Somatic Embryogenesis in Rosa hybrida L. cv. Barkarole.

Somatic Embryogenesis in *Rosa hybrida* L. cv. Barkarole.

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(Received July 18, 1996)

SUMMARY

Embryogenic callus was derived from the leaf primordia or pith of the shoot tip on a medium containing 10.0 μM BAP and 1 μM GA3. It had meristematic cells, globular embryos and parenchymas, and was maintained on a medium supplemented with 30 g/l sucrose, 10.0 μM BAP, 0.1 μM GA3 and 2.0 g/l gellan gum. Embryogenesis from embryogenic callus was enhanced on a medium containing 60 g/l sucrose, 10.0 μM BAP, 1.0 μM GA3 and 2.0 g/l gellan gum. Many embryos were isolated in a liquid medium containing 0.01 μM BAP and 10.0 μM GA3 which was shaken rotationally at 100 rpm. Cultures 1 to 2 mm in length consisted of individual embryos; those over 2 mm in length consisted of a mass of embryos able to divide into individual embryos without difficulty.

Res. Bull. Fac. Agr. Gifu Univ. (61) : 25−30, 1996

INTRODUCTION

Tissue culture of roses may be accomplished by any of three procedures; shoot tip culture[1], shoot regeneration [2, 3] and embryogenesis[4−7]. Embryogenesis is a promising procedure for genetic engineering and for rapid proliferation in nurseries. There are few reports on the embryogenesis of hybrid tea roses,

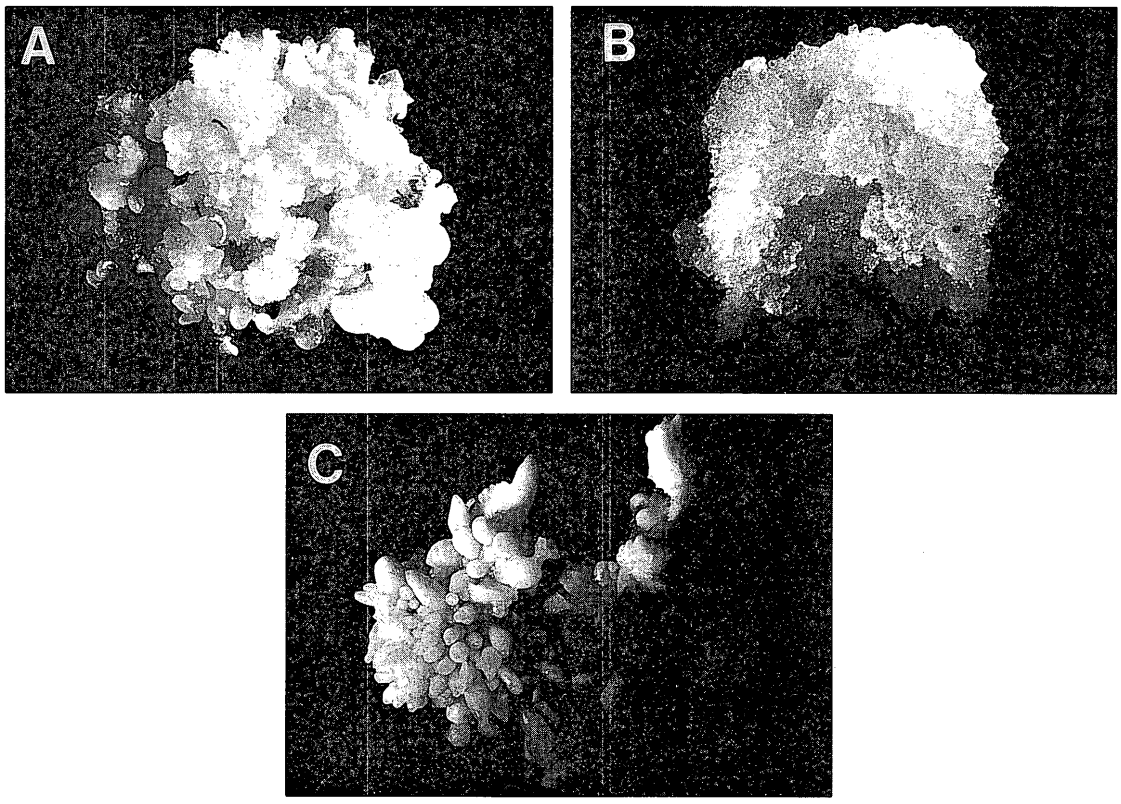

Fig. 1. Embryogenic callus (A) and callus (B) derived from *Rosa hybrida* cv. Barkarole. (C) is embryogenesis from embryogenic callus.

however. This study reports on the induction and successful maintenance of embryogenic callus and the embryogenesis derived from it.

