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Effects of Gelling Agents on Adventitious Organ Induction and Callus Growth in Some Plant Species

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Summary

Using organ segments and callus in various plant species, effects of gelling agents on adventitious organ induction and callus growth were examined. Bulblet induction in bulb-scale segments of *Lilium longiflorum,* adventitious bud induction from stem segments of *Torenia fournieri,* and bud induction from leaf segments of *Perilla frutescens* and *Centaurea cyanus* were promoted by Gelrite. Agarose and Bacto-agar were shown moderate effects, and agar was shown suppressing effects on adventitious organ induction. Callus growth of *L. longiflorum* and *Callistephus chinensis* were also stimulated by Gelrite. Therefore, Gelrite is most favourable gelling agents on adventitious organ induction and callus growth in plant species examined.

Key words: adventitious organ induction, callus growth, gelling agents, Gelrite

Introduction

In plant tissue culture, studies on adventitious organ differentiation, callus induction and protoplast culture were performed using solid medium. Agar, Bactor-agar, agarose or Gelrite were used as gelling agents, and kinds of gelling agents was affected adventitious bud induction, somatic embryogenesis, pollen embryogenesis[4, 12, 13] and protoplast division[6]. Thus we tried to examine the effects of gelling agents on adventitious organ induction and callus growth.

Materials and Methods

Materials

In adventitious bulblet induction, bulb-scale segments of *Lilium longiflorum* Thunb. were used as we previously described[2]. In adventitious bud induction, stem segments (5 mm in length) of *Torenia fournieri* Lind., leaf discs (1 cm in diameter) of *Perilla frutescens* Britton[8] and *Centaurea cyanus* L. were used as the explants. All of plant materials were grown on vermicurite for 2 months.

For lily callus induction, bulb-scale segments of *L. longiflorum* were cultured on the medium containing mineral salts of Murashige and Skoog[5] (MS medium), 4 % sucrose, 1

μM of naphthaleneacetic acid (NAA) and 1 μM of benzyladenine (BA). In the case of *Callistephus chinensis* Nees, hypocotyl segments (1 cm in length) were cultured on the MS medium with 3 % sucrose, 10 μM of NAA and 10 μM of BA.

Adventitious organ induction

For adventitious organ induction, 0.1 μM of NAA and 1 μM of BA in lily bulb-scale segments, 0.5 μM of BA in *Torenia* stem segments, 1 μM of BA in *Perilla* leaf discs and 1 μM of NAA and 0.1 μM of BA in *Centaurea* leaf discs were added to the MS medium with 3 % sucrose, as control medium.

As the stimulating treatments of adventitious organ induction, 1 or 10 μM of calcium ionophore A23187 (Calbiochem, USA) was added to the medium, or the explants were treated with N_2 stream for 30 or 60 min just after the excision of explants (anaerobic treatment).

The cultures were maintained under dark condition (for callus growth) or 16 hr long day photoperiod (for adventitious organ induction) and constant temperature at 25±2 °C.

Gelling agents

As gelling agents, 0.8 % agar (Wako, Japan), 0.8 % Bacto-agar (Difco, USA), 0.4 % agarose (type II, Sigma, USA) and 0.25 % Gelrite (Merck, USA) were used.

Results and Discussion

Adventitious organ induction

As shown in Table 1, adventitious bulblet induction in lily bulb-scale segments was promoted by application of A23187 and anaerobic treatment. When agar was used as gelling agents, the suppressing effects were shown. The most promotive results were obtained by Gelrite.

As we previously reported[2], the promotive effects of anaerobic treatment on bulblet induction was clear and this effects were further stimulated by use of Gelrite (Table 1). The application of calcium ionophore A23187 also promoted bulblet induction. This chemical increased intracellular Ca^{2+} levels, and we described that the promotion of bulblet

Table 1. Effects of gelling agents on bulblet induction in bulb-scale segments of *Lilium longiflorum*.

Gelling agent	No. of bulblets per explant		
	Control	+ A23187 (1 μM)	N_2 treatment (1 hr)
Agar (0.8 %)	1.6	4.8	4.4
Bacto-agar (0.8 %)	2.4	6.0	5.4
Agarose (0.4 %)	2.8	6.6	5.6
Gelrite (0.25 %)	3.2	7.6	6.2

The bulb-scale segments were cultured for 3 weeks on MS medium with 3 % sucrose, 0.1 μM of NAA and BA, and with or without 1 μM of A23187. The anaerobic treatment with 100 % N_2 stream for 1 hr were applied to the explants just after the excision. Agar, Bacto-agar, agarose or Gelrite were added to the medium as gelling agent.

induction by phospholipid was due to increment of intracellular Ca^{2+} [3].

In the case of adventitious bud induction, number of buds formed in the explants increased in order of agar < Bacto-agar < agarose < Gelrite (Table 2, 3, 4).

Adventitious bud initiation in *Torenia* stem segments was promoted by some chemicals

Table 2. Effects of gelling agents on adventitious bud induction in *Torenia* stem segments.

