Propagation of Ornamental Cultivars of Foxglove (*Digitalis purpurea*) in vitro©

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Two ornamental cultivars of foxglove (*Digitalis purpurea* L.) were used as donor plants. For callus induction, cotyledon segments obtained in vitro were cultivated on one-half strength Murashige and Skoog medium. Callus formation was superior with 'Dwarf Foxy' in comparison to 'Sutton's Apricot'. The effect of NAA and BAP on adventitious bud formation from callus segments was tested. Benzylaminopurine was essential for adventitious bud formation from callus. Adventitious bud formation and subsequent shoot growth were vigorous on media supplemented 1.0 or 3.16 μM BAP. The best result obtained from 'Dwarf Foxy' on the medium containing 3.16 μM BAP supplemented with 1.0 μM NAA.

INTRODUCTION

Foxglove (*Digitalis purpurea* L.) is a biennial plant with soft, hairy, toothed, ovate, and lance-shaped leaves in a basal rosette. The life span of the plant is two seasons (except annual-type cultivars). This plant is native to Europe and widely cultivated, and it is a common garden escape and is naturalized in the northern part of Japan. Foxglove belongs to the figwort family (*Scrophulariaceae*) and the whole plant is toxic. It contains various cardiac glycosides, and it is used in modern medicine to increase the force of the systolic contractions and prolong duration of the diastolic phase in congestive heart failure. Digitalis drugs lower venous pressure in hypersensitive heart ailments, elevate blood pressure in a weak heart, act as a diuretic, and reduce edema. Several reports on callus production for medical use of this species have been published (Hagimori et al, 1983, 1984; Onisei et al, 1992), however, there were few reports on in vitro propagation of ornamental cultivars. This study reports the successful regeneration of multiple buds on callus induced from cotyledon segment of foxglove.

MATERIALS AND METHODS

Mature seeds of two foxglove cultivars, 'Dwarf Foxy' (Sakata Co. Ltd., Japan) and 'Sutton's Apricot' (Suttons Seeds, U.K.) were used. After sterilization by sodium hypochrorite solution (0.5% available chlorine), seeds were sown on phytohormone-free Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with one-half concentrations of macro salts in a petri dish (sterilized polystyrene dish, 9 cm in diameter). Three weeks after sowing (2 weeks after germination), cotyledon segments (ca 5 mm long) were taken from in vitro seedlings and five segments were placed on 30 ml fresh medium in a 100 ml Erlenmeyer flasks. Those were inoculated on one-half strength MS medium supplemented with different concentrations of BAP (1.0, 3.16, 10.0, or 31.6 μM) combined with NAA (0, 1.0, or 10.0 μM). All media were supplemented with 30 g·liter^{-1} sucrose and solidified with Gellan gum 3 g·liter^{-1}. The pH of the medium was adjusted to 5.3 before autoclaving. All experiments were carried out in 3 replications, each with 45 segments.

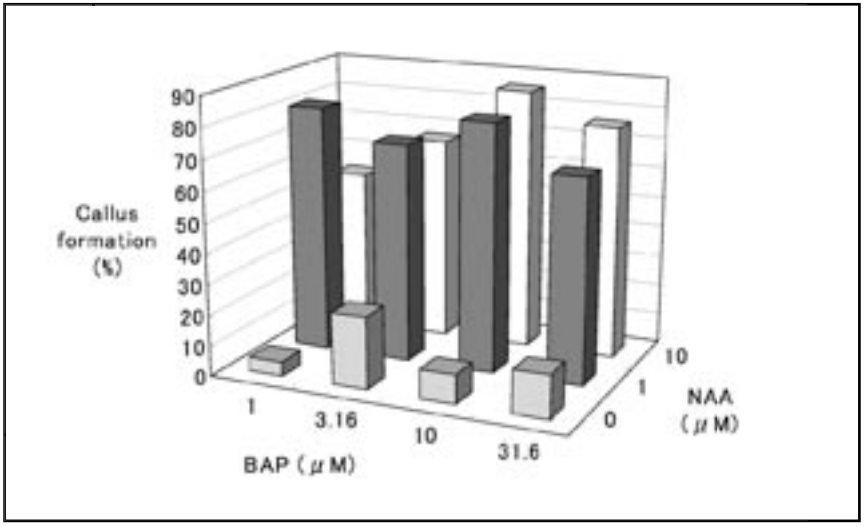

Figure 1. Effects of BAP and NAA for callus induction from cotyledon segments of *Digitalis purpurea* 'Dwarf Foxy'.

