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論　文（Original article）

Characterization of the cell walls prepared from *Populus alba* cells grown in boron deficient medium

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Abstract

A new cell line of *Populus alba* L., designated T-0B, which was able to grow in a medium without boron, was established from previously reported T-5B cells maintained in a low-boron medium (5 μM). The boron content in T-0B cell walls was decreased to about one-third the level in the walls of the original *Populus* cells maintained in medium containing 100 μM boron. Rhamnogalacturonan II dimers, cross-linked by borate esters, were also decreased. A sugar composition analysis of the cell walls revealed decreases in arabinosyl and galacturonosyl residues, and an increase in glucosyl residues, compared with the levels in the original cell walls. Furthermore, the amounts of xylosyl, galactosyl, glucosyl and galacturonosyl residues in the extracellular polysaccharides increased significantly. These results suggest that the interactions among cell wall polysaccharides were weakened by decreases in the boron-rhamnogalacturonan II cross-linkages resulting from boron deficiency. Consequently, cell wall polysaccharides, especially pectin, were released into the medium as extracellular polysaccharides.

Key words : Boron deficiency, cell wall, pectic polysaccharide, *Populus alba* L., rhamnogalacturonan II

Abbreviations: Ara = arabinose; CDTA = *trans*-1,2-cyclohexanediamine-*N,N,N′,N′*-tetraacetic acid monohydrate; Fuc = fucose; Gal = galactose; GalUA = galacturonic acid; Glc = glucose; GlcUA = glucuronic acid; HG = homogalacturonan; Man = mannose; RG-I = rhamnogalacturonan I; RG-II = rhamnogalacturonan II; Rha = rhamnose; Xyl = xylose

Introduction

Boron deficiency in plants causes many anatomical, physiological and biochemical disorders, such as inhibition of growth and abnormal development (Blevins and Lukaszewski, 1998). Recently, researchers have reported a close correlation between plant cell walls and boron (Loomis and Durst, 1992; Matoh et al., 1992; Hu and Brown, 1994). Most cellular boron is localized in the primary cell walls and cross-links two molecules of RG-II (Matoh et al., 1993; Kobayashi et al., 1996). The RG-II is the complex polysaccharide containing characteristic sugar residues such as apiose and 2-keto-3-deoxyoctonic acid. This may compose the pectin network, necessary for maintaining cell wall structure, with HG and RG-I in plant cell walls. The cross-link between RG-IIs is formed by borate cis-diol ester bonds at apiosyl residues in RG-II. These informations indicate that boron has a physiological function in cell walls. In fact, changes in the cell wall structure, such as cell wall swelling (Ishii et al., 2001), increases in the cell wall pore size and cell enlargement (Fleischer et al., 1999), which may be caused by boron deficiency, have been reported.

Recently, we reported a change in cell wall structure caused by boron deficiency in the cell line T-5B, which was maintained in a low-boron medium (Kakegawa et al., 2005). In these cells, the boron content in the cell walls is decreased and the ratio of monomeric RG-II (mRG-II) is increased. A sugar composition analysis of the cell walls after fractionation by sequential extraction revealed that pectic polysaccharides composed of Ara and GalUA could be extracted more easily from T-5B cells than from cells grown in medium containing sufficient boron (100 μM).

In the present study, we established and characterized a *Populus alba* cells grown in boron-deficient medium to clarify the effects of boron deficiency on the cell wall structure. According to the obtained results, we attempt to discuss the function of boron in plant cell walls.

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Materials and Methods

Culture methods and determination of cell growth

Cell suspension cultures of *Populus alba* L. were maintained in modified Murashige and Skoog (MS) medium containing 100 μM boron (Kakegawa et al., 2000), and used as the original cells for this study. A T-0B cells, which was able to grow and be subcultured in a medium without boron (0B medium), was isolated from previously established T-5B cells cultured in the medium containing 5 μM boron (Kakegawa et al., 2005) by habituation on 0B medium. The cell number was counted with a haemacytometer after enzymatic maceration (Kakegawa et al. 2000). The fresh weight was determined after vacuum filtration of cells. The dry weight of cell walls was estimated by using cell wall materials isolated as described below. Data were obtained from three independent experiments.

