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Production of Polysaccharides by *Silene vulgaris* Callus Culture Depending on Carbohydrates of the Medium

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Abstract—Sources of carbohydrate nutrition such as sucrose, glucose, and galactose, with the exception of arabinose, were shown to influence positively callus growth and polysaccharide (pectin silenan and acidic arabinogalactan) biosynthesis. Galactose was found to cause a stimulatory effect on yield and productivity of arabinogalactan. Low concentrations of sucrose failed to support the cell growth and polysaccharide biosynthesis. Increasing sucrose concentrations led to biomass accumulation but failed to enhance efficiency of the substrate utilization. The optimal medium for the campion cell culture growth was found to be one containing 30 g/liter of sucrose or a mixture of sucrose with glucose (in 15 g/liter). Increasing sucrose concentrations in the medium from 30 to 100 g/liter failed to significantly influence the polysaccharide yields while the polysaccharide productivity per liter of the medium grew due to promotion of culture productivity in biomass. Variations of the carbon sources in the nutrient media were shown to influence insignificantly the biochemical characteristics of arabinogalactan and silenan while an increase in the sucrose concentration to 50-100 g/liter led to a diminution of the galacturonic acid content in silenan and to changes in contents of the neutral monosaccharide residues in silenan and arabinogalactan.

Key words: *Silene vulgaris* (M.) G., cell culture, callus, pectin, silenan, arabinogalactan, plant polysaccharides, carbohydrates

The plant cell wall is a dynamic complex system; its composition can change under the influence of external factors such as phytohormones, sources of carbon, nitrogen, and calcium [1-3]. However, the physiological regulation of growth processes and polysaccharide biosynthesis have been studied insufficiently. Data concerning the action of various factors on the quantitative and qualitative composition of polysaccharides are not numerous in the literature.

Cell cultures are used for study of physiology and biochemistry of sugar metabolism in plant cells [4, 5]. Carbon excess or deficiency is known to cause different reactions of plant cells, and these factors effect significantly the plant metabolism, growth, and development [6]. Cell cultures can grow while consuming various carbohydrates (nearly 30 components have been tested), but the best growth was recorded, as a rule, on the following two carbon sources: sucrose and glucose [4, 7]. The carbon origin affects the polysaccharide biosynthesis in plant cell cultures changing amounts and compositions of the cell wall polysaccharides [2, 4].

The diverse spectra of physiological activities of plant polysaccharides, in particular, pectins and arabinogalactans are known (immunomodulating, anti-ulcer, antitoxic, antitumor) [8], and their studies are of great fundamental and applied interest.

The polysaccharides of the campion (*Silene vulgaris* (M.) G.) callus have been shown earlier [9] to be acidic arabinogalactan and a pectin named silenan. The quantitative and qualitative variations in the polysaccharide compositions during the growth cycle of the campion culture as well as an influence of hormonal factors on cell growth and producing polysaccharides have been studied [9-11].

The present work revealed sugar effects on growth of the campion callus and on biosynthesis and chemical parameters of the polysaccharides produced.

MATERIALS AND METHODS

Cultivation of the callus. The campion callus was grown in modified Murashige and Skoog's medium [12]. Sucrose, glucose, galactose, and arabinose at concentration of 30 g/liter; sucrose at concentrations of 10, 20, 40, 50, 75, and 100 g/liter were used as the carbon source. In addition, the following pairs of the sugars were employed:

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sucrose (15 g/liter) + glucose (15 g/liter) and sucrose (20 g/liter) + arabinose (10 g/liter). The control medium was 30 g/liter of sucrose. The callus was subcultured for 21 days at 26 ± 1°C in darkness.

The growth indexes of fresh and dry biomass as well as productivity per dry biomass (g/liter) were calculated at the end of culture cycle. Ten to fifteen calluses were taken in experiments for each factor. Fresh biomass was dried at 60°C for estimation of dry weight.

The growth indexes were calculated according to the formula: $I = (m_i - m_0)/m_0$, where m_0 and m_i (gram) are the initial and final callus weight. The value of the specific cell growth rate in relation to the dry biomass (μ, day^{-1}) was calculated accordingly to the formula [13]: $\mu = (m_i - m_0)/(m_0 t)$, where m_0 is the initial callus weight (gram), m_i is the final callus weight (gram), t is the cultivation time (days). The time of dry biomass doubling (T, day) was determined by the formula: $T = \ln 2/\mu$.

