Callus and Organ Formation in Tissue Cultures of Spinach (*Spinacia oleracea* L.)

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**Summary**

When hypocotyl segments of the spinach plant were cultured on medium containing IAA (15 mg/l) in the presence of GA, those cultivars in which the callus growth was profuse in terms of the concentrations of GA (10⁻⁷-10⁻⁴ M), e.g. ‘HOYO’, ‘TOKAI’ and ‘WAKAKUSA’, showed generally low bud-forming activity. Furthermore, in the cultivars which had relatively high bud-forming capacity on the medium containing IAA plus GA, e.g. ‘NIPPON’ and ‘TOKO’, callus proliferation of their hypocotyl tissue segments were low overall. Root formation derived from hypocotyl segments of ‘TOKO’ and ‘NIPPON’ were markedly enhanced by the simultaneous application of GA to the medium with IAA. While the effective concentrations of GA on bud formation of spinach hypocotyl segments were different among these cultivars, the bud formation was enhanced by the addition of GA at the concentrations ranging from 10⁻⁷ to 10⁻⁴ M, except in the case of ‘TOKAI’. Optimal concentrations of GA on the bud formation of ‘NIPPON’, ‘TOKO’ and ‘HOYO’ were 10⁻⁵, 10⁻⁶ and 10⁻⁴ M, respectively. The induction of bud formation of hypocotyl segments in the spinach plant may take place during a relatively early stage of the incubation.

**Introduction**

Spinach is a dioecious plant and open pollinated varieties are heterozygous. For developing F₁ varieties, homozygous parents are needed. However, it is very difficult to obtain homozygous lines of spinach(7). To preserve cultivars close to a homozygous line, and to employ techniques aimed at selection of a heat and disease resistant line, tissue cultures of the spinach plant may prove very useful. To date, only the callus formation(4,6) and suspension culture of the callus cells(3) have been introduced into the tissue cultures of spinach. In recent years adventitious bud formation has been achieved from excised tissues of spinach when cultured in media containing 5,6-C₁₂ IAA(8) or IAA plus GA (15). However, they failed to regenerate the plant. There are two problems regarding the tissue culture of spinach. Firstly, there is the difficulty in the techniques for the elimination of bacterial contamination on the seed surface. It would be desirable to use seed separated from the pericarp.

Furthermore, hypocotyl segments of spinach cultured on various media with auxins and cytokinins like NAA plus BA or 2,4-D plus kinetin produced only callus initiation, while adventitious bud formation scarcely occurred (14). The bud formation of the hypocotyl segments was stimulated by simultaneous application of IAA and GA(15), whereas application of GA to plant tissue cultures had no appreciable effect upon the stimulation of bud formation (1,13,16). In this investigation, we undertook to compare the callus and organ forming potential among the five cultivars of spinach and could obtain the flowering from the regenerated plants of the hypocotyl tissues cultured *in vitro*.

**Materials and Methods**

After removing the pericarp by hand, seeds
of *Spinacia oleracea* L. (cv. WAKAKUSA, NIPPON, TOKO, HOYO and TOKAI) were surface-sterilized in 0.5% sodium hypochlorite for 30 min. and washed with sterile distilled water. After that, they were planted on the basal media containing MS salts(9), 20 g/l sucrose and 7 g/l agar (pH 6.0±0.1). After a six–day incubation in the dark, hypocotyl segments were excised from the seedlings. Hypocotyl segments of about 6 mm in length were cultured on basal medium supplemented with various combinations of GA (10⁻⁷–10⁻⁴ M) and 15 mg/l IAA. For each medium tested there were 28 replicate explants and response was measured as described previously(15). All cultures were maintained at 25°C under continuous light of ca. 1,500 lx. (Toshiba White 20 W fluorescent lamps).

Rooted shoots were transplanted to medium containing 10 mg/l IBA.

**Results and Discussion**

1. **Callus Formation**
   Callus formation was observed in hypocotyl tissue segments of the five cultivars of spinach plants, but the percentages of callus formation and amount of callus growth were different according to the concentrations of GA added to 15 mg/l IAA medium.

