PERGAMON

Phytochemistry 59 (2002) 703–708

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Changes in cell wall polysaccharides of *Silene vulgaris* callus during culture

Elena A. Günter, Yury S. Ovodov*

Institute of Physiology, Komi Science Centre, The Urals Branch of the Russian Academy of Sciences, 50, Pervomaiskaya str., 167982 Syktyvkar, Russia

Received 24 August 2001; received in revised form 22 January 2002

Abstract

In *Silene vulgaris* (M.) G. cell culture three growth phases were distinguished, namely, a lag phase, an exponential phase and a stationary phase. Pectin termed silenan and an acidic arabinogalactan were isolated as cell wall polysaccharides of *S. vulgaris* callus at the different growth phases during culture. Production of silenan as the galacturonan (or rhamnogalacturonan) core was observed at the beginning of the exponential phase and at the stationary phase of the callus growth. Arabinogalactan, containing the galacturonic acid residues, is formed at the exponential phase followed by attachment to the core of silenan in the middle of the exponential phase. The arabinogalactan constituent of silenan appeared to be destroyed gradually at the stationary growth phase. The monosaccharide compositions of silenan and arabinogalactan were determined at various phases of the callus growth. Silenan was found to be formed in maximum amounts at the exponential phase of the cell growth. Insignificant alterations of the yields of acidic arabinogalactan were found during culture while total productivity per litre of medium and rate of production per day of arabinogalactan were found to be maximal at the exponential phase of growth.

Keywords: *Silene vulgaris* (Moench) Garcke; Caryophyllaceae; Callus; Cell wall polysaccharides; Arabinogalactan; Pectin; Silenan

1. Introduction

Biosynthesis of cell wall components and their alterations during plant development have been presented in reports by several authors (Fujino and Itoh, 1998; Yeo et al., 1998; Stolle-Smits et al., 1999). Much attention has been paid to the changes in the composition of the cell wall polysaccharides from suspension-cultured cells during culture. Noncellulosic components of the cell wall have been shown to undergo the degradation or interconversion associated with growth in several different species including *Vinca rosea* (Takeuchi and Komamine, 1978, 1980) and *Linum usitatissimum* (Goubet and Morvan, 1994). In vitro biosynthesis of polysaccharides was shown to be most active in the cell division and expansion phase (Takeuchi and Komamine, 1980; Goubet and Morvan, 1994; Geshi et al., 2000). No data on the kinetic of biosynthesis of polysaccharides in *Silene vulgaris* (M.) G. callus culture are present in literature.

The preliminary studies of *S. vulgaris* callus induced from the medicinal plant *S. vulgaris* indicated the synthesis of polysaccharides possessing immunomodulatory activity (Popov et al., 1999). *S. vulgaris* callus appeared to be an alternative to the plant as the source of this valuable product. We have described previously that the main polysaccharides of the callus were acidic arabinogalactan and pectin (named silenan) (Misharina et al., 1999). In addition, the influence of various factors on producing polysaccharides by *S. vulgaris* callus has been previously studied (Günter and Ovodov, 2001).

The aim of the present study was an investigation of variations in growth and the cell wall polysaccharide composition of *S. vulgaris* callus during culture.

2. Results and discussion

2.1. Growth of *S. vulgaris* callus culture

The growth curve was found to be typical for culture of cells namely for *Silene alba* cell culture (Lhernould et al., 1994). The kinetic of growth in terms of fresh weight (fr. wt) during a culture cycle is shown in Fig. 1. A 3 day

* Corresponding author. Tel.: +7-8212-241001; fax: +7-8212-24-1001.

E-mail address: ovoys@physiol.komisc.ru (Y.S. Ovodov).

0031-9422/02/$ - see front matter
PII: S0031-9422(02)00041-9

lag phase was followed by an exponential phase between the 3rd and 21st day and a stationary phase. The fresh cell weight reached its peak (518 g l^{-1}) on day 21. Changes in growth parameters in relation to dry weight (dry wt) during a culture cycle are shown in Fig. 1 and Table 1. The curve of dry wt was found to compose of three parts: a lag phase—from the beginning to the 3rd day of the culture, with a synthesis of 2.3 g of dry wt per litre of medium; an exponential phase—from the 3rd to the 18th day when there was a significant increase in the dry wt per litre of medium (12.0 g l^{-1}) and a stationary phase—from the 18th day onwards when destruction of biomass was observed. The cell growth rate and productivity of dry biomass per day were maximum during an exponential phase and attained 0.93 day $^{-1}$ and 0.76 g l^{-1} day^{-1}, respectively (Table 1). The time of dry biomass doubling reached minimal value (0.75 day) during an exponential phase.