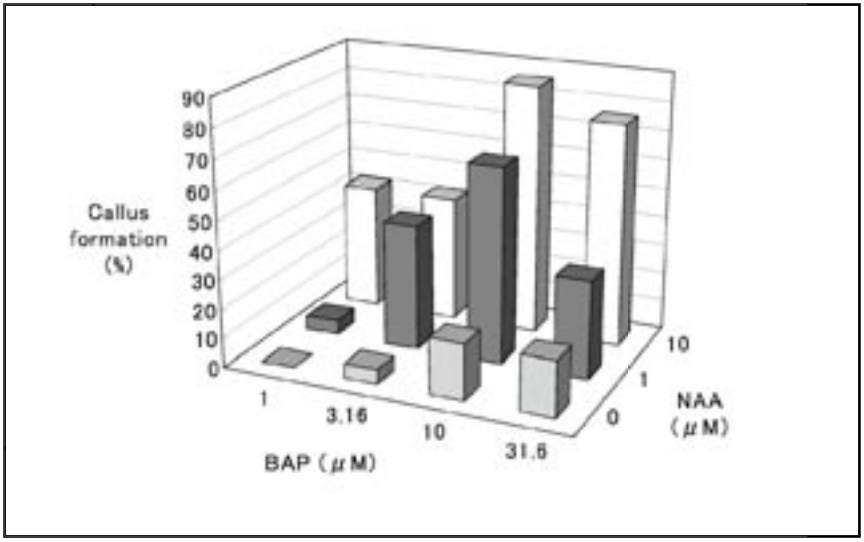

Figure 2. Effects of BAP and NAA for callus induction from cotyledon segments of *Digitalis purpurea* 'Sutton's Apricot'.

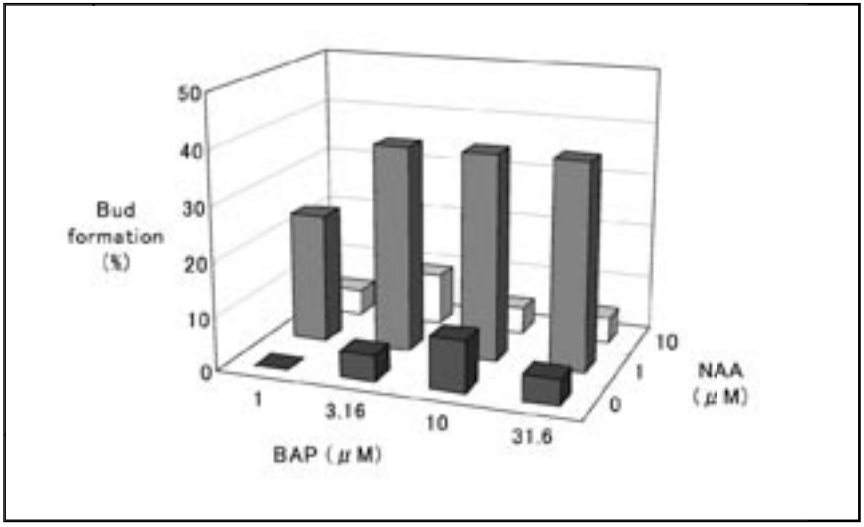

Figure 3. Effects of BAP and NAA for adventitious bud formation from callus of *Digitalis purpurea* 'Dwarf Foxy'.

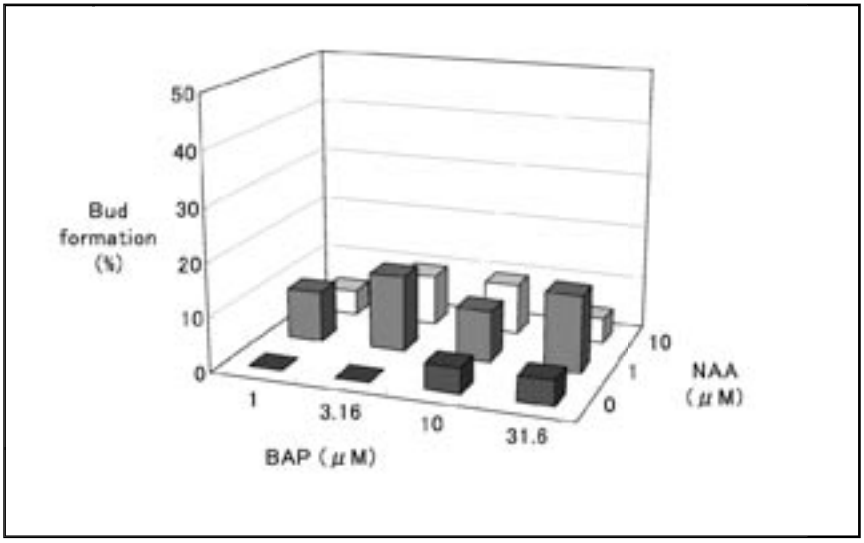

Figure 4. Effects of BAP and NAA for adventitious bud formation from callus of *Digitalis purpurea* 'Sutton's Apricot'.

