Useful methods: International survey for duckweed stock cultivation

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 the international survey for duckweed stock cultivation. 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

387-849.pdf

GUIDELINES

Fortunately duckweed research is indeed in bloom. Thus, it happens frequently that a new stock collection has to be established. This is a point to think about carefully because it will have consequences on the work for the following years. The local conditions as well as the available personnel and 1nancial resources have to be considered. Therefore, we thought of making a survey in order to learn how the basic requirements are met with across the globe. We wrote to the managers of the stock collections listed in the “Update of duckweed stock Collections” in Duckweed Forum 9: 11-12 (2021), surveying on the questions as given below. The responses received from the nine stock collections are summarized in the sequence as mentioned for the “Name of the Institution and the manager of the stock collection”, numbered 1 – 9 suf1xed with the two or three letter code used by the stock collection. Further on the two or three letter code is used to refer to the stock collection. We hope that this survey will be helpful for many existing and upcoming duckweed groups in carefully selecting the modalities for successfully maintaining a stock collection of the clones in their laboratory.

Name of the Institution and the manager of the stock collection (two or three letter code)

1. Friedrich Schiller University Jena, Matthias Schleiden Institute - Plant Physiology, Jena, Germany; Klaus-J. Appenroth (KJA)
2. University Greifswald, Greifswald, Institute of Botany and Landscape Ecology, Germany; Manuela Bog (BOG)
3. School of Life Sciences, Huaiyin Normal University, China; Olena Kishchenko (NB)
4. Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, China; Hongwei Hou (HHW)
5. Landolt Duckweed Collection, Zurich, Switzerland; Originally at the ETH Zurich, the last ca. 10 years in private rooms. Elias Landolt and Walter Laemmler (Information by K.J. Appenroth) (For this collection, there is no letter code and hence “Landolt” is being used for the same)
6. Rutgers Duckweed Stock Cooperative, Rutgers University, New Brunswick NJ, USA; Mike Chen (EL)
7. University of Debrecen, Faculty of Science and Technology, Institute of Biology and Ecology, Department of Botany, Hungary; Viktor Oláh (UD)
8. IPK Gatersleben: Ingo Schubert/ Manuela Nagel (we are using IS for Ingo Schubert)
9. Central University of Kerala, India; K. Sowjanya Sree (KSS)

Name of the nutrient medium/ Reference

1. KJA: N medium with enhanced phosphate content (0.15 mM - 1 mM) with and without glucose (25 mM). Appenroth Duckweed Forum 3: 180-186 (2015).
2. BOG: N medium according to Appenroth et al. 1996; selected clones on sugar containing N medium (25 mM, Z medium).
3. NB: SH medium; Schenk RU, Hildebrandt AC (1972) Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can J Bot 50(1):199– 204. https://doi.org/10.1139/b72-026.
4. HHW: B5, SH and MS medium.
5. Landolt: For many years different media were used, depending on the duckweed species; later commercial available MS medium was used for all clones.
6. EL: ½ SH medium as base medium, is also modi1ed by adding sucrose at 0.1% to 0.5% to promote growth or antibiotic cefotaxime at 100 µg/mL to control bacterial contamination for some duckweed strains.
7. UD: ISO 2005 modi1ed Steinberg medium / Environment Canada EPS 1/RM/37 2nd Ed. 2007 (https://www.canada.ca/content/dam/eccc/migration/main/faunescience-wildlifescience/ 1ad45620-6a99-470c-8fdc-d57351d47ec8/rm37-202nded-lemnaenglish-20-20u.pdf).
8. IS: Liquid nutrient medium N (Appenroth et al. Biologia Plantarum 38: 95–106 (1996)).
9. KSS: N medium with enhanced phosphate content (1 mM) with and without glucose (25 mM). Appenroth et al. Plant Biol. 38: 95-106 (1996).

MATERIALS

Type of glassware or plasticware used for maintaining the stock cultures; Size of the glassware or plastic ware (volume/diameter)

1. KJA: Erlenmeyer Easks with cotton wool stoppers; 100 ml-Easks with 75 ml medium.
2. BOG: Wide and narrow neck Erlenmeyer Easks with cotton stoppers; 100 ml.
3. NB: Petri dishes; 6 cm diameter.
4. HHW: Erlenmeyer Easks 100 ml, 250ml and 500 ml; petri dishes 7 and 9 cm in diameter.
5. Landolt: Test tubes; 20 ml with 5 – 7 ml medium, cotton wool stoppers.
6. EL: Glass baby jars 200 ml liquid medium and with plastic lid; petri dishes 60 x 15 mm.
7. UD: Erlenmeyer Easks 100 ml.
8. IS: Erlenmeyer Easks 100 ml; plastic tubes 50 ml; plastic petri dishes 9 cm diameter.
9. KSS: Erlenmeyer Easks 150 ml, 50 ml medium; with cotton wool stoppers.

Agar/ gelrite concentration in case of solid or semisolid media

	A	B
	KJA	0.45 % gelrite
	BOG	Only liquid medium
	NB	0.8 % agar
	HHW	1.0 % agar
	Landolt	0.9 % agar
	EL	0.8 % agar
	UD	Liquid
	IS	0.45 % gelrite
	KSS	Liquid

Temperature and light conditions of the stock culture room

16h

1 KJA: 17 °C; 30 µmol m^{-2} s^{-1} continuous white light by fluorescence tubes.

2 BOG: Room temperature and daylight; in winter additional light for *Wolffia* and *Wolffiella*.

3 NB: 24 ± 1°C (light period) and 20 ± 1°C (dark period) with a photon flux density of 100 µmol m^{-2} s^{-1} provided by cool white fluorescent bulbs in a 16 h light/8 h dark cycle.

4 HHW: 25 ±0.2°C, 85 µmol m^{-2} s^{-1} PAR, and a 16:00:00 photoperiod. 16h

5 Landolt: Room temperature (office) and day light, sometimes with an artificial illumination using a living room lamp.

6 EL: 15 °C and low light (less than 50 µmol m-2 s-1).

7 UD: 24 °C, 60 µmol m-2 s-1 white light irradiation, 16/8 h photoperiod.

8 IS: 24°C; 16 h white light, 100 µmol m^{-2} s^{-1}.

9 KSS: 17 °C; 30 µmol m^{-2} s^{-1} continuous white light by fluorescence tubes.

How often must the cultures be renewed?

10 KJA: Each four months. *Wolffia microscopica* each month.

11 BOG: Each 3 – 4 months.

12 NB: Each 6-8 weeks.

13 HHW: Each month.

14 Landolt: If I remember correctly from my several visits in the lab of Elias Landolt, the media need to be renewed each 2 months. Klaus-J. Appenroth.

15 EL: It depends. In brief, for most Lemna strains, the subculture cycle is one year; for most strains of other four genera—*Spirodela*, *Landoltia*, *Wolffiella*, and *Wolffia* the cycle is better less than one year, particularly *Wolffiella*, and *Wolffia* strains should be renewed around six months. A few "weak" strains are gathered in the nursery trays to renew every 1-3 months. This strategy might be called the 12-6-3 rule for easily keeping in mind.

16 UD: Each 2 weeks.

17 IS: Liquid ~8 weeks; solid ~6 months.

18 KSS: 2-4 months depending on the species.

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Stock collection at Landolt's duckweed collection, Zurich, Switzerland.

Stock collection of duckweeds at Friedrich Schiller University, Jena, Germany.

Stock collection at the Rutgers Duckweed Stock Cooperative, Rutgers University, New Brunswick, USA.