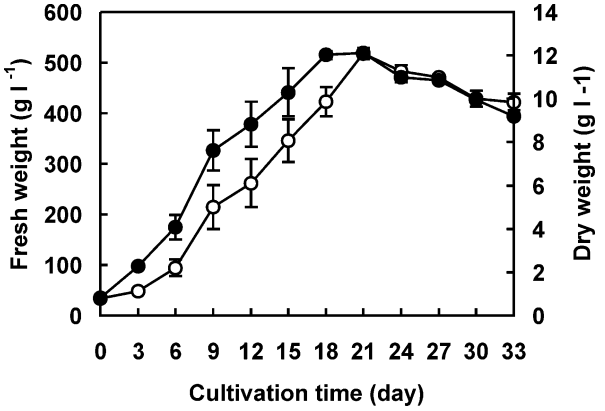

Fig. 1. Growth curve of the callus culture of *S. vulgaris*: (○) fr. wt, (●) dry wt. Bars indicate s.d.

2.2. The contents of the cell wall polysaccharides during culture

The highest yields (7–9%) of silenan were obtained during an exponential phase with the maximum yield on the 18th day of cultivation (Fig. 2A). A slight decrease of the yield was observed on the 9th day of cultivation (early exponential phase). This is likely to be related to a dilution effect, because the contents of silenan during this period increased insignificantly while the dry cell weight increased permanently. Takeuchi and Komamine (1980) have shown that biosynthesis of pectic substance was more active in the cell division phase than in the cell expansion phase. The productivity of silenan in the callus was found to increase continuously during the lag and the exponential phase (Fig. 2A). The highest productivity of silenan was observed from the 15th to the 24th day of time course with the maximum (0.7 g l^{-1}) on the 18th day of cultivation. The yield and productivity of silenan by the callus were decreased during a stationary phase probably due to the cessation of growth and following destruction of biomass and a degradation of arabinogalactan-rich pectic polysaccharide at that period. A higher production of pectin per day was obtained during an exponential phase of growth (0.025–0.037 g l^{-1} day^{-1}) (Table 1). Stolle-Smits (1999) has reported that more galactose-rich pectic polymers were deposited into the cell walls of green bean pods during elongation of cells, while the polymers were degraded during maturation of the pods.

Thus, yields, total productivity, and rate of production per day of silenan were found to be maximal during the exponential phase of cell growth. Biosynthesis of silenan corresponded well with the cell growth pattern.

Table 1
The growth and biosynthetic characteristics of *S. vulgaris* callus during cell culture

Cultivation time (day)	μ (day^{-1}) [a]	T (day) [b]	P_{bm} (g l^{-1} day^{-1}) [c]	P_a (g l^{-1} day^{-1}) [d]	P_p (g l^{-1} day^{-1}) [e]
3	0.60±0.03	1.16±0.06	0.49±0.03	0.034±0.004	0.037±0.007
6	0.67±0.12	1.05±0.18	0.54±0.09	0.039±0.004	0.035±0.007
9	0.93±0.13	0.75±0.10	0.76±0.10	0.030±0.0003	0.030±0.001
12	0.83±0.11	0.84±0.12	0.67±0.09	0.030±0.003	0.034±0.005
15	0.78±0.09	0.89±0.11	0.63±0.07	0.029±0.005	0.036±0.002
18	0.77±0.01	0.90±0.01	0.62±0.01	0.023±0.003	0.033±0.007
21	0.67±0.01	1.04±0.02	0.54±0.01	0.020±0.006	0.025±0.004
24	0.52±0.01	1.32±0.03	0.42±0.01	0.016±0.001	0.018±0.0002
27	0.46±0.003	1.51±0.01	0.37±0.003	0.017±0.004	0.012±0.005
30	0.38±0.004	1.84±0.02	0.30±0.004	0.014±0.001	0.009±0.001
33	0.31±0.01	2.21±0.04	0.25±0.004	0.013±0.001	0.008±0.001

The data obtained are expressed as mean values±s.d.