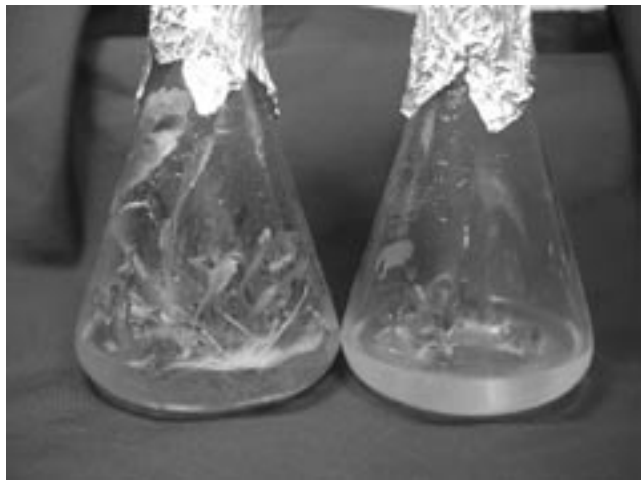

Figure 5. Growth of adventitious buds on callus derived from cotyledon segment of *Digitalis purpurea.* Callus, was cultured on one-half strength MS medium supplemented 3.16 μM BAP combined with 1.0 μM NAA. Left: 'Dwarf Foxy' Right: 'Suttons Apricot'.

After 4 weeks of culture, all induced calli were excised from explants, and were transplanted onto fresh one-half strength MS medium supplemented with the same concentrations of BAP combined with NAA in initial culture, or hormone-free one-half strength MS medium. After 6 weeks of callus culture, adventitious bud formation was evaluated. All experiments were carried out in 3 replications, each with 15 callus segments. All cultures were incubated at 22±1 °C under continuous illumination with florescent lamps at a photon flux density of 25 $\mu mol \cdot m^{2} \cdot s^{-1}$.

RESULTS AND DISCUSSION

Four to five days after cultivation, cotyledon segments began to form callus. Effects of NAA and BAP on initial callus formation are summarized in Fig. 1 ('Dwarf Foxy') and Fig. 2 ('Sutton's Apricot'). Callus formation was superior with 'Dwarf Foxy' in comparison to 'Sutton's Apricot'. Callus formation was stimulated in media supplemented with NAA, and was accelerated by increased NAA.

The results of plantlet regeneration from callus are summarized in Fig. 3 ('Dwarf Foxy') and Fig. 4 ('Sutton's Apricot'). On hormone-free medium, no formation of adventitious bud from calli occurred and those transplanted calli died shortly after inoculation (data not shown). After 3 weeks of callus culture, bud formation was observed on calli that were transplanted on the same medium as initial cultures. Bud formation rates from callus of 'Dwarf Foxy' were higher than those of 'Sutton's Apricot'. Subsequently shoot growth was also vigorous with 'Dwarf Foxy' (Fig. 5). Formation of adventitious buds and subsequent growth of shoots was vigorous on media that were supplemented with 1.0 or 3.16 μM BAP with 1.0 μM of NAA on both cultivars. Formation of adventitious buds on media supplemented with 10.0 or 31.6 μM BAP were also high but all buds on those media showed vitrification and those buds turned brown by the end of investigation (data not shown). Under

our experimental conditions, the best results with 'Dwarf Foxy' were obtained on medium containing 3.16 µM BAP supplemented with 1.0 µM NAA, for both shoot elongation and root formation from adventitious buds (Fig. 5).

Onisei et al (1992) observed a remarkable shoot proliferation on stem-tip culture of *D. purpurea* when the explants were transplanted on the same cytokinin-rich MS medium as initial culture (containing BAP 2 mg·liter^{-1}). Rucker (1982) reported that BAP concentrations above 10 mg·liter^{-1} or below 0.1 mg·liter^{-1} were insufficient or inhibiting for the induction of buds from leaf fragments of *D. purpurea*. In our research, BAP was also essential and effective for adventitious bud formation from callus derived from cotyledon.

Plantlet regeneration was not observed with 'Sutton's Apricot'. Further research is needed to improve methods for in vitro propagation of ornamental cultivars of foxgloves.

LITERATURE CITED

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