# The Effects of Xylooligosaccharides on Tissue Culture of Trees©

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**Morphogenetic effects of xylooligosaccharides on in vitro cultured forest trees were studied. The presence in the culture medium of xylooligosaccharides at a low concentration promoted growth of cultured shoot primordia of *Betula mandshurica* var. *japonica* (syn. *B. platyphylla* var. *japonica*). Acidic xylooligosaccharides (aldouronic acids) at low concentration were effective in promoting rooting of in vitro cultured *Cryptomeria japonica* and *Pinus thunbergii* shoots. Purified acidic oligosaccharides, aldobiouronic acid, and aldotetraouronic acid were effective for rooting in vitro shoots of *C. japonica*. Shoot primordium differentiation of *B. mandshurica* var. *japonica* was promoted by xylooligosaccharide mixture. Bud differentiation from callus of *Populus sieboldii* was promoted by the xylooligosaccharide mixture and aldouronic acids. Those functions of xylooligosaccharides could be used as new plant growth regulators in the field of tree propagation.**

## INTRODUCTION

Forest biomass could be used economically only if all components are used for various purposes properly. For example, recent bio-ethanol production from cellulose component of wood can be economically feasible when other components, hemicellulose and lignin, are also used as functional materials. Here, we attempt to assess the plant growth regulatory function of xylooligosaccharides produced from hemicellulose of birch. There is a report that acidic xylooligosaccharide has effects on callus and somatic embryo induction in vitro culture of common mallow and cotton (Katapodis et al., 2002).

## MATERIALS AND METHODS

Wood of *Betula mandshurica* var. *japonica* (syn. *B. platyphylla* var. *japonica*) was steamed at 13 $kg \cdot cm^{-2}$ for 15 min, and then the water soluble xylan hydrolyzate was extracted with water. This water extract was decolorized and purified by using synthetic adsorbents (Amberite XAD 2 and 7) and various types of cation and anion

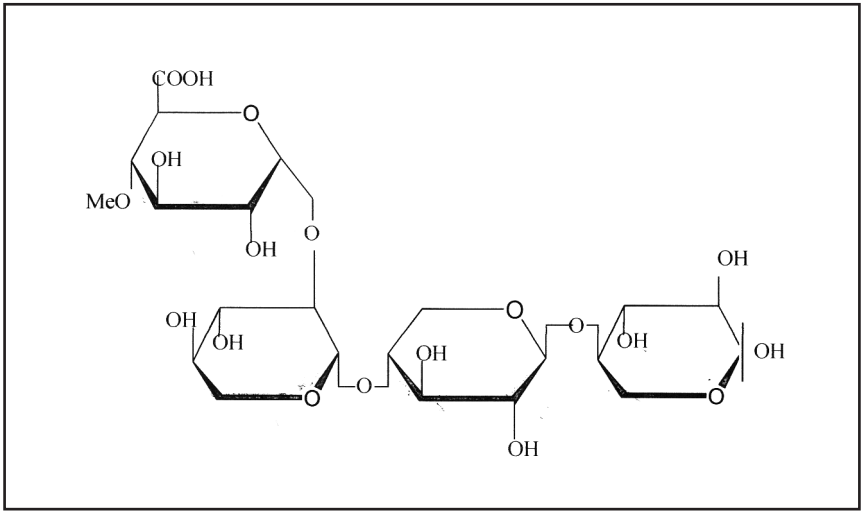


**Figure 1.** Chemical structure of aldotetraouronic acid.

ion exchange resins (C-111, $H^+$; SA-11A, $Cl^-$; A-168, $H^+$). The purified xylooligosaccharides were separated into neutral and acidic fractions using a strongly basic anion exchanger (OAc-form). The neutral fraction was a mixture of xylose and xylooligosaccharides from xylobiose (Xyl2) to xylooctaose (Xyl8).

The acidic fraction was further fractionated into four aldouronic acids: aldobio-(GlcAXyl), aldotrio-(GlcAXyl2), aldotetrao-(GlcAXyl3) (Fig. 1), and aldopentaouronic acids (GlcAXyl4) by ion exchange chromatography (Ishihara et al.,1996).

For bioassay of xylooligasaccharides in vitro cultured *B. mandshurica* var. *japonica*, *Populus sieboldii* Miq., *Pinus thunbergii* Parlatore, and *Cryptomeria japonica* (L. fil.) D. Don were used.

