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Histological Identification of Somatic Embryogenesis from Excised Root Tissues of Spinach (*Spinacia oleracea* L.)

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In spinach (*Spinacia oleracea* L.) tissue culture, there are several studies on plantlet regeneration through different pathways via adventitious bud formation[1-3] or somatic embryogenesis[4,5] that occur in cell masses on the medium containing various kinds of auxins and gibberellic acid (GA_3). Sasaki *et al.*[1] and Satoh *et al.*[3] histologically observed the regenerating cell masses, and clarified that the spinach regenerants were derived from adventitious buds. However, there is no histological observation on somatic embryogenesis from cell masses of spinach. In this report, the histological study identified that the spinach regenerants obtained from root cultures were derived from somatic embryos.

Spinach (*Spinacia oleracea* L. cv. Jiromaru) seeds were aseptically sowed on the solid medium (pH 5.7) containing Murashige and Skoog's (MS) medium[6] with 20 g/*l* sucrose and 8 g/*l* agar. Cultures were kept at 25°C in the dark. For induction of cell masses, root segments 8 mm in length were excised from 10-day-old seedlings, and placed horizontally on a primary culture medium which contained MS inorganic salts, 30 g/*l* sucrose, 10 or 30 μM 1-naphthaleneacetic acid (NAA), 0 to 100

Table 1. Effect of NAA combined with GA_3 on cell mass and plantlet formation from excised root segments of spinach (*Spinacia oleracea* L.)*1.

Growth regulators (μM)		No. of explants	% of explants forming cell mass*2	No. of cell masses producing plantlets	% of plantlet formation*3
NAA	GA_3				
10	0	50	100	0	0
10	0.01	50	100	5	10
10	0.1	50	100	5	10
10	1	50	100	17	34
10	10	50	100	11	22
10	100	50	100	5	10
30	0	50	100	0	0
30	0.01	50	100	2	4
30	0.1	50	100	8	16
30	1	50	100	13	26
30	10	50	100	16	32
30	100	50	100	14	28

*1 Values were calculated with data of 2 replicated experiments.
*2 (No. of explants forming cell mass/no. of explants inoculated) $\times$100
*3 (No. of cell masses producing plantlet/no. of cell masses inoculated) $\times$100

Fig. 1 Plantlets formed from root-derived cell masses of spinach.
Cell masses were induced on primary culture medium and transferred to the liquified secondary culture medium without growth regulators.

