rpc00055

Arabidopsis thaliana gnom 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, 1 mg/L 2,4-D, 0.8% (w/v) agar, pH 5.8 (medium no. 43) [Materials III]
- Culture conditions: 23℃, dark [Methods II]
- Subculture: 28-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* gnom cell line (rpc00055) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Arabidopsis gnom cell line was established from a root of *Arabidopsis thaliana* (L.) Heynh. *gnom* mutant (Ueda *et al*. 2004). *GNOM* encodes a GDP/GTP exchange factor for small G-proteins of the ADP ribosylation factor class (Geldner *et al*. 2003). The *gnom* mutant plants show severe growth arrest, but the gnom cell culture proliferate normally like wild-type cells (Ueda *et al*. 2004). The gnom cells are grown on a modified Murashige and Skoog (mMS) medium supplemented with 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), and solidified with 0.8% (w/v) agar, pH 5.8. Our gnom cell culture has been maintained in the dark at 23℃ and subcultured at 28-day intervals.

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4℃)

A) MS salt mix

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

B) Sucrose

C) KH_2PO_4 (100 mg/mL)

KH_2PO_4	100 mg/mL

D) B5_VT

Nicotinic acid	0.4 mg/mL
Pyridoxine·HCl	0.4 mg/mL
Thiamine·HCl	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) Agar, powder

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

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 mMS medium (medium no. 43)

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

MS salt mix	1 bag (1 L)
Sucrose	20 g

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

KH_2PO_4 (100 mg/mL)	3.4 mL
B5_VT	5 mL
2,4-D (0.2 mg/mL)	5 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 8 g of agar 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 28-day-old culture with a forceps and place the cells onto fresh mMS medium.

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

3. Incubate cell cultures under the dark condition at 23℃.

Notes

- We send gnom 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 gnom callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of gnom 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 seven pieces of gnom callus (about 5-mm in diameter) on 30 mL of mMS medium in a 9-cm Petri dish, and culture them for 28 days.

References

Geldner N, Anders N, Wolters H, Keicher J, Kornberger W, Muller P, Delbarre A, Ueda T, Nakano A, Jürgens G (2003) The *Arabidopsis* GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. Cell 112: 219–230. DOI: 10.1016/S0092-8674(03)00003-5

Ueda T, Uemura T, Sato MH, Nakano A (2004) Functional differentiation of endosomes in Arabidopsis cells. Plant Journal 40: 783–789. DOI: 10.1111/j.1365-313X.2004.02249.x

Appendix A: Formulation of culture medium

Table A.1. modified Murashige and Skoog medium (medium no. 43)

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	510
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
Nicotinic acid	2
Pyridoxine·HCl	2
Thiamine·HCl	20
myo-Inositol	200
Sucrose	20000
2,4-D sodium monohydrate	1.18
Agar	8000