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Changes of major wall polysaccharides and glycoproteins of tobacco cells in response to excess boron

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Abstract

Effects of excess concentrations of boron on major cell wall components of tobacco cells (*Nicotiana tabacum* L. cv. Burley 21) were studied. Pectin, xyloglucan, hydroxyproline-rich glycoproteins (extensin), and arabinogalactan proteins were characterized. Results showed that increased boron supply resulted in significant decrease in cell and cell wall dry weights. Also, high concentrations of boron reduced relative amounts of major wall components. Compared with the normal conditions, increase in certain sugars in pectin (e.g., xylose and its methylated derivative) and decrease in glucose, arabinose, and rhamnose in those treated cells with higher concentrations of boron was significant. The content of hydroxyproline-rich glycoproteins decreased when tobacco cells were supplied with higher concentrations of boron. In these cells, increased ratio of protein to glycan part and increase in hydroxyl lysine among other amino acids were noticeable. Amount of arabinogalactan proteins also decreased as boron supply increased. Excess boron did not alter the composition and concentration of amino acids of arabinogalactan; however, it remarkably increased xylose and glucose but decreased galactose and arabinose concentrations.

Key Words: arabinogalactan protein, boron, cell wall polysaccharides, extensin, *Nicotiana tabacum*

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Introduction

Boron (B) is an essential micronutrient for normal growth of higher plants. B's cross-linking with apiose residues (and probably other cis-diol containing sugars) in the pectic polysaccharide rhamnogalacturonan II (RGII) has been widely accepted as its primary role.

However, there are increasing evidences showing that B is essential even for organisms lacking cell walls, supporting a function for B beyond the cell envelope (1, 2). Evidence for a possible role of B in other processes such as the maintenance of plasma membrane function and several metabolic pathways continue to mount (3).

Even in connection with its beneficial effects on human health, it has been suggested that boron could possibly affect cell membranes and the way signals are transmitted across these membranes. As such, boron has also effect on the balance and absorption of calcium, magnesium and phosphorus (4).

Plant researchers have observed a close relationship between B nutrition and the primary cell wall. In the past, the plant cell was considered as an inanimate rigid box. It is now recognized as a dynamic structure that plays important roles in controlling the growth and differentiation of plant cells (5). By definition, only primary cell walls are extensible. In the primary walls of growing plant cells, cellulose microfibrils are tethered together by cross-linking glycans (hemicelluloses). This assembly is embedded in matrix polysaccharides and glycoproteins (6). Ultrastructural and physical properties of the cell wall are known to be affected by B deficiency, partly due to B-dependent conformation of pectin or formation of borate esters with hydroxyl groups of glycoproteins (7). Whereas Boron-deficient tissues are fragile, plants grown on high B levels may have unusually flexible or resilient tissues. Mechanical properties of growing cells can be modified by cross links between major components of their walls. For instance, reduction and degradation of xyloglucan (XG) and increase in methyl-esterification of pectin enhance wall extensibility (8, 9). Calcium binding of de-esterified pectin (10), and increase in isodityrosine residues of hydroxyproline-rich glycoproteins (HRGP) are processes involved in wall stiffening (11).

The present study has characterized major cell wall components of tobacco in normal conditions (0.1 mM) and in the presence of excess B (10 and 20 mm). Suspension cultures are highly homogenous systems that mainly consist of cells with primary cell walls. Moreover, they produce polysaccharides which are the same as those produced by intact plants (12, 13). Although the effect of B deficiency on wall polysaccharides, particularly pectin, has been widely reported, few studies have investigated the potential impact of excess B on wall components other than pectin, i.e. glycoproteins. The present study is concerned with examining the effects of excess concentrations of B on the quantity and quality of major wall polysaccharides and glycoproteins in primary cell walls of tobacco cells.

