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Influence of Phytohormones on Monosaccharide Composition of Polysaccharides from Wheat Suspension Culture

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Abstract

Plant polysaccharides with technical and physiologic traits attract researchers by their high physiological activity in regulation of the growth, development and protective reactions. Cell cultures allow to regulate chemical composition of synthesized substances by changing media composition and are widely used to enhance or change the biosynthesis of metabolites. The aim of this study was to investigate the influence of phytohormones 2,4-dichlorphenoxyacetic acid (2,4 –D) and abscisic acid (ABA) of culture medium on chemical composition of polysaccharides (PS), extracted from cells and extracellular liquid of wheat suspension culture. It was shown for the medium with ABA that monosaccharide composition of extracellular PS mainly represented by glucose (87%), whereas PS isolated from cells were rich for xylose and glucuronic acid. Monosaccharide composition of extracellular PS from media with 2,4-D showed 6-fold increase of arabinose, 8-fold – of galactose, 5-fold – of xylose and glucuronic acid, compared to extracellular PS from ABA medium. Composition of cellular PS from media with 2,4-D were mainly similar to ABA and differed by the increased amount of mannose (3-fold), and galacturonic acid (2,5-fold). Thus, regulative effect of the use of two different types of phytohormones was demonstrated on the biosynthesis of variously composed polysaccharides.

1. Introduction

Currently, cell biology is of a considerable interest in production of new biologically active compounds. Like native plants, cell cultures synthesize a wide range of polysaccharides, such as pectins, arabinogalactan, galactans, arabinans, xyloglucans etc. [1]. Plant cell cultures have a number of advantages over traditional raw materials (no organismic control, regardless of the climatic conditions, the ability to optimize and standardization process, the homogeneity of the system, a greater percent yield), serves as a convenient model system for studying the structure and biosynthesis of polysaccharides. Furthermore, tissue culture cells could potentially be of practical significance for the production of physiologically active polysaccharides [2, 3].

Plant polysaccharides are known for their important technical and physiologic traits. They attract researchers by their high physiological activity in regulation of the growth, development and protective reactions [4, 5, 6].

Our earlier investigations have shown that the ability to maintain morphogenic potential of long-term cultivated tissues of wheat and barley is accompanied by appearance of extracellular polysaccharides [7, 8]. In addition, we have determined high level of biological activity of polysaccharides obtained from suspension culture [9].

One of the advantages of plant cell cultures is the possibility to regulate chemical composition of synthesized biologically active substances by changing media composition [10]. Recent investigations in polysaccharides obtainment is concentrated in investigation of strategies for the improvement of biotechnological methods targeted to media optimization, culture appropriateness and etc. [11, 12].

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It is well known that phytohormones in tissue culture are widely used to enhance or change the biosynthesis of metabolites [13]. Recent studies have shown that auxins are able to enhance susceptibility to bacterial invasion [14] and induce upregulation of biosynthetic genes [15]. It was reported that abscisic acid is capable of altering cell wall properties and compositions [16].

Therefore, the aim of this study was to investigate the influence of phytohormones; synthetic auxin – 2,4-Dichlorphenoxyacetic acid (2,4-D), inducing cell division and growth, and abscisic acid (ABA) mostly used for regulation of stress responses, on to chemical composition of polysaccharides (PS), extracted from extracellular liquid and cells of wheat suspension culture.

2. Materials and methods

Wheat cell suspension culture were grown in two variants of Murashige and Skoog medium [17]: first – with 1.0 mg/l abscisic acid (ABA) and second – with 5.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D).