Analysis of the sugar compositions of the nutrient medium. The agar medium was melted, a mixture of acetonitrile and water was added followed by mixing, and agar flakes were removed by filtration. The filtrate was analyzed by HPLC. The sugars were determined using an HPP 5001 chromatograph (Czechoslovakia) fitted with a CGC column (150 × 3 mm), containing the sorbent Separon NH_2, 7 μ; the eluent was a mixture of acetonitrile–water (70 : 30 v/v) at flow rate of 1 ml/min. The sugars were detected using a RIDK102 differential refractometer.

The sugar consumption by the callus culture was estimated using the economic and metabolic coefficients [13]. The economic coefficient (Y) corresponding to a quantity of dry biomass (gram) per 1 g of the sugar consumed was calculated in relation to sugars metabolized according to the formula: $Y = (x - x_0)/(s_0 - s)$, where x_0 is the initial concentration of the biomass (g/liter), x is the final concentration of the biomass (g/liter), s_0 is the initial concentration of substrate (g/liter), s is the final concentration of substrate (g/liter).

Metabolic coefficient (Q) demonstrating the substrate consumption rate by the growing culture was estimated as the ratio $Q = (s_0 - s)/\Delta tx$, where s_0 is the initial concentration of substrate (g/liter), s is the final concentration of substrate (g/liter), x is the biomass concentration (g/liter), t is time (days).

Polysaccharides were isolated from the callus as described earlier [9].

General analytical methods. Total content of glycuronic acids in polysaccharide fractions was estimated using the reaction with 3,5-dimethylphenol in the presence of concentrated sulfuric acid [14], and total protein content was determined according to the Lowry method [15]. Total content of carbohydrates was determined using the reaction with phenol in the presence of sulfuric acid [16]. Spectrophotometric measurements were run on an Ultrospec 3000 instrument (England). GLC was performed with a Hewlett-Packard 4890A chromatograph (USA) fitted with an RTX-1 (0.25 mm × 30 m) capillary column with argon as a carrier gas using a flame-ionization detector and HP 3395A integrator.

Complete acidic hydrolysis. Polysaccharide fractions (2.0-2.5 mg each) were hydrolyzed with 2 M trifluoroacetic acid (TFA, 1 ml) at 100°C for 3-4 h; the hydrolyzates were evaporated in vacuum with methanol to complete removal of TFA. *Myo*-inositol (0.5 mg/ml) was used as the internal standard. Monosaccharides were identified by GLC as the corresponding alditol acetates [17].

The arithmetic means and standard deviations were calculated using statistical data treatment. The data verification was evaluated using the *t*-criterion of Student.

RESULTS

Carbohydrates such as sucrose, glucose, and galactose were able to maintain growth of the isolated campion culture [18]. Calluses have a high specific rate of cell growth and high productivity of biomass, as well as significant growth indexes (Table 1, Fig. 1a).

The productivity of dry biomass per liter of the nutrient substrate containing glucose and galactose was found to be a little lower than on media containing sucrose or the pair sucrose and glucose (Fig. 1a). The parameters of growth on the medium containing sucrose (20 g/liter) + arabinose (10 g/liter) were lower than those in the control (sucrose, 30 g/liter). The culture growth was substantially inhibited on medium with arabinose and necrotic coloration of tissues was observed.

The data of quantitative and qualitative analysis of sugars in the medium are given in Table 2. Consumption of sucrose, glucose, and galactose by the culture occurs with an equal rate, as it is obvious from the amounts of corresponding sugars (S) in the culture medium. An insignificant hydrolysis of sucrose into the constituent sugars glucose and fructose was observed in the medium.

The economic coefficient (Y) calculated in relation to sugar metabolizing and corresponding to quantity of dry biomass (gram) per 1 g of sugar consumed has a tendency to decrease during the growth cycle of the culture. Coefficients of the 21st day of cultivation on sucrose, glucose, and galactose were found to differ insignificantly (0.41-0.60) and to achieve maximum on the medium containing the pair of sucrose and glucose (0.60).