Isolation and sequential extraction of cell walls

The cell wall material was prepared according to kakegawa et al. (2005). The cell wall material was sequentially extracted with 50 mM CDTA, 50 mM Na_2CO_3, 1 M KOH and 4 M KOH to give each soluble fraction as described by Selvendran and O'Neill (1987). The 4M KOH-insoluble residue was collected as the cellulose fraction.

Determination of the boron content and the ratio of boron-RG-II dimers (B-dRG-II) and mRG-II in cell walls

Following digestion of the cell wall samples with concentrated HNO_3, the boron content was determined with an inductively coupled plasma-mass spectrometer (SII SPQ8000A or SPQ9000; Seiko Instruments Inc., Chiba, Japan) as previously described (Kakegawa et al., 2005). The amounts of B-dRG-II and mRG-II were determined by size exclusion chromatography after enzymatic digestion of cell walls according to Ishii et al. (2001). Three separate experiments were done to obtain the data.

Preparation of extracellular polysaccharides (ECPs)

ECPs were precipitated by pouring 5 volumes of ethanol into a cell-free medium of original, T-5B and T-0B cell cultures at 4°C for 16 h. The precipitate was collected and redissolved in distilled water. After dialysis against distilled water for 24 h, ECPs were obtained by freeze-drying.

Determination of the sugar content

The amounts of total sugar and uronic acid were determined by the phenol-sulfuric acid method (Dubois et al., 1956) and the *m*-hydroxybiphenyl assay (Blumenkrantz and Asboe-Hansen, 1973), respectively. For analysis of the 4 M KOH-insoluble fraction, samples were hydrolyzed by H_2SO_4 before colorimetric assay (Kakegawa et al.,2005)

Glycosyl composition and linkage analysis

The compositions of the neutral and acidic glycosyl residues in the soluble fractions were determined as trimethylsilyl ethers of methyl glycosides (Selvendran and Ryden, 1990). For the 4 M KOH-insoluble fraction, the H_2SO_4 hydrolyzed samples were neutralized by $Ba(OH)_2$ before methanolysis. The derivatives were analyzed by gas-liquid chromatography according to York et al. (1986). Data were obtained from two independent experiments. Glycosyl linkage analysis was performed according to York et al. (1986).

Results and Discussion

A newly established *Populus* cell line that tolerated boron deficiency (T-0B cells) were maintained in MS-medium without boron. An abnormal cell shape and plasmolysis were abundantly observed (data not shown). Growth suppression was also indicated by the decreased cell number, fresh weight and dry weight of cell walls compared with the original cells (Table 1). The boron content in the T-0B cell walls was decreased to about 34% of the level in the original cell walls (Table 1) and was lower than that in the T-5B cell walls (Kakegawa et al., 2005). Consistent with the decrease in the cell wall boron, the ratio of mRG-II was increased in T-0B cell walls (Table 1). Since the T-5B cells only showed a slight suppression of growth (Kakegawa et al., 2005), the boron-deficient conditions appear to have a more critical

Table 1. Comparison of T-0B and original cells

	Cell line	
	Original	T-0B
Cell number ($x10^5$ cells/ml medium)	75.9 ± 2.6	52.9 ± 0.5
Fresh weight (g/ml medium)	0.31 ± 0.01	0.21 ± 0.06
Cell wall (mg/g fresh weight)	15.38 ± 1.68	11.71 ± 0.34
Boron content in cell wall (μmol /g cell wall)	2.73 ± 0.14	0.92 ± 0.05
Monomeric RG-II (% of total RG-II)	10 ± 3	42 ± 5

Data are the mean values ± SD (n=3) obtained from three separate experiments.

effect on T-0B cells. However boron was not completely eliminated from the cell walls of T-0B cells, despite the use of a boron-deficient medium. This may result from the absorption of boron derived from the glassware used for the culture, since RG-II instantly forms borate cis-diol esters when boron is supplied (Ishii et al., 2001).

