Journal of Plant Biology, March 2004, 47(1) : 21-26

Long-Term Effects of Growth Regulators on Growth and Turnover of Symplastic and Apoplastic Sugars in the Suspension Subculture of Kidney Bean

Up-Dong Yeo[1]*, Dev Mani Pandey[1], and Kyong-Ho Kim[2]
[1]Faculty of Biological Science, Chonbuk National University, Chonju 561-756, Korea
[2]National Honam Agricultural Experiment Station R.D.A., Iksan 570-080, Korea

Suspension cells of kidney bean were grown for 42 d in MS medium supplemented with growth regulators (2.0 mgL^{-1} 2,4-D and 0.5 mgL^{-1} kinetin) or without. At the stationary growth phase (42 d), the sugars were fractionated into the symplastic (ethanol and starch) and apoplastic [low-molecular pectin (lm-pectin), high-molecular pectin (hm-pectin), hemicellulose, and cellulose] sugars. The neutral sugars (NS) of hm-pectin and hemicellulose fractions were analyzed by GLC. The growth of the suspension cells in the liquid MS media, in terms of settled cell volume (SCV), remained similar, to the end of the experiment, irrespective of the presence or absence of growth regulators, indicating the non-necessity of the exogenous growth regulators for the subculture. Total sugar (TS) of the ethanol fraction and NS of the lm-pectin of the suspension cells grown in the medium with growth regulators were higher than in the medium without growth regulators. However, starch content in the starch fraction and uronic acid (UA) content of the lm-pectin fraction did not exhibit any differences. From these results, it was suggested that the growth regulators modulated the structure of the cell wall polysaccharide. Analysis of the NS composition of the hm-pectin fractions revealed that the Rha, Arb, and Gal contents in the presence of growth regulators were higher than in the absence, while the Xyl, Man, and Glc contents in the presence of growth regulators were higher than in the absence, indicating the turnovers of rhamnogalacturonan and/or arabinogalactan. On the other hand, analysis of NS composition of hemicellulose fractions revealed that the Ara and Glc contents in the presence of growth regulators was higher than in the absence, whereas Xyl and Glc contents were nearly consistent, indicating the turnovers of arabinaogalactan I or II. The cellulose contents remained similar, irrespective of the presence (19.1%) or absence (18.7%) of growth regulators.

Keywords: cellulose, cell-wall polysaccharide, hemicellulose, high-molecular pectin, low-molecular pectin, *Phaseolus vulgaris*

The cell wall is a major structural element of plant cells and plays an important role in protection of protoplasts from mechanical forces and in the mechanical support of plant tissues and organs. Cell wall polysaccharides turn-over extensively during plant growth and development (Labavitch, 1982; Taiz, 1984; Masuda, 1990; Sakurai, 1991, 1998; Hoson, 1993). The crystalline cellulose polysaccharide in the matrix is a major factor in regulation of cell shape and rigidity. But cellulose synthesis and its regulation mechanisms remain unclear (Delmer et al., 1993; Delmer, 1999). Kokubo et al. (1991) had reported that three isogenic barley mutants that developed fragile culms produced lower amount of cellulose than corresponding normal strains. According to Yeo et al. (1995), the cellulose contents of normal barley strains under *in vitro* culture conditions were close to those of their corresponding mutant strains. Albersheim et al. (1997) suggested that the structure of cell-wall polysaccharides might define their mode of synthesis. Nishitani and Masuda (1980) have reported that auxin stimulates the turnover of galactose (Gal)-containing polysaccharides during cell elongation. However, detailed information on long-term effects of growth regulators on the cell-wall polysaccharides in the subculture of suspension cells of kidney bean is not available. Therefore, the present work was undertaken to investigate the long-term effects of growth regulators on the turnover of symplastic and apoplastic (cell-wall) sugars in the subculture of suspension cells of kidney bean.