Substrate consumption rate by the growing culture is expressed by metabolic coefficient (Q). The metabolic coefficient of glucose remains approximately at the same level during culture, while those of sucrose and the pair of sucrose with glucose have a tendency to diminish. The metabolic coefficient of sucrose (30 g/liter) is higher than that of glucose (30 g/liter) at the beginning of the

Table 1. Growth parameters of *S. vulgaris* callus in the presence of various carbohydrates

Sugar	Sugar concentrations, g/liter	Growth index		μ, day^{-1}	T, day
		of fresh biomass	of dry biomass		
Glc	30	11.8 ± 2.2*	13.8 ± 2.9	0.66 ± 0.14	1.10 ± 0.22
Gal	30	11.7 ± 5.3	12.7 ± 4.5	0.60 ± 0.21	1.31 ± 0.51*
Ara	30	0*	0.5 ± 0.1*	0.02 ± 0.003*	32.79 ± 4.36*
Suc	10	5.3 ± 1.5*	4.1 ± 1.0*	0.19 ± 0.05*	3.90 ± 1.67*
Suc	20	10.9 ± 3.2*	10.9 ± 3.1*	0.52 ± 0.15*	1.49 ± 0.68*
Suc (control)	30	14.8 ± 3.2	15.0 ± 2.5	0.72 ± 0.12	0.99 ± 0.16
Suc	40	14.7 ± 2.9	16.5 ± 3.6	0.78 ± 0.17	0.92 ± 0.20
Suc	50	14.3 ± 3.9	17.4 ± 4.0	0.83 ± 0.19	0.87 ± 0.18
Suc	75	12.1 ± 2.6*	12.6 ± 2.3*	0.60 ± 0.11*	1.20 ± 0.27*
Suc	100	8.4 ± 1.2*	8.5 ± 1.1*	0.41 ± 0.05*	1.73 ± 0.22*
Suc + Glc	15 + 15	14.6 ± 7.2	12.8 ± 4.9	0.61 ± 0.23	1.26 ± 0.37*
Suc + Ara	20 + 10	6.8 ± 1.2*	6.6 ± 1.0*	0.32 ± 0.05*	2.24 ± 0.32*

Note: μ is the specific cell growth rate based on dry biomass, day^{-1}; T is time of biomass doubling, days. * Differences are reliable at $p < 0.05$. Suc, sucrose.

culture growth (3 days). The coefficients are closely related for various sugars on the 21st day of the callus cultivation.

The economic and metabolic coefficients of the medium with galactose were found to be nil on the 3rd day of growth and they begin to increase only later.

The campion callus culture failed to grow on the medium with arabinose: the consumption of arabinose by callus from the medium was absent, and the metabolic coefficient was nil (Tables 1 and 2, Fig. 1a).

The optimal sucrose concentrations for the callus growth are shown in Tables 1 and 2 and Fig. 1 (b and c). The productivity is increased in proportion to an augmentation of the sucrose concentrations in the medium excluding the highest concentrations (75 and 100 g/liter) (Fig. 1b). Increasing sucrose concentrations shows a larger effect on the increase in dry biomass than fresh biomass (Fig. 1, b and c). The best parameters of the culture growth are observed for the media containing 30, 40, and 50 g/liter of sucrose.

The white, friable, moderately hydrated callus of campion is obtained when the contents of sucrose are 10-30 g/liter. The callus is less hydrated at 40 and 50 g/liter of sucrose, a compact yellow callus arises at 75 and 100 g/liter of sucrose, and necrotic focuses arise at 100 g/liter of sucrose.

Amounts of unconsumed sucrose (S) enhance with increasing the sucrose concentrations in the medium (Table 2). The economic coefficient (Y) on the 21st day of cultivation on sucrose virtually did not change on varying sucrose content in the medium from 10 to 40 g/liter (0.42-0.52), while it was 1.27 at 50 g/liter sucrose.

The rates of consumption of carbohydrates by the growing culture are changed with increasing sucrose concentration in the medium. The metabolic coefficient (Q) during the culture achieves a maximum in the middle of the exponential phase of the cell growth at low sucrose concentration of 10-20 g/liter; the coefficient has a tendency to decrease at sucrose content of 30 g/liter; it remains at the same level approximately and shows low values at 40-50 g/liter of sucrose. The coefficients of the 21st day callus cultivation are nearly the same for various sucrose concentrations.

A reduction in the pH of the medium was observed during callus cultivation on various sugars excluding arabinose, where increasing pH was observed in the middle of the exponential phase of growth.