Materials and methods

Cell cultures and extraction of polysaccharides

A rapid growing cell line in the form of callus was established from leaves of tobacco (*Nicotiana tabacum* L. cv. Burley 21). The calli was maintained in our laboratory for

more than 100 subcultures from which suspension cultures were established. Both calli and suspensions were grown in a modified LS medium containing 3% sucrose and 0.1 mM B as boric acid, pH 5.8. The cells were grown on a rotary shaker at 123 rpm in darkness and were sub-cultured every 7 days in their logarithmic growth phase (14). Exactly 1 g of 7-day-old cells was transferred to three flasks containing 0.1, 10 and 20 mM of B and were allowed to grow for 7 days, then harvested by suction filtration. Filtered cells were used to prepare cell walls and the filtrates were used to extract arabinogalactan proteins (AGP). Filtered cells were washed with 20 volumes (v/w) of water and 0.5 mM $CaCl_2$, then suspended in 4 volumes (v/w) of 99.5% EtOH and filtered. The filtered cake was washed successively by suspension in 10 volumes (v/w) of 99.5% EtOH twice (1 h each) and then suspended in the same portion of a mixture of $CHCl_3$: MeOH (2:1, v/v) overnight and finally suspended in acetone for 1 h. The residue was air-dried and used as a cell wall preparation (15). Contamination of wall polysaccharides with starch was removed by incubation with 300 units of porcine pancreas α-amylase (EC 3.2.1.1, Sigma) in Na-acetate buffer (50 mM, pH 6.5) over a period of 2 h at 37˚C. Pectin was extracted three times (20 min each) with EDTA (50 mM in 50 mM Na-phosphate buffer, pH 6.8, 95˚C) and was dialyzed against deionized water (2 L, 24 h, 3-4 changes), before lyophilization. Hemicellulose was dissociated with 17.5% NaOH and 0.02% $NaBH_4$, 25˚C three times for a total time of 24 h. It was neutralized with acetic acid and dialyzed against deionized water (2 L, 24 h with 4-5 changes). This was followed by centrifugation at ×10,000 g for 20 min. Water-soluble hemicellulose was designated as hemicellulose B (HB). In order to partially release the remaining hemicellulosic substances entrapped within cellulose microfibrils, the alkali-insoluble residue was initially treated with trifluoroacetic acid (TFA). After the extraction of TFA-soluble hemicelluloses, the residue was washed with EtOH: Et_2O (1:1, v/v), dried in air and designated as cellulose (8).

Ion-exchange and size exclusion chromatography

Fractionation of pectin and HB was performed on a DEAE-Sephadex A-25 anion exchange column (2.6×10 cm, Pharmacia Biotech, Uppsala, Sweden). The column was equilibrated with Na-phosphate buffer (10 mM, pH 7.2) and adsorbed materials were eluted sequentially with 10, 100 and 500 mM Na-phosphate buffer, pH 7.2. Each tube (3 mL) was assayed for total sugar (TS) and uronic acid (UA) contents (8). Carbohydrate-containing fractions were pooled, lyophilized, and subsequently chromatographed on a Sepharose CL6-B column (1.6×90cm, Pharmacia Biotech, Uppsala, Sweden) (16, 17). Fractionation of cell filtrate was also performed on DEAE-Sephadex A-25 column equilibrated and washed with Na-phosphate buffer (10 mM, pH 6.0) followed by sequential elution of adsorbed materials with 0.05, 0.1, 0.2, 0.3, 0.5 M NaCl, and 0.5 M NaOH (18). Tubes were assayed for protein content in addition to measurement of total sugar and uronic acid contents.

Analytical methods

Total sugars (TS) were assayed through the phenol-sulfuric acid method (19), using glucose as a standard. Uronic acids (UA) was determined using a modified *m*-hydroxydiphenyl method (15) and galacturonic acid as the reference material.

Protein contents were assayed according to the method modified by Darber (20). Degree of methyl esterification (DME) of uronic acids was determined colorimetrically (15). Xyloglucan (XG) content was determined by the iodine staining method. Molecular weights of HB and XG were determined using dextran standards of 10, 77 and 500 KDa (Sigma Chemical Co, St. Louis, MO, USA) (9). Hydroxyproline was measured according to the method proposed by Smith and others (21).

