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Liquid Culture and Short Induction Period Enhanced Somatic Embryogenesis in Cucumber (*Cucumis sativus* L.)

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Transformation technology is important for analyzing newly isolated genes or producing new plant varieties. An efficient transformation system requires optimal culture conditions for Subsequent plant regeneration. Selection of transformed calli in liquid medium is beneficial in reducing the frequency of escape[1-3] and chimeric plants[1-2]. Cucumber (*Cucumis sativus* L.) is an important vegetable crop in Japan. Genetic transformation of this species is desirable for the development of new varieties that are resistant to pests and diseases. Regeneration systems in cucumber have been reported[4-7] over the last few years. These reports included regeneration systems using liquid medium[6,7], but these culture systems employed solid-state media during the initial culture phase. Recently, it was reported that embryogenesis using a liquid medium from initial culture in sunflower[8] and melon[9] enhanced regeneration frequency compared to previous reports which employed a solid medium during all culture phases. In this report, we present evidence that a combination of a liquid medium and a brief induction period of one week enhanced somatic embryogenesis in cucumber.

Peeled cucumber seeds (cv. Shinhokusei No. 1, TOKIWA KENKYU NOUJO CO. Ltd., Japan) were sterilized and cultured on Murashige and Skoog (MS) medium[10] gelled with 0.8% agar, for 1 day at 25°C, in the dark. Semi-opened cotyledons were cut into four pieces and cultured on MS medium containing 1-naphthaleneacetic acid (NAA; 1 mg/*l*), 2, 4-dichlorophenoxyacetic acid (2, 4-D; 1 mg/*l*) and benzyladenine (BA; 0.5 mg/*l*) for induction of somatic embryos[4]. Medium state (solid or liquid) and various induction periods (1, 2, 3, or 4 weeks) of somatic embryos were investigated (**Table 1**). Liquid cultures were placed on a rotary shaker at 60 r.p.m., and the medium changed at one week interval. All cultures were incubated at 25°C under a 16 hr photoperiod (*ca.* 2000 lux). After four weeks from initial culture, induced somatic embryos from all treatments were separated from explants and transferred onto hormone-free MS solid medium (0.8% agar) for germination. In a preliminary experiment, somatic embryos were germinated and elongated within 2 weeks after transferring onto MS agar medium. Hence, the number of transplanted somatic embryos and plantlets grown from somatic embryos were counted after 4 and 6 weeks, respectively, from initial culture. Each treatment consisted of 20 explants and replicated two times.

Table 1 summarizes the results of somatic embryos and plantlets obtained. Liquid medium or combinations of liquid and solid media induced somatic embryos and regenerated plantlets. The

Table 1. Effects of medium state and induction period on embryogenesis of cucumber (*Cucumis sativus* L.).

Culture period and medium state*1				Number of somatic embryos induced*2	Number of plantlets grown*3	Germination raito
1 W	2 W	3 W	4 W	(A)	(B)	(B/A×100)
CEL	MSL	MSL	MSL	67.5±12.5	13.0±1.0	19.3
CEL	CEL	MSL	MSL	69.0±7.0	2.5±0.5	3.6
CEL	CEL	CEL	MSL	36.5±10.5	1.5±1.5	4.1
CEL	CEL	CEL	CEL	40.5±6.5	0.5±0.5	1.2
CEA	MSL	MSL	MSL	19.5±0.5	2.5±0.5	12.8
CEA	CEL	MSL	MSL	22.5±5.5	2.0±0	8.9
CEA	CEL	CEL	MSL	25.0±2.0	1.0±0	4.0
CEA	CEL	CEL	CEL	12.5±1.5	1.5±0.5	12.0
CEA	MSA	MSL	MSL	5.5±1.5	2.0±0	36.4
CEA	CEA	MSL	MSL	16.5±7.5	1.5±1.5	9.1
CEA	CEA	CEL	MSL	27.0±1.0	1.0±0	3.7
CEA	CEA	CEL	CEL	14.0±4.0	1.0±1.0	7.1

Each treatment consisted of 20 explants and repeated two times. After 4 weeks culture, induced embryos were transferred to MS ager without phytohormone to enhance germination.

