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# Improved Culture Conditions for *in vitro* Clonal Propagation of Scallion (*Allium chinense* G. DON)

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A modified formulation of nitrogen supplements ($NH_4^+/NO_3^-=1/5$) in Schenk and Hildebrandt (SH) medium containing 10 $\mu$M IAA and 5-10 $\mu$M benzylaminopurine (BAP) improved multiple shoot formation from shoot tip explants of scallion (*Allium chinense* G. DON). Multiple shoots were also induced from callus on a modified SH medium containing 0~0.1 $\mu$M IAA and 10 $\mu$M BAP. Propagated shoots were rooted and the resulted plantlets transferred to the soil successfully.

Scallion (*Allium chinense* G. DON) has a spindle shaped bulb cultivated throughout much of the world. Planting stock is obtained through conventional vegetative propagation of field grown bulbs. The number of propagules obtained from one original bulb by the conventional method is limited. In addition, virus infection which may be accumulated in this material can cause reduction in both bulb size and final yield. There are reports telling that field grown scallions are mostly infected with viruses in Japan.[1] Introduction of virus free scallion in the cultivation increased in yield more than 30%.[1] Thus, the situation with scallion is the same as that with garlic (*Allium sativum*).[2,3] Therefore, clonal propagation of *Allium* species by tissue culture techniques has been attempted extensively.[2,3] *In vitro* propagation of scallion was also reported.[1,4] However, there were problems with the growth rate of explants and efficiency of regeneration. We here report improved culture conditions which give rapid clonal propagation of scallion through *in vitro* culture techniques.

## Materials and Methods

Scallions (cv. Rakuda) were obtained from local farms in Sanrihama, Fukui-ken, Japan and cultivated in a greenhouse of our laboratory. The bulbs were washed throughly in tap water, rinsed briefly with 70% ethanol and sterilized in NaOCl (1% as Cl) for 15 min. Shoot tip tissues, including the apex and leaf primordia located on the base of the bulb, were excised into 1-2 $mm^3$ sections and served as explants. Linsmaier and Skoog (LS),[5] Gamborg B 5 (B 5),[6] Nitsch[7] and Schenk and Hildebrandt (SH)[8] formulations were used to examine an effect of media. Routinely, a modified SH solid medium was used in experiments; 300 mg/$l$ of $(NH_4)_2HPO_4$ was used instead of 300 mg/$l$ of $NH_4H_2PO_4$. Gellan gum (0.2%) was used as a gelling agent of the medium. All media were sterilized by autoclaving. All explants and calli were cultured at 25°C in a photoperiod of 16 hr/day of fluorescent light (1,500 lux).

## Results and Discussion

*Direct multiple shoot formation from shoot tip explant*

Direct shoot formation was reported to be induced from about a half of shoot tip explants of scallion.[4] We first examined the effect of medium on shoot elongation to improve culture conditions. When shoot tip explants were inoculated on various solid media supplemented with 10 $\mu$M IAA and 10 $\mu$M BAP, the explants showed better growth of shoots on SH medium than on LS, Nitsch and B 5 media. Hence we used SH medium as a basal formulation in further experiments.

Shoot induction from shoot tip explants and subsequent multiple shoot formation in *Allium* species are affected by auxin and cytokinin.[3,4] Ohsawa *et al.* reported that 10 $\mu$M IAA and 10 $\mu$M BAP, or 10 $\mu$M BAP alone induced multiple shoots from shoot tip explants of scallion but NAA was less efficient than IAA.[4] In our experiment with SH basal formulation, 10 $\mu$M IAA and 5-10 $\mu$M BAP resulted in better multiple shoot formation; more than 4 shoots per explant were obtained after 4-week culture. However, other combinations of IAA (0, 1, 5, 10 and 50 $\mu$M) and BAP (1, 5, 10 $\mu$M) also gave multiple shoot formation, but they resulted in less number of shoots per explant.

When the ratio of $NH_4^+$ to $NO_3^-$ of SH medium containing 10 $\mu$M IAA and 10 $\mu$M BAP was modified (original ratio of SH medium, 1/10), the growth and number of shoots arisen from the shoot tip explant were enhanced at the 1/5 ratio of $NH_4^+/NO_3^-$ (**Fig. 1**). Therefore, the modified SH medium, in which $NH_4H_2PO_4$ was replaced with $(NH_4)_2HPO_4$ (300 mg/*l*, 2.3 mM), was used in further experiments. Dunstan and Short reported the modified B 5 medium (BDS medium) with 1/3.6 ratio of $NH_4^+/NO_3^-$ for callus growth of onion.[9] They also used the BDS medium successfully for shoot induction and development in cultures of onion[10] and leek.[11] Thus, the optimal ratio of $NH_4^+/NO_3^-$ seems to be ca. 1/5 for induction and development of multiple shoots in cultures of *Allium* species.

Multiple shoots obtained after 4-week culture of the explants on the modified SH medium containing 10 $\mu$M IAA and 10 $\mu$M BAP were divided into several pieces, each of which developed multiple shoots again on the same medium. Thus, shoots were propagated by repeating these procedures.