For this purpose callus tissue were placed in liquid culture medium at ratio 200–300 mg on 30–40 ml of media and cultured on a shaker at 140 rpm at a temperature of 26 ± 2 °C and 16-hour photoperiod. The resulting cell suspension culture were collected after 10 days, filtered and extracellular liquid concentrated on rotary evaporator IKA WERKE for isolation of PS. Cellular PS were obtained through water extraction from filtrated cells and evaporated. Both types of polysaccharides were extracted by 70% ethanol [18] and precipitated by centrifugation at 10.000 rpm 10 min at 8 °C. Total sugar amount was determined by Dubois method [19]. Precipitate was dissolved in 0.5 ml of ultra pure water and hydrolyzed by 4.0M trifluoroacetic acid (TFA) for carbohydrate analysis. Separation and detection of monosaccharides was carried out by high performance anion exchange chromatography ICS 5000 (Thermo Scientific, Dionex, USA) on CarboPac PA-20 column with electrochemical detector (PAD, Dionex) in pulse-amperometric regime. Elution buffers are 200 mM NaOH, 1 M NaOAc, 0.1 M NaOH. The column is preequilibrated with 200 mM NaOH and eluted with linear gradient at flow rate of 0.4 ml/min at 30 °C.

3. Results and discussion

Wheat cell suspension cultures were cultured in media containing two types of phytohormones - ABA and 2,4-D, and PS were extracted from the extracellular liquid suspension (liquid part) and the (filtered and dried cells) cells of suspension (Tables 1 and 2). HPLC analysis of the monosaccharide composition of the polysaccharides (PS) derived from suspension culture of wheat cells allowed to identify that extracellular PS and cellular PS contain the following monosaccharides and uronic acids: arabinose (Ara), galactose (Gal), xylose (Xyl), mannose (Man), galacturonic (GalUA) and glucuronic (GlcA) acid. Results of the analysis of chromatograms of 29-variants of wheat callus cells and extracellular liquid, differing from each other by cultivation medium and cultivation time showed that the quantitative composition of PS monosaccharides varies significantly.

Monosaccharide composition of polysaccharides isolated from the extracellular liquid medium with ABA, regardless of the concentration (0.1, 0.5, 1.0 mg/l) is composed of glucose (87%), arabinose (5%) and xylose (5%), and has the following

Table 1
Characteristics of polysaccharide fractions isolated from extracellular liquid and cells of suspension in the presence of various plant hormones (in %)

Auxins / Fractions, output in %	ABA (extracellular liquid)	ABA (cells)	2,4 -D (extracellular liquid)	2,4 -D (cells)
Arabinose	1-5	2-3	11-30	2-4
Xylose	2-5	62-66	10-33	40-81
Galactose	1-2	1-2	6-20	2-3
Glucose	86-91	11-14	19-53	5-49
Mannose	3	1-2	3	1-11
Glucuronic acid	1	9-10	3-42	1-2
Galacturonic acid	1	8	0	3-21

Table 2
Composition of monosaccharaides in PS, obtained from extracellular liquid and cells of suspension, grown on media with ABA and 2,4-D (mkg/ml)

Monosaccharaides, mkg/ml	ABA, (extracell. PS)	ABA, (cells)	2,4-D (extracell. PS)	2,4-D (cells)
Ara	1.4	1	8	1.25
Gal	0.7	0.6	5.4	0.9
Xyl	1	30.2	5.2	25.2
Glc	24	5.9	12.43	11.1
Man	0.5	0.6	0.3	1.2
GlcUA	0.2	4.3	1.4	0.3
GalUA	0.1	3.85	0.03	3.1

ratio Ara: Gal: Xyl: Glc: Man: GlcA: GalUA – 10: 4: 10: 160: 0.4: 0.02 (Fig. 1a). Monosaccharides of cellular PS grown in medium with ABA differed by the increased content of xylose (63%), glucose (14%), glucuronic acid (9%) and galacturonic acid (8%) in proportions Ara: Gal: Xyl: Glc: Man: GlcA: GalUA – 10: 8: 220: 48: 2: 32: 28 (Fig. 1b). On Figs. 2 and 3 there are chromatograms that reflect the distinct features of monosaccharide composition of extracellular PS and cell PS of suspension culture, grown on media with ABA, analysis of which are presented in Fig. 1.