*1 CE: MS medium+1 mg/*l* NAA+1 mg/*l* 2, 4-D+0.5 mg/*l* BA
MS: MS medium without phytohormone
L: liquid medium
A: medium gelled with agar (0.8%)
1 W: first week, 2 W: second week, 3 W: third week, 4 W: fourth week

*2 Counted after 4 weeks from initial culture. The number is the mean of two experiments (mean ±S.D.).

*3 Counted 2 weeks after culture on MS agar medium. The number is the mean of two experiments ±deviation (mean ±S.D.).

highest number of embryos and plantlets were induced by liquid culture alone for 4 weeks. The number of embryos and plantlets induced were reduced remarkably when liquid medium and solid medium were combined. As induction period in liquid medium was increased, the number of somatic embryos and plantlets were gradually reduced. A short period of induction (1 week) combined with liquid-state media in all culture phases was the best culture condition; the mean numbers of induced somatic embryos and regenerated plantlets were 67.5 and 13.0 per 20 explants, respectively.

Liquid culture of sunflower[8] and melon[9] stimulated shoot organogenesis or embryogenesis effectively. In this study we also showed that liquid culture was superior to solid medium for induction of somatic embryogenesis in cucumber. It is not clear why the liquid culture promoted embryogenesis and subsequent plantlet differentiation more effectively in this species. However, one plausible explanation is that the liquid medium provided more efficient contact between explants and medium, hence explants could make better use of phytohormones and untrients contained in the medium.

After 1 week from initial culture, hemisphere-like structures appeared on the surface of explants. Somatic embryos at various stages from globular stage to germinated somatic embryos were also observed on the surface of explants after 3 to 4 weeks of culture.

Somatic embryos in liquid culture are generally released into the liquid medium[9,10]. In the present study, embryogenic calli or somatic embryos in liquid culture adhered to the explant (**Fig. 1-a**) and were not released into the medium. These somatic embryos could be easily detached

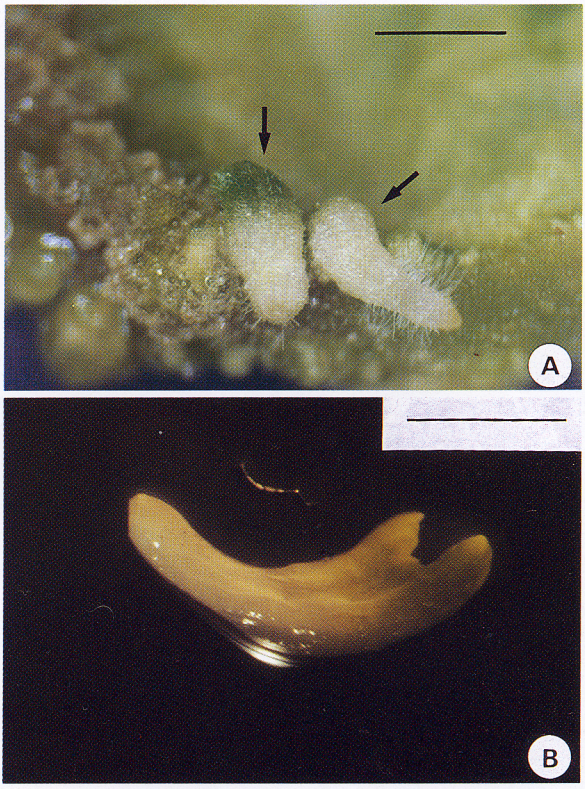

Fig. 1 Somatic embryogenesis in liquid culture of cucumber,
A: Induced somatic embryo adhering to surface of explant.
Arrow; induced somatic embryo.
bar stands for 5 mm.
B: Somatic embryo at the torpedo stage.

from the explants with a pair of forceps. Somatic embryos separated from explants were transferred onto MS medium (0.8% agar) without any phytohormone (**Fig. 1-b**). About 1.2-36.4% of induced somatic embryos were able to germinate and elongate into shoots. Further studies on the germination of somatic embryos are required to establish an optimal culture system for the application of this technique in the regeneration of transformed cucumber plants.

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《和文要約》

液体培養と短期間の不定胚誘導によるキュウリ不定胚形成の促進

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キュウリの不定胚誘導に及ぼす不定胚誘導期間(1, 2, 3, 4 週間)と培地の形状(液体または固形)の影響について検討した．誘導される不定胚数及び植物体数は，培養全期間を液体培養で行った場合に最も多く，また不定胚誘導期間が短いほど得られる幼植物体数が多かった．