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Research Note

Somatic embryogenesis from filaments of *Vitis vinifera* L. and *Vitis labruscana* Bailey

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Summary: Somatic embryogenesis from filaments of grape was investigated. The combination of 1 µM 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 µM N-(1,2,3-thiadiazol-5-yl)-N'-phenylurea (TDZ) or 10 µM 2,4-D and 10 µM TDZ was suitable for somatic embryogenesis from filaments. The filaments of 8 grape cultivars including recalcitrant genotypes were cultured on a half strength MS basal medium supplemented with 1 µM 2,4-D and 1 µM TDZ. Two-step culture (liquid medium followed by solid medium) gave better results for somatic embryogenesis than one-step culture (solid medium only). Induction of embryogenic callus (EC) was achieved with 5 of 8 cultivars including recalcitrant ones by the two-step culture method, indicating that the filament culture enlarges the number of the EC inducible cultivars.

K e y w o r d s : Somatic embryogenesis, filament culture, *Vitis*.

Introduction: Grapevine, the most important fruit crop worldwide, is infected by some diseases despite repeated spraying of chemicals in Japan due to the damp climate. Thus, disease–tolerant grapes are highly desirable. However, the introduction of useful genes of wild species into cultivated species, *i.e.* genetic improvement by traditional hybridization, takes a long time. Genetic transformation systems can introduce useful genes not only from wild species but also from different species if useful genes are revealed. In grapevine, transformants could be obtained using embryogenic callus (EC) or somatic embryos by *Agrobacterium* infection (MARTINELLI and MANDOLINO 1994, YAMAMOTO *etal* . 2000). YAMAMOTO *et al*. (2000) demonstrated that the introduction of the rice chitinase gene enhanced the resistance to some fungal diseases.

EC and/or somatic embryos of grape have been induced from anthers (HIRABAHASHI and AKIHAMA 1982, NAKANO *etal* . 1997), unfertilized ovules (MULLINS and SRINIVASAN 1976, NAKAJIMA *et al*. 2000), ovaries (NAKANO *et al*. 1997), leaves (HIRABAYASHI 1985, MATSUTA and HIRABAYASHI 1989, MATSUTA 1992, NAKANO *et al*. 1997) and tendrils (SALUNKHE *et al*. 1997). The number of cultivars from which EC and/or somatic embryos can be produced is increasing, but is still limited. Besides, rates of somatic embryogenesis were low for many cultivars cultivated in Japan.

In preliminary experiments we cultured anthers attached with the fragment of filament. It was observed that EC was also produced from the filaments. For roses, it was reported that the filament was effective to induce friable EC as explants, compared with other organs (NORIEGA and SÖNDAHL 1991). To the best of our knowledge, there has been no report of the filament culture of grape. Thus, we examined the filament culture of 8 cultivars including recalcitrant ones. In this paper, we describe the somatic embryogenesis from the filaments of recalcitrant grape cultivars.

Material and Methods: Eight grape cultivars (two *Vitis vinifera* L. cvs, 6 *Vitis labruscana* Bailey cvs) listed in Tab. 2 were used. Flower buds were collected 14-21 d before anthesis from vineyard-grown grapevines, and then sterilized for 20 min with sodium hypochlorite (1 % activated chlorine) containing a few drops of Tween 20, and then rinsed twice with sterile water. Filaments were excised under a stereoscopic microscope.

Filaments of Muscat of Alexandria were cultured on a 0.85 % agar medium which consisted of a half strength MS basal medium supplemented with 1-10 µM auxin (2,4-dichlorophenoxyacetic acid (2,4-D) or 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)) and 1-10 µM cytokinin (N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU) or N-(1,2,3-thiadiazol-5-yl)-N'-phenylurea (TDZ)). Filaments of 8 cultivars were cultured on a 1/2 MS medium supplemented with 1 µM 2,4-D and 1 µM TDZ. The culture media were adjusted to pH 5.8 at 60 °C with 0.1 M Tris-HCl to avoid callus browning (BORNHOFF and HARST 2000) and autoclaved. Cultures were done in two ways: Method 1 - on a solid medium (0.85 % agar) only and method 2 - in a liquid medium for one month and then transferred to the solid medium. Explants of liquid medium were agitated continuously in a rotary shaker (60 rpm). All explants were cultured at 26 °C in the dark. Filaments were subcultured on the same medium for 4-8 weeks intervals till 6 months. Then, calli were transferred to a 1/2 MS hormone-free medium and cultured for three months to induce embryos. Sixty explants were used in each experiment. Induced embryogenic calli were maintained on a 1/2 MS medium containing 1 µM 2,4-D, 5 % maltose instead of sucrose and 3 % agar (NAKAJIMA *et al*. 2000).