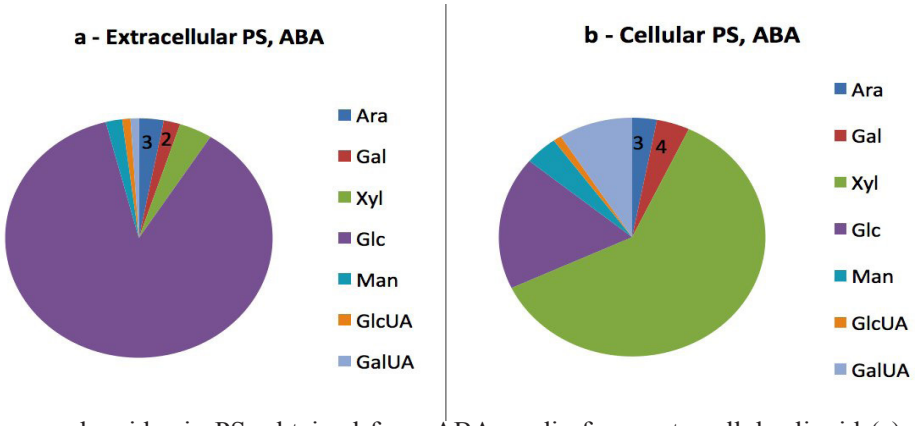

Fig. 1. Ratio of monosaccharaides in PS, obtained from ABA media from extracellular liquid (a) and from cells of suspension (b).

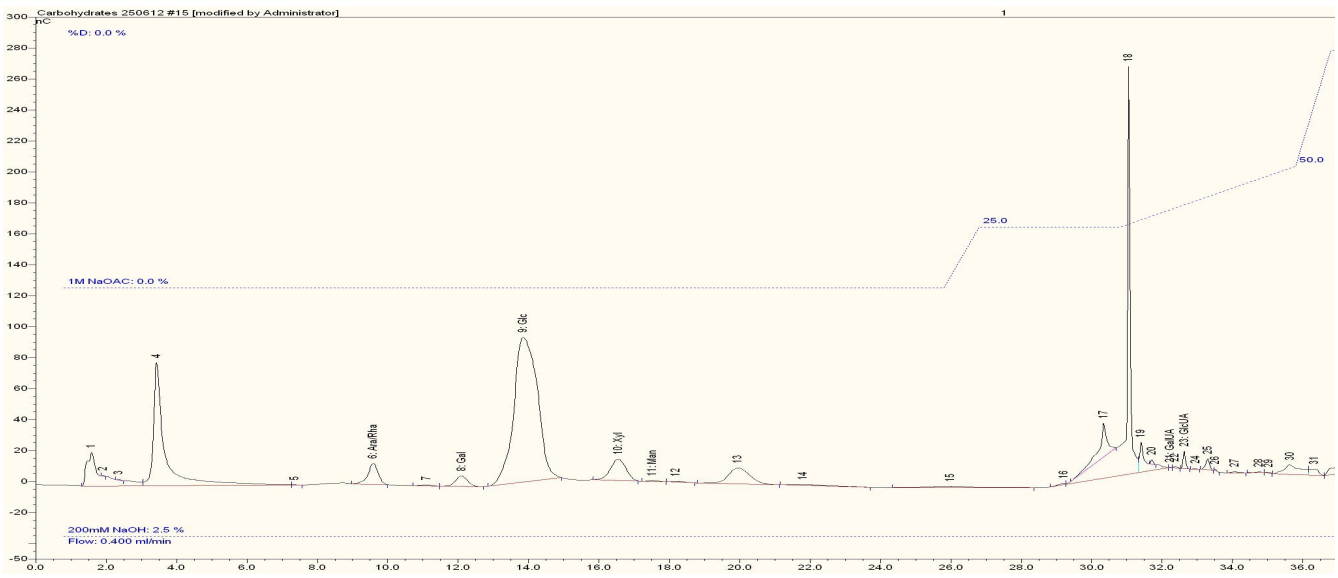

Fig. 2. Monosaccharide composition of extracellular PS from media with ABA.