Silenan and arabinogalactan have been isolated from campion callus cultivated on media with different carbon sources [18]. Yields and productivities of silenan are similar for calluses growing on sucrose, glucose, and galactose in concentration of 30 g/liter (Fig. 2a). Biosynthetic parameters increase up to 10.6% and 0.83 g/liter on the medium with the pair of sucrose and glucose (15 g/liter each). The percentages of silenan in the callus cultivated on arabinose and on the mixture of sucrose (20 g/liter) with arabinose (10 g/liter) were comparable with those for the callus grown on sucrose, glucose, and galactose (Fig. 2a). Nevertheless, 7- and 1.5-fold decrease in the silenan productivity per liter of medium containing arabinose or the mixture of sucrose

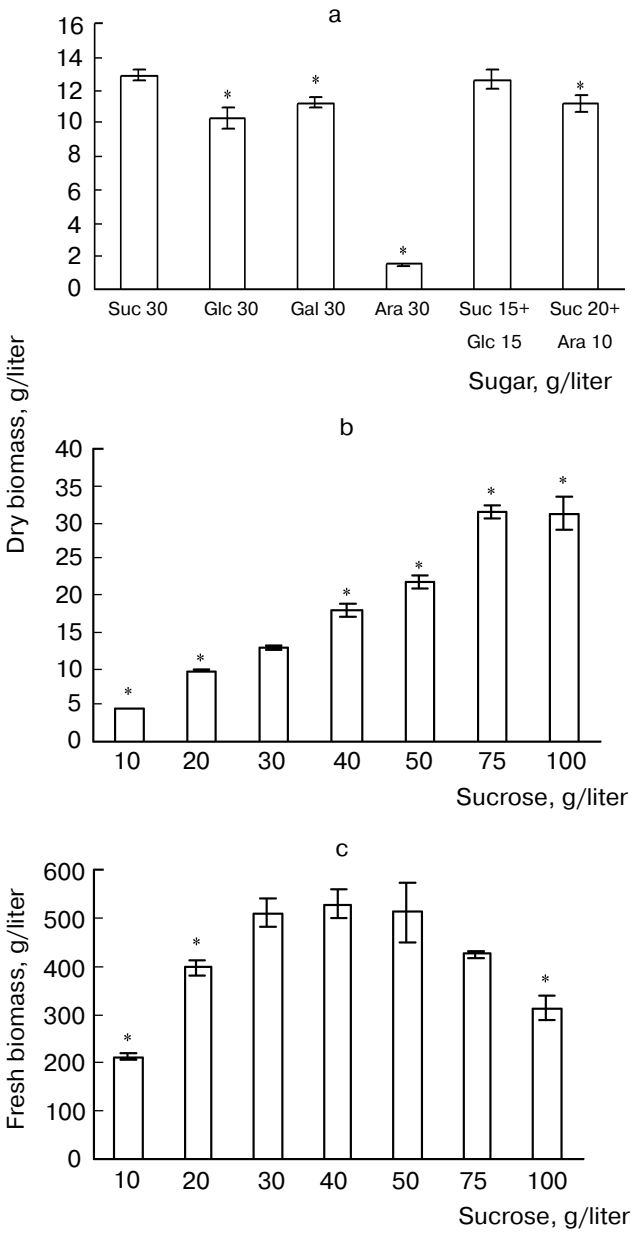

Fig. 1. Influence of various sugars (a) and sucrose (b, c) on growth of *S. vulgaris* callus. * Differences are reliable at $p < 0.05$. Control, sucrose (30 g/liter).

with arabinose was observed in comparison with the control (sucrose) that is related with decrease in productivity of cultures in biomass (Fig. 2b).

The yields and productivities of acidic arabinogalactan are maximal or minimal in the presence of galactose or arabinose, respectively, as the carbon sources. Biosynthetic characteristics of arabinogalactan on media with glucose as well as on the mixtures of sucrose with glucose or arabinose are comparable with those of the control (Fig. 2).

Data concerning the influence of sucrose concentrations on biosynthetic parameters of the campion callus are shown in Fig. 3. A decrease in the silenan yield in comparison with the control (30 g/liter of sucrose) was observed at the sucrose concentrations of 10 and 20 g/liter, while increasing sucrose concentrations from 30 to 100 g/liter in the medium failed to influence reliably the pectin yields (Fig. 3a). The silenan productivity per liter of the substrate decreases at sucrose concentrations below that of the control, and it achieves the maximum at 75 g/liter of sucrose (Fig. 3b).