GLC Analysis

Sugar composition of polysaccharides was determined by a GLC system (Model 17A, Shimadzu Cooperation) equipped with a capillary column (DB-17, J&W Scientific, 30 m×0.320 mm i.d.). Oven temperature was set to a range from 200˚C to 230˚C at 1˚C min-1. Alditol acetate derivatives of sugars were prepared using the method devised by Sakurai and Nevins (1997). KDO (3-Deoxy-d-manno-2-octulosonic acid) was determined colorimetrically (22).

Extraction and characterization of HRGP and AGP

Hydroxyproline-rich glycoproteins (extensin), were eluted from the cells with 100 mM AlC_{l3} (10 volume, v/w). Trichloroacetic acid was added (10%, w/v) and centrifuged at ×15,000 g for 45 min at 4˚C. Hydroxyproline-rich supernatant was extensively dialyzed before lyophilization (23). The concentration of AGPs was determined with ß- Glucosyl Yariv reagent (1,3,5-tris[4-ß-D-glucopyranosyloxyphenylazo]-2,4,6-trihydroxybenzene, Bio Supplies, Parkville, Australia) by single radial gel diffusion (24). Both glycoproteins were subjected to further analysis to determine their sugar and amino acid compositions.

Determination of amino acids

Samples were hydrolyzed with 6 M HCl in sealed tubes at 110˚C for 24 h. The hydrolysates were filtered through 0.45 µm cellulose acetate filters and applied to amino acid analyzer (L-8500 A, Hitachi, Japan).

Statistical analysis

All of the experiments were carried out with at least three independent repetitions in triplicate. All values are shown as the mean ± SD. Statistical analysis was performed using Student´s t-Test. Differences were considered significant at the 0.05 level.

Results

Compared with cells grown in normal concentration (0.1 mM) of B, increase in B concentration significantly reduced cell growth (Fig. 1).

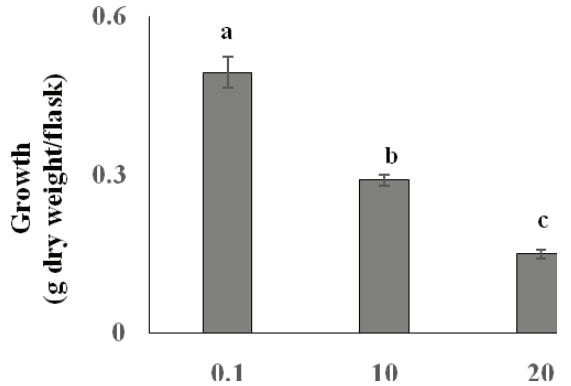

Figure1. Effect of different concentrations of boron on the growth of tobacco cells

Note. Data are presented as the means± SD with n = 3. Bars with different letters are significantly different at p≤0.05, according to the Student's t-test

While cellulose content of tobacco cell walls did not change in presence of 10 mM of B, treatment with 20 mM of B significantly decreased the content of cellulose (Table1).

The contents of pectin and hemicelluloses

were markedly reduced in 10 mM and 20 mM of B, compared with 0.1 mM B-treated cells (Table 1). Treatment of tobacco cells with 10 and 20 mM of B also increased the ratio of uronic acids to neutral sugars of pectin (Table 2).

Table 1. Major wall components of tobacco cells treated with different concentrations of boron (B).

B supply (mM)	Cell wall (% of FW)	Pectin	HB	TFA-SF	Cellulose
		(µg.mg cell wall^{-1})			
0.1	4.3±0.1^{a}	220±12^{a}	129±10^{a}	270±11^{a}	202±9^{a}
10	2.5±0.1^{b}	116±10^{b}	47±2^{c}	245±10^{b}	209±10^{a}
20	2.0±0.1^{b}	115±6^{b}	55±4^{b}	274±14^{a}	124±10^{b}

Note. HB = Hemicellulose B; TFA-SF = TFA-soluble fraction. Data are means ± SD with n = 3. Different letters indicate significant differences at p≤0.05, according to the Student's t-test.