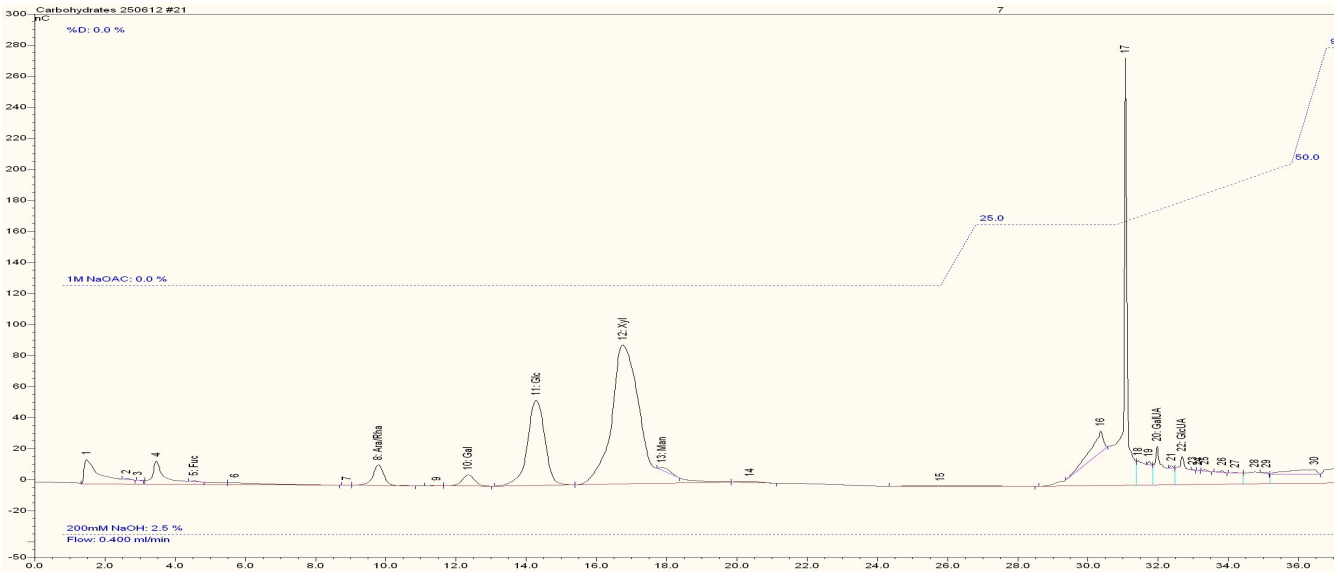

Fig. 3. Monosaccharide composition of PS from cells of suspension with ABA.

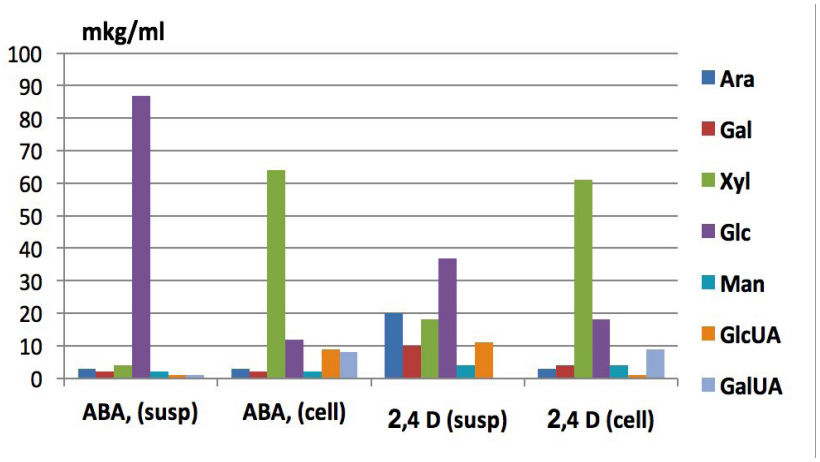

Fig. 4. Comparative monosaccharide composition of extracellular and cellular PS extracted from culture media with ABA and 2,4-D.

