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Callus Induction and Plant Regeneration from Petal Explants of Rose (*Rosa hybrida*)

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Rose is one of the most important floricultural crops in the world and the production of new cultivars in this plant has been a major issue for many years. Modern roses have been mainly developed through spontaneous or artificially induced bud sports[1], but the recent progress of plant biotechnology provides the alternative applications such as tissue culture[2,3] and gene manipulation technologies[4] for plant breeding. In our laboratory, we have investigated the use of abundant somaclonal variations induced in tissue cultures and attempted to establish an efficient system for the selection of useful genetic variations for improving important traits such as disease resistance in various crop plants[5]. Also in rose plants, we have elaborated an effective method for tissue culture and clarified the conditions suitable for plant regeneration from the leaf explant-derived calli[6]. To further develop our culture system, the present work describes an utilization of petal tissues as a culture material for successful callus induction and subsequent plant regeneration in rose plants.

Fully opened flowers were harvested from rose plants (*Rosa hybrida* cv. Carl Red) grown in a greenhouse for 2-3 months and used in the following culture experiment. The receptacles, sepals, and outermost petals were removed from flowers, and the remaining petals were excised and dipped into 70% ethanol for 30 sec and then into a 1% sodium hypochlorite solution for 90 sec for surface-sterilization. After rinsing with sterilized distilled water, petals were cut into smaller segments (1×1 cm) and then placed on a Murashige-Skoog[7] (MS) medium supplemented with 3% sucrose and different concentrations of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) and solidified with 0.3% Gelrite (Merk, NJ., USA). The medium was adjusted to pH 5.7 with 1 N NaOH before autoclaving. Culture bottles were tightly sealed with Parafilm and incubated at 26± 1°C in the dark.

After 7-10 days of incubation, pale yellow calli were induced from the decolorized edge portions of red-color petal explants of Carl Red in all the combinations of NAA and BAP concentrations (**Fig. 1-A**). These calli proliferated slowly, became brownish, and produced adventitious roots (5-10 roots from each petal explant) 14-16 days after incubation (**Fig. 1-B**). The root elongation ceased 30-40 days after incubation, and subsequently the bugle-like structures (BLS), as shown in **Fig. 1-C**, were produced apart from the adventitious roots in the callus tissues approximately 50 days after incubation. The formation of BLS was frequently observed in calli cultured in the presence of 0.25-1.0 μg/m*l* of NAA and 0.0025-0.015 μg/m*l* of BAP (**Table 1**). Especially, the BLS formation was highest in frequency (62% in average of four separate experiments) and in number (15-20 per explant) in combination of 0.75 μg/m*l* NAA and 0.01 μg/m*l* BAP. In a previous

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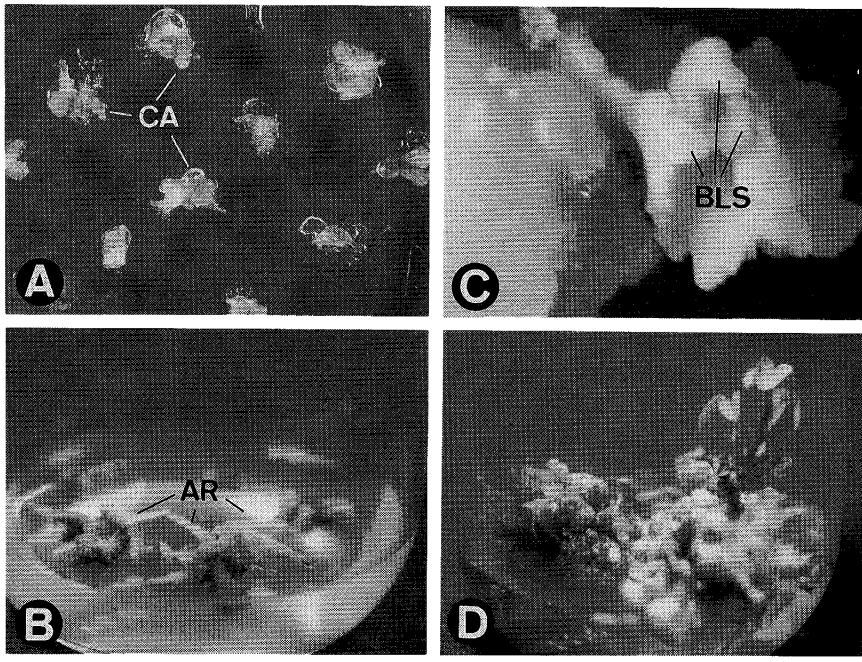