Figure 1 shows the composition of major sugar residues in the soluble cell wall fractions prepared from the original and T-0B cells. A marked decrease in GalUA residues was observed, predominantly in the Na_2CO_3-, 1 M KOH- and 4 M KOH-soluble fractions. The amount of Ara residues also decreased in the 4 M KOH-soluble fraction. In contrast, the Glc residues in the 1 M KOH-soluble fraction increased by about 3-fold compared to the corresponding fraction of the original cell walls. No significant difference was observed in other minor sugar residues and 4 M KOH-insoluble fraction (data not shown).

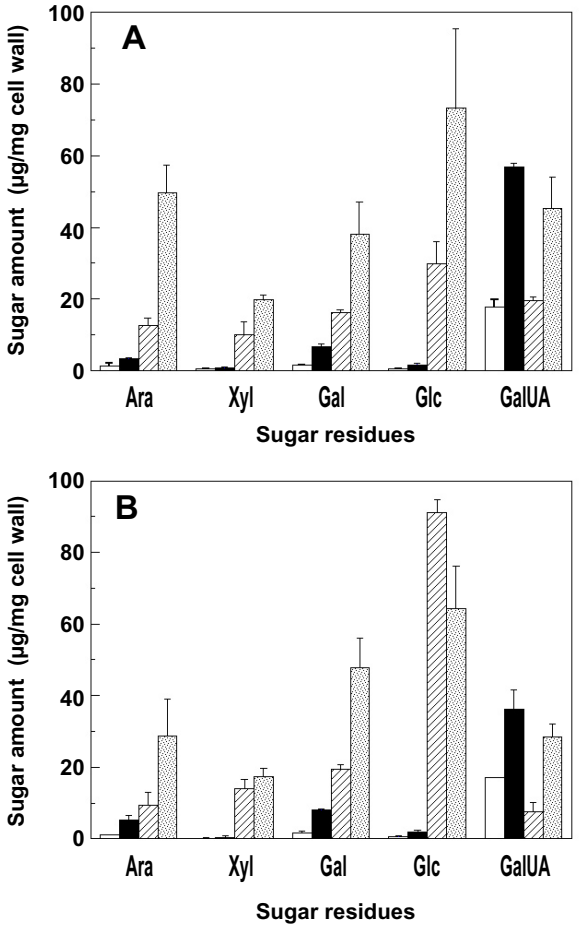

Fig. 1. Glycosyl residue compositions in each cell wall fraction obtained by sequential extraction of cell walls isolated from the original (A) and T-0B (B) cells. Open bars: CDTA-soluble fraction. Solid bars: Na_2CO_3-soluble fraction. Hatched bars: 1 M KOH-soluble fraction. Shaded bars: 4 M KOH-soluble fraction. Data are the means ± SD of values obtained from two separate experiments.

Decreases in Ara and GalUA were also observed in the T-5B cells (Kakegawa et al., 2005). In that case, the decreased amounts of GalUA and Ara in the 4 M KOH-soluble fraction appeared in the CDTA- and 1 M KOH-soluble fractions, respectively, at similar amounts. Hence, it could be supposed that the pectic polysaccharides composed of GalUA and Ara became more easily extractable due to weakness of the cross-linkages between the pectic polysaccharides resulting from the decrease in borate cross-linkages. However GalUA and Ara were lost from the cell walls of T-0B cells (Fig. 1). These results indicate that leakage of pectic polysaccharides from the cell walls to the medium was induced by the further decrease in borate cross-linkages.

The compositions of the major glycosyl residues in the ECPs are shown in Fig. 2. No significant changes were observed between the original and T-5B cells. In contrast, the amounts of most sugar residues were increased in the medium from T-0B cells. In particular, Xyl, Gal, Glc and GalUA each increased by about 2-fold compared with the original cells. No conspicuous changes in the contents of minor sugar residues were observed (data not shown). These results suggest that most of the polysaccharides derived from the cell walls eluted into the medium due to weakening of the interactions between them resulting from the decrease in borate cross linkages. However, increases in most of the sugar residues of ECPs were also shown in addition to Ara and GalUA, which decreased among the cell wall polysaccharides. This result may arise from differences in the turnover activities in each cell wall polysaccharide.