Gelling agent	No. of buds per explant		
	Control	+ A23187 (10 μM)	N_2 treatment (30 min)
Agar (0.8 %)	12.8	14.2	22.2
Bacto-agar (0.8 %)	20.1	23.4	24.1
Agarose (0.4 %)	22.2	24.0	26.2
Gelrite (0.25 %)	30.7	35.1	32.6

The stem segments were cultured for 2 weeks on MS medium with 3 % sucrose and 0.5 μM of BA, and with or without A23187. The anaerobic treatment with 100 % N_2 stream for 30 min was applied to the explants just after the excision. Agar, Bacto-agar, agarose or Gelrite was added to the medium as gelling agent.

Table 3. Effects of gelling agents on adventitious bud induction in *Perilla* leaf discs.

Gelling agent	No. of buds per explant		
	Control	+ A23187 (10 μM)	N_2 treatment (30 min)
Agar (0.8 %)	3.2	2.8	3.8
Bacto-agar (0.8 %)	4.0	4.6	4.9
Agarose (0.4 %)	4.2	4.4	4.6
Gelrite (0.25 %)	6.4	7.4	8.6

The leaf discs were cultured for 4 weeks on MS medium with 3 % sucrose and 1 μM of BA, and with or without A23187. The anaerobic treatment with 100 % N_2 stream for 30 min was applied to the explants just after the excision. Agar, Bacto-agar, agarose or Gelrite were added to the medium as gelling agent.

Table 4. Effects of gelling agents on adventitious bud induction in *Centaurea* leaf discs.

Gelling agent	No. of buds per explant		
	Control	+ A23187 (10 μM)	N_2 treatment (30 min)
Agar (0.8 %)	2.2	2.6	3.2
Bacto-agar (0.8 %)	3.8	4.4	3.8
Agarose (0.4 %)	3.6	4.4	3.6
Gelrite (0.25 %)	5.4	7.6	7.2

The leaf discs were cultured for 4 weeks on MS medium with 3 % sucrose, 1 μM of NAA and 0.1 μM of BA, and with or without A23187. The anaerobic treatment with 100 % N_2 stream for 30 min was applied to the explants just after the excision. Agar, Bacto-agar, agarose or Gelrite were added to the medium as gelling agent.

Table 5. Effects of gelling agents on growth in *Lilium* and *Callistephus* callus.

Gelling agent	Growth rate (%)	
	Lilium	*Callistephus*
Agar (0.8 %)	420	440
Bacto-agar (0.8 %)	650	570
Agarose (0.4 %)	480	600
Gelrite (0.25 %)	860	920

Callus of *Lilium* was cultured for 8 weeks on MS medium with 3 % sucrose, 1 μM of NAA and 1 μM of BA. Callus of *Callistephus* was cultured for 4 weeks on MS medium with 3 % sucrose, 10 μM of NAA and 10 μM of BA. Agar, Bacto-agar, agarose or Gelrite was added to the medium as gelling agent. The increments in fresh weights were measured and growth rates were calculated.

and treatments such as cyclic AMP[1, 10], calcium ionophore A23187[11], traumatic acid[10], additional wounding[7] and anaerobic treatment[9]. Changes of gelling agents from agar to Gelrite also stimulatively in this material (Table 2). Some micro elements of Gelrite seemed to be affected bud induction.

In the case of *Perilla* leaf discs, adventitious bud was induced by addition of BA to culture medium[8]. Application of A23187 was not effective in agar medium, but promotive effects were shown in Bacto-agar, agarose and Gelrite medium (Table 3). The anaerobic treatment also promotively on bud induction and Gelrite was further stimulated this response (Table 3).

In *Centaurea,* adventitious bud initiation has not been reported. In this material, application of NAA was essential for bud induction, and the induction was promoted by Gelrite medium, application of A23187 or anaerobic treatment (Table 4).

Callus growth

Effects of gelling agents on callus growth were examined using lily and *Callistephus* calluses. As shown in Table 5, callus growth were promoted by Gelrite medium. Therefore, Gelrite was also effective on cell division as well as adventitious organ induction.

Gelrite was purified from oligosaccharides released from *Pseudomonas elodea,* and the major components were glucose, rhamnose and uronic acid. The stimulating effects of Gelrite was thought to be due to the micro elements, but its elements was unknown.

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各種植物の不定器官分化とカルス増殖に及ぼす培地固化剤の影響

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摘　　要

植物の組織・細胞培養においては，固形培地あるいは液体培地を使用する．固形培地の固化剤としては各種のゲル化剤が用いられているがそれらの不定器官分化や細胞増殖に対する影響については全く報告がない．そこで，鉄砲ユリの球根分化，トレニアの茎切片や青ジソとヤグルマギクの葉切片における不定芽分化，鉄砲ユリとアスターのカルス増殖に対する培地固化剤の影響について検討した．固化剤としては，寒天，バクトアガー，アガロース及びゲルライトを使用した．その結果，不定器官分化，カルス増殖の双方ともにゲルライトの使用が最も良い結果を示した．寒天では分化及び増殖のいずれもが若干抑制され，アガロース及びバクトアガーでは中間の結果であった．従って，今回調べた植物種ではゲルライトの使用が最も好ましいと結論された．