MATERIALS AND METHODS

Induction of embryogenic callus

Shoot segments of *Rosa hybrida* L. cv. Barkarole with a lateral bud were disinfected in sodium hypochlorite solution (1% available chlorine) for 15 min followed by three rinses in sterile distilled water. The tip was cut from a shoot segment and inoculated onto a medium containing of Murashige and Skoog' s salts and vitamins (MS medium), 30 g/l sucrose, 10.0 μM 6-benzylaminopurine (BAP), 1.0 μM gibberellin A3 (GA3) and 2.0 g/l gellan gum. This was adjusted to pH 5.7.

Maintenance of embryogenic callus and embryogenesis from embryogenic callus on solid medium

Small masses of embryogenic callus from the leaf primordia or pith were subcultured at six-week intervals on to a fresh medium similar in composition.

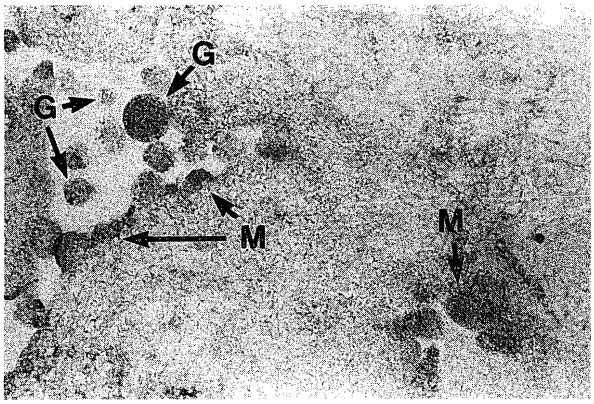

Fig.2. Histomorphological observation of embryogenic callus. G:Globular embryo M: Meristematic cells

Embryogenic calluses 5 mm in diameter were explanted onto a variety media. The medium consisted MS medium plus 30 to 100 g/l sucrose, 0 to 100 μM BAP, 0 to 10 μM GA3 and 2.0 g/l gellan gum. Embryogenic calluses were cultured for six weeks at 25℃in a 16 hr photoperiod provided by fluorescent lights giving 3000 lx. A minimum of 20 cultures were tested.

Embryogenesis from embryogenic callus in liquid medium

Embryogenic calluses 3 mm in length were cultured on several mediums consisted MS medium plus 60 g/l sucrose and 0 to 100 μM BAP and GA3. An explant was put into 40 ml of medium in a 100 ml Erlenmeyer flask and shaken rotationally at 100 rpm. These were maintained at 25℃ in the dark for six weeks. A minimum of 10 cultures were tested.

RESULTS AND DISCUSSION

After 6 weeks, a creamy yellow embryogenic callus distinguishable from non-embryogenic callus (Fig.1) was first observed at the base of the shoot. This callus appeared to originate from the leaf primordia or pith of the shoot tip and had meristematic cells, globular embryos and parenchymas (Fig. 2).

Somatic embryos of hybrid tea roses until now were induced from a variety of materials; filament[6], stem segment [7], leaf [4] and shoot tip (as in this study).

The embryogenic callus was derived on a medium containing 10.0 μM BAP and 1.0 μM GA3. BAP and GA3 were selected as the factors inducing embryogenic callus based on the reports of Rout et al. [7] and Noriega and Sondahl[6]. These hormones appeared to play an important role in embryogenesis as well as *in vivo*[8], although the relation between somatic embryogenesis and BAP or GA3 had been not obvious. These embryogenic calluses were used for maintenance and embryogenesis as follows.

Maintenance of embryogenic callus and embryogenesis from embryogenic callus on solid medium

Embryogenesis from embryogenic callus was not enhanced by 30 g/l sucrose, but the embryos differentiated in 48% of explants on the medium containing 60g/l sucrose (Table 1, Fig. 1(C)). Embryogenesis was enhanced by 10.0 μM BAP and also by 1.0 μM GA3. The differentiated embryos were characterized by white, small or no cotyledons, and elongated hypocotyls (Fig. 1(C)).

Table 1. Effect of sucrose concentrations on maintenance of embryogenic callus and embryogenesis.