Table 2. Total sugar (TS), uronic acid (UA) and degree of methyl esterification (DME) of pectin of tobacco cellstreated with different concentrations of boron (B).

B supply (mM)	TS ⅋ of Pectin)	UA	DME (% of UA)
0.1	59 ± 2.36^{b}	41 ± 1.64^{b}	20 ± 0.8^{b}
10	57 ± 2.28^{b}	43 ± 1.72^{b}	25 ± 1.0^{a}
20	52 ± 2.08^{a}	48 ± 1.92^{a}	23 ± 0.9^{a}

Note. Data are presented as the means± SD with n = 3. Different letters show significant differences at p≤0.05, according to the Student's t-test.

As appeared in Table 2, degree of methyl eterification of uronides was also increased in 10 and 20 mM of B, compared with normal concentration (0.1 mM) of B Analysis of sugar composition of pectin showed that treatment with 10 and 20 mM of B reduced the contents of Glc, Rha, and Ara but increased the contents of methyl xylose and methyl fucose of tobacco cell walls (Table 3).

Xyloglucan content of HB was considerably reduced in 20 mM of B; yet it did not change in 10 mM of B, compared with the control condition (Fig. 2)

Xyloglucan content of trapped hemicelluse (TFA-SF), however, was significantly reduced in presence of both 10 and 20 mM of B, compared with the control condition (Fig. 2). Average molecular weights of both hemicelluloses and its xyloglucan in 10 and 20 mM B-treated cells were higher than those of the cells treated with 0.1 mM B (Fig. 3).

Table 3. Glycosyl composition of pectic fractions of the cell wall of tobacco cells.

	Glycosyl composition (µg.mg pectin^{-1})								
B supply (mM)	Me-Fuc	Me-Xyl	Rha	Ara	Xyl	Man	Glc	Gal	KDO
0.1	N	0.02±0.00	27.29±0.33	48.20±0.58	N	2.13±0.03	8.92±0.11	20.66±0.25	0.40±0.00
10	N	1.96±0.02	23.71±0.28	48.84±0.59	1.55±0.02	3.97±0.05	8.44±0.10	22.45±0.27	0.32±0.00
20	0.80±0.01	4.51±0.05	16.62±0.20	26.82±0.32	1.00±0.01	1.20±0.01	1.28±0.02	17.85±0.21	0.38±0.00

Note. Glycosyls were determined as their alditol acetate derivatives by GLC, KDO was determined colorimetrically. Data are presented as the means± SD (n = 3). N not detected.

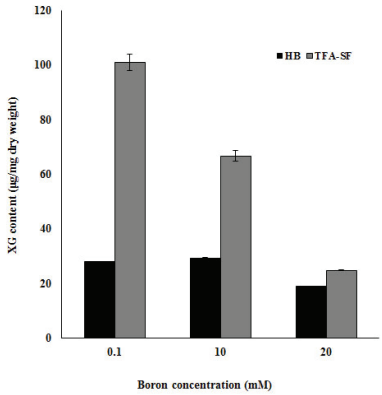

Figure 2. Xyloglucan content of hemicellulose B and TFA-soluble fractions in the walls of tobacco cells treated with different concentrations of B.

Note. Data are presented as the means± SD (n = 3). Bars with different letters are significantly different at p≤0.05, according to the Student's t-test. XG = Xyloglucan; HB = hemicellulose B; TFA-SF = TFA-soluble fraction.

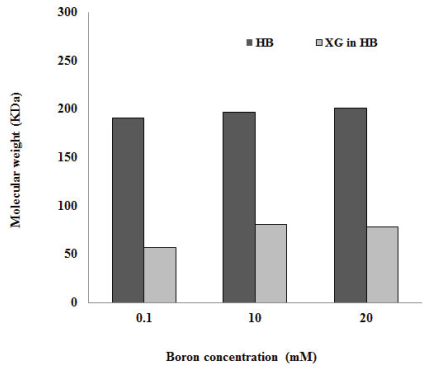

Figure 3. Mass average molecular weight of hemicellulose B (HB) and xyloglucan of HB tobacco cells treated with different concentrations of B.