[a] μ (day^{-1}): the dry cell growth rate.

[b] T (day): time of dry biomass doubling.

[c] P_{bm} (g l^{-1} day^{-1}): productivity of dry biomass.

[d] P_a (g l^{-1} day^{-1}): the acidic arabinogalactan production by the callus.

[e] P_p (g l^{-1} day^{-1}): the silenan production by the callus.

An insignificant alteration of the yields of acidic arabinogalactan was found during cultivation. The data obtained are given in Fig. 2B. The contents of arabinogalactan were 6–8% the callus dry wt previously treated with methanol and chloroform. Optimal biosynthesis of acidic arabinogalactan was at the exponential phase of the *S. vulgaris* cell growth, followed by modification of this polysaccharide. Conversely, the relative level of β-1,3-, β-1,4-, β-1,6-galactans in the cell walls of *L. usitatissimum* suspension culture was found to be maximum during lag and exponential phase (Goubet and Morvan, 1994; Geshi et al., 2000). These variations were likely to be related to the activity of different enzymes and the pH optimum for synthesis enzymes that participate in biosynthesis of polysaccharides during development of cells. Goubet and Morvan (1994) have reported that two peaks of galactan synthase activity detected that catalyze the synthesis of β-1,4-galactan at pH 8 during the lag phase and the synthesis of β-1,3-, β-1,6-galactans at pH 5 during the growth phase; both activities are negligible at the end of the growth phase.

The productivity of the callus in biosynthesis of ara binogalactan per litre of medium was found to increase with the culture age up to the 21st day (0.5 g l^{-1}) then productivity appeared to be rather constant (0.4–0.5 g l^{-1}). A similar tendency was shown for biosynthesis of polysaccharides by cell cultures of *Panax notoginseng* (Zhang et al., 1996) and *Panax quinquefolium* (Zhong and Wang, 1998). The maximum acidic arabinogalactan production per day was obtained during an exponential phase (0.029–0.039 g l^{-1} day^{-1}) (Table 1). Thus, although concentration of polysaccharide in the callus was shown to change insignificantly during a culture cycle, total productivity per litre of medium and rate of production per day of arabinogalactan were found to be maximum during an exponential phase, i.e. a phase of cell division and expansion. This phenomenon was observed due to improvement of the cell growth during culture. The polysaccharide (primary metabolite) as the cell wall component was permanently biosynthesized by the cells. The same phenomenon was also claimed in the case of *P. ginseng* cells (Liu and Zhong, 1996) and *P. notoginseng* cells (Zhang and Zhong, 1997).

2.3. The compositions of the cell wall polysaccharides during cultivation

Changes of the sugar compositions of silenan and arabinogalactan during culture are shown in Fig. 3 and

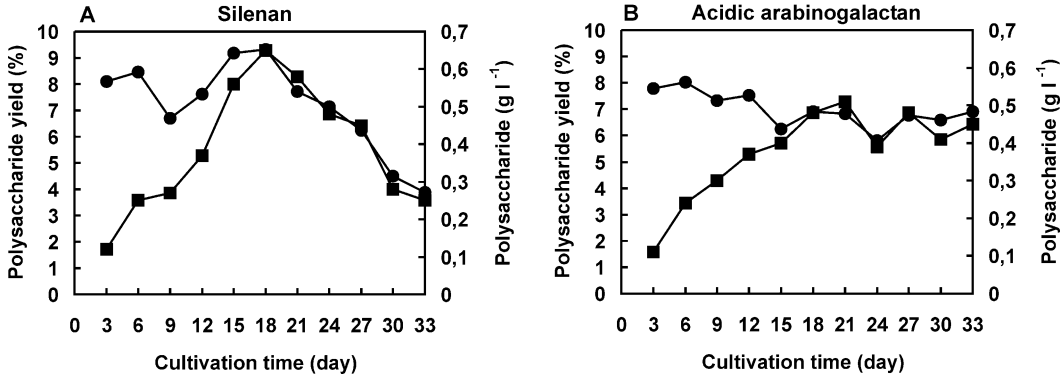

Fig. 2. Variations in the yields (●) and productivity (■) of silenan (A) and acidic arabinogalactan (B) in *S. vulgaris* callus during culture. The data are mean values from two separate experiments.