*Corresponding author; +82-63-270-3362
e-mail y520419@moak.chonbuk.ac.kr

Abbreviations: Arb, arabinose; Fuc, fucose; Gal, galactose; Glc, glucose; hm-pectin, high molecular pectin; lm-pectin, low molecular pectin; Man, mannose; MOPS, 3-(N-morpholino)propanesulfonic acid; NS, neutral sugar; Rha, rhamnose; SCV, settled cell volume; TFA, trifluoroacetic acid; TS, total sugar; UA, uronic acid; Xyl, xylose

MATERIALS AND METHODS

Suspension Culture

The seeds of kidney bean (*Phaseolus vulgaris* L. cv. Kangnangkong 1) were soaked in running tap water for 1 d and germinated in a mixture of sand and vermiculite (1:1, v/v). The epicotyl segments of the 7 d old seedlings were sterilized with 70% ethanol for 30 s and with 1% sodium hypochlorite solution for 10 min. The epicotyl segments of 2 × 3 mm were transferred into a 100-mL flask containing 25 mL of liquid MS (Murashige and Skoog, 1962) medium supplemented with 2.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin, 3% sucrose, and 1.1% agar. pH of the medium was adjusted to 5.8 with 1 M KOH and it was autoclaved at 121°C for 20 min. Culture conditions included a white fluorescent light (15 μE $m^{-2}s^{-1}$) in a shaking incubator (25 ± 1°C) with rotation of 120 rpm. After 30 d of culture, the suspension cells (clusters) were *in vitro* sedimented by standing for 10 min. The 3 mL of settled cell volume (SCV), ca 0.3 g fresh weight of the suspension cells were transferred into a 100-mL flask containing 25 mL of the medium supplemented with the growth regulators (2.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin) or without. Cell homogeneity was maintained by sub-culturing at the 2 weeks-intervals. The homogeneous cells (0.3 g) were cultured for 42 d (stationary phase) in the media supplemented with the growth regulators or without. The SCV was measured for 6 weeks. The flasks were placed into a shaking incubator with a completely randomized design. After 42 d of culture, the suspension cells were centrifuged at 1,000*g* for 10 min with Beckman J2-MC Centrifuge (USA) and washed twice with distilled water and the fresh weight was measured and fixed with 80% ethanol at 80°C for 15 min and stored in a refrigerator (4°C) until use.

Fractionation of Symplastic and Apoplastic Sugars

The fractionation was performed with a modified method of Sakurai et al. (1987). The ethanol samples were centrifuged at 1,000*g* for 10 min. The supernatant was designated the ethanol fraction. This ethanol or methanol fraction contains mono- and oligosaccharides such as glucose, fructose, and sucrose (Wakabayashi et al., 1991). The pellet was hydrated in deionized water for 10 min and homogenized with a mortar and pestle and centrifuged. The residue was washed twice with 10 mL of 50 mM MOPS/KOH buffer (pH 7.0). The pellet was resuspended in 10 mL of 50 mM MOPS/KOH buffer (pH 7.0) and boiled for 1 min. After cooling at room temperature, the suspension was treated with 4 μL (20 units) of porcine pancreatic α-amylase (Type I-A, Sigma, USA) in 50 mM MOPS/KOH buffer (pH 7.0) for 2 h at 37°C to remove starch and was subsequently centrifuged at 1,000*g* for 10 min. The supernatant was designated the starch fraction. The pellet was washed twice with deionized water. The supernatant was combined to the starch fraction. The ethanol and starch fractions were named the symplastic fractions. The homogenate was boiled for 10 min and centrifuged at 1,000*g* for 10 min and this step was repeated three times. The pooled supernatant was designated the low-molecular pectin (lm-pectin) fraction. Pectin substances (high-molecular pectin) from cell wall were extracted with 50 mM EDTA in 50 mM sodium phosphate buffer (pH 6.8) at 100 for 15 min and centrifuged at 1,000*g* for 10 min. This step was repeated three times. The pooled supernatant was designated the high-molecular pectin (hm-pectin) fraction. Next, hemicellulose substances were extracted three times with 17.5% NaOH containing 0.02% $NaBH_4$ for 18 h at 25°C and centrifuged at 1,000*g* for 10 min. The supernatant was designated the hemicellulose fraction. The residue was washed twice, each time with 0.03 M acetic acid and a mixture of ethanol and diethyl ether (1:1, v/v) to remove any phenolic compounds. The washed residue was dried for 48 h at 40°C. The dried material was designated the cellulose fraction.

