rpc00017

Spinacia oleracea Spi-I-1 callus culture

Components

- A 9-cm plastic Petri dish, containing cells placed on semi-solid medium

Notice

- Subculture the cells to fresh medium immediately after arrival [Notes I].
- Do not store the cell culture in a refrigerator and a freezer.
- Maintain aseptic conditions of the cell culture, and work in a laminar flow cabinet.

Method

- Culture medium: mMS medium, 0.5 mg/L NAA, 1 mg/L BAP, 1.2% (w/v) agar, pH 6.5 (medium no. 68) [Materials III]
- Culture conditions: 27℃, dark [Methods II]
- Subculture: 28–56-day intervals [Methods I]

Citation of cell line

When results obtained by using this cell line are published in a scientific journal, it should be cited in the following manner: "*Spinacia oleracea* Spi-I-1 cell line (rpc00017) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Spinach Spi-I-1 cell line was established from *Spinacia oleracea* L. (Nakagawa *et al.* 1985). The Spi-I-1 cells are grown on a modified Murashige and Skoog (mMS) medium supplemented with 0.5 mg/L 1-naphthaleneacetic acid (NAA) and 1 mg/L 6-benzylaminopurine (BAP), and solidified with 1.2% (w/v) agar, pH 6.5. Our Spi-I-1 cell culture has been maintained in the dark at 27℃ and subcultured at 28–56-day intervals.

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4℃)

A) KCl

B) L-Glutamine

C) NZ-Amine Type A

D) $CaCl_2 \cdot 2H_2O$

E) $MgSO_4 \cdot 7H_2O$

F) KH_2PO_4

G) Sucrose

H) MS_micro_1

H_3BO_3	0.62 mg/mL
$MnSO_4 \cdot 5H_2O$	2.41 mg/mL
$ZnSO_4 \cdot 7H_2O$	0.86 mg/mL
KI	0.083 mg/mL
$Na_2MoO_4 \cdot 2H_2O$	0.025 mg/mL
$CuSO_4 \cdot 5H_2O$	0.0025 mg/mL
$CoCl_2 \cdot 6H_2O$	0.0025 mg/mL

I) MS_micro_2

$FeSO_4 \cdot 7H_2O$	2.78 mg/mL
Na_2-EDTA	3.73 mg/mL
Heat at 80℃ for 3–4 hours for chelating Fe	

J) MS_VT

Nicotinic acid	0.5 mg/mL
Pyridoxine·HCl	0.5 mg/mL

Thiamine·HCl	0.1 mg/mL
Glycine	2 mg/mL

K) MS_inositol

myo-Inositol	40 mg/mL

L) NAA (1 mg/mL)

NAA·K	1.2 mg/mL

Potassium 1-naphthylacetate, FUJIFILM Wako Pure Chemical Corporation (#161-04021)

M) BAP (1 mg/mL)

6-Benzylaminopurine	1 mg/mL

Dissolve 6-benzylaminopurine in small volume of KOH (1 N), and fill up with distilled water

N) Agar, powder

Agar, powder, Junsei Chemical (#24440-1201)

O) NaOH (1 N)

Glassware and equipment

A) Erlenmeyer flask (100 mL), capped with two layers of aluminum foil

B) Forceps, sterilized before use

Preparation of mMS medium (medium no. 68)

1. Dissolve the following chemicals in approximately 800 mL of distilled water.

KCl	1.4 g
L-Glutamine	1 g
NZ-Amine Type A	1 g
$CaCl_2 \cdot 2H_2O$	0.44 g
$MgSO_4 \cdot 7H_2O$	0.37 g
KH_2PO_4	0.17 g
Sucrose	20 g

2. Add following stock solutions, and fill up to approximately 950 mL with distilled water.

MS_micro_1	10 mL
MS_micro_2	10 mL
MS_VT	1 mL

MS_inositol	2.5 mL
NAA (1 mg/mL)	0.5 mL
BAP (1 mg/mL)	1 mL

3. Adjust the pH of the solution to 6.5 with NaOH (1 N), and fill up to 1 L with distilled water.

4. Pour 40 mL of the medium into a 100-mL flask containing 0.48 g of agar.

5. Autoclave the flask at 121℃ for 20 min.

Methods

1. Pick up an appropriate amount of callus cells from a 28–56-day-old culture with a forceps and place the cells onto fresh mMS medium.

2. Incubate cell cultures under the dark condition at 27℃.

Notes

- We send Spi-I-1 cells on semi-solid mMS medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh mMS medium immediately after arrival.

- In order to maintain Spi-I-1 callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of Spi-I-1 cells is affected by culture conditions, such as a room temperature, aeration conditions of the culture and so on, an amount of cells transferred to fresh medium and the subculture intervals may vary from one lab to another. We usually inoculate four to seven pieces of Spi-I-1 callus (about 3–5-mm in diameter) on 40 mL of mMS medium in a 100-mL flask, and culture them for 28–56 days.

References

Nakagawa H, Tanaka H, Oba T, Ogura N, Iizuka M (1985) Callus formation from protoplasts of cultured *Spinacia oleracea* cells. Plant Cell Reports 4: 148–150. DOI: 10.1007/BF00571303

Appendix A: Formulation of culture medium

Table A.1. modified Murashige and Skoog medium (medium no. 68)

Chemical	Concentration (mg/L)
KCl	1400
L-Glutamine	1000
NZ-Amine Type A	1000
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
KH_2PO_4	170
H_3BO_3	6.2
$MnSO_4 \cdot 5H_2O$	24.1
$ZnSO_4 \cdot 7H_2O$	8.6
KI	0.83
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
$FeSO_4 \cdot 7H_2O$	27.8
Na_2-EDTA	37.3
Nicotinic acid	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.1
Glycine	2
myo-Inositol	100
Sucrose	20000
NAA·K	0.6
6-Benzylaminopurine	1
Agar	12000