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Useful methods 3: Media for in vitro-cultivation of duckweed

In 1 collection

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Duckweed

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ABSTRACT

This protocol details about various media for in vitro-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

375-831.pdf

GUIDELINES

There is a large number of cultivation media in use for duckweed. This simply shows that most duckweed can adapt to a broad range of conditions. This does not mean that physiological responses are always the same. In order to support scientific standards for the duckweed community, we compiled the most useful nutrient media here in form of lab protocols, including the stock solutions used. All protocols are for autotrophic cultivation. If mixotrophic cultivations are required, either glucose ([M] 50 millimolar (mM)) or sucrose ([M] 25 millimolar (mM)) should be added. We have never added vitamins or other organics as green plants produce it themselves.

Final concentrations are given in molar concentrations – and should be given in scientific publications. Only then different nutrient media can be compared and the data do not depend e.g. on the amount of crystal water of the substances.

Most of the original protocols were published before FeEDTA or FeNaEDTA were available on a commercial basis. In most cases, these substance are much easier to handle than other sources of iron. Only when possible chelating effects may have an influence on the physiological effects under investigation (e.g. flowering) one should take care. All nutrient media should be autoclaved before use. The numbers in brackets are the molecular weight/ mass in g mol^{-1}, which might help in calculations.

MATERIALS

Materials

- KNO_3
- KH_2PO_4
- $K_2HPO_4x3H_2O$
- $MgSO_4x7H_2O$
- $Ca(NO_3)_2x4H_2O$
- H_3BO_3
- $ZnSO_4x7H_2O$
- $Na_2MoO_4x2H_2O$
- $MnCl_2$ x $4H_2O$
- FeNaEDTA
- EDTA-Na_2
- NH_4NO_3
- $CaCl_2x2H_2O$
- Fe(III)EDTA
- $MnSO_4xH_2O$
- KI
- $CuSO_4x5H_2O$
- KNO_3
- $CoCl_2x6H_20$
- Iron(III)tartrate
- KCl

Modified Steinberg-Medium

1

CITATION

R. A. Steinberg. Mineral requirements of Lemna minor. Plant Physiology.

LINK

https://doi.org/10.1104/pp.21.1.42

Note

Modified 1998 by R. Altenburger, Dept. of Chemical Ecotoxicology, UFZ Centre for Environmental
Research, Leipzig. Germany. Described in ISO standard (ISO 20079).

Note

This nutrient medium is recommended for investigating the effects of substances that might be chelated, e. g. heavy metals. Therefore, the concentration of chelators is so low that the optimal growth of duckweed is not possible. If you want to use this medium but want to have higher growth rates, the FeEDTA and $EDTANa_2$ concentrations should be increased fivefold. $EDTANa_2$ was added to keep the same ration of chelated and free EDTA as in the original protocol.

From stock solution 1 to 3, use 20 mL per litre ready-to-use media.

	A	B	C	D	E
	Stock	Compound	Stock concentration		Final concentration
	1	KNO3 (101.1)	173 mM	17.50 g/L	3.46 mM
		KH2PO4 (136.1)	33 mM	4.50 g/L	0.66 mM
		K2HPO4 x 3H2O (228.2)	0.63 g/L	3.6 mg/L	72 µM
	2	MgSO4 x 7H2O (246.5)	20.5 mM	5.00 g/L	0.41 mM
	3	Ca(NO3)2 x 4H2O (236.1)	62.5 mg/L	14.75 g/L	1.25 mM
	4	H3BO3 (61.8)	0.388 mM	120 mg/L	1.94 µM
		ZnSO4 x 7H2O (287.5)	0.126 mM	180 mg/L	0.63 µM
		Na2MoO4 x 2H2O (241.9)	36 µM	44 mg/L	0.18 µM
		MnCl2 x 4H2O (197.9)	0.182 mM	180 mg/L	0.91 µM
	5	FeNaEDTA (367.0)	0.562 mM	1.031 g/L	2.81 µM
		EDTA-Na2 (372.24)	0.244 mM	0.454 g/L	1.22 µM

2 From stock solution 4 and 5, use only 5 mL per litre.

3 Adjust the pH to pH 5.5, e.g. by using 1 % (v/v) HCl.

Murashige-Skoog-Medium (1/10 MS-medium)

4 Reference:

CITATION

Toshio Murashige, Folke Skoog. A revised medium for rapid growth and bio assay with tobacco tissue cultures. Physiologia Plantarum.