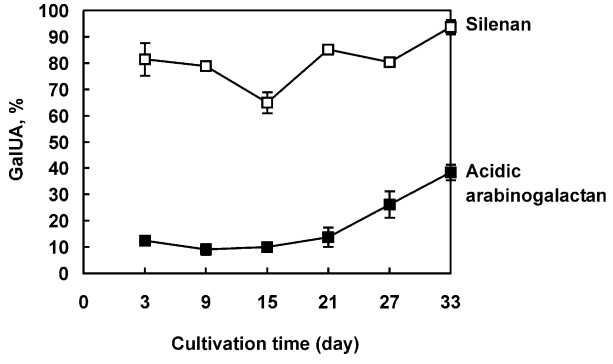

Fig. 3. Changes in the amounts of galacturonic acid (GalUA) in silenan (□) and acidic arabinogalactan (■) in *S. vulgaris* callus during culture. Bars indicate s.d.

Table 2
The neutral sugar composition of silenan in callus culture of *S. vulgaris* during cell culture

Cultivation time (day)	Gal/Ara	Monosaccharides (%)[a]						
		Gal	Ara	Rha	Xyl	Man	Glc	Total
3	1.7	2.9	1.7	0.9	0.5	0.7	1.6	8.3
9	1.0	4.5	4.4	1.0	1.1	1.1	1.7	13.8
15	1.3	3.7	2.8	1.2	1.9	1.9	2.6	14.1
21	1.2	2.9	2.4	1.4	0.4	0.9	1.9	9.9
27	1.6	2.5	1.6	1.6	0.8	0.8	1.0	8.3
33	1.9	2.6	1.4	1.4	1.0	0.8	1.3	8.5

The data obtained are mean values from two experiments.
[a] Molar percentages.

Tables 2 and 3. The relative amounts of galacturonic acid in the isolated silenan covered the range of 79–94% during the culture age with a decrease to a minimum on day 15 to 65% at the period of elongation of the cells (Fig. 3). These data are in agreement with the results of Fujino and Itoh (1998) who showed that the uronic acid contents of pectic polysaccharides in the elongating regions were lower than those in the non-elongating regions of *Pisum sativum* cells. The total amounts of neutral sugars rose until the 15th day of cultivation when upon they decreased. The residues of D-galactose and L-arabinose were shown to be the main neutral sugars in this polysaccharide. The residues of rhamnose, xylose, mannose and glucose were also detectable (Table 2). The galactose/arabinose ratio was changed during culture as follows: high during the lag phase (ca. 1.7), rapidly droping to ~1 during exponential then rising to ~2 during the stationary phase. These alterations may imply changes in the arabinogalactan side chains of silenan. Decrease of the galactose/arabinose ratio during the exponential phase appeared to demonstrate that arabinogalactan was attaching to the backbone of silenan at this period followed by release of the arabinose residues rather than the galactose residues from the side arabinogalactan chains of the rhamnogalacturonan during the stationary phase. These data indicated a location of the arabinose residues at the outer surface of the ramified regions of the macromolecule. Fujino and Itoh (1998) have shown that the ratio of galactose/arabinose increased for non-elongating regions of *P. sativum* cells.

Stolle-Smits et al. (1999) has reported that exponentially growing cell walls contained large amounts of neutral monosaccharide-rich pectic polymers (rhamnogalacturonan). In addition, the level of branched rhamnogalacturonan remained constant, while the level of linear homogalacturonan steadly increased during elongation of green bean pod cells.

Gel filtration of the silenan fractions obtained on days 3, 15, 21 and 33 on a Sephacryl S-500 column resulted in the homogeneous polysaccharide fractions each of which showed a single peak only. K_{av} of the fractions obtained was shown to change as follows: high during the lag phase, decreased during exponential phase and increased during stationary phase. These results demonstrated that the alterations of molecular mass of the macromolecule are observed during the cell growth. Low and intermediate molecular weight silenan is produced during the lag phase; high molecular weight silenan is formed during the exponential phase while the molecular mass declines again during the stationary phase. A similar tendency was observed during development of green bean pod cells (Stolle-Smits et al., 1999).