*Callus formation and regeneration of shoots from callus*

Callus was induced from shoot tip explants on the LS solid medium containing 10 $\mu$M 2,4-D and

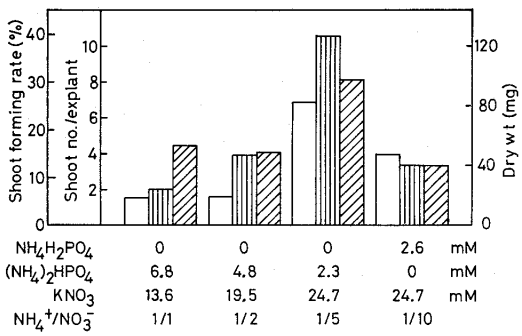


**Fig. 1.** Effects of $NH_4^+/NO_3^-$ ratio on multiple shoot formation. Various ratios of $NH_4^+/NO_3^-$ in SH medium were formulated by $NH_4H_2PO_4$, $(NH_4)_2HPO_4$ and $KNO_3$. Explants (about 30) were cultured for 4 weeks on the media containing 10 $\mu$M IAA and 10 $\mu$M BAPwith modified nitrogen compositions. □; shoot forming rate in %, [(number of explants with shoot)/(total number of explants)]×100. ▥; average number of shoots formed per explants which formed shoots. ▩; average dry weight/explants.

**Table 1.** Effect of IAA on shoot regeneration from callus.

| IAA ($\mu$M) | No. of callus used | No. of callus forming shoot | No. of shoots formed per culture[a] |
|---|---|---|---|
| 0 | 9 | 8 (89)[b] | 9.7 |
| 0.1 | 9 | 6 (67) | 11.4 |
| 10 | 9 | 3 (33) | 9.1 |

Calli cultured on the modified SH medium containing 10 $\mu$M 2,4-D and 10 $\mu$M BAP were transferred and cultured for 6 weeks on the modified SH media containing 10 $\mu$M BAP and IAA indicated.
[a] Average number of shoots per piece of callus from which shoot regeneration was observed.
[b] Figures in parentheses indicate % of callus regenerating shoot.

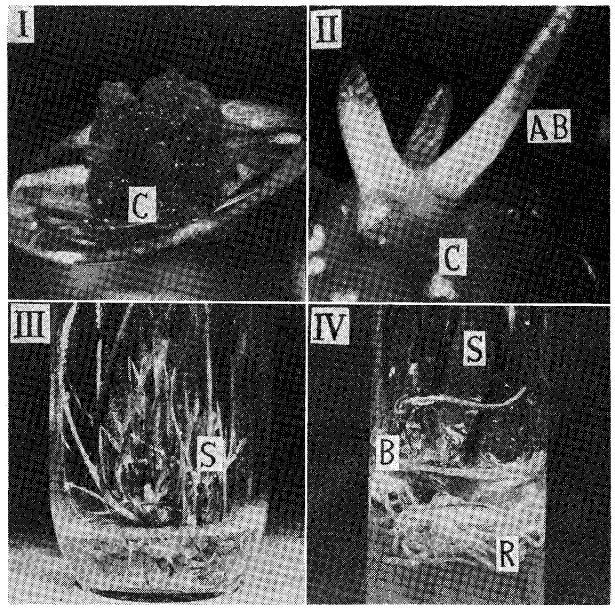


**Fig. 2.** Regeneration of plantlet from callus. I, callus on the modified SH medium containing 10 $\mu$M 2,4-D and 10 $\mu$M BAP; II, initial stage of adventitious shoot formation on the modified SH medium containing 0.1 $\mu$M IAA and 10 $\mu$M BAP; III, multiple shoots on the same medium as II; IV, root and bulb formation on the modified SH medium containing 1 $\mu$M IAA and 10 $\mu$M kinetin. AB, adventitious bud; B, bulb; C, callus; R, root; S, shoot.

10 $\mu$M BAP but no shoot formation was observed on this medium. This consisted with the previously reported observation.[4] Callus obtained was then transferred and subcultured on the modified SH medium containing 10 $\mu$M 2,4-D and 10 $\mu$M BAP, since callus proliferated on the modified SH medium better than on the LS medium. This is probably due to favorable nitrogen composition, as described previously.[9] Callus thus obtained was yellow and globular.

When callus was transferred to the modified SH medium containing 10 $\mu$M BAP in the presence or absence of IAA, shoots were regenerated as shown in **Table 1** and **Fig. 2**. Increasing concentrations of IAA reduced number of callus pieces with adventitious shoots, and optimal IAA concentration for shoot number was 0.1 $\mu$M among concentrations tested although multiple shoots were obtained on the medium without IAA (**Table 1**). The result obtained here seems to be mostly similar to those with other *Allium* species.[3] Thus we could establish improved culture conditions for multiple shoot induction and development of scallion directly from shoot tip explants and through callus culture.

*Rooting of shoots and transplanting to the soil*

Roots were formed from a shoot transferred on the modified SH medium containing 1 $\mu$M NAA and 10 $\mu$M kinetin within 4 weeks. Regenerated plantlets were transplanted to small pots with soil successfully and produced bulbs. When regenerated plantlets were transferred onto the modified SH medium without any hormone, they grew well and then formed small bulbs within 4 weeks even in a culture vessel (**Fig. 2**). Characterization of *in vitro* propagated plants is now in progress.

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ラッキョウ (*Allium chinense* G. Don) の *in vitro* クローン増殖法における培養条件の検討

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ラッキョウ (*Allium chinense* G. Don) の茎頂組織の外植片から multiple shoot を効率よく得るため，その培養条件の改良を試みた．10$\mu$M IAA と 5～10 $\mu$M BAP (benzylaminopurine) を含み，窒素の形態を $NH_4^+/NO_3^-=1/5$ に変えた改変 Schenk and Hildebrandt (SH) 培地を用いると multiple shoot の形成を改善することができた．ラッキョウカルスからの multiple shoot の形成は，0～0.1 $\mu$M IAA と 10 $\mu$M BAP を含む改変 SH 培地が効果的であった．いずれの shoot からも発根培地で容易に根を誘導することができ，さらに得られた幼植物を圃場に移植し栽培するとりん茎の肥大が認められた．