Yields of arabinogalactan are very similar (5-8%) at sucrose concentrations of 20-100 g/liter, while yields are reliably reduced at 10 g/liter of sucrose (Fig. 3a). Productivity of arabinogalactan per liter of the medium is maximal at 75 g/liter and is reduced in comparison with the control at 10 g/liter (Fig. 3b).

An investigation of sugar compositions of polysaccharides from the callus cultivated in the presence of various carbon sources demonstrated that silenan contains large quantities of the D-galacturonic acid residues (70-86%); the main neutral sugar components are the residues of galactose, arabinose, and rhamnose (Table 3). The arabinose/galactose ratios were close to 1 : (1.0-1.8), and 1 : 2.9 in the presence of arabinose. Increase in the galacturonic acid content in silenan occurs in the medium with arabinose. Increase in the relative contents of arabinose and galactose residues is observed in the medium with the mixture of sucrose and arabinose (Table 3).

Arabinose and galactose are the dominating neutral components of arabinogalactan in the ratio 1 : (3.5-6.0). The quantities of galacturonic acid in arabinogalactan increase in the presence of galactose in the culture medium.

Silenan samples from callus cultured on different sucrose concentrations were shown to have the similar qualitative sugar compositions and to be distinguished in the ratio of monosaccharide residues and galacturonic acid contents (Table 4). Quantities of the galacturonic acid residues decrease to 58-65% in the silenan samples obtained in the presence of 50-100 g/liter of sucrose. The arabinose/galactose ratio is 1 : (1.1-1.8) at sucrose concentrations of 10-50 g/liter, and this ratio is 1 : (0.7-0.8) in the presence of 75 and 100 g/liter of sucrose. Increasing contents of the arabinose and galactose residues in silenan was observed using 40 and 50 g/liter of sucrose while quantities of the other neutral monosaccharide residues did not change. The galactose residue content in silenan was reduced in the presence of 75 and 100 g/liter of sucrose.

The samples of the acidic arabinogalactan vary only in the ratio of the monosaccharide residues. The arabinose/galactose ratios are 1 : (4.3-5.3) and 1 : (2.8-3.8) in the presence of 10-30 and 40-100 g/liter of sucrose, respectively, and the galacturonic acid content is 9-15%. The relative numbers of the galactose and arabinose

Table 2. Use of sugars by campion callus culture

Carbon source, g/liter	Metabolizing sugar	S, g/liter			Y			Q		
		Period of cultivation, days								
		3	12	21	3	12	21	3	12	21
Glc, 30	glucose	28.5	20.1	4.9	2.76	0.80	0.41	0.12	0.13	0.11
Gal, 30	galactose	29.5	19.3	7.1	0	0.63	0.48	0	0.11	0.10
Ara, 30	arabinose	36.8	35.5	35.3	0.15	0.24	0.24	0	0	0
Suc, 10	sucrose	9.2	2.8	0.8	5.81	0.46	0.42	0.03	0.14	0.09
	glucose	0	1.2	0.6						
	fructose	0	1.7	0.5						
Suc, 20	sucrose	19.4	8.9	2.4	1.54	0.34	0.51	0.12	0.28	0.09
	glucose	0	2.8	0						
	fructose	0	2.9	0						
Suc, 30	sucrose	28.0	17.3	4.6	0.97	0.78	0.48	0.25	0.11	0.09
(control)	glucose	0	0.7	0						
	fructose	0	0.8	0						
Suc, 40	sucrose	39.9	28.3	7.5	2.1	1.03	0.52	0.11	0.07	0.09
	glucose	0.9	0	0						
	fructose	0.7	0	0						
Suc, 50	sucrose	48.2	37.4	32.7	2.30	1.25	1.27	0.06	0.06	0.04
	glucose	1.6	0	0						
	fructose	1.6	0.5	0.8						
Suc (15) +	sucrose	13.3	7.0	5.2	0.54	0.51	0.60	0.37	0.19	0.08
Glc (15)	glucose	14.5	7.7	3.8						
	fructose	0	1.0	0.3						

Note: S, sugar contents in the medium during cultivation, g/liter; Y, economic coefficient; Q, metabolic coefficient. Mean values from a series of experiments are given.

residues in arabinogalactan increase in the presence of 75-100 g/liter of sucrose.

