rpc00043-3n-3-2.1 (2017.10.19; revised 2024.1.29)
RIKEN BRC plant cell line documentation

rpc00043

Nicotiana tabacum 3n-3 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: MS medium, 0.9% (w/v) agar, pH 5.7 (medium no. 34) [Materials III]
- Culture conditions: 27℃, 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: "*Nicotiana tabacum* 3n-3 cell line (rpc00043) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Tobacco 3n-3 cell line is a crown-gall line that was obtained by infecting mesophyll protoplasts of *Nicotiana tabacum* L. cultivar Petit Havana SR-1 with *Agrobacterium tumefaciens* strain A208 (Nakagawa *et al*. 1987). The 3n-3 cells are grown on a phytohormone-free Murashige and Skoog (MS) medium solidified with 0.9% (w/v) agar, pH 5.7. Our 3n-3 cell culture has been maintained in the dark at 27℃ 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) MS_VT

Nicotinic acid	0.5 mg/mL
Pyridoxine·HCl	0.5 mg/mL
Thiamine·HCl	0.1 mg/mL
Glycine	2 mg/mL

D) MS_inositol

myo-Inositol	40 mg/mL

E) Agar, powder

F) KOH (1 N)

Glassware and equipment

A) Erlenmeyer flask (200 mL), capped with two layers of aluminum foil

B) Forceps, sterilized before use

Preparation of MS medium (medium no. 34)

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.

MS_VT	1 mL
MS_inositol	2.5 mL

3. Adjust the pH of the solution to 5.7 with KOH (1 N), and fill up to 1 L with distilled water.

4. Pour 80 mL of the medium into a 200-mL flask containing 0.72 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-day-old culture with a forceps and place the cells onto fresh MS medium.

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

Notes

- We send 3n-3 cells on semi-solid MS medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh MS medium immediately after arrival.

- In order to maintain 3n-3 callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of 3n-3 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 three to five pieces of 3n-3 callus (about 10-mm in diameter) on 80 mL of MS medium in a 200-mL flask, and culture them for 28 days.

References

Nakagawa S, Tjokrokusumo DS, Sakurai A, Yamaguchi I, Takahashi N, Syōno K (1987) Endogenous levels of gibberellins, IAA and cytokinins in tobacco crown gall tissues of different morphologies. Plant & Cell Physiology 28: 485–493. DOI: 10.1093/oxfordjournals.pcp.a077319

Appendix A: Formulation of culture medium

Table A.1. Murashige and Skoog medium
(medium no. 34)

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	170
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	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.1
Glycine	2
myo-Inositol	100
Sucrose	30000
Agar	9000