   Namely, in both cultivars NIPPON and TOKAI, the growth of callus occurred on the medium containing 15 mg/l IAA alone, whereas the application of GA to the medium had no effect on the callus growth. At high concentrations of 10⁻⁵–10⁻⁴ M, addition of GA to the medium was rather inhibitory to callus forma-
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2. Adventitious root formation

When hypocotyl segments of spinach plant were cultured on medium containing 15 mg/l of IAA plus $10^{-7}$-$10^{-4}$ M of GA, root formation was observed in all media regardless of the presence of GA (Fig. 2). Namely, root formation in cv. WAKAKUSA occurred on the medium containing $10^{-7}$-$10^{-6}$ M of GA and on the medium without GA. Moreover, in both cultivars NIPPON and TOKO, root formation was also frequently induced by addition of $10^{-7}$-$10^{-6}$ M GA to the IAA medium. By contrast, the application of GA at $10^{-5}$ and $10^{-4}$ M enhanced root formation of cv. HOYO and TOKAI respectively, while their root-forming activities were relatively weak as compared with those of cv. WAKAKUSA. Although the inhibition of in vitro root formation by GA has been reported in many plant species (2, 5, 10), the results obtained were somewhat different depending on the plant materials (11, 12, 17). Therefore, the action of exogenous GA on root formation of the spinach plant may also be found to differ among the cultivars.

3. Adventitious bud formation

When hypocotyl segments of five cultivars
in spinach plants were cultured on medium containing various concentrations of GA supplemented with 15 mg/l IAA for 16 weeks (Fig. 3), the adventitious bud formation in cv. WAKAKUSA was obtained by the addition of $10^{-5}-10^{-6}$ M GA, and 20% of the explants formed buds (Fig. 4-A).

The bud formation of cv. NIPPON was promoted by the presence of GA at the concentrations from $10^{-7}$ to $10^{-8}$ M, and about 50 % of the explants formed buds (Fig. 4-B). Forty percentages of the explants of cv. TOKO also formed buds on medium containing $10^{-6}$ M GA (Fig. 4-C). Furthermore, the bud formation of cv. HOYO was stimulated by the presence of $10^{-4}$ M GA (Fig. 4-D). On the contrary, bud formation of cv. TOKAI was scarcely observed, regardless of the presence of GA. Thus, effect of exogenous GA on adventitious bud formation of hypocotyl segments was different depending on the cultivars of spinach plants. Also, the optimal concentrations of GA were found to differ among the cultivars.

Namely, the optimal concentrations of GA on the bud formation of cv. NIPPON, TOKO and HOYO were $10^{-5}$, $10^{-6}$ and $10^{-4}$ M respectively. On the other hand, the bud formation of both cultivars NIPPON and WAKAKUSA were initiated after about 5 or 6 weeks of incubation. The percentages of bud formation were increased until about 7 to 8 weeks after the incubation, as shown in Fig. 5.

Recently, Mii et al. (8) reported that application for a short term (20 days) of 5,6-Cl$_2$ IAA greatly stimulated bud formation of hypocotyl tissues in the spinach plant. These results imply that the induction of bud formation takes place from the hypocotyl tissue segments in a relatively early stage of the incubation.

Rooted shoots obtained from these tissues cultured (Fig. 6) were transplanted to medium containing 10 mg/l of IBA under continuous light conditions, and exhibited flowering at 6 weeks after the transplanting (Fig. 7). As shown above, the cultivars which responded to GA with callus proliferation showed low bud-forming activity in the hypocotyl tissue culture. However, in the cultivars in which relatively high bud-forming capacity was observed, such as cv. NIPPON and TOKO, callus proliferation of the tissue segments was reduced under the presence of GA.

From these results, the effect of GA on callus growth of hypocotyl tissues of spinach was found to be different depending on the cultivars. The cultured hypocotyl tissue seems to have produced a satisfactory result as to bud formation when callus proliferation was repressed by the addition of GA.

Literature Cited

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組織培養におけるホウレンソウ（*Spinacia oleracea* L.）のカルス及び器官形成

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摘 要

ホウレンソウ胚軸切片をIAA（15 mg/l）とGA（10^-7 10^-4 M）を含む培地で培養した場合、GA添加により旺盛なカルス増殖を示す品種‘豊葉’、‘東海’、‘若草’の不定芽形成は一般に低かった。また、カルス増殖の少ない品種‘日本’、‘東湖’の不定芽形成は比較的高かった。不定根形成は‘東湖’、‘日本’及び‘若草’品種で高い形成率を示し、‘日本’、‘東湖’及び‘東海’における不定根形成がGA添加により促進された。不定芽形成に対するGAの効果は品種により異なったが、‘東海’を除く4品種の不定芽形成は10^-7-10^-4 M濃度のGA添加により促進された。得られた不定芽組織から幼植物体を再生することが出来た。