Measurement of Sugar Content of Each Fraction

The total sugar (TS) content of the symplastic (ethanol and starch) fractions and apoplastic (lm-pectin, hm-pectin, hemicellulose, and cellulose) fractions were determined by a phenol-sulfuric acid method (Dubois et al., 1956). Before determination, the cellulose fraction was hydrolyzed with 7.5 M H_2SO_4 over ice bath and with 1 M H_2SO_4 for 1 h at 100. The neutral sugar (NS) and uronic acid (UA) contents in lm-pectin, hm-pectin, and hemicellulose fractions were determined by a phenol-sulfuric acid method (Dubois et al., 1956) and an *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973).

Total sugar (TS, μg mL^{-1}) = $91 \times A_{490}$

Neutral sugar (NS, μg mL^{-1}) = $92.2 (A_{490} - 0.464A_{520})$

Uronic acid (UA, μg mL^{-1})

= $112.4 \times (A_{520} - 0.0272 A_{490})$

Analysis of NS Composition in Hm-Pectin and Hemicellulose Fractions

The alditol acetate derivatives of hm-pectin and

hemicellulose fractions were analyzed by GLC. The hemicellulose fraction was neutralized with a half volume of glacial acetic acid. The hm-pectin and neutralized hemicellulose fractions were dialyzed against deionized water with seamless cellulose tubing (18/32) for 2 d. Three mL of each fraction was taken in a screw-capped tube and dried with a stream of filtered air at 50°C. The dried polysaccharides were hydrolyzed by 2 mL of 2 M TFA containing 300 μg of *myo*-inositol as an internal standard in a screw-capped test tube for 1 h at 121°C and then, dried by air flow at 50°C. The hydrolyzed monosaccharides were reduced by $NaBH_4$ and acetylated with acetic anhydride in the presence of 1-methylimidazole as a catalyst (Bleckeney et al., 1983). The acetylated monosaccharides were dissolved in 100 μL of acetone and 1 μL was introduced into a GLC system (M600D, Young-Lin Instrument Co. Ltd, Korea) equipped with a flame ionization detector and a capillary column (SP-2380, Supelco, USA). The oven temperature was raised from 180°C to 230°C at a rate of 4°C/min. The sugar content (%) was determined by the ratio of peaked area.

Statistics

Using t-test, data of three replications were analyzed.

RESULTS

Growth of the Suspension Cells

Growth in the subcultures of suspension cells of kidney bean was investigated, in terms of SCV. During the whole culture duration/period, the growth response did not exhibit any difference between media with growth regulator (2.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin) or without. The growth rate was high, to 14 d (logistic phase). However, this increase did not continue onto 21 d. Thereafter, the growth rate started to decrease and remained similar, onto 42 d (stationary phase) (Fig. 1), indicating the non-necessity of the exogenous growth regulators for the subculture.

Comparison of TS Contents in Symplastic, Ethanol and Starch Fractions

TS contents of the symplastic, ethanol and starch fractions of the suspension cells grown in the medium with the growth regulators or without were measured at the 42 d of culture, stationary phases. TS content

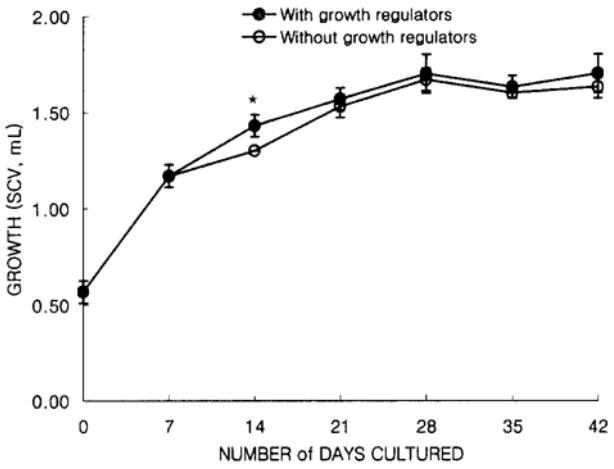

Figure 1. Growth response (SCV, mL) of kidney bean suspension cells in the liquid MS media supplemented with the growth regulators (2.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin) or without. Bars represent SE, n=3. *Significant difference at P=0.05.

