rpc00003

Vitis VR 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, 0.05 mg/L 2,4-D, 0.2 mg/L kinetin, 1.2% (w/v) agar, pH 6.1 (medium no. 3) [Materials III]
- Culture conditions: 27℃, dark [Methods II]
- Subculture: 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: "*Vitis* VR cell line (rpc00003) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Grape VR cell line was established from anthers of *Vitis* interspecific hybrid cultivar Bailey Alicante A (Yamakawa *et al.* 1983a, b). The VR callus cells accumulate higher amount of anthocyanins than VW callus cells (rpc00004). The VR cells are grown on a modified Linsmaier and Skoog (mLS) medium supplemented with 0.05 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.2 mg/L kinetin, and solidified with 1.2% (w/v) agar, pH 6.1. Our VR cell culture has been maintained in the dark at 27℃ and subcultured at 56-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) LS_VT_modified

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

D) 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)

E) Kinetin (0.2 mg/mL)

Kinetin 0.2 mg/mL
Dissolve kinetin in small volume of KOH (1 N), and fill up with distilled water

F) Agar, powder

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

G) KOH (1 N)

Glassware and equipment

A) Erlenmeyer flask (300 mL), capped with two layers of aluminum foil

B) Forceps, sterilized before use

Preparation of mLS medium (medium no. 3)

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.

LS_VT_modified	2.5 mL
2,4-D (0.2 mg/mL)	0.25 mL
Kinetin (0.2 mg/mL)	1 mL

3. Adjust the pH of the solution to 6.1 with KOH (1 N), and fill up to 1 L with distilled water.

4. Pour 100 mL of the medium into a 300-mL flask containing 1.2 g of agar.

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

Methods

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

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

Notes

- We send VR 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 VR callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of VR 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 two to three pieces of VR callus (about 8-mm in diameter) on 100 mL of mLS medium in a 300-mL flask, and culture them for 56 days.

- It is important to subculture good healthy cells. Yellow and pink VR cells are the most suitable, but red cells are too aged.

References

Yamakawa T, Ishida K, Kato S, kodama T, Minoda Y (1983a) Formation and identification of anthocyanins in cultured cells of *Vitis* sp. Agricultural and Biological Chemistry 47: 997–1001. DOI: 10.1080/00021369.1983.10865764

Yamakawa T, Kato S, Ishida K, Kodama T, Minoda Y (1983b) Production of anthocyanins by *Vitis* cells in suspension culture. Agricultural and Biological Chemistry 47: 2185–2191. DOI: 10.1080/00021369.1983.10865938

Appendix A: Formulation of culture medium

Table A.1. modified Linsmaier and Skoog medium (medium no. 3)

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
Thiamine·HCl	1
myo-Inositol	100
Sucrose	30000
2,4-D sodium monohydrate	0.059
Kinetin	0.2
Agar	12000