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Plant Regeneration from Anther Culture of *Vitis vinifera* via Embryogenesis

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Grapes are one of main fruits in Yamagata. It is necessary for the genetic improvement of grapes to establish an ideal method of plant regeneration from callus. Plant regeneration via embryogenesis has been reported by using the cultivation of anthers,[1,2,4-6] leaves,[3,7] stems,[8] and unfertilized ovules.[9,10] However, a satisfactory method of controlling somatic embryogenesis has not been obtained. I report here embryogenesis from cultured anthers of *V. vinifera*.

Cabernet Sauvignon was used in this study. Inflorescences were collected just before flowering and chilled at 4℃. They were surface-sterilized with sodium hypochlorite (1% available chlorine) containing Tween 20 (a few drops) and rinsed 3 times with sterile distilled water.

The anthers were aseptically excised from the flowers and cultured in 9 cm Petri dishes containing 20 ml of medium for a month. A half-strength MS (1962) medium[11] was used as a basal medium and supplemented with 2% sucrose, 0.75% agar, various concentrations of NAA or 2, 4-D and 6-benzylaminopurine (BA), as shown in **Table 1** (Primary media). The pH was adjusted to 5.8 before autoclaving. The cultures were maintained at 25℃ in the dark.

When the anthers were cultured, most of them soon turned brown. After 2 weeks, yellowish callus tissues appeared from the swollen anthers, but the rate was low in each combination with growth regulators (**Table 1**). Callus proliferation was more vigorous on the media with NAA than those with 2, 4-D. The effect of chilling on callus formation was not definite, but a long-term treatment was inhibitory (**Table 1**).

A prolonged culture led to browning of callus tissues, especially on the media with 2, 4-D. Calli obtained from anthers cultured for a month on the primary media with 2, 4-D were transplanted to the media supplemented with NAA, BA and casein hydrolysate, maintenance media to prevent callus browning as shown in **Table 2**. However, the effect of the treatment was not appreciable. After another month of culture, the calli were transplanted on the media lacking casein hydrolysate and thereafter subcultured on them every month. Calli obtained on the primary media with NAA were subcultured on the maintenance media every month.

After 3 months of subculture, somatic embryo from calli was formed only on the medium containing NAA (0.02 mg/*l*) and BA (2.0 mg/*l*). Although the rate of callus formation was low, three of 4 calli formed on this medium produced somatic embryos (**Table 2**). This result suggests that the calli formed on this medium are mostly embryogenic. When they were subcultured further, a large number of somatic embryos were produced (**Fig. 1A**).

High concentration of NAA was necessary to induce embrygenic callus according to the previous reports.[1,2] However, high concentration of NAA was not suitable in my experiment. In many previous studies, anthers containing uninucleated microspores have been used, but in the present study I used mature anthers. Therefore, it is considered that the difference in hormone sensitivity was related to the stage of anthers.

Table 1. Effect of the medium and chilling on callus formation from cultured anthers.

Growth regulators			No. of calli/No. of anthers	
NAA	2, 4-D	BA	CS 1	CS 2
0.02 mg/*l*		0.2 mg/*l*	3/21	0/21
0.02		2.0	4/21	1/21
0.2		0.2	6/21	0/21
0.2		2.0	4/21	2/21
1.0		0.2	6/21	1/21
1.0		2.0	5/21	3/21
	0.2 mg/*l*	0.2	5/21	2/21
	0.2	2.0	1/21	1/21
	1.0	0.2	4/21	1/21
	1.0	2.0	8/21	4/21

Inflorescences were chilled for 86 hr (CS 1) and 134 hr (CS 2) at 4°C.