DISCUSSION

The campion callus was shown to grow on carbon sources such as sucrose, glucose, and galactose but failed to grow on arabinose as the sole carbon source. Sucrose and glucose support a high rate of cell growth.

Easy interconversion of glucose, sucrose, and fructose provides involving any of these general substrates in the main pathways of degradation—glycolytic and pentose phosphate pathways. Information concerning the utilization of other carbon sources is extremely limited. It is known that the pentoses (arabinose, xylose, and ribose) failed to support growth of plant cells [4, 7].

The campion callus culture is galactose-adapted and shows a high growth rate which appeared to be connected with a high activity of UDP-galactose-4-epimerase (EC 5.1.3.2) as a key enzyme in the utilization of galactose to maintain the required level of galactose involved in the main energetic metabolism [4, 7].

There are hydrolytic and non-hydrolytic mechanisms of sucrose consumption by cells. In the first case, hydrolysis of sucrose into glucose and fructose occurs in the environmental medium or on the cell surface, in the second the molecules of sucrose permeate through the

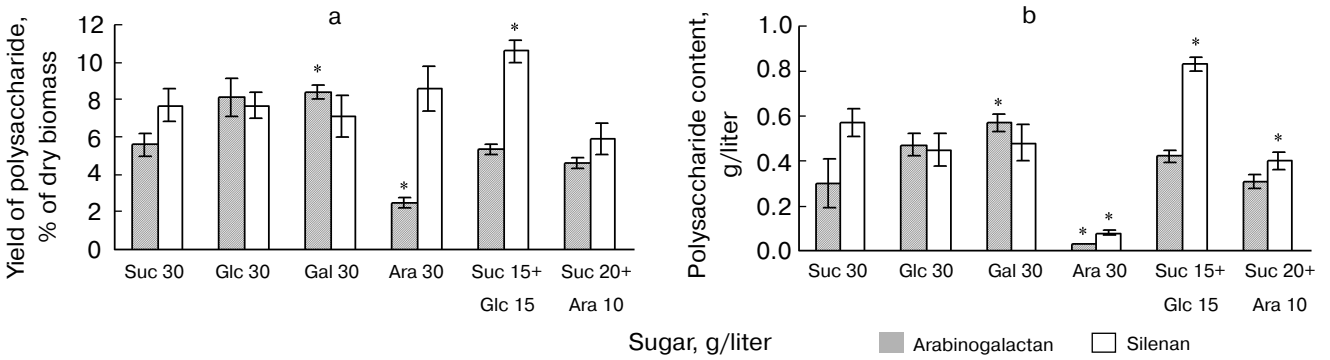

Fig. 2. Influence of sugars on yields (a) and productivities (b) of polysaccharides in *S. vulgaris* callus. * Differences are reliable at $p < 0.05$. Control, sucrose (30 g/liter).

