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Direct Induction of Adventitious Buds in *Torenia* Seedlings

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When organ segments were used as explants, the bud initiation was usually induced by cytokinin alone as reported in *Torenia*[1], *Perilla*[2] and *Rudbeckia*[3]. In long stem segments of *Torenia*, adventitious buds were formed in the marginal regions, namely 5 mm from the cut ends of explants, and the additional wounding caused significant increase in the number of buds[4]. Therefore, the wounding was thought to play some role in organogenesis. To examine the effects of wounding on adventitious bud initiation, development of experimental systems for bud initiation from non-wounded explants seemed to be required. Thus, we tried to examine the effects of cytokinin on adventitious bud induction directly from intact *Torenia* seedlings.

Although exgenously applied concentration of phytohormone had attracted the researchers, attention in many previous reports, incorporated concentrations of phytohormone were thought to be important for physiological responses. Therefore, incorporated cytokinin levels in cultivated seedlings were also examined.

Seeds of *Torenia fournieri* Lind. were sterilized with NaOCl (available choline 1%), washed with sterilized water and sown in a medium containing Murashige and Skoog's mineral salts and vitamines[5], 3% sucrose and 0.3% Gelrite (Merck) (MS medium). Some cytokinins such as benzyladenine (BA), kinetin (K), or zeatin (Z) was added to the MS medium. Zeatin was filter-sterilized, and applied to the medium. Cultures were maintained under 16-h long-day photoperiods (at 500 μmol $\cdot$ m^{-2} $\cdot$ s^{-1}) at a constant temperature of 25°C. After 5 weeks of culture, the percentages of seedlings with adventitious buds and the number of adventitious buds per seedling (total number of buds formed per total cultured seedlings) were observed. More than 100 seeds were observed in each treatment. All experiments were repeated at least 3 times and the standard errors were calculated.

Extraction and analysis of BA in the seedlings were performed as follows. The seedlings cultured for 5 weeks were homogenized with a chilled mortar and pestle in liquid nitrogen. The homogenates were soaked in 80% methanol with 10 mg/l of butylated-hydroxytoluene for 24 h at 4°C. The solution was filtrated through 4 layers of gauze and 2 layers of Miracloth (Calbiochem-Behring) and centrifuged at 15,000 $\times$ g for 30 min. The resultant supernatant was extracted twice with *n*-hexane and then twice with *n*-buthanol. The fraction of *n*-buthanol was taken to dryness (rotary vacuum-evaporated, 40°C) and then dissolved acidic water (pH 3.5). The sample was passed through cation exchange column (AG50W-X4; bed volume, 50 ml; Bio-Rad), the eluate was injected onto HPLC on Shimadzu shim-pack CLC-ODS and eluted with 1 mM tetra-butylammonium (pH 2.8) (solvent A) and CH_3CN (solvent B) at a flow rate of 1.45 ml $\cdot$ min^{-1} according to the following gradient program: 0 to 30 min., a linear gradient from 90% A plus 10% B to 20% A

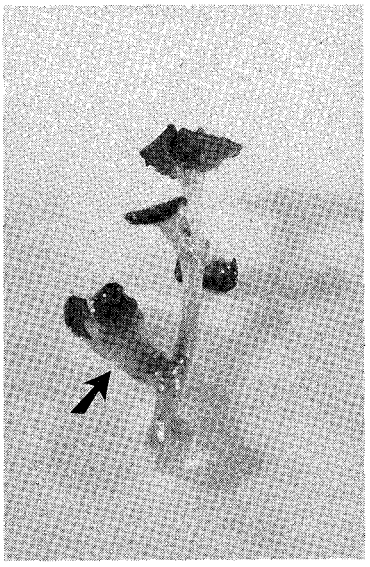

Fig. 1 Direct adventitious bud induction in *Torenia* seedlings.
Seeds of *T. fournieri* were sown to the MS medium with 10 μM of BA, and cultured for 5 weeks. Arrow indicates adventitious buds. The bar shows 1 mm.