Hydroxyproline-rich glycoprotein contents of tobacco cells decreased along with increasing B supply (Table 4).

Amino acid and sugar compositions of HRGP eluted from tobacco cells treated with different concentrations of B (n=3).

Increase in protein portion of HRGP along with increase in concentration of B

Tabel 4. Amino acid and sugar compositions of HRGP eluted from tobacco cells treated with different concentrations of boron (B; n=3).

	B supply (mM)		
	0.1	10	20
Amino acids[a] (mol%)			
Hyp	13.8 ± 0.08	14.7 ± 0.09	10.5 ± 0.06
Ser	4.4 ± 0.03	4.4 ± 0.03	4.3 ± 0.03
Pro	8.8 ± 0.05	6.3 ± 0.04	6.6 ± 0.04
Gly	4.5 ± 0.03	6.0 ± 0.04	7.3 ± 0.04
Ala	4.5 ± 0.03	2.0 ± 0.01	7.3 ± 0.04
Val	7.2 ± 0.04	9.5 ± 0.06	9.4 ± 0.06
Tyr	5.8 ± 0.03	2.4 ± 0.01	2.8 ± 0.02
Hyl	7.1 ± 0.04	11.3 ± 0.07	24.1 ± 0.14
Lys	12.1 ± 0.07	9.3 ± 0.06	5.3 ± 0.03
Monosaccharides[b] (mol%)			
Me-Xyl	N[c]	N	N
Rha	24.6 ± 0.15	34.2 ± 0.21	30.1 ± 0.18
Ara	36.4 ± 0.22	38.8 ± 0.23	34.5 ± 0.21
Gal	35.2 ± 0.21	23.2 ± 0.14	31.3 ± 0.19
Protein[d] (%)	77.1 ± 0.46	89.1 ± 0.53	90.9 ± 0.55
Sugar[e] (%)	22.9 ± 0.14	10.9 ± 0.07	9.1 ± 0.05
Amount[f] (µg)	69.8 ± 0.42	50.2 ± 0.30	59.9 ± 0.36

Note. [a] Only main amino acid residues are shown, [b] Determined as alditol acetate derivatives by GLC [c] Not detected. [d] Percentage of HRGP weight accounted for as protein by Bradford method, [e] Percentage of HRGP weight accounted for as sugar by GLC, [f] Amount of HRGP against 1 mg dry weight of cell wall.

was noticeable. (Table 4). Of amino acid constituents of protein portion of HRGP, hydroxylsine exhibited the most increase.

Amounts of AGP that were released to the media were quantified by radial gel diffusion. They were reduced by treatments containing higher concentrations of B (Table 5).

There were no significant changes in amino acid contents. As shown in Table 5, whereas contents of glucose and xylose were significantly increased by B treatments, decrease in galactose, rhamnose and arabinose was remarkable

Table 5. Content and composition of AGP in tobacco cells treated with different concentrations of boron (B; n=3).