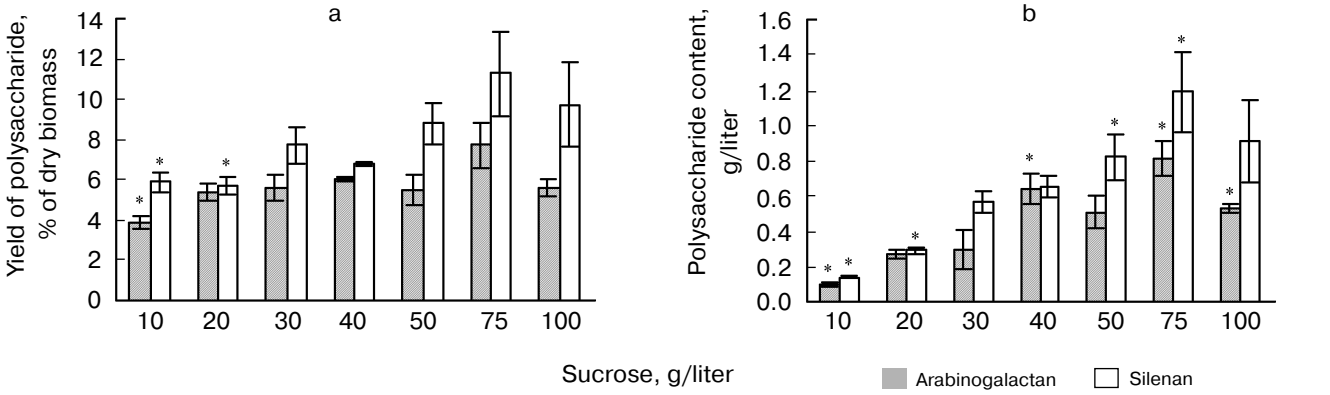

Fig. 3. Influence of sucrose concentrations on yields (a) and productivities (b) of polysaccharides in *S. vulgaris* callus. * Differences are reliable at $p < 0.05$. Control, 30 g/liter sucrose.

cell walls and its inversion occurs intracellularly as described for the callus of carrot [4, 5].

Minute amounts of glucose and fructose in the medium were detected only in the case of the campion callus culture, thus indicating an insignificant hydrolysis of sucrose and a prevalence of non-hydrolytic mechanism of the sucrose uptake by the campion culture cells.

Intensive consumption of sucrose, glucose, and galactose by cells was found to occur during cultivation. The economic coefficient in relation to carbohydrates has a tendency to decrease during the growth cycle of the campion culture, thus demonstrating a drop in effectiveness of using substrate during cultivation. An exception is the medium containing the mixture of sucrose and glucose, which has a constant economic coefficient during cultivation. An increasing of an adaptation period of the culture to the substrate appeared to come on the medium with galactose.

The sucrose concentrations from 2 to 3.4% are usually used for cell cultivation; however, these are non-optimal amounts for all the cell cultures. Physiological changes in cells in response to the deprivation of carbohydrates have been studied using suspension cultures of sycamore and rice [6, 19]. An absence of sugar was shown to cause a stop of cell growth, a rapid consumption of the cellular carbohydrates, and a diminution in the respiration rate, a degradation of lipids and proteins, and a decrease of enzymic activities. Changes in metabolism are obviously involved in the adaptation mechanism of plant cells in response to carbohydrate starvation. A diminution of the enzymic activities connected with the carbohydrate metabolism and respiration protects cells against a stress by an initiation of biosynthetic mechanisms to conserve energy. Similar metabolic changes occur in plants during senescence. Similar physiological changes appeared to occur during growth of the campion cells in the presence of low concentrations of sucrose causing an inhibition of the cell growth and biosynthesis of polysaccharides.

Increasing sucrose concentrations in the medium lead to biomass accumulation while efficiency of the substrate utilization is reduced. Similar observations have been noted for other cell cultures [4, 20, 21]. A higher

Table 3. Characterization of the polysaccharide fractions isolated from the campion callus cultivated in the presence of various carbon sources

Content, mole %**	Suc		Glc		Gal		Ara		Suc + Glc		Suc + Ara	
	AG	SV	AG	SV	AG	SV	AG	SV	AG	SV	AG	SV
GalA***	11.1	70.0	14.4	77.4	22.5	79.0	11.8	85.9*	10.8	68.3	8.3	76.3
Gal	43.6	1.6	35.7	3.2	43.1	3.2	30.0	6.4	46.5	1.9	47.7	4.4
Ara	8.2	0.9	9.7	1.8	11.8	1.8	5.3	2.2	7.7	1.2	13.8	4.2
Rha	2.8	0.6	2.9	1.2	3.1	1.2	2.3	1.4	2.1	1.0	3.0	1.9
Glc	1.8	1.5	3.4	1.4	3.8	0.9	2.8	1.2	4.1	1.7	2.7	1.8
Xyl	2.0	0.5	4.3	0.8	3.6	1.0	3.2	0.8	5.6	0.5	2.6	0.9
Man	1.5	0.7	1.7	1.2	1.8	0.7	2.3	1.1	1.6	0.7	5.1	1.5
Protein***	8.7	12.9	15.7	13.7	14.5	13.0	13.4	3.8	12.9	11.8	25.4	18.5

* Differences are significant at $p < 0.05$.
** Mean values from series experiments are given.
*** Weight percentages. Mean values from three experiments are given. AG, acidic arabinogalactan; SV, silenan; GalA, D-galacturonic acid. Control, 30 g/liter sucrose.

