Micropropagation of the Male Sterile 'Soushun' Japanese Cedar©

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INTRODUCTION

Japanese cedar [*Cryptomeria japonica* (L. fil.) D. Don] is one of the most important timber tree species in Japan. More than 10% of the Japanese population is suffering from Japanese cedar allergies and this is a serious problem in Japan (Goto et al., 2004). In order to reduce pollen production, plus trees with reduced male flowers or no-pollen tree have been selected. 'Soushun' is one of the male sterile Japanese cedar lines. There is no pollen in their male flowers. 'Soushun' is the most desirable line to reduce pollen production and is in high demand for new forestation. In general, Japanese cedar is clonally propagated by cutting, but cutting production is time consuming and it is difficult to establish a short-term propagation routine. In contrast, the micropropagation (tissue culture) technique needs smaller explants than the traditional methods and thus enables faster propagation. We are developing a micropropagation method for the male sterile Japanese cedar. In our method, we established sterilized rooted plants as the sterile scion stocks. They grow under in vitro conditions and the shoots obtained from them are rooted on agar medium. Rooted shoots are acclimatized in a greenhouse. Shoots are obtained from stock continuously in a 3–4 month cycle.

There are two problems in our micropropagation method for Japanese cedar. One is the low rooting rate. In the usual propagation methods, depending on lines, most of Japanese cedar plus tree lines represent high rooting rate, but in vitro shoots show low rooting rates and take a long time to root in spite of auxin treatment. Another problem is that acclimatized plants grow not vertically but horizontally. This is thought to be caused by horizontal growth of shoots of the sterile scion stocks. These two problems cause low yields of plants.

Tanaka et al. (2005) reported that CO_2 enrichment treatment promoted in vitro *Eucalyptus* plantlets rooting and growth. Quality of light also affects photosynthesis and other developmental and biochemical processes, but very little work has been done on the specific effect of light quality regulating growth and physiology under in vitro culture conditions (Lee et al., 2007).

We studied the effects of CO_2 enrichment and light quality for Japanese cedar rooting and growth of the sterile scion stocks under in vitro culture condition to establish an efficient clonal propagation method of Japanese cedar.

MATERIALS AND METHODS

Plant Materials. Current shoots were excised from 2-year old cuttings grown in a greenhouse and washed in running tap water containing detergents. The explants were surface disinfected with 70% ethanol for 1 min followed by a surface sterilization with 7.5% hydrogen peroxide containing Tween 80 for 15 min and rinsed three times with sterilized distilled water. The explants were transferred to initiation medium, modified Gresshoff and Doy (GD) solid medium (Sommer et al., 1975) supplemented with 5 μM benzyladenine, 2% sucrose, and 0.7% agar. The pH of the medium was adjusted to 5.7 before autoclaving. The shoots were maintained in ½ strength Woody Plant (WP) (Lloyd and McCown, 1980) solid medium with 2% sucrose and 0.7% agar. After 2 months of culture, the shoots were transferred to rooting medium, ¼ strength WP solid medium supplemented with 20 μM indolebutyric acid. After 6 weeks of culture, the rooted shoots were transferred into perlite containing ½ strength WP liquid medium with 2% sucrose. We defined the rooted plants as the sterile scion stock.

Growth of the Sterile Scion Stocks. The rooted shoots were transferred to perlite containing liquid medium. Culture vessels were placed under three kinds of light conditions: red (600–700 nm), mixture of 80% red and 20% blue (400–500 nm), and fluorescent light. Photon flux density was 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on red and mixture light, and 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on fluorescent light. Cultures were maintained at 25 °C with 16-h photoperiod and CO_2 concentration was elevated to 1000 ppm. After 3 months of culture, fresh weight and dry weight of shoots and roots were measured and the useful vigorous shoots elongated over 3 cm were counted.

Rooting Treatment. Three- to five-cm shoots were excised from the sterile scion stock and placed in glass culture vessels containing rooting medium. The caps of vessels had 2 openings (1 cm in diameter) and they were covered with filter membranes (Milli seal, Millipore, Tokyo, Japan). The culture conditions were the same as growth measurement treatment, but under ambient CO_2 concentration was used as control. For each treatment, 15 shoots were used and the experiments were replicated twice. After 6 weeks of rooting treatment, rooting rate was measured.

RESULTS

Growth of the Sterile Scion Stocks. Growth of the scion stocks is shown in Table 1. Under fluorescent light growth was the highest fresh weight and dry weight, although the number of elongated shoots over 3 cm was highest under fluorescent light. The number of vigorous shoots was highest under red light. The scion stocks grown under each light condition are shown in Fig. 1. The scion stocks grown under fluorescent light had a lot of shoots but were shorter. In contrast, the scion stocks grown under red and mixture light had less shoots but they elongated better.

Rooting Treatment. The rooting rates of 6-week cultures are shown in Table 2. Rooting rate was highest under red light with or without CO_2 enrichment. Fluorescent light showed the poorest rooting rate. There was no accelerative effect of CO_2 enrichment on rooting.

