rpc00111

Arabidopsis thaliana YG1-c 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: mLS medium, 0.2 mg/L 2,4-D, 0.4% (w/v) gellan gum, pH 5.8 (medium no. 71) [Materials III]
- Culture conditions: 27°C, dark [Methods II]
- Subculture: 14–21-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: “*Arabidopsis thaliana* YG1-c cell line (rpc00111) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan.”

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Introduction

Arabidopsis YG1-c cell line was established by subculturing the YG1 suspension culture on a semi-solid culture medium. The parent YG1 cell line (rpc00050) was derived from a seedling of *Arabidopsis thaliana* (L.) Heynh. accession Columbia (Nishikiori *et al*. 2011, Yoshikawa *et al*. 2021). The YG1-c cells are grown on a modified Linsmaier and Skoog (mLS) medium supplemented with 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), and solidified with 0.4% (w/v) gellan gum, pH 5.8. Our YG1-c cell culture has been maintained in the dark at 27°C and subcultured at 14–21-day intervals.

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4°C)

A) MS salt mix

Murashige and Skoog Plant Salt Mixture, FUJIFILM Wako Pure Chemical Corporation (#392-00591)

B) Sucrose

C) BY2_P

KH_2PO_4	80 mg/mL

D) LS_VT_modified

Thiamine·HCl	0.4 mg/mL
myo-Inositol	40 mg/mL

E) 2,4-D (0.2 mg/mL)

2,4-D sodium monohydrate	0.236 mg/mL

(2,4-Dichlorophenoxy)acetic acid sodium salt monohydrate, Sigma-Aldrich (D6679)

F) Gellan gum

G) KOH (1 N)

Glassware and equipment

A) Petri dish (9 cm diameter, 2 cm height), sterile

B) Forceps, sterilized before use

C) Surgical tape

    3M™ Micropore™ Surgical Tape, 12.5 mm × 9.1 m, 3M Japan Limited (#1530-0)

Preparation of mLS medium (medium no. 71)

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

MS salt mix	1 bag (1 L)
Sucrose	30 g

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

BY2_P	2.5 mL
LS_VT_modified	2.5 mL
2,4-D (0.2 mg/mL)	1 mL

3. Adjust the pH of the solution to 5.8 with KOH (1 N), and fill up to 1 L with distilled water.

4. Add 4 g of gellan gum to the medium.

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

6. Pour 30 mL of the medium into a 9-cm Petri dish.

Methods

1. Pick up an appropriate amount of callus cells from a 14–21-day-old culture with a forceps and place the cells onto fresh mLS medium.

2. Seal the Petri dishes using two rounds of surgical tape.

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

Notes

- We send YG1-c cells on semi-solid mLS medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh mLS medium immediately after arrival.

- In order to maintain YG1-c callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of YG1-c 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 nine pieces of YG1-c callus (about 3-mm in diameter) on 30 mL of mLS medium in a 9-cm Petri dish, and culture them for 14–21 days.

- Suspension cultures can be derived from YU-1-c callus cultures by subculturing them into a liquid culture medium. The suspension culture method is the same as rpc00050 YG1.

References

Nishikiori M, Mori M, Dohi K, Okamura H, Katoh E, Naito S, Meshi T, Ishikawa M (2011) A host small GTP-binding protein ARL8 plays crucial roles in tobamovirus RNA replication. PLoS Pathogen 7: e1002409. DOI: 10.1371/journal.ppat.1002409

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Appendix A: Formulation of culture medium

Table A.1. modified Linsmaier and Skoog medium (medium no. 71)

Chemical	Concentration (mg/L)
KNO_3	1900
NH_4NO_3	1650
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
KH_2PO_4	370
H_3BO_3	6.2
$MnSO_4 \cdot 4H_2O$	22.3
$ZnSO_4 \cdot 7H_2O$	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
Thiamine·HCl	1
myo-Inositol	100
Sucrose	30000
2,4-D sodium monohydrate	0.236
Gellan gum	4000