It can be assumed, that ABA stimulate synthesis and secretion to apoplast hemicelulloses – glucans, xyloglucans and arabinoxylans. At the same time, cells under the effect of ABA retain high amount of xylans, xyloglucans, and some amount of glucuronoarabinoxylans, and pectins. These results are in accordance with data in literature about exogeniously applied ABA, which stimulates the accumulation of pectic arabinan, in root meristem of Arabadopsis [20]. Arabinans are known to play role in cell-to-cell adhesion [21], mechanical property of the cell wall [22], endo-arabinase activity [23]. It was determined that ABA induce fast and enhanced induction of reactive oxygen species and altered expression of cell wall modification genes, including pectin-esterase and xyloglucan enotransglycosylase [16]. It was reported that ABA is able to induce expression of cell wall loosening gene in Arabidopsis [24]. We suppose that these mechanisms could be possibly involved into the synthesis of determined by us PSs under the ABA impact.

Analysis of PS monosaccharides from extracellular liquid on media with 2,4 D showed the increase in quantity of arabinose on 6 times, galactose 8 times, xylose 5 times and glucuronic acid 6 times, compared to extracellular PS from ABA media. (Table 2, Fig. 4). On 2,4-D medium the content of arabinose of extracellular PS ranged from 11 to 30%, xylose – from 10 to 33%, galactose – from 6 to 20% of and glucuronic acid – from 5.0 to 42%. However, it was found that absolutely all variants of extracellular PS of 2,4-D were lack of galacturonic acid, and had very low amounts of mannose. The ratio of monosaccharaides in extracellular PS from medium with 2,4-D was: Ara: Gal: Xyl: Glc: Man: GlcA: GalUA – 10: 6: 10: 14: 1: 6: 0 (Fig. 5a).

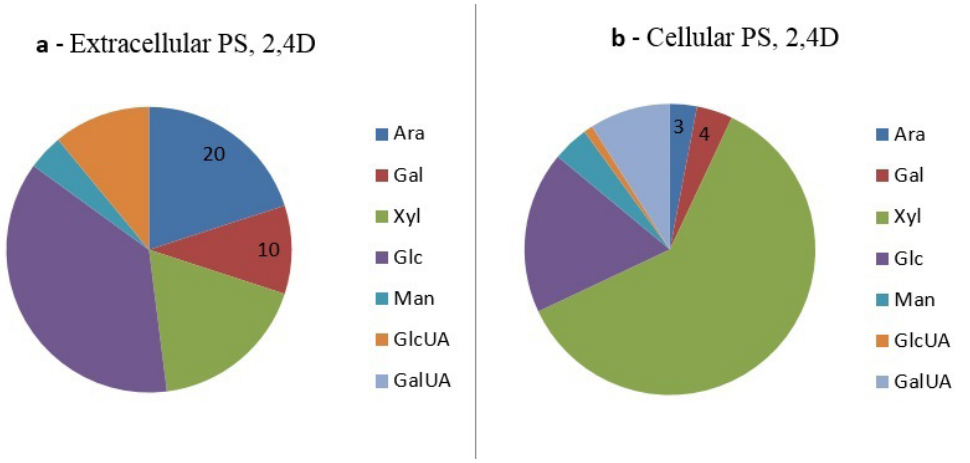

Fig. 5. The ratio of monosaccharidies of PS from medium with 2,4 D – extracellular liquid (a) and cells of suspension (b).