## RESULTS AND DISCUSSION

When the xylooligosaccharide mixture was added to the medium, the growth was promoted up to 80 ppm concentrations. Rooting rate of in vitro cultured shoots of *P. thunbergii* was the highest at the 10 ppm level of the xylooligosaccharide mixture in the root induction medium (RIM) (Table 1). The aldouronic acids were also effective on the high rooting rate of in vitro cultured shoots of *P. thunbergii.* Aldobiouronic acid and aldotetraouronic acid were effective for rooting of in vitro cultured shoots of *C. japonica* (Table 2, Fig. 2). The xylooligosaccharide mixture was effective for shoot primordium differentiation of *B. mandshurica* var. *japonica* in vitro culture. The xylooligosaccharide mixture and aldouronic acids are also effective for bud differentiation from in vitro cultured callus of *P. sieboldii.*

Xylooligosaccharide has been on sale as health care and beauty products (a functional food). It can promote the proliferated bifidobacterium in the intestine and improving the function of intestine, effective as a cure for constipation and diarrhea, promoting the absorption of Ca and Fe and preventing osteoporosis, reducing blood fat and cholesterol and protecting the liver, and increasing immunity.

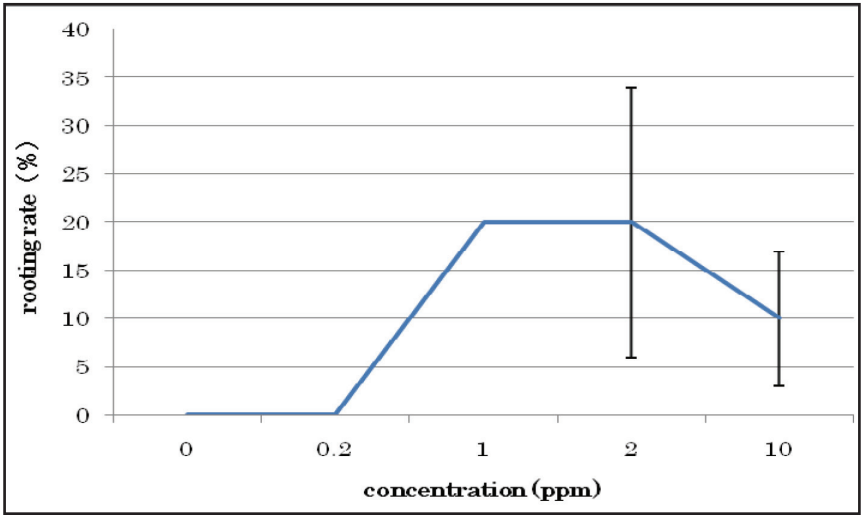


**Figure 2.** Effects of aldobiouronic acid on rooting of *Cryptomeria japonica* shoots (one month culture on root induction medium, N = 10).

**Table 1.** Effects of xylooligosaccharide mixture on rooting of *Pinus thunbergii* shoots after 2 months culture.

| Concentration (ppm) | Average shoot length (mm ± SD) | Rooting rate (%) |
|---|---|---|
| 0 | 36.2±17 | 20 a[x] |
| 10 | 34.0±17 | 50 b |
| 50 | 27.8±13 | 30 a |
| 100 | 33.6±18 | 20 a |

Root induction medium, three replication initial shoot length 20 mm.
[x] a, b responses with the same letter are not significantly different at P = 0.05 by Duncan new multiplication range test.

**Table 2.** Effects of aldotetraouronic acid on rooting of *Cryptomeria japonica* shoots.

| Concentration | Rooting rate (%) | | |
|---|---|---|---|
| (ppm) | 2 months | 3 months | 4 months |
| 0 | 0 | 7 | 7 |
| 0.1 | 7 | 20 | 67 |
| 0.5 | 13 | 40 | 80 |
| 1 | 13 | 40 | 60 |
| 5 | 20 | 40 | 53 |

Root induction medium, N = 15.

However, so far its use in the agronomic industry has not been commercialized. There may be the possibility of xylooligosaccharide use as plant growth regulators and its additive high value can save the cost of the production of bio-ethanol by improving the total value of forest biomass.

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