Culture techniques for seagrasses

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Seagrass plants: Healthy plant modules with apical shoots collected in the wild.

Seagrass seeds: Dark seeds with a hard seed coat collected from flowering shoots in the wild during the species flowering season

Apparatus: Growth Chamber, Aeration system, Hot plate

Agar medium: 2 g of agar in 100 ml boiling seawater in a Erlenmeyer over a hot plate for 2-5 minutes until agar is dissolved.

Glass-ware: Aquaria (> 30L), Erlenmeyers

Plastic-ware: Plastic containers, assay tubes, Magenta® vessels

Culture of plant modules collected in the field

1. Collect plants composed of at least 3 modules (i.e. 1 apical shoot plus 2 shoots and respective internodes and roots) and transport to the laboratory in seawater. Place the plants in an aerated aquarium until transfer to the culture media.

2. Prepare one of the following culture media in glass/plastic containers:
i) a monophasic medium of seawater (natural or artificial);
ii) a biphasic medium with natural sediments (preferably sterilized sand) and seawater;
iii) a biphasic medium with an agar-solidified layer (2% agar w/v with seawater as solvent) and seawater.
Attention: Plant the seagrass modules in the agar medium immediately before the complete solidification of the agar, which should be allowed to cool down first.

3. Distribute the containers with the plants in the three different culture media per large volume (> 30L) aquaria filled with aerated seawater. Keep the aquaria in a growth chamber at a light intensity, photoperiod, temperature and salinity similar to the field conditions, or adjust according to the purpose of the experiment.

4. Remove randomly without replacement four plants from each media after the designated time period for the experiment. Determine plant survival and other significant plant variables (e.g. rhizome elongation rate, shoots and internodes production), according to the purpose of the experiment.

Culture of seedlings germinated in vitro *from seeds*

1. Clean the seeds by gently submerge them for 10 min. in a 10% (v:v) commercial bleach solution made up with autoclaved seawater to avoid contamination and overgrowth of algae.

2. Place the seeds individually in sterilized assay tubes containing biphasic media of sandy sediment and seawater (previously autoclaved).

3. Place the assay tubes in a growth chamber at field temperature and light and optimal germination conditions of salinity usually consisting of hyposaline shock (e.g. 1 PSU for *Zostera noltii*, 16 PSU for *Cymodocea nodosa*), and allow seeds to germinate, i.e. until the rupture of the seed coat and emergence of the cotyledon.

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4. After the emergence of the first chlorophyll-bearing leaf (seedling stage), transfer the plants to new sterilized assay tubes containing sterile sandy sediment and full strength seawater. Add a nutritional solution of macro- and micro-nutrients, PES culture medium (Provasoli 1968), as 1:3 of PES:Seawater.

5. Maintain the seedlings in a growth chamber at field temperature, salinity and light conditions, and transfer them to bigger culture vessels (Magenta® – G7 300 ml Sigma Co.) when necessary using the same medium and PES solutions as described above.

Additional information:

Cabaço S, Alexandre A, Santos R (2006) *Survival and growth of the seagrass Zostera noltii in different culture media. In: Gambi MC, Borg JA, Buia MC, Di Carlo G, Pergent-Martini C, Pergent G, Procaccini G (eds) Proceedings of the Mediterranean Seagrass Workshop, Malta 29 May - 4 June 2006. Biologia Marina Mediterranea, 13 (4):24-28.*

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