Tissue Culture of *Salix pet-susu*: A Fast-Growing Biomass Resources Tree©

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INTRODUCTION

Willows (*Salix* species) are a fast-growing species for effective forest biomass production. They are widely distributed in the world but occur most abundantly in the cooler parts of the Northern Hemisphere. Among many willow species, *S. pet-susu* Kimura is one of the fastest growing species in the Hokkaido Island of Japan. For multiuse of all components of wood, components like hemicellulose or lignin in addition to cellulose can also be used for many functional materials. We have attempted to assess the plant growth regulatory function of xylooligosaccharides produced from the hemicellulose of hardwoods like *Salix*. In the course of study, we established an in vitro culture system of *S. pet-susu* for in vitro assessing of regulatory products and molecular tree breeding. There have been several reports of tissue culture of other *Salix* species (Bergman et al., 1985; Stoehr et al., 1989; Neuner and Beiderbeck, 1993; Liesebach and Naujoks, 2004). Although several papers have been reported on the tissue culture of trees such as poplar, this is the first report for tissue culture of *S. pet-susu*.

MATERIALS AND METHODS

Branches of *S. pet-susu* at the arboretum of Forestry and Forest Products Research Institute (FFPRI) Hokkaido branch in Sapporo were collected in June 2008. Surface sterilization of stem segments was done using 0.1% benzalkonium chloride for 10 min., 70% ethyl alcohol for 1 min, and 1% sodium hypochroride for 7 min., then washed well twice with sterile water for eliminating microorganism. For initial culture, 1/2 LP media (different in the combination of hormones such as 10 μM benzylaminopurine (BAP), thidiazuron or zeatin, and 0.03 μM NAA) (Aitken-Christie and Thorpe,1984) were compared. For subculture and rooting of the shoots, 1/2 Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 1 μM IBA was used. For callus induction, Woody Plant Medium (Lloyd and McCown, 1981) containing BAP and 2,4-D or NAA was used. Culture condition was maintained at the constant temperature of 25 °C under 16-h photoperiod of 70 $\mu M \cdot m^{-2} \cdot s^{-1}$ by fluorescent lamps.

RESULTS AND DISCUSSION

Axillary buds were induced from the dissected segments (2 cm length) of branches of *S. pet-susu* in all the media tried. However, BAP and zeatin were better for

bud induction than thidiazuron. Benzylaminopurine was also the best cytokinin for *S. viminalis, S. caprea, S. dasyclados,* and two hybrids (Bergman et al., 1985). Induced buds were allowed to elongate to shoots in the same medium without subculture. Shoots subcultured to the rooting medium rooted easily; coppice shoots were initiated after 2 months of culture and about 10 coppice shoots were induced at the base of plantlets in vitro after 3.5 months culture without subculture (Table 1, Fig. 1). In vitro coppice shoots production may be a good method for propagation of *S. pet-susu*. Callus was induced from shoot segments and 2,4-D was effective for callus induction (Table 2) which is in accordance with the result of *S. exigua* (Stoehr et al., 1989). However, subculture of callus is still difficult so far. We wish to apply the in vitro culture of *S. pet-susu* for the morphological response screening test of biological active wood components like xylooligosaccharide. There may be the possibility of xylooligosaccharide as plant growth regulators and its additive high value can save costs in the production of bioethanol by improving the total value of forest biomass.

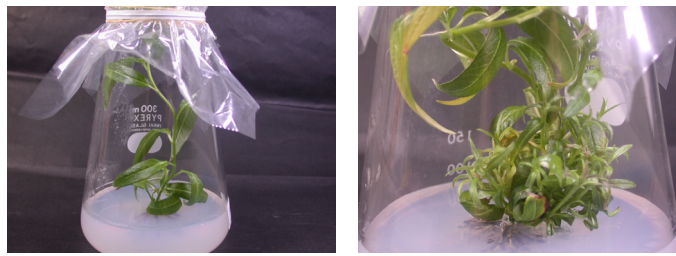

Figure 1. Coppice shoots induced from in vitro cultured *Salix pet-susu* ($^1/_2$ MS medium containing 1μM IBA). Left: 1 month culture; right: 3 months cultutre.

Table 1. Number of coppice shoots induced from in vitro cultured *Salix pet-susu* in rooting medium.

month	Average coppice shoot number (mm±SE)	Average coppice shoot length (mm±SE)
0	0±0 a	0±0 a
1	0±0 a	0±0 a
2.5	6.7±2 b	26±2.6 b
3.5	9.5±1 c	39±5 b

$^1/_2$ MS medium, 4 replication, initial shoot length 20 mm.
a,b,c: Responses with the same letter are not significantly different at P = 0.05 by Duncan new multiplication range test.

Table 2. Effects of different media on callus induction from stem segments of *Salix pet-susu*.

Auxin concentration (μM)	Callus induction rate (%)	Average callus diameter (mm)±SE
NAA 0.54	20	6
NAA 2.7	60	7±1.5
2,4-D 0.45	100	8.4±1.8
2,4-D 2.3	100	8.8±0.9

WPM medium containing 0.44 μM BAP, N = 5.
Observed after 3 weeks culture. Subcultured calli on the same media died after 1 month.

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