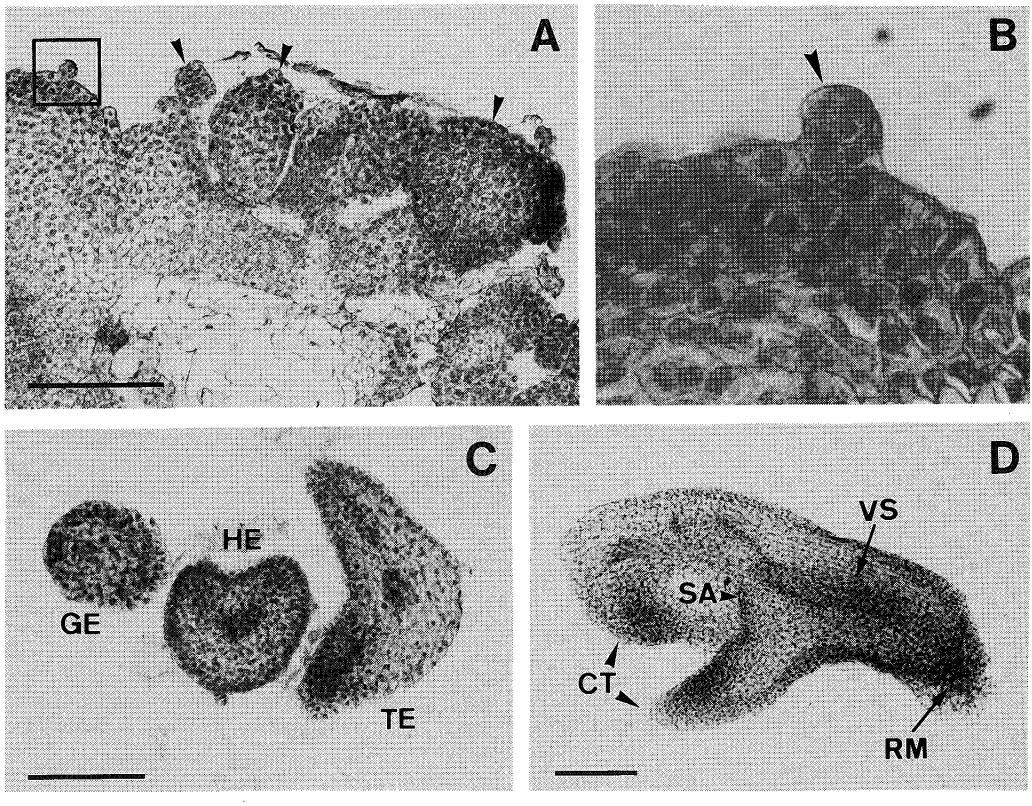

Fig. 2 Somatic embryogenesis in spinach.
A: Somatic embryos (arrowheads) producing on the cell mass after 4 weeks of primary culture; B: Enlarged view of a globular embryoid shown in open square area in A. Globular embryoid (arrowhead) forming from epidermal layers; C: Somatic embryos at globular (GE), heart-shaped (HE) and torpedo-shaped (TE) stages, forming on the cell mass after 3 days of secondary culture; D: A developing somatic embryo with cotyledon (CT), shoot apex (SA), vascular system (VS) and root meristem (RM) after 7 days of secondary culture. Bars represent 200 μm.

μM gibberellic acid (GA_3) and 8 g/l agar. After 4 weeks of culture, the cell masses formed from the explants were excised and transferred without subdivision for inducing somatic embryos to a solid or liquid medium (secondary culture medium) without growth regulator. The other elements in the secondary culture medium were the same as those in the primary culture medium. The frequencies of cell mass and plantlet formation were calculated after 4 weeks of primary culture and 2 weeks

of secondary culture, respectively. All histological specimens were made according to the usual paraffin method. Serial sections 8 μm thick were cut with a microtome and stained with hematoxylin.

The results of preliminary experiments showed that plantlets were formed from cell masses in spinach root cultures on the culture medium containing auxins, NAA in particular, combined with GA_3, but not formed on the culture medium with auxins alone. The effects of NAA and GA_3 on the cell mass and plantlet formation are presented in **Table 1**. The results indicate that GA_3 was necessary for spinach-plantlet regeneration, and the concentration of GA_3 slightly affected the frequency of plantlet formation.

When the cell masses obtained on the primary culture medium for 4 weeks were transferred to the liquified secondary culture medium, many individually isolated plantlets were formed (**Fig. 1**). Therefore, it seemed that the regenerants obtained were derived from somatic embryos. Sasaki *et al.*[1] and Satoh *et al.*[3] confirmed through the histological studies that the spinach regenerants formed on the hypocotyl-derived cell masses were derived from adventitious shoots. On the other hand, although there are several reports on spinach plant regeneration via somatic embryo formation[4,5], histological observation on somatic embryos derived from cell masses has not been performed. Consequently, to clarify the morphogenetic pathways of plantlets regenerated in this experiments, we histologically observed the root-derived cell masses. The results showed that many somatic embryos formed on the cell masses after 4 weeks of primary culture (**Fig. 2-A** and **B**), and that globular, heart-shaped, torpedo-shaped (or older) and mature embryos were recognized on the secondary culture medium (**Fig. 2-C** and **D**). From these results, many plantlets shown in **Fig. 1** were confirmed to be derived from somatic embryos.

References

1) Sasaki, H., Y. Saito, H. Yada, 1987. J. Hokkaido Univ. Educ., **38**: 1-9 (in Japanese).
2) Al-Khayri, J. M., F. H. Huang, T. E. Morelock, T. A. Busharar, E. E. Gbur, 1991. Plant Science, **78**: 121-127.
3) Satoh, T., T. Abe, T. Sasahara, 1992. Plant Tissue Culture Letters, **9**: 176-183.
4) Mutoh, M., T. Ichihashi, K. Sugimoto, 1992. Misc. Publ. Inst. Agrobiol. Resour., **4**: 137-161 (in Japanese).
5) Xiao, X., M. Branchard, 1993. Plant Cell Reports, **13**: 69-71.
6) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.

《和文要約》

ホウレンソウ根組織片における不定胚形成の組織学的同定

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ホウレンソウの芽生えから取り出した根組織片をNAAとGA_3とを各種濃度で組み合わせて添加した初代培地に置床し，培養4週間後に生長調節物質無添加の二次培地へ移植すると，小植物体が多数形成された．これらの再生した小植物体は，不定胚に由来することが明らかになった．