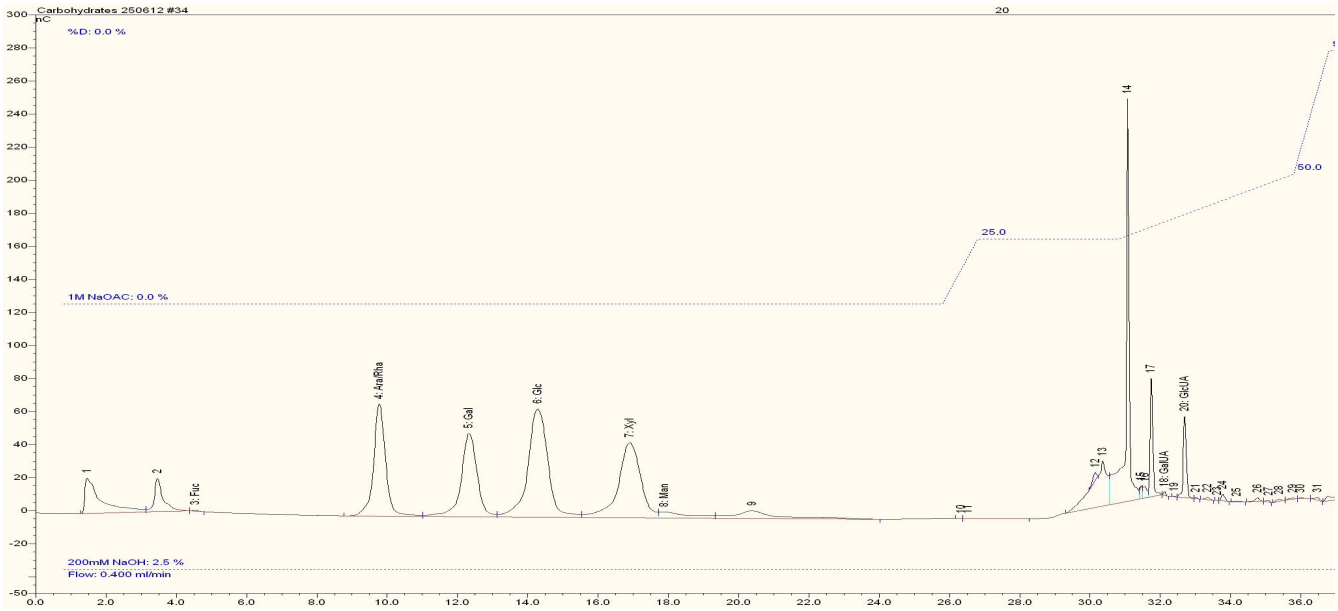

Fig. 6. Monosaccharide composition of PS, extracted from extracellular space of suspension culture with 2,4-D.

In the analysis of the monosaccharide composition of cell PS from cultured in media supplemented with 2,4-D, showed an increase of xylose (68.6%), mannose (10.4%) and galacturonic acid (8%), and reducing the amount of arabinose (2.7%) and galactose (2.5%), compared to extracellular PS grown with 2,4-D. The proportion of monosaccharides of dry cells cultured on 2,4-D was -Ara: Gal: Xyl: Glc: Man: GlcA: GalUA – 10: 8: 230: 18: 35: 7: 27 (Fig. 5b). Figures 6 and 7 presents chromatograms of HPLC, reflecting the distinctions of monosaccharide composition of extracellular PS and PS from cells of suspension cultures, cultivated on media with 2,4-D, analysis of which is presented also in Table 2.

We assume that 2,4-D induce the increased output of arabinogalactans, arabinoxylans, glucuronarabinoxylans into extracellular space. There is an evidence that auxin treatment induces expansins and pectin methilesterase (PME) activity in roots [25], influence activity of extracellular class III peroxidases, which play role in secondary cell wall remodeling, polymerization, and mediation of cross-linking between lignins, polysaccharides and proteins [26]. In detail, it was revealed that 2,4 D in *Catharanthus roseus* cell suspension cultures reduced covalently-bound peroxidase and enhanced ionically bound peroxidase activity [27].

Consequently, we suppose that 2,4 D in wheat suspension culture induce increased amount of extracellular PS predominantly arabinans, arabinogalacans can occur possibly through PME, expansins and peroxidase activity regulation.

Meanwhile, cellular PSs under 2,4-D influence deposit xylans, xyloglucans, pectins, mannans.