LINK

https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Note

This medium is especially popular for stock cultivations. Several of the components in stock solution 6 might not be required at all, e.g. KI. Landolt and Kandeler (1987) cited this version of the MS-medium according to Jacobs (1947) and mentioned that it is very rich of nitrate and potassium. They also criticized the complete lack of sodium and the low concentrations of trace elements.

	A	B	C	D	E
	Stock	Compound	Stock concentration		Final concentration
	1	KNO3 (101.11)	752 mM	76.0 g/L	2.06 mM
		NH4NO3 (80.04)	824 mM	65.95 g/L	1.88 mM
	2	CaCl2 x 2H2O (147.02)	120 mM	17.6 g/L	0.3 mM
	3	MgSO4 x 7H2O (246.48)	60 mM	14.79 g/L	0.15 mM
	4	KH2PO4 (136.09)	50 mM	6.80 g/L	125 µM
	5	Fe(III)EDTA (367.0)	4 mM	1.468 g/L	10 µM
	6	H3BO3 (61.83)	4 mM	247 mg/L	10 µM
		MnSO4 x H2O (169.02)	4 mM	676 mg/L	10 µM
		ZnSO4 x 7H2O (287.5)	1.2 mM	345 mg/L	3 µM

	A	B	C	D	E
		KI (166.0)	0.2 mM	33.2 mg/L	0.5 µM
		Na2MoO4 x 2H2O (241.95)	0.04 mM	9.7 mg/L	0.1 µM
		CuSO4 x 5H2O (249.68)	0.004 mM	1 mg/L	0.01 µM

From each stock solution 2.5 ml per litre final medium.

N-Medium

5 Ref:

CITATION

K.-J. Appenroth, S. Teller, M. Horn (1996). Photophysiology of turion formation and germination in Spirodela polyrhiza.. Biologia Plantarum.

LINK

10.1007/BF02879642

Note

This medium was developed in our Institute and used for several decades for all duckweed species. The original low concentration of phosphate ([M] 60 micromolar (µM)) was used to induce turions formation. As this is rather a special case, we increased the phosphate concentration to [M] 150 micromolar (µM) as given in the table. We never found nutrient media that supported a faster growth of duckweed than this one. When this nutrient medium should be used for stock cultivation, we increase the KH2PO4 concentration to even [M] 1 millimolar (mM) , add [M] 50 millimolar (mM) glucose and solidify by 0.9 % agar. Even after several decades of cultivation, the plants do not miss the trace elements that are not supplied. Most probably, even in chemicals of p.A.- quality, these elements are present as contaminations.

	A	B	C	D	E
	Stock	Compound	Stock concentration		Final concentration
	1	KH2PO4 (136.1)	30 mM	4.083 g/L	0.15 mM
	2	Ca(NO3)2 . 4H2O (236.2)	0.2 M	47.23 g/L	1 mM
	3	KNO3 (101.1)	1.6 M	161.8 g/L	8 mM

	A	B	C	D	E
		H3BO3 (61.83)	1 mM	61.8 mg/L	5 µM
		MnCl2.4H2O (197.9)	2.6 mM	514.5 mg/L	13 µM
		Na2MoO4.2H2O (241.95)	80 µM	9.4 mg/L	0.4 µM
		MgSO4.7H2O (246.48)	0.2 M	49.30 g/L	1 mM
	4	Fe(III)EDTA (345.07)	5 mM	1.725 g/L	25 µM
	or				
		FeNaEDTA (MW 367.1)	5 mM	1.835 g/L	25 µM

6 Use 5 mL for 1 L from each stock solution nutrient medium. The nutrient medium might be adjusted to pH 5.5 but this is normally omitted.

Schenk-Hildebrand medium (SH-medium)

7 Reference:

CITATION

Schenk, R. U. and A. C. Hildebrandt (1972). Medium and Techniques for Induction and Growth of Monocotyledonous and Dicotyledonous Plant Cell Cultures.. Canadian Journal of Botany.

LINK

https://doi.org/10.1139/b72-026

Note

SH-Medium is not even described in Landolt and Kandeler (1987). However, following recommendation by Dr. Ann Stomp, USA Elias Landolt introduced this medium in his lab and was very satisfied with it for stock cultivation. This medium is also commercially available. As in many other cases, several of the trace elements (stock solution 3) might have no function for duckweed cultivation.