Fig. 1 Callus induction and plant regeneration from petal explants of rose (*R. hybrida* cv. Carl Red).
Petal explants were prepared from flowers of rose grown in a greenhouse and cultured on a MS medium containing 0.75 μg/m*l* NAA and 0.01 μg/m*l* BAP. Calli (CA) were induced from petal explants 7 days (A), and adventitious roots (AR) were generated from calli 14 days after incubation (B). Bugle-like structures (BLS) were produced apart from adventitious roots in calli 50 days after incubation (C) and differentiated to shoots by transferring to a MS medium containing 1.0 μg/m*l* BAP (30 days after transfer) (D).

Table 1. Effect of plant growth regulators on callus induction and subsequent morphogenesis from petal explants of rose (*R. hybrida* cv. Carl Red).

Concentrations of BAP (μg/m*l*)	Concentrations of NAA (μg/m*l*)						
	0.05	0.1	0.25	0.5	0.75	1.0	1.5
0.001	C	C	CR	CR	CR	CRS	CR
0.0025	C	C	CR	CRS	CRS	CR	CRS
0.005	C	CR	CRS	CRS	CRS	CRS	CR
0.0075	C	CR	CRS	CR	CRS	CRS	CRS
0.01	CR	CR	CR	CRS	CRS	CRS	CR
0.015	CR	CR	CRS	CRS	CR	CR	CR
0.02	C	C	CR	CR	CR	CR	CR

Petal explants were cultured on MS medium containing NAA and BAP, and morphological changes, such as callus induction (C), adventitious root (R), and shoot formation (S) were recorded 40 days after incubation.

work[6], we demonstrated that a similar BLS was produced in calli derived from leaf explants of this cultivar and the shoots could be differentiated from the structures when the calli were transferred to the shoot formation medium (MS medium containing 1.0 μg/m*l* BAP). In addition, the root formation was shown to be initiated when excised shoots were cultured on 1/2-strength MS medium containing 1 μg/m*l* indole-3-acetic acid[6]. Consequently, the petal callus-derived BLS was first cultured on the shoot formation medium (at 26°C under a continuous illumination of 4,000 lux) and then transferred to the root formation medium. Under these culture conditions, more than 80% of