	Sucrose concentration (g/l)		
	30	60	100
Embryogenesis frequency(%)	7.7a	48.0b	29.7c
Embryogenic callus frequency (%)	29.7b	29.3b	0.0c
Entire browning (%)	28.6b	9.2a	27.5 b
Partial browning (%)	34.1 b	8.7 a	23.3 b
Weight (g)	1.88 b	2.16 b	0.90 a

All mediums contained 0 to 100 μ M BAP, 0 to 10 μ M GA3 and 2.0 g/l gellan gum.

Difference letters represent significant differences, according to Duncan's multiple range test at 5 % level.

Table 2. Effect of BAP concentrations on maintenance of embryogenic callus.

	BAP concentration (μ M)				
	0.0	0.1	1.0	10.0	100.0
Embryogenic callus frequency (%)	10.2 a	17.3a	36.8 b	42.5 b	0.0 a
Entire browning (%)	2.3 a	12.4 a	7.5 a	5.6 a	76.2 b
Partial browning (%)	14.4	33.2	22.6	21.8	21.8
Weight (g)	2.16 b	2.14 b	2.03 b	2.61 b	0.39 a

All mediums contained 0 to 10 μ M GA3, 30g/l sucrose and 2.0 g/l gellan gum.

Difference letters represent significant differences, according to Duncan's multiple range test at 5 % level.

Table 3. Effect of GA3 concentrations on maintenance of embryogenic callus.

	GA3 concentration (μ M)				
	0.0	0.01	0.1	1.0	10.0
Embryogenic callus frequency (%)	13.7	29.8	35.9	17.8	9.9
Entire browning (%)	26.3	19.9	15.9	24.8	23.7
Partial browning (%)	26.3	17.7	15.9	25.8	35.9
Weight (g)	1.70	1.98	2.28	1.63	1.72

All mediums contained 0 to 100 μ M BAP, 30g/l sucrose and 2.0 g/l gellan gum.

Noriega and Sondahl[6] suggested that the early stages of embryo differentiation were better achieved after transferring to a highly osmotic medium. They induced mature embryos with two expanded cotyledonary leaves from embryogenic callus. In this study, the ineffectiveness of the medium on embryogenesis might have induced abnormality of the embryos. Burger et al. [9], from their study of immature embryo culture, mentioned the necessity of both BAP and NAA for cotyledon expansion and shoot formation; and Rout et al.[7] referred to the effectiveness of L-proline in the induction of normal embryogenesis. Experiments concerning the enhancement of normal embryogenesis and plantlet formation are presently in progress.

The embryogenic callus frequency at 30 g/l sucrose was the same as that at 60 g/l sucrose (Table 1). As 60g/l sucrose also induced embryogenesis, it was decided that the addition of 30 g/l sucrose was effective for the maintenance of embryogenic callus.

Embryogenic callus was maintained at a significantly high level on the medium containing 1.0 or 10.0 μ M BAP (Table 2). GA3 had no effect on the maintenance of embryogenic callus, but callus frequency and its weight were high at 0.1 μ M GA3 (Table 3).

Comparing 1.0 μ M with 10.0 μ M BAP on the medium containing 0.1 μ M GA 3 , 10.0 μ M BAP showed a significantly high frequency and weight of embryogenic callus. The callus accordingly was suitably maintained on the medium supplemented by 30g/l sucrose, 10.0 μ M BAP, 0.1 μ M GA3 and 2.0 g/l gellan gum.

Embryogenesis from embryogenic callus in liquid medium

Many embryos were isolated in liquid medium by shaking rotationally. Cultures 1 to 2 mm in length consisted of individual embryos, and those over 2 mm in length consisted of a mass of embryos (Fig. 3) which was able to divide into embryos without difficulty.

A supplement of 0.01 μ M BAP to the medium stimulated the isolation of individual embryos (Table 4), as did a high concentration of GA3 (Table 5).