Table 1. Long-term effects of growth regulators (2.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin) on total sugar contents in the symplastic, ethanol and starch fractions of kidney bean suspension cells at the stationary phases. Data show mean ± SE, n=3. **Significant difference at P=0.01.

Treatment	Ethanol fraction (mg/FW)	Starch fraction (mg/FW)
With growth regulators	2.01 ± 0.07**	5.97 ± 0.11
Without growth regulators	0.83 ± 0.01	5.47 ± 0.09

(2.91 ± 0.07) of the ethanol fractions of the suspension cells grown in the medium with the growth regulators was higher than that (0.83 ± 0.01) of suspension cells grown in the medium without. TS content of the starch fractions did not exhibit any difference between the media with growth regulators (5.97 ± 0.11) or without (5.47 ± 0.09) (Table 1).

Comparison of Total Sugar Contents of Apoplastic Fractions

NS and UA contents of the apoplastic, lm-pectin, hm-pectin, and hemicellulose fractions and TS content of cellulose fraction of the suspension cells grown in the media with growth regulators or without were measured. NS content (2.65 ± 0.02) of the lm-pectin fraction of the suspension cells grown in the medium with the growth regulators was higher than that (1.62 ± 0.02) of the suspension cells grown in the medium without ($P<0.05$). However, the UA content did not exhibit any difference between the media with the growth regulators or without. And also, NS and UA

Table 2. Long-term effects of growth regulators (2.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin) on sugar contents in the apoplastic (lm-pectin , hm-pectin, hemicellulose, and cellulose) fractions of kidney bean suspension cells at the stationary growth phases (42d). NS - neutral sugar, UA uronic acid. Data show mean ± SE, n=3. *Significant difference at P=0.05.

Treatment	Apoplastic fractions (mg/FW)							
	lm-pectin		hm-pectin		Hemicellulose		Cellulose	Total
	NS	UA	NS	UA	NS	UA		
With growth regulator	2.65 ± 0.02*	0.22 ± 0.01	3.23 ± 0.08	1.00 ± 0.02	7.44 ± 0.14	1.05 ± 0.01	3.68 ± 0.06 (19.1%)	19.27 ± 0.20*
Without growth regulator	1.62 ± 0.04	0.20 ± 0.00	2.94 ± 0.14	0.97 ± 0.02	7.74 ± 0.13	1.12 ± 0.02	3.35 ± 0.09 (18.7%)	17.94 ± 0.22

Number percentages in parenthesis represent the cellulose content to the total amount.

contents of the remaining apoplastic, hm-pectin and hemicellulose fractions of the suspension cells did not exhibit any differences between the media with the growth regulators or without. Similarly, the cellulose contents remained similar, irrespective of the presence (19.1%) or absence (18.7%) of growth regulators (Table 2).

Comparison of NS Composition in Non-Cellulosic, Hm-pectin and Hemicellulose Fractions

To analyze the structural turnover of the non-cellulosic polysaccharides of the suspension cells between the media with growth regulators or without, the acetylated monosaccharides of the hm-pectin and hemicellulose fractions of them were analyzed by GLC. In the hm-pectin fractions, the Rha (10.1%), Arb (30.3%), and Gal (18,2%) contents in the presence of growth regulators were lower than those (20.3, 42.7, and 27.7%, respectively) in the absence, while the Xyl (13.8%), Man (13.5%), and Glc (14.0%) contents in the presence of growth regulators were higher than those (0.0, 2.0, and 8.0%, respectively) in the absence. In the hemicellulose fractions, the Arb (58.4%) and Glc (32.2%) contents in the presence of growth regulators was higher than those (Arb, 50.2% and Glc, 26.4%) in the absence, whereas the Xyl (10%) and Glc (8%) contents were nearly consistent, irrespective of the presence or absence of the growth regulators. Due to small amounts of Fuc in the hm-pectin and Rha, Fuc, and Man in the hemicellulose fractions, they were not detected (Table 3).