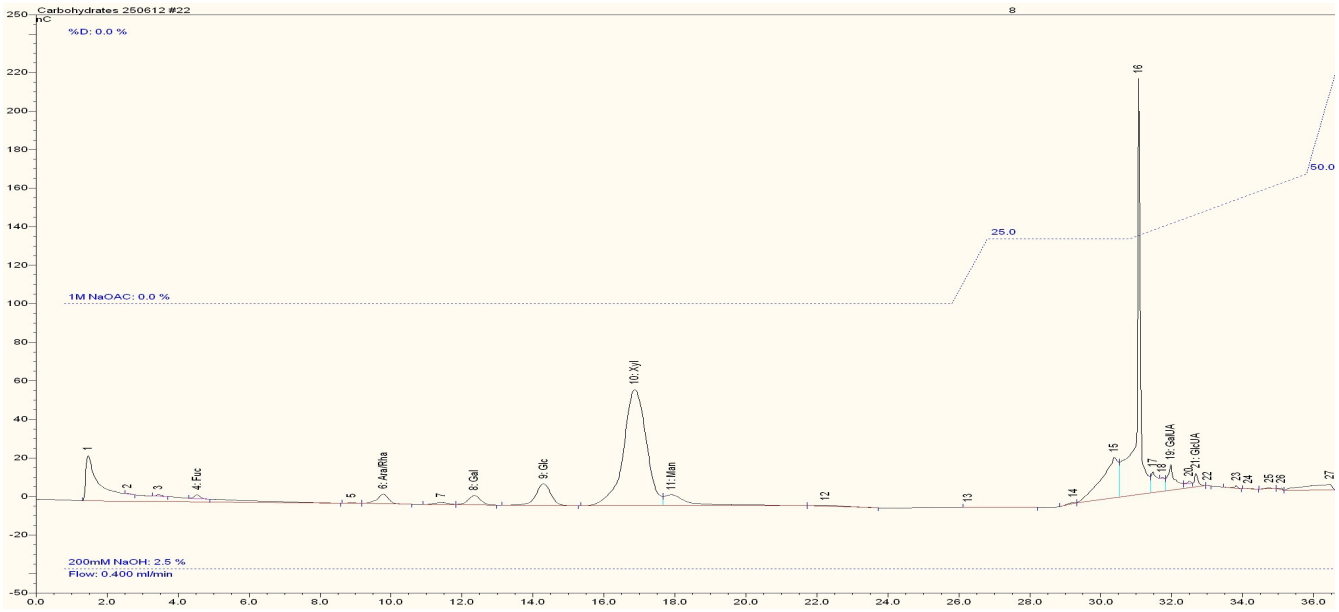

Fig. 7. Monosaccharide composition of PS, extracted from cells of suspension culture on media with 2,4-D.

It was recentely reported that auxins modulate expression of cell wall related genes and remodeling of hemicellulose xyoglucan side chain structure [28]. Therefore, we suppose that 2,4-D in cell suspension culture of wheat stimulate biosynthesis of xylans, xyloglucans, pectins and mannans for strengthening the cell wall of wheat callus cells and enhance interplays between auxin signaling, cell wall biosynthesis and remodeling.

In general, HPLC data confirm the results obtained earlier in our studies by Gas chromatography that extracellular polysaccharides, extracted from suspension culture of wheat with 2,4-D, consist of arabinoxylans, xyloglucans and arabinogalactans [29].

4. Conclusion

Overall, cellular PS from media with both types of phytohormones are the same in monosaccharide composition and mainly presented by xylose, glucose. However, cellular PS differ by the uronic acid content in these two media: on ABA media they have increased amount of glucuronic and galacturonic acids, whereas on 2,4-D media there is a presence only of galacturonic acid. Therefore, cellular PS are presented in both media by xyloglucan and xylan pectins, containing uronic acids. We suppose that ABA and 2,4-D play as triggers for strengthening cell wall via increased xylans production.

PS secreted into extracellular liquid are different in both type of culture media. Monosaccharide composition of extracellular PS in ABA medium consists mainly of glucose, consequently, we can assume that the ABA promotes output of β-glucans to extracellular environment.

Levels of arabinose and galactose in extracellular PS from 2,4-D medium were significantly higher than in extracellular PS on ABA, as well as they were also rich for xylose, glucose and glucuronic acid. Thus, we can conclude that 2,4-D induce the output to extracellular space high amounts of – arabinogalactans, arabinoxylans, arabinoglucuronans, xyloglucans.

Our results for the first time confirm that it is possible to regulate accumulation and secretion of biologically active polysaccharides by phytohormones in model system of wheat embryogenic cell suspension culture elaborated in our laboratory.

Our results revealed that investigated plant phytohormones are capable to cause the secretion different types of PS into extracellular space in wheat suspension cultures, which is very useful for the obtainment of valuable PSs with different possible physiologic activities.

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