Table 3
The neutral sugar composition of acidic arabinogalactan in callus culture of *S. vulgaris* during cell culture

Cultivation time (day)	Gal/Ara	Monosaccharides (%)[a]						
		Gal	Ara	Rha	Xyl	Man	Glc	Total
3	3.9	40.2	10.2	2.6	5.0	2.3	3.8	64.1
9	3.3	42.9	13.1	2.9	3.6	2.0	3.9	68.4
15	4.1	48.8	11.9	2.7	2.9	2.1	3.2	71.6
21	4.1	41.4	10.0	2.9	2.8	1.4	2.7	61.2
27	4.7	32.7	6.9	2.9	1.5	1.6	1.5	47.1
33	4.5	26.8	6.0	2.9	1.7	1.7	1.8	40.9

The data obtained are mean values from two experiments.
[a] Molar percentages.

The total amounts of galacturonic acid in the acidic arabinogalactan fraction were found to change during cultivation of the callus. The galacturonic acid contents were shown to vary in the range of 9–14% from the 3rd to the 21st day of the culture age. Then galacturonic acid amounts were increased up to 38% with culture age (Fig. 3) likely due to forming pectin with low galacturonic acid contents. Conversely, the uronic acid contents in the 1,4-glucan fraction of *Vinca rosea* suspension culture were shown to decrease with culture age (Takeuchi and Komamine, 1978).

These differences appeared to be connected with the nature of the polysaccharides investigated. Arabinogalactan of *Silene vulgaris* contained galacturonic acid and represented the component of pectic substances of this plant. The arabinogalactan ramified region of the silenan macromolecule appeared to be utilized during the vegetative period of the plant as well as during callus culture to afford arabinogalactan more rich in galacturonic acid residues. The galacturonan, which is poor in neutral monosaccharide residues at the end of the vegetative stage, seemed to be involved into the plant protopectin system. Conversely, the 1,4-glucan fraction of *Vinca rosea* containing the residues of glucuronic acid is hemicellulose, and is included in the plant protopectin system at the end of vegetation as the whole macromolecule since the glucose residues seemed to be the energetic resources of this system.

The total contents of neutral sugars in arabinogalactan were found to decrease during the culture age. The main neutral sugars in this fraction were D-galactose and L-arabinose with negligible amounts of rhamnose, xylose, mannose and glucose detected (Table 3). The contents of galactose and arabinose decreased to the end of a culture cycle (stationary phase of growth) while the level of other neutral sugars was virtually constant. The galactose/arabinose ratio was close to ca. 4–5. Thus, biosynthesis of acidic arabinogalactan is optimal at the exponential phase of the *S. vulgaris* cell growth.

Gel filtration of the arabinogalactan fractions obtained on days 3, 15, 21 and 33 on a Sephacryl S-500 column yielded the homogeneous polysaccharide

fractions each of which showed a single peak only. K_{av} values of the fractions obtained were shown to change as for those of the silenan fractions. A similar tendency in the molecular mass alterations of arabinogalactan as well as silenan was observed during the cell culture.

Thus, silenan and arabinogalactan were shown to be produced by the *S. vulgaris* callus during culture. Both polysaccharides were isolated from the callus at the exponential phase. Silenan contained high quantities of galacturonic acid at the beginning of the exponential phase of the callus growth. Arabinogalactan appeared to attach to the rhamnogalacturonan core of silenan in the middle of the exponential phase to give a genuine pectin of *S. vulgaris* composed of the linear and ramified regions. Silenan is further transformed into galacturonan (or rhamnogalacturonan) at the stationary growth phase. Simultaneously, the arabinogalactan constituent of silenan appeared to be gradually destroyed and used by the callus during culture.

3. Experimental

3.1. General

The callus of *S. vulgaris* was obtained as described earlier (Misharina et al., 1999) and has been maintained at the Department of Molecular Immunology and Biotechnology of the Institute. The callus cells were cultivated in modified Murashige and Skoog's (1962) medium that contained 15 g l^{-1} sucrose, 15 g l^{-1} galactose, 8 g l^{-1} agar, 6-benzylaminopurine, and 2,4-dichlorophenoxyacetic acid. The cells were subcultured at 25 °C in the darkness. Total galacturonic acid was estimated colorimetrically with 3,5-dimethylphenol (Usov et al., 1995). The sugar contents were determined by the phenol-sulfuric acid procedure (Dubois et al., 1956). Spectrophotometric measurements were run on a Ultrospec 3000 instrument (UK).