	B supply (mM)		
	0.1	10	20
Amino acids[a] (mol%)			
Ala	10.7 ± 0.06	10.1 ± 0.06	11.4 ± 0.07
Asp	9.1 ± 0.05	9.4 ± 0.06	8.9 ± 0.05
Hyp	7.0 ± 0.04	7.2 ± 0.04	7.4 ± 0.04
Pro	6.4 ± 0.04	6.0 ± 0.04	5.4 ± 0.03
Thr	4.9 ± 0.03	5.3 ± 0.03	5.4 ± 0.03
Ser	8.1 ± 0.05	8.1 ± 0.05	9.1 ± 0.05
Glu	6.7 ± 0.04	6.7 ± 0.04	7.4 ± 0.4
Gly	8.6 ± 0.05	8.4 ± 0.05	9.5 ± 0.06
Val	7.5 ± 0.05	6.1 ± 0.04	7.1 ± 0.04
Ile	3.5 ± 0.02	2.5 ± 0.02	3.5 ± 0.02
Leu	6.4 ± 0.04	5.7 ± 0.03	5.4 ± 0.03
Tyr	3.4 ± 0.02	3.2 ± 0.02	3.7 ± 0.03
Phe	4.2 ± 0.03	5.1 ± 0.03	4.2 ± 0.02
Hyl	6.7 ± 0.04	8.8 ± 0.05	7.6 ± 0.03
Lys	4.4 ± 0.03	3.0 ± 0.02	2.6 ± 0.05
Arg	2.5 ± 0.02	1.6 ± 0.01	1.5 ± 0.02
Monosaccharides[b] (mol%)			
Gal A	3.4 ± 0.02	3.1 ± 0.02	4.6 ± 0.03
Ara	48.6 ± 0.29	35.8 ± 0.21	30.8 ± 0.18
Gal	18.4 ± 0.11	10.9 ± 0.07	12.3 ± 0.07
Rha	1.2 ± 0.01	1.9 ± 0.01	0.1 ± 0.00
Xyl	13.4 ± 0.08	18.7 ± 0.11	27.7 ± 0.17
Glc	11.7 ± 0.07	24.9 ± 0.15	21.5 ± 0.13
Level of AGP [b]	15.8 ± 0.09	14.0 ± 0.08	13.2 ± 0.08

Note. [a]Determined as alditol acetate derivatives by GLC, [b] µg/ mg dried medium

Discussion

A noticeable reduction was observed in dry weight of the tobacco cells in higher concentrations of B. Primary cell walls are considered as a mixture of biopolymers, essentially polysaccharides, among which cellulose, XG and pectin are of particular importance. A noticeable reduction was observed in the dry weight of the tobacco cells at higher concentrations of B. Primary cell walls are considered as a mixture of biopolymers, essentially polysaccharides, among which cellulose, XG and pectin are of particular importance. In the cells which were treated with higher concentrations of B, reduction of cell dry weight was accompanied by reduction of major wall components e.g.,

pectin, cellulose, and HB. The sum of wall components was lower than expected, and this was more pronounced in the cells treated with high concentration of B, compared with the grown in control conditions. It may be accounted for by production of low molecular weight compounds e.g., proteins, lipids polyamins, and phenolics which have been washed out during extraction and further dialysis (3). Identification of these components was beyond the scope of the present study.

Pectin is thought to fill the interstices within the cellulose/XG network, whereas cellulose and XG are known to form a network mediated most likely by non-covalent linkages (25). Primary cell wall pectins are characterized by a high quantity of neutral sugar side chains, mostly composed of Ara and Gal that are mainly branched at O-4 of Rha residues. The structural classes of the pectic polysaccharides include homogalacturonan, xylogalacturonan, apiogalacturonan, RG-II, and RG-I (25). Several findings suggest that the cell wall composition and architecture, mainly related to the alterations in cross-linked glycans and pectins, play a central role in the responses of plants to various environmental signals (26). The pectin/cellulose interactions have been suggested by other researchers (27, 28, 29). In the cell walls of *Nicotiana plumbaginifolia*, Iwai et al. (30) reported that arabinose-rich pectins are strongly associated with cellulose hemicelluloses complexes. Some researchers suggested interactions between cellulose and pectic galactan side chains in apple cell walls (27). In the present study, arabinose content of pectin of tobacco cells was significantly decreased in higher concentrations of B. This may result in a loosen pectin-cellulose interaction. Artificial composites created with pectins or isolated pectic domains have shown that pectins can also bind to cellulose microfibrils through their neutral sugar side chains (29).