Table 4. Characterization of the polysaccharide fractions isolated from *S. vulgaris* callus cultivated in the presence of different sucrose concentrations

Content, mole %**	Sucrose concentration, g/liter											
	10		20		40		50		75		100	
	AG	SV	AG	SV	AG	SV	AG	SV	AG	SV	AG	SV
GalA***	15.1	78.5	13.8	80.5	10.7	71.3	11.5	65.3*	9.3	58.5*	9.3	57.8*
Gal	34.3	3.2	35.8	2.9	47.5	4.9	39.8	5.3	52.3	2.1	49.6	2.6
Ara	8.1	1.8	7.8	2.8	12.5	3.8	12.1	3.9	14.4	2.6	17.5	3.7
Rha	2.8	1.2	2.6	1.6	3.0	1.4	2.8	1.7	2.6	1.1	3.0	1.4
Glc	0.9	1.7	2.7	1.6	4.3	1.0	3.4	1.8	4.8	1.0	3.0	2.2
Xyl	2.2	0.8	2.6	1.1	3.4	0.6	2.8	0.7	6.1	0.5	6.2	0.9
Man	1.3	1.0	0.9	1.2	2.4	0.9	3.6	1.3	4.5	0.5	4.2	1.2
Protein***	24.1	9.5	22.4	15.7	17.2	17.2	16.8	19.9	18.8	21.5	24.0	21.5

* Differences are significant at $p < 0.05$.
** Mean values from a series of experiments are given.
*** Weight percentages. Mean values from three experiments are given. AG, acidic arabinogalactan; SV, silenan. Control, 30 g/liter sucrose.

biomass accumulation in the media containing large amounts of sugars occurs due to prolongation of growth period, while growth rate failed to depend on sugar concentration in the medium [7, 22].

Prevention of campion callus growth and change in its consistency at high sucrose concentrations appeared to be initiated by high osmotic pressure (osmotic stress) at the high sucrose contents in the medium that was noted for other cell cultures [7, 20, 23].

Thus, our observations on physiological modifications in the campion callus culture in relation to changes in sugar nutrition are in agreement with the data obtained for other cultures (sycamore, stevia, catharantus, perilla, etc.) [4, 7, 19, 22].

In spite of intensive study of the influence of sugar nutrients on growth of cultivated cells, limited data are known concerning their action on biosynthesis and composition of polysaccharides in the cell walls. At the same

time, we have found that sources of carbohydrate nutrition such as sucrose, glucose, and galactose with an exception of arabinose influence positively the polysaccharide biosynthesis in campion callus culture. Galactose in comparison with the control causes a stimulatory effect on yield and productivity of arabinogalactan, which are minimal in the presence of arabinose as the sole carbon source. At the same time, the percentage of silenan in callus on medium with arabinose failed to differ from that for various media containing other carbohydrates. Arabinogalactan appeared to be consumed for biosynthesis of the side chains of silenan, while *de novo* biosynthesis of the arabinogalactan molecules appeared to be limited due to the cells failing to utilize arabinose.

Increasing galactose contents in the cell walls has been shown to occur on cultivation of cells on medium with galactose [2]. However, we failed to find such changes.

Variations in the carbon sources in nutrient media influence insignificantly the biochemical characteristics of silenan and arabinogalactan. Nevertheless, we observed increasing contents of galacturonic acid residues and arabinose/galactose ratios due to increase in the galactose residue contents on the media with arabinose, where cell growth is absent and necrosis is initiated. These changes seemed to demonstrate production of pectin consisting of high amounts of the galacturonic acid residues and containing the galactose-rich side chains as observed earlier during necrosis of cells [9].

Increasing the sucrose concentration up to 50-100 g/liter leads to a decrease in the galacturonic acid residues in silenan, while a considerable increase in galactose and arabinose residues is observed at 50 g/liter of sucrose. Pectin composed of a linear backbone and branching regions appeared to be formed in the presence of sucrose of this concentration. A decrease in amounts of galactose residues in silenan and a reduction in arabinose/galactose ratio at further increase in sucrose concentration in the medium to 75 and 100 g/liter seemed to be connected with a reaction of the cells to osmotic stress. A reduction in amounts of galactose residues in cell walls caused by a high osmotic pressure was observed earlier for tobacco cell culture [24].

Thus, modifications of sugar constituents in the media may be used for regulation of biosynthesis and enhancing production of polysaccharides by cell cultures.

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