Tissue Culture of Male Sterile Sugi Cedar (*Cryptomeria japonica*) for the Solution of the Pollen Allergy Problem©

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INTRODUCTION

Sugi cedar (*Cryptomeria japonica* D. Don) is an important conifer tree for industrial plantation production in Japan. Forty five percent of man-made forests in Japan is comprised of sugi cedar. However, a pollen allergy problem caused by sugi cedar and hinoki cypress is currently serious. For the solution of pollinosis, production of non-pollen male sterile clones by tissue culture is considered as one option. A complete pollen sterile sugi cedar was found in Toyama prefecture, Japan (Taira et al., 1993). By the year 2007, more than 20 individual clones of pollen sterile sugi cedar have been found in Toyama, Niigata, Fukushima, Aomori, Kanagawa, and Ibaraki prefectures, Japan. Here we describe the screening of surface sterilization, initial culture, and subculture media for tissue culture of male sterile sugi cedar.

MATERIALS AND METHODS

Eleven original male sterile clones of sugi cedar and three male sterile hybrids produced by crossing between individuals which possessed sterile gene as hetero allele. Surface sterilization of leafy shoots was carried out using several chemicals including benzalkonium chloride, sodium hypochloride, ethyl alcohol, and hydrogen peroxide. For initial culture, ½ LP (Quorin and Lepoivre's medium) or CD (Campbell and Durzan medium) (both containing 5 $g \cdot L^{-1}$ activated charcoal) media were compared. Culture condition was at 25 °C constant temperature under a 16-h photoperiod of 70 $\mu M \cdot m^{-2} \cdot s^{-1}$ by fluorescent lamp.

RESULTS AND DISCUSSION

Sequential surface sterilization using 0.1% benalkonium chloride for 15~20 min., 1% sodium hypochroride for 10 min, 70% ethyl alcohol for 2 min, and 5% hydrogen peroxide for 10 min then washed well with sterile water was effective for eliminating microorganisms. Axillary buds were induced from 2-cm-long, dissected-shoot explants of male sterile sugi cedar clones, 'Toyama 1', 'Toyama 3', 'Shindai 5', 'Shindai 8', F1 'Hayatsuki 1', and F1 'Ryowa 6'. Among the cytokinins tested, zeatin was relatively effective for bud induction for all clones. More buds were induced in the medium containing zeatin than that containing BAP with male sterile clone 'Shindai 5'. Shoots were developed from buds on the medium 1/2 LP containing 5 $g \cdot L^{-1}$ activated charcoal. These materials will be used for micropropagation of male sterile sugi cedar in order to reduce its pollen in the air (Table 1; Fig. 1).

Table 1. The effects of different cytokinins on bud induction of *Cryptomeria japonica.*

Cytokinin (10 μM)	No. of induced buds / explant ±SE[x,y]
Zeatin	6.4 ± 0.9
BAP	4.8 ± 0.7

[x]Ten explants were used for each treatment.

[y]Cultured on 1/2 LP medium containing 0.03 μM NAA for 1 month.

Figure 1. Shoot elongation of *Cryptomeria japonica* in vitro.

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