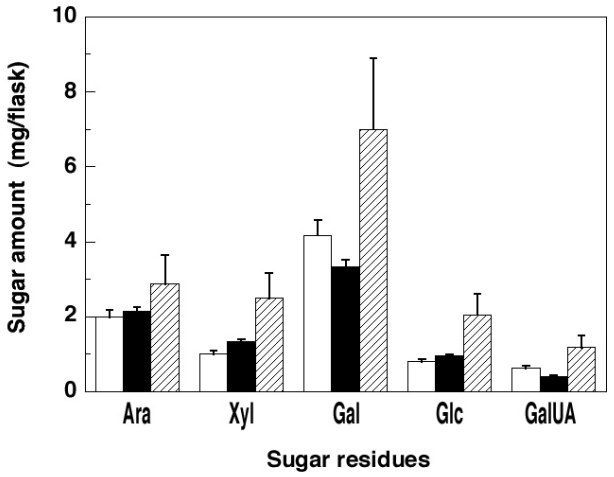

Fig. 2. Glycosyl residue compositions of the extracellular polysaccharides (ECPs) of the original (open bars), T-5B (solid bars) and T-0B (hatched bars) cells. Data are the means ± SD of values obtained from two separate experiments.

According to Fleischer et al. (1998), cell death is caused by the weakening of the cell walls in the boron deficient medium and B-dRG-II is required for a formation of pectin network. Actually, the cell death was observed in the non-tolerant original cells transferred into media containing low concentrations of boron (Kakegawa et al., 2005). Therefore, T-0B and T-5B cells may have acquired a particular mechanism of tolerance to a low boron condition, which is essential to maintain the cell wall structure. In the case of T-0B, the Glc residues in the 1M KOH-soluble fraction were increased (Fig. 1). Since the sugar linkage analysis showed the presence of 4-linked Glc as a major component (33.6% of total sugars, Table 2), it was indicated that 1,4-glucan was predominantly contained in the 1 M KOH fraction. This newly synthesized 1,4-glucan may be used to compensate for the loss of pectic polysaccharides and sustain the cell wall network.

In this report, we have shown differences in the cell walls between the original cells and the T-0B cells, as well as a comparison with the previously reported T-5B cells. The results revealed that boron deficiency has a conspicuous effect on the interactions among pectic polysaccharides, especially HG and arabinan, through changes to the cross-linkages of RG-IIs. These findings indicate the importance of boron for the formation and maintenance of a pectic polysaccharide network. Further analysis of this cell line is necessary to elucidate the role of the newly synthesized 1,4-glucan, which may participate in the tolerance to boron deficiency.

Table 2. Glycosyl linkage analysis of 1 M KOH-soluble fraction in T-0B cell walls.

Glycosyl linkage		Sugar composition (mol%)
Rha	2,4-	2.1
Fuc	T-	2.8
Ara	T-	2.6
	2-	4.8
	2,5-	2.9
Xyl	T-	3.8
	2-	10.2
	2,4-	2.3
Man	4-	4.2
Gal	T-	3.4
	2-	4.9
	4-	4.1
Glc	T-	1.6
	4-	33.6
	4,6-	16.6

T-: Terminal residue.

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References

Blevins, D. G. and Lukaszewski, K. M. (1998) Boron in plant structure and function. Annu. Rev. Plant physiol. Plant Mol. Biol., **49**, 481-500.

Blumenkrantz, N. and Asboe-Hansen, H. M. (1973) New method for quantitative determination of uronic acids. Anal. Biochem., **54**, 484-489.

Dubois, M., Gilles, K. A., Hamilton, J. K., Robers, P. A.and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. Anal. Chem., **28**, 350-356.