Results and Discussion: The effects of phytohormones on the induction of somatic embryogenesis were examined using the filaments of cv. Muscat of Alexandria (Tab. 1). Within one month of culture, calli were produced from the filaments in all media examined. After 6 months of culture, the produced calli were transferred to a hormone-free medium. If somatic embryos were induced from callus, it was counted as embryogenic callus. If 2,4-D was used as auxin, TDZ gave better results than CPPU to induce EC (Tab. 1). If 2,4,5-T was used as auxin, both cytokinins were also effective to induce EC. As for the concentration, 1 µM was effective in any combination of auxin and cytokinin. Among the examined media, 1 µM 2,4-D and 1 µM TDZ or 10 µM 2,4-D and 10 µM TDZ were most suitable for somatic embryo-

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Table 1

Effects of phytohormone combinations on the somatic embryogenesis induction from filaments of cv. Muscat of Alexandria

Phytohormone (μM)				Embryo induction
2,4-D	2,4,5-T	TDZ	CPPU	rate (%)
10		10		20.0
10		1		11.7
1		1		20.0
10			10	1.7
10			1	0.0
1			1	6.7
	10	10		1.7
	10	1		0.0
	1	1		10.0
	10		10	5.0
	10		1	3.3
	1		1	5.0

genesis. Therefore, the combination of 1 μM 2,4-D and 1 μM TDZ was used in the following experiment. Matsuta (1992) and Nakano *et al*. (1997) reported that the combination of 2,4,5-T and CPPU was suitable to induce somatic embryogenesis from leaf callus of cv. Koshusanjaku (*V. vinifera*). In this study, the combination of 2,4-D and TDZ was more effective than that of 2,4,5-T and CPPU in inducing EC from the filaments. Further investigation is necessary to determine whether this is due to the different origin of explants or whether it is a genotype specific phenomenon.

The filaments of 8 cultivars were cultured in a liquid or solid medium, which consisted of a 1/2 MS medium supplemented with 1 μM 2,4-D and 1 μM TDZ (Tab. 2). Method 2 enabled somatic embryogenesis in 5 of 8 cultivars, whereas method 1 enabled somatic embryogenesis in 3 of 8 cultivars. Furthermore, induction rate of EC was higher if method 2 was used. EC has been maintained on the 1/2 MS medium (3 % agar and 5 % maltose instead of sucrose) supplemented with 1 μM 2,4-D, which is the same medium for the maintenance of embryogenic callus of cv. Kyoho (Nakajima *et al*. 2000). Embryogenic ability was maintained for more than

Table 2

Induction rate (%) of somatic embryogenesis from filaments of 8 cultivars

Cultivar	Species	Embryo induction rate	
		Method 1	Method 2
Muscat of Alexandria	*V. vinifera*	13.8	13.3
Neo Muscat	*V. vinifera*	28.3	38.3
Aki Queen	*V. labruscana*	0.0	0.0
Campbell Early	*V. labruscana*	1.7	5.0
Delaware	*V. labruscana*	0.0	13.3
Kyoho	*V. labruscana*	0.0	0.0
Muscat Bailey A	*V. labruscana*	0.0	10.0
Pione	*V. labruscana*	0.0	0.0

one year. Stamp and Meredith (1988) reported that the effectiveness of the solid versus the liquid-solid culture procedure of anthers varied with genotype and growing condition (greenhouse or vineyard). In this experiment, we only used filaments of anthers originating from vineyard-grown grapevines. Further investigation is necessary to determine the effectiveness of the liquid-solid culture in using greenhouse-grown filaments, too.

Somatic embryogenesis has not been produced in cv. Muscat of Alexandria from anthers (Hirabayashi and Akihama 1982) and leaves (Hirabayashi 1985), and in cvs Campbell Early and Muscat Bailey A from leaves (Hirabayashi 1985, Nakano *et al*. 1997), anthers (Hirabayashi and Akihama 1982, Nakano *et al*. 1997) and ovaries (Nakano *etal* . 1997). By using filaments, somatic embryogenesis could be induced in cvs Muscat of Alexandria, Campbell Early and Muscat Bailey A for the first time. With method 2, somatic embryo induction rates of cvs Delaware and Neo Muscat were about 3-10 times higher than indicated by Nakano *etal* . (1997) using ovaries as explants.

In this study, the effectiveness of filaments as explants to induce somatic embryogenesis in grape was demonstrated. Filament culture might enlarge the number of the EC inducible cultivars and increase the induction rate of somatic embryogenesis in some cultivars.

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