Effective isolation of embryos from embryogenic

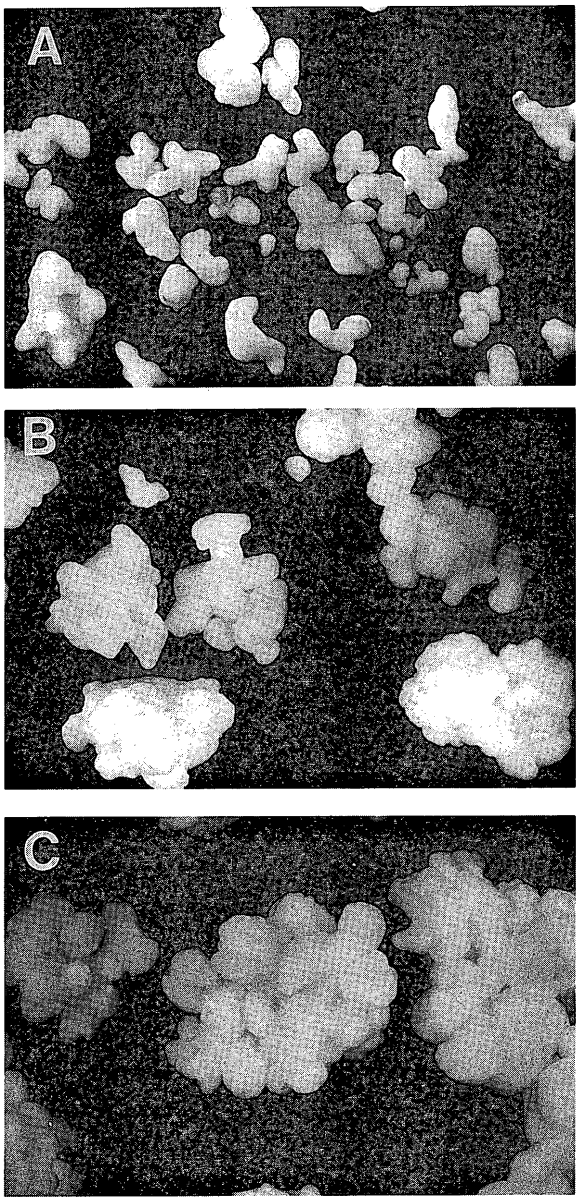

Fig. 3. Embryogenesis in liquid medium by shaking rotationally.
A: Embryos 1 to 2 mm in length
B: Embryos 2 to 4 mm in length
C: Embryos over 4 mm in length

Table 4. Effect of BAP concentrations on number of isolated embyros.

	BAP concentration (μM)				
	0.0	0.01	0.1	1.0	10.0
Number of embryos (1 to 2 mm)	30.0bc	43.9c	21.9ab	11.9a	28.1bc
Number of embryos (2 to 4 mm)	11.2c	12.9c	8.1bc	1.9ab	1.3a

All mediums contained 0 to 10 μM GA3 and 60g/l sucrose. Difference letters reresent significant differences, according to Duncan's multiple range test at 5% level.

Table 5. Effect of GA3 concentrations on number of isolated embryos.

	GA3 concentration (μM)				
	0.0	0.01	0.1	1.0	10.0
Number of embryos (1 to 2 mm)	19.1a	23.0ab	24.3ab	25.5ab	37.4b
Number of embryos (2 to 4 mm)	7.2	5.7	5.2	8.5	8.5

All mediums contained 0 to 10 μM BAP and 60g/l sucrose. Difference letters represent significant differences, according to Duncan's multiple range test at 5% level.

callus, therefore, was achieved through shaking rotationally in a liquid medium supplemented with 60 g/l sucrose, 0.01 μM BAP and 10.0 μM GA3.

The multiplication rate of roses was low (2.0 to 3.0 times over four weeks) according to Curie et al.[1]. Over 40 embryos were isolated from embryogenic callus 3 mm in diameter for six weeks (Table 4). If a plantlet develops from an embryo as Noriega and Sondahl[6] and Rout et al.[7] maintain, this procedure will make an efficient propagation method.

Matthew et al.[5] reported plant regeneration from protoplast via embryogenesis. We believe firmly that the embryogenic callus in this study is suitable for use in the protoplast system.

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バラ‘バルカロール’からの体細胞胚形成

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(1996年7月18日受理)

要　　約

茎頂組織を10.0μM BAPと1.0μM GA3を添加したMS培地で培養した結果，茎頂髄組織あるいは葉原基由来のEmbryogenic callusが誘導された。Embryogenic callusを組織学的に観察した結果，柔組織のなかに球状胚や分裂組織が認められた。

Embryogenic callusはショ糖30 g/l，BAP 10.0μM，GA3 1.0μM，ゲランガム 2.0 g/lを添加したMS培地で増殖させることができた。Embryogenic callusからの体細胞胚形成はショ糖 60 g/l，BAP 10.0μM，GA3 1.0μM，ゲランガム 2.0 g/lを添加したMS培地で促進された。MS培地にショ糖 60 g/l，BAP 0.01μM，GA3 10.0μMを添加した液体培地で回転振とう培養した結果，多数の体細胞胚が単離できた。

岐阜大農研報（61）:25－30, 1996