Fleischer, A., Titel, C. and Ehwald, R. (1998) The boron requirement and cell wall properties of growing and atationary suspension-culktured *Chenopodium album* L. cells. Plant Physiol. **117**, 1401-1410.

Fleischer, A., O'Neill, M. A. and Ehwald, R. (1999) The pore size of non-graminaceous plant cell walls is rapidly decreased by borate ester cross-linking of the pectic polysaccharide rhamnogalacturonan II. Plant Physiol., **121**, 829-838.

Hu, H.,and Brown, P. H. (1994) Localization of boron in cell walls of squash and tobacco and its association with pectin. Plant Physiol., **105**, 681-689.

Ishii, T., Matsunaga, T. and Hayashi, N. (2001) Formation of rhamnogalacturonan II-borate dimer in pectin determines cell wall thickness of pumpkin tissue. Plant Physiol., **126**, 1698-1705.

Kakegawa, K., Edashige, Y. and Ishii, T. (2000) Metabolism of cell wall polysaccharides in cell suspension cultures of *Populus alba* in relation to cell growth. Physiol. Plant., **108**, 420-425.

Kakegawa, K., Ishii, T. and Matsunaga, T. (2005) Effect of boron deficiency in cell suspension cultures of *Populus alba* L. Plant Cell Rep., **23**, 573-578.

Kobayashi, M., Matoh, T. and Azuma, J. (1996) Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. Plant Physiol., **110**, 1017-1020.

Loomis, W. D. and Durst, R. W. (1992) Chemistry and biology of boron. Bio Factors, **3**, 229-239.

Matoh, T., Ishigaki, K., Mizutani, M., Matsunaga, W., and

Takabe, K. (1992) Boron nutrition of cultured tobacco BY-2 cells. I. Requirement for and intracellular localization of boron and selection of cells that tolerate low levels of boron. Plant Cell Physiol., **33**, 1135-1141.

Matoh, T., Ishigaki, K., Ohno, K. and Azuma, J. (1993) Isolation and characterization of boron-polysaccharide complex from radish roots. Plant Cell Physiol., **34**, 639-642.

Selvendran, R. R. and O'Neill, M. A. (1987) Isolation and analysis of cell walls from plant material. Methods Biochem. Anal., **32**, 25-153.

Selvendran, R. R. and Ryden, P. (1990) Isolation and analysis of plant cell walls. In Day P. M. (ed.)*" Methods in Plant Biochemistry vol. 2 Carbohydrates"*, *Academic press, London*, 549-579.

York, W. S., Darvill, A. G., McNeil, M. and Albersheim, P. (1986) Isolation and characterization of plant cell wall and plant cell components. Methods Enzymol., **118**, 3-40.

ホウ素飢餓培地で増殖可能なギンドロ培養細胞から調製した細胞壁の特性

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要 旨

ホウ素を含まない培地で増殖可能なギンドロ培養細胞系 (T-0B) を確立し、その特性を十分なホウ素を含む培地 (100μM) で培養された細胞 (Original) と比較した。T-0B 細胞の細胞壁に含まれるホウ素量は Original 細胞の約三分の一に減少していた。それにともないホウ素によって架橋されたラムノガラクツロナンⅡ二量体の減少とラムノガラクツロナンⅡ単量体の増加も観察された。細胞壁多糖類の構成糖分析により T-0B ではアラビノース、ガラクツロン酸の減少、および非セルロース性グルコース残基の増加が顕著であることが明らかになった。また、細胞外多糖の構成糖分析ではほとんどの糖残基の増加が確認され、特にキシロース、ガラクトース、グルコース、及びガラクツロン酸残基は Original 細胞の約 2 倍に増加していた。これらの結果からホウ素欠乏によるホウ素・ラムノガラクツロナンⅡ架橋の減少によって細胞壁多糖類間の結合が弱くなり本来細胞壁に保持されるべき多糖が細胞外多糖として培地中に分泌されたと推測された。

キーワード：ホウ素欠乏、細胞壁、ペクチン、*Populus alba L.*、ラムノガラクツロナンⅡ

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