DISCUSSION

In the present research, the growth response of the suspension cells in the media with growth regulators (2.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin) or without remained similar, in terms of SCV, onto the end (42 d) of subculture), irrespective of the presence or absence of growth regulators (Fig. 1). The results indicated that exogenous growth regulators were not necessary for the subculture, meaning that the suspension cells will synthesize the endogenous growth regulators for growth. Further, it needs to quantify the in situ synthesis of growth regulators.

The TS content of the symplastic, ethanol fraction of suspension cells grown in the medium with the growth regulators was higher than in the medium without (Table 1). This result indicates that the exogenous growth regulators modulate not only the symplastic sugar metabolism but also the apoplastic (cell-wall) sugar metabolism.

NS content of the lm-pectin fraction of the suspension cells grown in the medium with the growth regulators was higher than in the medium without. However, NS content of the hm-pectin fraction of the suspension

Table 3. Long-term effects of growth regulators (2.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin) on the composition of NS in the non-cellulosic (hm-pectin and hemicellulose) fractions of kidney bean suspension cells at stationary growth phases (42 d). Arb, arabinose; Fuc, fucose; Gal, galactose; Glc, Glucose; Man, mannose; Rha, rhamnose; Xyl, xylose. ** Significant difference at P=0.01.

Treatment	Hm-pectin fraction (%)							Hemicellulose fraction (%)						
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
With growth regulators	10.1	0.0	30.3	13.8**	13.5**	18.2	14.0**	0.0	0.0	58.4**	9.9	0.0	7.7	32.2**
Without growth regulators	20.3**	0.0	42.0**	0.0	2.0	27.7**	8.0	0.0	0.0	50.2	9.8	0.0	5.4	26.4

cells did not exhibit any difference between the media with the growth regulators or without (Table 2). On the contrary, analysis of the changes in the structure of pectic polysaccharides in the NS of the hm-pectin fraction, using GLC analysis of the acetylated monosaccharides, revealed that the Rha, Arb, and Gal contents in the presence of growth regulators were lower than in the absence, while the Xyl, Man, and Glc contents were higher than in the absence. The Fuc content was immeasurable by GLC, due to its small quantity (Table 3). In support of our findings, Kato and Nevins (1982) have reported that Rha, Ara, Gal, and UA are the components of the rhamnogalacturonan I or II. From our results we suggest that among pectic polysaccharides, rhamnogalacturonans and/or arabinogalactans are synthesized natively in the medium without the growth regulators and their structure is modulated in the medium with the growth regulators. On the other hand, NS and UA contents of the hemicellulose fraction of the suspension cells also did not exhibit any differences between the media with growth regulators or without (Table 2). But, for analysis of the changes in the structure of hemicellulosic polysaccharides, GLC analysis of the acetylated monosaccharides in the hemicellulose fractions revealed that the Ara and Glc contents in the presence of growth regulators was higher than those in the absence, whereas the Xyl (10%) and Gal (>10%) contents were nearly consistent, irrespective of the presence or absence. The Fuc content was immeasurable by GLC (Table 3). In agreement to our results, in the suspension cells of kidney bean, Chai et al. (1998) were also unable to detect Fuc, due to the small quantity of xyloglucan. The amount of Xyl was reported to be 65 to 78% of the total amount of hemicelluloses in intact barley culms of three isogenic lines (Kokubo et al., 1991). According to Basic et al. (1988), xyloglucans usually have the Gal and Fuc residues in in vivo dicot plants. This is in contrast to our findings where hm-pectin contained more Gal, as compared to the hemicellulose fraction. It might due to either modification or turnover of the Gal-containing polysaccharides, i.e., arabinaogalactan I or II into other polysaccharides by stimulation of the growth regulators. In support to our results, Labavitch and Ray (1974) reported that auxin causes modification of xyloglucan in dicot plants. Similarly, Nishitani and Masuda (1980) have reported that auxin stimulates the turnover of Gal-containing polysaccharides during cell elongation. A quantitative analysis of hemicellulosic polysaccharides revealed that active changes of Gal and Xyl in the cell wall during in vivo cell extension and maturation, suggesting that xyloglucan is synthesized (Nishitani and Masuda, 1979; Terry et al., 1981). In contrast to the findings of Basic et al. (1988), we were unable to detect Fuc residue in the apoplastic, hm-pectin and/or hemicellulose fractions.