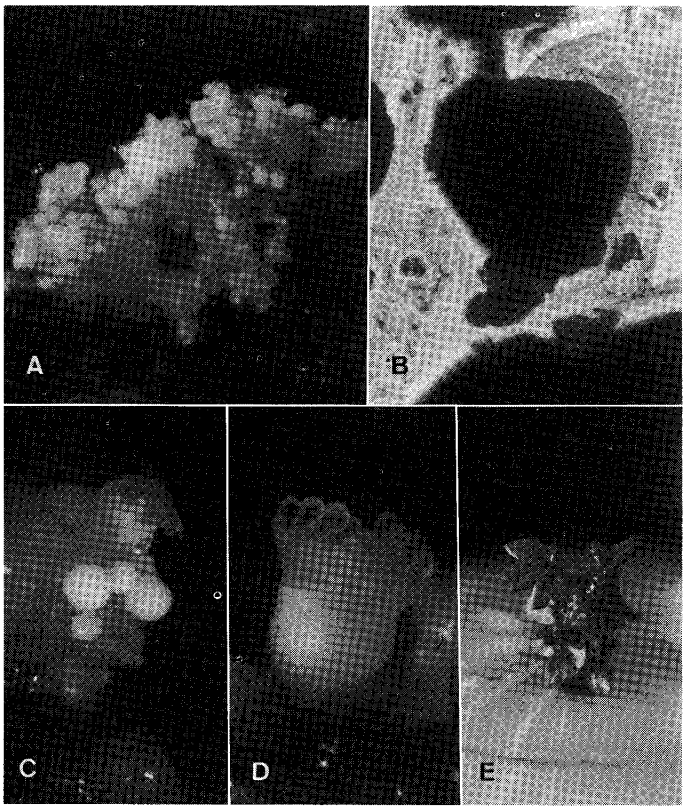

Fig. 1. (A) Somatic embryos produced on MS/2 medium containing NAA (0.02 mg/*l*) and BA (2.0 mg/*l*); (B) Heart-shaped embryo; (C), (D) Germination of somatic embryos; (E) Plantlet regenerated from somatic embryo.

Somatic embryos were formed on the brown calli and exhibited a characteristic pattern of development different from that of zygotic embryos. Most of the somatic embryos were globular and did not develop further. Few embryos were heart-shaped. Somatic embryos germinated without becoming torpedo-shapes (**Fig. 1 B, C, D**).

Table 2. Embryo production from anther-derived calli (CS 1).

Primary media			Maintenance media			No. of calli producing embryos/No. of calli
NAA	2, 4-D	BA	NAA	BA	(CH)*	
0.02 mg/*l*		0.2 mg/*l*	0.02 mg/*l*	0.2 mg/*l*		0/3
0.02		2.0	0.02	2.0		3/4
0.2		0.2	0.2	0.2		0/6
0.2		2.0	0.2	2.0		0/4
1.0		0.2	1.0	0.2		0/6
1.0		2.0	1.0	2.0		0/5
	0.2 mg/*l*	0.2	0.02	0.2	(250 mg/*l*)	0/5
	0.2	2.0	0.02	2.0	(250)	0/1
	1.0	0.2	0.1	0.2	(250)	0/4
	1.0	2.0	0.1	2.0	(250)	0/8

* Maintenance media were supplemented with casein hydrolysate (CH), only when the calli cultured for a month on the primary media with 2, 4-D were transplanted.

When transplanted to a basal agar medium, most of the embryos failed to produce normal plantlets. Attempts to produce normal plantlets by supplementation of NAA (0.1 mg/*l*) or BA (0.1, 0.5, 1.0 mg/*l*) were unsuccessful. The most effective treatment to obtain many normal plantlets was two weeks of chilling at 4℃ (**Fig. 1E**).

In a preliminary study, Kyoho and Olympia were also used. Callus formation from their anthers was superior to Cabernet Sauvignon. However, somatic embryo formation was not observed.

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≪和文要約≫

ブドウ葯からの不定胚形成および植物体再生

村山　徹

山形県立農業試験場

ブドウ（品種 Cabernet Sauvignon）の葯を低温処理後，NAA 0.02 mg/*l*，BA 2.0 mg/*l*，sucrose 2% を含む 1/2 MS 培地で培養した．継代培養すると，3か月後に褐変したカルスから，不定胚が形成された．不定胚は，低温処理することによって，植物体を再生した．