisation

Marchantia spores can be obtained usually in three months using Microboxes. The lid of Microboxes has a specially designed filter that allows high gas exchange, limited dehydration and blocks the entry of contaminants such as fungi into the culture. We are using the TP4000+TPD4000 Microboxes with a green XXL+ filter.

EXTERNAL LINK

marchantia far red spore microboxes

MATERIALS TEXT

44 mm Jiffy-7 peat pellets (#32170142, Jiffy)

Microboxes with green XXL+ filters (TP4000-TPD4000, SACO2, Belgium)

1 Put 15 “Jiffy 7” pellets in a Microbox, add 800 mL of water, close the lid and autoclave (A and B in Figure).

2 After autoclaving the Microbox with the “Jiffy 7” pellets, work in a flow hood.

3 Put at least one 10 mm x 10 mm thallus fragment or 3 gemmae per pellet using sterile tweezers (C and D in Figure).

4 Add another 150-200 mL of sterile water, close the lid and place at 21 °C under continuous light with light intensity of 150 µmol/m^2/s (E in Figure).

5 After one month transfer plants under a 16 h light / 8 h dark regime, with light intensity of 150 µmol/m^2/s supplemented with far red light (peak emission: 700-750 nm, spectral photon flux density: 2400 µmol/m2/s, distance from plants: 32 cm) (F in Figure).

6 After approximately 4 weeks mature male and female reproductive organs are produced (G and H in Figure).

7 In a flow hood, open the lid of the Microbox, and using a pipette add 5-10 mL of sterile water on top of the male and female reproductive organ (I and J in Figure). After a few seconds you will be able to see the sperm as a cloudy exudate in the water drop. For fertilisation, use the pipette to "move" the reproductive organs in order to touch each other.

8 Alternatively, for fertilisation, transfer the water drop from the male gametophore with a disposable sterile plastic pipette on the top of the female reproductive organ (pic in step 11).

9 After one 4 weeks mature sporangia are ready for collection (K in Figure). Place 1-3 mature sporophytes in a 1.5 mL Eppendorf tube with 5 silica gel beads (L in Figure) and store at 4 °C if the spores are to be used within 1-2 months. For longer storage, allow the sporophyte to desiccate for a week at 4 °C and then store at -80 °C.

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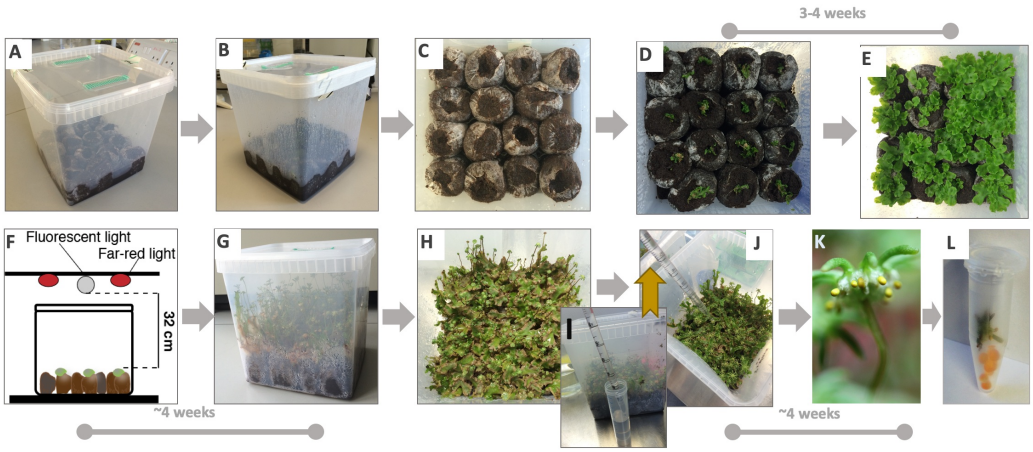

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