Although this study was not concerned with analyzing sugar linkages, occurrence of methy-xylose in side chains of pectin molecules reduces the possibility of pectin interacting with cellulose/hemicelluloses complex. Degree of methyl-esterification of uronic acids of 10 and 20 mM B-treated tobacco cells was higher than that of 0.1 mM B-treated ones. The kind of sugars and degree of esterification of uronic acids with methyl groups have been postulated to alter the reology of pectin and accessibility of wall modifying enzymes (31). Formation of a pectin network involves ionic bridging of the nonestrified carboxyl groups by calcium ions. When blocked by methyl-esterified groups, the carboxyl groups cannot participate in this type of inter-chain network formation (1). However, in methylatedglycan chain both the hydrogen bonds and the hydrophobic contacts between methyl groups are important parameters for an efficient association of the chains (32).

Xyloglucan may contribute to the formation of macromolecular architecture, and to the determination of the mechanical properties of the cell wall. Loosening of the cell wall leading to cell expansion occurs by coordinated breakage of hydrogen bonds between xyloglucan and cellulose microfibrils and of xyloglucan tethering (33). Moreover, difficulty of XG extraction suggestes that XG has also some covalent binding with pectin and other wall polymers (34, 25). The lower XG content of 10 and 20 mM B-treated cells indicates the reduction of viscous interactions between cellulose and matrix polysaccharides in these treatments (8).

Glycolipids and glycoproteins have been suggested to be putative B-binding structures (3). Hydroxyproline rich glycoprotein is highly insoluble and its major function appears to strengthen cell wall (21). Although each type of extensin has unique characteristics, sugars and amino acid moieties of extension of tobacco cells in the present study shared similarities with previous reports (35, 11, 21). Amounts of HRGP extracted from the cells decreased as B supply increased. With regard to the effect of B on wall proteins of certain pollen tubes, research suggests that in the presence of B HRGPs might be modified (36). This alteration could be due to insolubility of HRGPs in the wall in the presence of B or to formation of a linkage between excess B and HRGPs, or both. It has been reported that HRGPs are slowly insolubilized into the cell wall by a covalent link (11). Furthermore, it has been shown that HRGPs are not covalently bound to the walls of B deficient nodules of soybean roots, suggesting that HRGP is a candidate for borate cross linking in primary cell walls (37). Coincidently, decrease of extractable HRGP from those cells treated with 10 and 20 mM of B can be attributed to tighter interactions between B and HRGP in their walls, compared to 0.1 mM B-treated ones. Yet, the reason behind increase of certain amino acids (e.g., glycine and hydroxylysine) and decrease of lysine in HRGP of 10 and 20 mM B-treated cells remains unclear and needs to be uncovered by further detailed studies.

Consistent with other reports, high amounts of alanine, serine, hydroxyproline, Ara, and Gal and low amounts of Rha and uronic acids were detected in AGP of tobacco cells (18, 35). The amounts of AGP released to the medium of tobacco cells decreased as the concentration of B in growth medium increased. Zhu et al. (38) reported that the level of AGP was greatly reduced in the plasma membranes and culture medium of growth-limited NaCl-adapted tobacco cells. They argued that this observation supports the notion that AGPs play a role in cell expansion. A direct role of B in maintaining membrane structure through cis-diol complexation with glycoproteins and, in particular, those that are glycosylphosphatidylinositol anchored (e.g., AGP), has been suggested (39). Therefore, it can be suggested that in higher concentrations of B, occurrence of more complexation of B with AGP and HRGP limits the solubility of these glycoproteins and their release to the medium, resulting in reduced growth of the cells. Kasajima and Fujiwara (40) showed induced expression of a gene belonging to zinc finger family transcription factors in response to high B in *Arabidopsis*. High B concentration may trigger a mechanical cascade of signals extending into the cytoplasm via the cell wall-plasma membrane-cytoskeleton continuum, with the possible involvement of AGP. This hypothesis is supported by the fact that B deprivation leads to an altered polymerization pattern of cytoskeletal proteins assembly (41). Therefore, it is plausible that reduction of the growth and other physiological responses of tobacco cells to high concentrations of B are resulted from changes in the activity of certain genes. Further experiments are needed to clarify the impact of B on the activity of different glycan transferase genes and change in the quantity and composition of wall polysaccharides and glycoproteins.

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