Pectin and hemicellulose synthesis during differentiation of vascular tissue in *Phaseolus vulgaris* callus and in the hypocotyls were regulated by arabinan synthetase during division and extension growth, leading to xylan synthetase during the period of secondary thickening of the cell wall (Bolwell and Northcote, 1981). Hormonal regulation of these enzymes might be responsible for the modulation in the amount of hm-pectin and hemicellulose polysaccharides.

The cellulose contents remained similar, irrespective of the presence (19.1%) or absence (18.7%) of growth regulators (Table 2). In agreement to our results, Yeo et al. (1995) reported that cells oriented towards cell division, such as calli and suspension cells of barley, produce more non-cellulosic polysaccharides and less cellulose (16-17%) than differentiated cells do. And also, Yeo et al. (1999) reported that the non-embryogenic and embryogenic calli of celery produce less cellulose (12-13%). According to Takeuchi and Komamine (1978), the suspension cells of *Vinca rosea* contained a less amount (25%, w/w) of cellulose during cell division, expansion and stationary phase. Therefore, the possible reason for the lower content of cellulose in the suspension cells is the reduced rate of its synthesis. Growth regulators could not modulate its rate of synthesis. However, the low level of cellulose biosynthesis of the in vitro cultured cells, the callus and suspension cells of barley was affected by several biochemical factors such as carbon sources (sucrose, maltose, and UDPG), reduced reagents (ascorbic acid and glutathione) and physical factors such as artificial pressure and high gravity (Yeo et al., 1998) and the low level of kidney bean suspension cells was also affected by $CaCl_2$ (Yeo and Kim, 2002)

From the our observements we conclude that the exogenous growth regulators have no effect on the growth in the subculture of suspension cells of kidney bean and the exogenous growth regulators have modulated the structural turnover of the symplastic and apoplastic sugars during the growth of suspension cells, oriented to cell division. These results support the suggestion of Albersheim et al. (1997), that the structure of cell-wall polysaccharides might define their mode of synthesis.

ACKNOWLEDGEMENTS

This research was supported in part by research

funds of Chonbuk National University, Chonju, Korea.

Received December 24, 2003; accepted January 8, 2004.

LITERATURE CITED

Albersheim P, Darvill A, Roberts K, Stachelin A,Varner JE (1997) Do the structures of cell wall polysaccharides define their mode of synthesis? Plant Physiol 113: 1-3

Basic AP, Harris H, Stone BA (1988) Structural and function of cell wall. *In* The Biochemistry of Plants, 14: Acad Press, San Diego, pp 297-376

Bleckeney AB, Harros PJ, Henry RJ, Stone BA (1983) A simple and rapid preparation of alditol acetates for monosaccharide analysis. Carbohy Res 113: 291-299

Blumenkrantz N, Asboe-Hansen G (1973) New method for quantitative determination of uronic acids. Anal Biochem 54: 484-489

Bolwell GP, Northcote DH (1981) Control of hemicellulose and pectin synthesis during differentiation of vascular tissue in bean (*Phaseolus vulgaris*) callus and in bean hypocotyls. Planta 152: 225-233

Chai Y-K, Kim K-H, Yeo U-D, Sakurai N (1998) Long-term effects of growth regulators and nitrogen sources on proliferation and turnover of cell wall polysaccharides in suspension culture of kidney bean (*Phaseolus vulgaris* L.). Kor J Plant Tiss Cult 25: 477-485