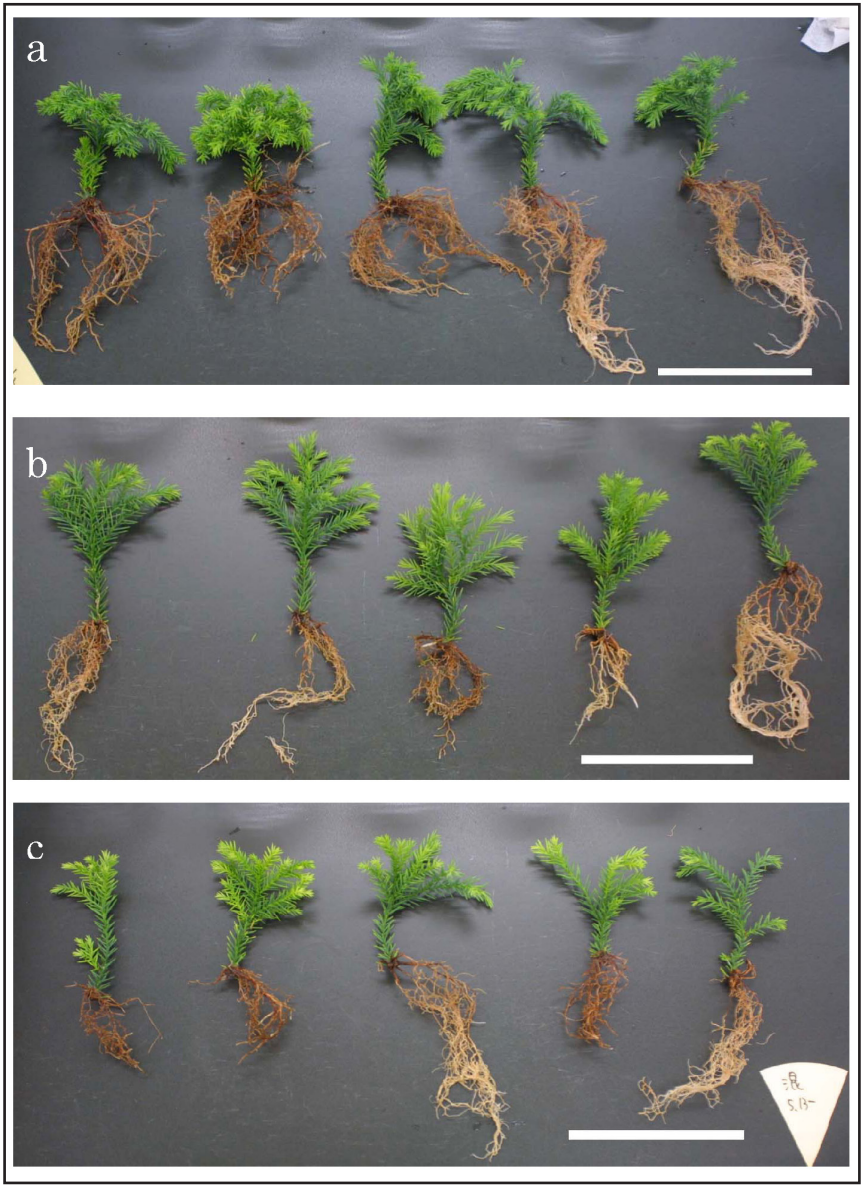

Figure 1. Morphological traits of the sterile scion stocks grown under fluorescent light (100 µmol·m^{-2}·s^{-1}) is shown in (a), red light (50 µmol·m^{-2}·s^{-1}) in (b), mixture light (50 µmol·m^{-2}·s^{-1}) in (c). Scale bar = 10 cm.

Table 1. Effects of light condition on the 3 months growth of the sterile scion stock.

Light type	Fresh weight (g)		Dry weight (g)		Total elongated shoots (no.)	Vigorous shoots (no.)
	Top	Root	Top	Root		
White (100 μmol·m^{-2}·s^{-1})	3.44 a	2.25 a	0.84 a	0.28 a	8.38 a	1.47 b
Red (50 μmol·m^{-2}·s^{-1})	2.13 b	1.21 b	0.44 b	0.13 b	6.35 b	2.1 a
Blue + red (50 μmol·m^{-2}·s^{-1})	1.36 c	0.74 b	0.29 c	0.09 b	5.7 b	1.8 ab

Table 2. Effects of light conditions and CO_2 concentration on rooting.

	Rooting rate (%)		
CO_2 concentration	Fluorescent (100 μmol·m^{-2}·s^{-1})	Red (50 μmol·m^{-2}·s^{-1})	Blue + red (50 μmol·m^{-2}·s^{-1})
Control	50	96.7	70.0
1000 ppm	43.3	90.0	80.0

DISCUSSION

The yields of vigorous shoots were largest on the scion stocks grown under red light, but the number of shoots was low (2.1/plant). Therefore we must establish more efficient light conditions to yield a higher number of vigorous shoots.

On rooting treatment, red light also showed the highest rooting rate. Therefore application of red light is thought to be efficient for rooting.

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