Useful methods 4: Stock cultivation of duckweed

In 1 collection

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Duckweed

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We use this protocol and it's working

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ABSTRACT

This protocol details about stock cultivation of duckweed. It contains protocols from the The International Steering Committee on Duckweed Research and Application (ISCDRA) Newsletter. A complete list of these news letters can be found here.

ATTACHMENTS

376-832.pdf

GUIDELINES

In the previous issue of “Duckweed Forum” (DF 3, 180-186 (2015)) we described several media standardized for duckweed cultivation. These are liquid media used for optimal cultivation of duckweeds at high growth rates. For long-term cultivation such high growth rates are not desired because aliquots of the cultures would have to be transferred into fresh nutrient medium between 2 weeks and perhaps 2 months, depending on clone and species. Cultivation of stocks thus means, at first, to reduce the rate of growth.

MATERIALS

Materials

- Agar-based medium
- nutrient medium
- KH_2PO_4
- Glucose
- sucrose
- 100 ml-Erlenmeyer flask
- Petri dishes
- parafilm

Stock cultivation of duckweed

1

Note

The first measure is to reduce the temperature. The optimal growth rates are measured under standardized temperature, at 25 °C (DF 3, 59-62 (2015)).

For stock cultivation we reduce the temperature to 18 °C. All the duckweed species can deal with this temperature, of course with a dramatically reduced growth rate. Even 15 °C is possible. The late Elias Landolt mentioned that clones collected from tropical climates cannot tolerate 5 °C for a longer time.

2 The next important environmental factor is the light intensity. Reduce the light intensity from 100 µmol m^{-2} s^{-1} continuous white light (standardized for optimal growth rate) to ca. 30 µmol m^{-2} s^{-1}.

Note

We observed that duckweeds are able to deal with low light intensity only when the temperature is also reduced. At 25 °C several species (e.g. from the genus *Wolffiella*) die at such low light intensities. This light intensity seems to be close to the photon compensation point.

3

Note

It is also very useful to reduce the water availability. This can be done by replacing the liquid medium
by Agar based medium. Instead of Agar also a kind of synthetic Agar, GELRITE, can be used.

Prepare these semisolid media with a defined nutrient medium to supply the macro and micro nutrients to plants.

Note

We normally use N-medium (DF 3, 182-183 (2015)).

4 Increase the concentration of KH_2PO_4 to [M] 1 millimolar (mM).

Note

At the low concentration used in liquid medium (e.g. [M] 60 micromolar (µM)), the survival of plants on Agar would be for a very short duration. However, also other media are possible. Often MS-medium is used because this medium is commercially available as a ready-to-use mixture.

5 

Add solid Agar to nutrient medium, normally at a concentration of 0.9%. For sensitive clones, addition of as low as 0.7 % Agar is possible. Gelrite is used at a concentration of ca. 0.45%.

6 Prepare 1 L Agar suspension, heat it in a microwave oven to 80 °C and pour 50 mL to 75 mL of it into each of the 100 ml-Erlenmeyer flask secured with cotton wool stoppers.

7 Autoclave the flasks. It is indeed a very large amount of Agar in each flask having the advantage that the
life time of stock cultures is very long ca. 4 to 5 or even 6 months.

8 In other stock collections, use standard glass test tubes, fill it with 5 mL Agar medium and close with cotton wool stoppers.

Note

Alternatively, sterile plastic Petri dishes could also be used. The diameter of the Petri dishes might be 9 cm (standard quality, very cheap) or much smaller, down to a diameter of 3 cm in order to spare medium and space.

9 In these cases, autoclave the Agar medium, normally in 1-L flask. Then pour into sterile Petri dishes in a laminar flow box.

10 Inoculate the plants, then close the Petri dishes by parafilm to prevent faster rate of drying of the Agar layer.

11 In order to control the sterility (in terms of pure, uncontaminated cultures) of plants it is useful to add low molecular weight carbohydrates.

Note

Glucose ([M] 50 millimolar (mM)) or sucrose ([M] 25 millimolar (mM)) are commonly used. We learned recently, that in some cases (e.g. Wolffia) after sterilization, [M] 50 millimolar (mM) glucose is too high for their regeneration and plants often die. In this case, but perhaps also in general, [M] 25 millimolar (mM) glucose or [M] 25 millimolar (mM) sucrose are better used in Agar or gelrite media. Fructose is not recommended.

12 Species of the genus *Spirodela* (*S. polyrhiza*, *S. intermedia*) tend to grow in several layers, one above the other. As a consequence, the younger fronds in the upper most layers lose their contact to water supplying Agar and die thereafter. For these two species, we use liquid medium without sugar. The disadvantage is that the contaminations cannot be recognized and in each case a sterility test is necessary before using these plants. When sugar is added, the survival of the fronds is too short.

13 For *S. polyrhiza* there exists another opportunity for their long-term preservation. Harvest the turions, survival organs, from bottom of the flasks.

14 Store turions in a fridge (5 °C , darkness) for several years.

15 Initiate the turion formation inoculating plants in a liquid medium with low phosphate concentrations, e.g. [M] 60 micromolar (µM) .

Note

The Inoculated plants and their emerging daughters use the available phosphate for vegetative growth and after a certain time period, the drastically reduced phosphate concentration (e.g. to [M] 2 micromolar (µM)) induces turion formation. Addition of glucose ([M] 50 millimolar (mM)) is recommended to accelerate this process and to increase the turion yield.