Delmer DP (1999) Cellulose biosynthesis exciting times for a difficult field of study. Annu Rev Plant Physiol Plant Mol Biol 50: 245-276

Delmer DP, Ohana P, Gonen L, Benzamin M (1993) In vitro synthesis of cellulose in plants: still a long way to go! Plant Physiol 103: 307-308

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28: 350-356

Hoson T (1993) Regulation of polysaccharide breakdown during auxin-induced cell wall loosening. J Plant Res 106: 369-381

Kato Y, Nevins DJ (1982) Structure of a pectic polysaccharide fraction from Zea shoot. Plant Physiol 89: 792-797

Kokubo A, Sakurai N, Kuraishi S, Takeda K (1991) Culm brittleness of barley (*Hordeum vulgare* L.) mutants is caused by smaller number of cellulose molecules in cell wall. Plant Physiol 97: 509-514

Labavitch JM, Ray PM (1974) Relationship between promotion of xyloglucan metabolism and induction of elongation by IAA. Ibid: 499-502

Labavitch JM (1982) Cell wall turnover in plant development. Annu Rev Plant Physiol 32: 385-406

Masuda Y (1990) Auxin-induced cell elongation and cell wall changes. Bot Mag Tokyo 103: 3455-370

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol 15: 473-493

Nishitani K, Masuda Y (1979) Growth and cell wall changes in azuki bean epicotyls. I. Changes in wall polysaccharides during intact growth. Plant Cell Physiol 20: 63-74

Nishitani K, Masuda T (1980) Modification of cell wall polysaccharides during auxin-induced growth in azuki bean epicotyls. Plant Cell Physiol 21: 169-181

Sakurai N (1991) Cell wall functions in growth and development - physical and chemical points of view. Bot Mag Tokyo 104: 235-251

Sakurai N (1998) Dynamic function and regulation of apoplast in the plant body. J Plant Res 111: 133-148

Sakurai N, Tanaka S, Kuraishi S (1987) Changes in wall polysaccharides of squash (*Cucurbita maxima* Duch.) hypocotyls under water stress condition. I. Wall sugar composition and growth as affected by water stress. Plant Cell Physiol 28: 1051-1058

Taiz L (1984) Plant cell expansion: Regulation of cell wall mechanical properties. Ann Rev Plant Physiol 35: 585-657

Takeuchi Y, Komamine A (1978) Changes in the composition of cell wall polysaccharides of suspension-cultured *Vinca rosea* cells during culture. Physiol Plant 42: 21-28

Terry ME, Bonner BA, Jones RL (1981) Soluble cell wall polysaccharides released from pea stems by centrifugation. I. Effect of auxins. Plant Physiol 67: 531-537

Wakabayashi K, Sakurai N, Kuraishi S (1991) Effect of ABA on synthesis of cell-wall polysaccharides in segments of etiolated squash hypocotyls II. Levels of UDP-neutral sugars. Plant Cell Physiol 32: 427-432

Yeo U-D, Soh W-Y, Tasaka H, Sakurai N, Kuraishi S, Takeda K (1995) Cell-wall polysaccharides of callus and suspension-cultured cells from three cellulose-less mutants of barley (*Hordeum vulgare* L.). Plant Cell Physiol 36: 931-936

Yeo U-D, Soh W-Y, Nakagawa N, Sakurai N, Takeda K (1998) Long-term effects of several biochemical and physical factors on cellulose synthesis in callus and suspension cultures of normal and mutant barley (*Hordeum vulgare* L.) strains producing less cellulose. J Plant Biol 41: 219-226

Yeo U-D, Han J-Y, Choi Y-E, Soh W-Y, Nakagawa N, Sakurai N (1999) Turnover of cell-wall polysaccharides during somatic embryogenesis and development of celery (*Apium graveolens* L.). J Plant Biol 42: 8-15

Yeo U-D, Kim K-H (2002) Regulation of cell-wall polysaccharide components by $CaCl_2$ in suspension culture of kidney bean (*Phaseolus vulgaris*). J Plant Biol 45: 90-95