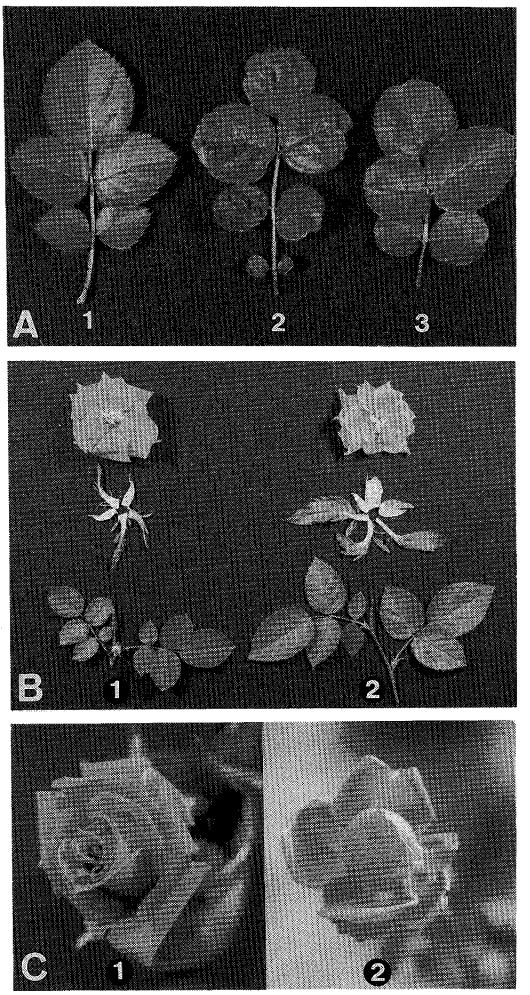

Fig. 2 Occurrence of somaclonal variations in regenerated plants derived from petal calli of *R. hybridia* cv. Carl Red.
A, original leaves(1), and rough-surface, round-shape(2) and flat round-shape leaves (3) ; B, regenerants carrying variations dually in sepals and petals; C, normal(1) and variant flower possessing petals with decreased number and pale red color(2). Compare flower shape and petal color tint between variant regenerants and normal plant.

BLS produced shoots 30-40 days after the first transfer (**Fig. 1-D**) and numerous roots were consequently formed from the shoots 20-30 days after the second transfer. The regenerated plants were transplanted to potted soil and acclimatized in a moistened growth chamber for a week. These regenerants grew normally under field conditions.

In the present study, we examined the occurrence of somaclonal variation in these regenerants in relation to morphological characteristics of the leaf and floral portions. **Fig. 2** shows some obvious variations which occurred in leaves (**A**), petals (**B** and **C**), and sepals (**B**) of regenerated plants. These variations were observed in approximately 5% (8 plants) of total 358 regenerants obtained in this study; five regenerants carried variant leaves, and two plants simultaneously induced the variations in color (pale red) and shape of petals (**Fig. 2-B**) and the remaining one in color and number of petal (**Fig. 2-C**). There was no variation in plant growth habit.

In *R. hybrida*, some workers have reported the successful induction of embryogenic or organogenic calli from various explants prepared from different tissues or organs, such as leaf[6,9,11],

stem[9], root[11], internode[11], and immature embryo[8]. These investigations were carried out using basically similar conditions (MS medium supplemented with NAA and BAP) for tissue culture. Recently, embryogenic calli were induced from filament[10] and petal explants[11] of *R. hybrida* cultivars, by the addition of 2, 4-D and zeatin and 2, 4-D and BAP, respectively. Nevertheless, the present study demonstrated that the use of NAA and BAP was effective for inducing calli capable of producing BLS from petal explants of Carl Red. This condition was essentially identical to the system used for culturing leaf explants of this cultivar[6]. These results suggest that the culture conditions established in our laboratory would be commonly applicable to explants prepared from different tissues or organs of this cultivar.

It is known that the occurrence of variant plants can be attributed to chromosomal abnormality or gene mutation induced through tissue culture[3]. Arene *et al.*[11] extensively examined the effective plant regeneration of different tissue-derived calli of *R. hybrida* and described the occurrence of dwarfed variants in addition to morphological changes of petals. Somaclonal variations were also observed in petals and leaves of the present regenerants of Carl Red, and the ratio of occurrence of somaclonal variation was comparable to the data of Arene *et al.*[11]. Although the stable propagation of these variations were not elucidated in this study, these results might be attractive for improving floral characteristics and growth habit of rose plants. Carl Red is one of the most popular cultivars in Japan. However, this cultivar is highly sensitive to phytopathogenic fungi such as the powdery mildew and black spot[12]. Recently, Chatani *et al.*[13] inoculated leaf callus-derived regenerants of this rose cultivar with the powdery mildew pathogen and selected somaclonal variants moderately resistant to the pathogen. These results suggest that plant regeneration through calli could be an attractive system for integrating useful morphological and physiological variations to rose plants.

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