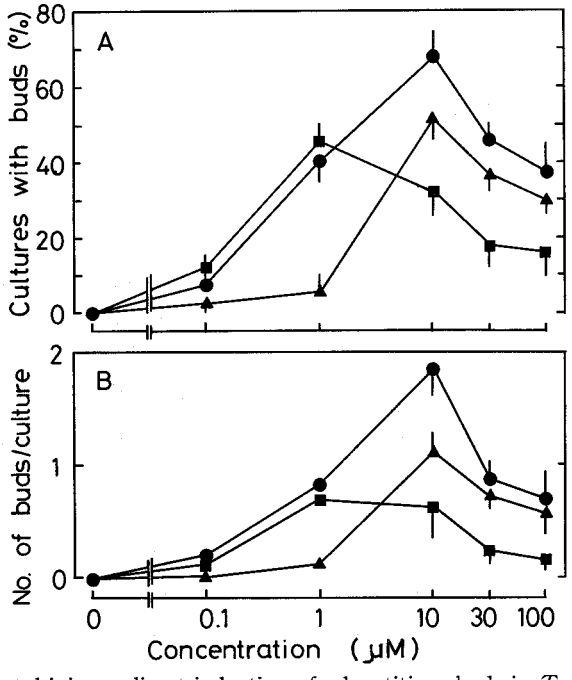

Fig. 2 Effect of cytokinin on direct induction of adventitious buds in *Torenia* seedlings.
Seeds of *T. fournieri* were sown to the MS medium with various concentrations of BA (●), kinetin (▲) or zeatin (■). After 5 weeks of culture, seedlings with adventitious buds (A) and the number of buds per total cultured seedling (B) were measured. For each treatment, at least 100 seeds were used. Experiments were repeated 3 times and the standard errors were calculated.

plus 80% B. More than 100 seeds were used in each treatment. All experiments were repeated at least 3 times and the standard errors were calculated.

When seedlings of *Torenia* were cultured on the medium with cytokinin, direct adventitious buds

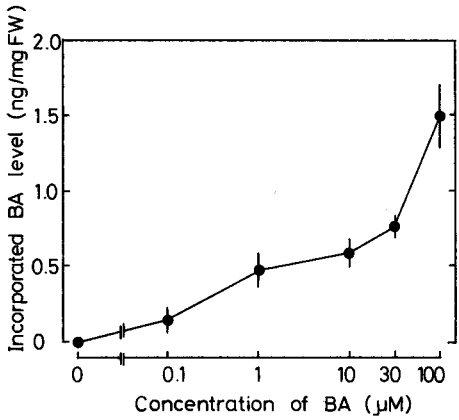

Fig. 3 Correlation between concentrations added to the medium and incorporated level of BA in *Torenia* seedlings.
Seeds of *T. fournieri* were sown to the MS medium with various concentrations of BA. After 5 weeks of culture, the seedlings were hervested and the incorporated BA levels were measured. For each treatment, at least 100 seeds were used. Experiments were repeated 3 times and the standard errors were calculated.

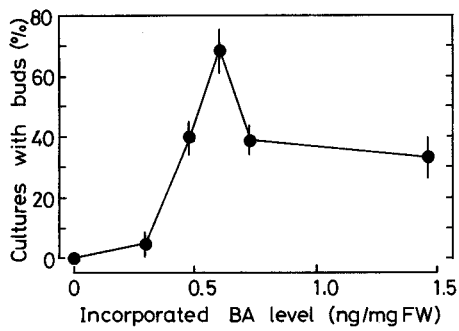

Fig. 4 Correlation between incorporated BA levels and direct adventitious bud induction in *Torenia* seedlings.
Seeds of *T. fournieri* were sown to the MS medium with various concentrations of BA. After 5 weeks of culture, seedlings with adventitious buds and BA levels were measured. For each treatment, at least 100 seeds were used. Experiments were repeated 3 times and the standard errors were calculated.

could be induced. Almost all hypocotyls of seedlings formed buds (**Fig. 1**). As shown in **Fig. 2-A**, buds were formed at 0.1 μM in BA or Z, not in K, and the best result in BA and K at 10 μM, and Z at 1 and 10 μM were obtained, respectively. Frequency of adventitious bud induction was highest at 10 μM of BA in all cases. Adventitious buds were also induced by cytokinin in *Torenia* stem segments[6], and similar responses to cytokinin were expressed by both materials. The number of adventitious buds per total cultivated seedlings was also increased by increasing the concentration of cytokinin to 10 μM (**Fig. 2-B**).

Next, we tried to examine the correlation between concentrations added to the medium and incorporated levels of BA in the seedlings. The levels of BA incorporated were gradually increased with increasing concentrations of BA in the medium (**Fig. 3**). Incorporated BA levels also closely correlated with bud induction in the seedlings (**Fig. 4**). The cytokinin levels were very limited in the highest bud-forming response, and the best result was obtained when the BA level was about 0.6 ng per mg fresh weight (**Fig. 4**).

The direct adventitious buds could be successfully attained in *Torenia* seedlings cultivated on the medium with cytokinin. This system provides a useful means for experiments on the effects of wounding on adventitious bud initiation.

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《和文要約》

トレニア実生からの直接不定芽誘導

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トレニア(*Torenia fournieri* Lind.)の種子をサイトカイニンを含む培地に播種したところ，実生の胚軸から直接不定芽を誘導することができた．サイトカイニン添加培地で育成した実生へのサイトカイニンの取り込み量を測定したところ，最も高い不定芽誘導反応をもたらすサイトカイニン取り込み量はきわめて限られた範囲にあった．