	A	B	C	D	E
	Stock	Compound	Stock concentration		Final concentration

	A	B	C	D	E
	1	CaCl2·2H2O (147.02)	136 mM	20 g/L	0.68 mM
	2	KNO3 (101.1)	2.48 M	250 g/L	12.4 mM
		MgSO4·7H2O (246.48)	162 mM	40 g/L	0.8 mM
		(NH4)H2PO4 (115.03)	260 mM	30 g/L	1.3 mM
	3	MnSO4·H2O (169.02)	5.92 mM	1 g/L	30 µM
		H3BO3 (61.83)	8.1 mM	0.5 g/L	40 µM
		ZnSO4·7H2O (287.6)	0.35 mM	0.1g/L	1.74 µM
		KI (166)	0.6 mM	0.1 g/L	3 µM
		CuSO4·5H2O (249.68)	80.1 µM	0.02 g	0.4 µM
		Na2MoO4·2H2O (241.95)	41 µM	0.01 g	0.2 µM
		CoCl2·6H2O (237.96)	42 µM	0.01 g	0.21 µM
	4	FeNaEDTA (367.1)	5.39 mM	1.98 g/L	0.26 mM
		Na2EDTA (372.24)	0.55 µM	0.204 g/L	2.75 µM

8 Use 5 mL from each stock solution for 1 L ready-to-use nutrient medium.

Hoagland medium

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Note

Reference: We describe the nutrient medium as given by Landolt and Kandeler (1987), p. 60-62 as the original paper was published already 1920. This might be the reason why so many modifications of this medium exists, e.g. Venkataraman et al. (1970).

	A	B	C	D	E
	Stock	Compound	Stock concentration		Final concentration
	1	KNO3 (101.1)	2 M	202 g/L	10 mM
	2	Ca(NO3)2 x 4H2O (236.15)	0.8 M	189 g/L	4 mM

	A	B	C	D	E
	3	KH2PO4 (136.1)	0.2 M	27.2 g/L	1 mM
	4	MgSO4 x 7H2O (246.5)	0.4 M	98.6 g/L	2 mM
		ZnSO4 x 7H2O (287.5)	0.3 mM	86.2 mg/L	1.5 µM
		H3BO3 (61.8)	10 mM	618 mg/L	50 µM
		MnCl2 x 4H2O (197.91)	2 mM	396 mg/L	10 µM
		CuSO4 x 5H2O (249.68)	80 µM	20 mg/L	0.4 µM
		Na2MoO4 x 2H2O* (241.9)	1 mM	242 mg/L	5 µM
	5	Iron(III)tartrate** (555.9)	4 mM	2.2 g/L	20 µM

* Used instead of MoO_3

** This substance is difficult to solve. Heat shortly and stir over night. Venkataraman et al. (1970) replaced this substance by Iron(III)citrate and often added Na_2EDTA, 10 µM to the final medium.

10 Use 5 mL from each stock solution for 1 L of ready-to-use nutrient medium.

Bonner-Devirian medium

11

Note

Reference: Bonner, J., and P. S. Devirian: Growth factor requirements of four species of isolated roots. Amer. J. Bot. 26: 661-665 (1939)

Note

This nutrient medium has rather low concentrations. We have only good experiences with this
nutrient medium cultivating *Lemna trisulca*.

	A	B	C	D	E
	Stock	Compound	Stock concentration		Final concentration
	1	KNO3 (101.1)	0.168 M	17 g/L	168 mM
	2	Ca(NO3)2 x 4H2O (236.15)	0.2 M	47.2 g/L	1 mM
	3	KH2PO4 (136.1)	29.4 mM	4 g/L	0.147 mM
		KCl (74.55)	161 mM	12 g/L	0.8 mM
	4	MgSO4 x 7H2O (246.5)	0.4 M	98.6 g/L	2 mM
		ZnSO4 x 7H2O (287.5)	0.3 mM	86.2 mg/L	1.5 µM
		H3BO3 (61.8)	10 mM	618 mg/L	50 µM
		MnCl2 x 4H2O (197.91)	2 mM	396 mg/L	10 µM
		CuSO4 x 5H2O (249.68)	80 µM	20 mg/L	0.4 µM
		Na2MoO4 x 2H2O (241.9)	1 mM	242 mg/L	5 µM
	5	Iron(III)tartrate* (555.9)	4 mM	2.2 g/L	20 µM

* This substance is difficult to solve. Heat shortly and stir over night. Venkataraman et al. (1970) replaced this substance by Iron (III) citrate and often added Na2EDTA, 100 µM to the final medium to study flower induction of *Wolffia microscopica*.

12 Use 5 mL from each stock solution for 1 L of ready-to-use nutrient medium.