3.2. Measurements and calculation of the growth parameters

The cell growth was determined by measurement of fr. wt and dry wt of biomass. Fresh cells were dried at 60 °C. Cell weights were estimated by the gravimetric method. The value of the cell growth rate (μ, day $^{-1}$) was calculated according to the equation:

$$\mu = (m_i - m_0)/(m_0 t),$$

where m_0 is the initial dry cell weight (g l^{-1}), m_i is the final dry cell weight (g l^{-1}), t is the cultivation time (days).

Time of dry biomass doubling (T, day) was obtained as follows:

$$T = \ln 2/\mu$$

The productivity of dry biomass per day (P_{bm}, g l^{-1} day^{-1}) was calculated according to the equation:

$$P_{bm} = (m_i - m_0)/t$$

The data were given as mean from two separate experiments ± s.d.

3.3. Isolation of polysaccharides

The callus (1 g) was treated with boiling methanol and chloroform. The residual material was extracted with water (1.0 l) at 50 °C for 2 h. An aqueous solution was concentrated, the residual material was centrifuged and 2 volumes of 96% ethanol were added to the supernatant to precipitate a crude polysaccharide fraction. The mixture obtained was centrifuged and the precipitate was dissolved in distilled water followed by dialysis against distilled water. The solution obtained was lyophilized to furnish the purified acidic arabinogalactan. The residual material was treated with diluted HCl (up to pH 4 as final concentration, 0.7 l) at 50 °C for 3 h, the mixture was filtered, and the plant material obtained was extracted with 0.7% aqueous ammonium oxalate (1.3 l) at 68 °C for 2 h. The solution was treated as described above to afford the purified silenan. The yields were calculated from the dry weight of the callus treated with methanol and chloroform. The productivity of the callus in relation to biosynthesis of polysaccharides per litre of medium (g l^{-1}) was estimated. The acidic arabinogalactan and pectin productions by the callus per day (P_a and P_p, g l^{-1} day^{-1}) were calculated. The data obtained are expressed as the mean from two separate experiments.

3.4. Complete acidic hydrolysis

All the obtained polysaccharide fractions (2 mg of each) were hydrolyzed with 2 M trifluoroacetic acid (1.0 ml) at 100 °C for 3–4 h in the sealed tubes. The acid was removed by the repeated coevaporation with methanol. The neutral sugars were quantified by gas–liquid chromatography as the corresponding alditol acetates using *myo*-inositol as the internal standard (York et al., 1985). The molar ratios were calculated from the peak areas.

3.5. Size exclusion chromatography

Gel filtration was carried out on a Sephacryl S-500 column (1.5×49 cm, a void volume 46.5 ml, eluent was 0.01 M NaCl at the flow rate of 0.45 ml min^{-1}). The 1.8 ml fractions were collected. The elution of polysaccharide

was controlled by the positive reaction of the eluate for carbohydrates by the phenol-sulfuric acid procedure (Dubois et al., 1956). The polysaccharide fraction was dissolved in a minimal volume of 0.01 M NaCl, applied onto a Sephacryl S-500 column and eluted with 0.01 M NaCl. The arabinogalactan fractions obtained on days 3, 15, 21 and 33 (9.0, 9.7, 8.1 and 5.6 mg, respectively) were applied onto the column to yield purified polysaccharide fractions as follows: 3-I, yield 6.8 mg, K_{av} 0.17; 15-I, yield 8.0 mg, K_{av} 0.06; 21-I, yield 6.8 mg, K_{av} 0.23; 33-I, yield 4.0 mg, K_{av} 0.42. The silenan fractions obtained on days 3, 15, 21 and 33 (6.3, 9.6, 9.6 and 10.1 mg respectively) were applied onto the column to yield purified polysaccharide fractions as follows: 3-II, yield 5.6 mg, K_{av} 0.45; 15-II, yield 5.0 mg, K_{av} 0.04; 21-II, yield 6.6 mg, K_{av} 0.14; 33-II, yield 5.4 mg, K_{av} 0.43.

Acknowledgements

This work was supported by the grant No.00-04-48063 of the Russian Foundation for Basic Research and by the grant No. 8.1.13 of the Scientific Council: "Chemistry and Technology of the Renewing Plant Material Processing" (Ministry of Industry and Science of the Russian Federation).

References

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Fujino, T, Itoh, T., 1998. Changes in pectin structure during epidermal cell elongation in pea (*Pisum sativum*) and its implications for cell wall architecture. Plant Cell Physiol. 39, 1315–1323.

Geshi, N., Jorgensen, B, Scheller, H.V., Ulvskov, P., 2000. *In vitro* biosynthesis of 1,4-β-galactan attached to rhamnogalacturonan I. Planta 210, 622–629.

Goubet, F., Morvan, C., 1994. Synthesis of cell wall galactans from flax (*Linum usitatissimum* L.) suspension-cultured cells. Plant Cell Physiol. 35, 719–727.

Günter, E.A., Ovodov, Y.S., 2001. Biotechnologic production of polysaccharides by *Silene vulgaris* callus. Biotech. News Intern. 6, 14–15.

Lhernould, S., Karamanos, Y., Priem, B., Morvan, H., 1994. Carbon starvation increases endoglycosidase activities and production of "unconjugated *N*-glycans" in *Silene alba* cell-suspension cultures. Plant Physiol. 106, 779–784.

Liu, S., Zhong, J.-J., 1996. Effect of potassium ion on cell growth and production of ginseng saponin and polysaccharide in suspension cultures of *Panax ginseng*. J. Biotechnol. 52, 121–126.

Murashige, T., Skoog, S.A., 1962. Revised medium for rapid growth and bioassays with tobaco tissue cultures. Physiol. Plant. 15, 473–479.

Misharina, E.A., Ovodova, R.G., Bushneva, O.A., Ovodov, Y.S., 1999. Formation of the callus of *Silene vulgaris* (Moench) Garcke *in vitro*. Rast Resursy 35, 88–95.

Popov, S.V., Popova, G.Y., Ovodova, R.G., Bushneva, O.A., Ovodov, Y.S., 1999. Effects of polysaccharides from *Silene vulgaris* on phagocytes. Intern. J. Immunopharmacol. 21, 614–622.

Stolle-Smits, T., Beekhuizen, J.G., Kok, M.T.C., Pijnenburg, M., Recourt, K., Derksen, J., Voragen, A.G.J., 1999. Changes in cell wall polysaccharides of green bean pods during development. Plant Physiol. 121, 363–372.

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.

Takeuchi, Y., Komamine, A., 1980. Turnover of cell wall polysaccharides of a *Vinca rosea* suspension culture. I. Synthesis and degradation of cell wall components. Physiol. Plant. 48, 271–277.

Usov, A.I., Bilan, M.I., Klochkova, N.G., 1995. Polysaccharides of algae, 48. Polysaccharide composition of several calcareous red algae: isolation of alginate from *Corallina pilulifera* P. et R. (Rhodophyta, Corallinaceae). Bot. Marina 38, 43–51.

York, W.S., Darvill, A.G., McNeil, M., Stevenson, T.T., Albersheim, P., 1985. Isolation and characterization of plant cell walls and cell-wall components. Methods Enzymol. 118, 3–40.

Yeo, U.-D., Kohmura, H., Nakagawa, N., Sakurai, N., 1998. Quantitative and qualitative changes of cell wall polysaccharides during somatic embryogenesis and plantlet development of asparagus (*Asparagus officinalis* L.). Plant Cell Physiol. 39, 607–614.

Zhang, J.-H., Zhong, J.-J., 1997. Hyperproduction of ginseng saponin and polysaccharide by high density cultivation of *Panax notoginseng* cells. Enzyme Microbial Technol. 21, 59–63.

Zhang, J.-H., Zhong, J.-J., Yu, J.-T., 1996. Effect of nitrogen source on cell growth and production of ginseng saponin and polysaccharide in suspension cultures of *Panax notoginseng*. Biotechnol. Progress 12, 567–571.

Zhong, J.-J., Wang, D.-J., 1998. Effect of nitrogen source on the production of ginseng saponin and polysaccharide by cell cultures of *Panax quinquefolium*. Process Biochem. 33, 671–675.