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Isolation of oligosaccharide fraction from wheat cell culture and determination of its monosaccharide composition

Abstract. Oligosaccharides, being a part of plant cell wall, can be secreted to the intercellular space and perform «signaling» activity on plant tissues – high physiological activity in very low concentrations. Oligosaccharide (OS) fraction was obtained from extracellular liquid wheat cell culture by size exclusion chromatography. Monosaccharide composition of extracellular OS has been determined by HPLC Ionic chromatography. It was shown that extracellular OS contain glucose, xylose, arabinose, galactose, fructose and rhamnose monosaccharide residues.

Key words: wheat cell culture, oligosaccharide, monosaccharides.

Introduction

Recently, attention of researchers is reasonably attracted by oligosaccharides – fragments of mushroom polysaccharides and plant cell wall. Oligosaccharide is any residue from polysaccharides linked by glycoside bonds. Some selected oligosaccharides can have a «signalling» effect on plant tissues at very low concentrations. Such oligosaccharides are called «oligosaccharines» [1]. To date, five groups of biologically active oligosaccharides (oligosaccharines – OS) are well known; oligoglucoside β -glucan and oligomers of chitin (chitosan) of the mushroom cell wall, lipooligosaccharides produced by *Rhizobium*, and two groups of plant oligosaccharides – pectins and xyloglucans [2]. OS are characterized by high biological activity at very low concentrations: 2-3 times lower than that for the known phytohormones. They induce in plants a number of eliciting protective responses against plant pathogens: the formation of phytoalexins, proteinase inhibitors, β -glucanase, chitinase, as well as the synthesis of callose and lignin [2,3]. Thus, pectin OS induce formation of flowers or plants vegetative shoots from explants in culture tobacco and inhibit root formation [4,5], and also inhibit stretching of etiolated pea stem segments induced by auxin [6]. Xyloglucan (XG) OS constitute the least studied OS group on biological activity. Antiauxin effect of nona- (XG9) and pentasaccharide xyloglucan (XG5) fragments of the cell wall have been showed in the segments *Acerpaeudoplatarvus* pea stalks [7] and in the culture of carrot isolated protoplasts [8]. Also, it is noted that oligosaccharines stimulate growth,

basal metabolism, and division of plant cells [9]. Currently oligosaccharines are poorly investigated and of a great interest for further research of their role in signal transduction of plants [10]. The total content of oligosaccharides in plant tissues varies in a range of 10^{-7} - 10^{-5} M [11]. Therefore, isolation and purification of oligosaccharides from native plant tissue extract is challenged by their low content and require a huge amount of plant material. Chemical and enzymatic hydrolysis of plant polysaccharides does not allow to obtain an exact replicate of biological active molecule's stereochemical characteristics [12, 13]. In this regard, plant cell culture methods are known for their potential in producing biologically active substances, which make them suitable for search of OS in intercellular space of liquid plant cell cultures [14].

Materials and methods

Cell suspension culture was obtained from long-term wheat callus tissues. For this purpose callus tissues were placed in liquid culture medium [15] at a ratio 200-300 mg on 30-40 ml of media and cultured on a shaker at 140 rpm at 26 ± 2 °C and 16-hour photoperiod. Extracellular liquid was collected and filtered, then concentrated on rotary evaporator IKA «WERKE».

Isolation of oligosaccharides was carried out by ethanol precipitation [16,17] and centrifugation at 10,000 rpm 10 minutes at 8 °C. Total sugar amount was determined by Dubois method [18]. Oligosaccharide's fraction was obtained by size-exclusion column chromatography with Biogel P2 sorbent,

calibrated with Blue Dextran (2000000 Da), maltoheptaose (1153 Da) and sucrose (342,3 Da). Monosaccharide composition was identified by the method of Ionic High Performance Liquid Chromatography (HPLC), by Dionex ICS 5000 chromatographic equipment (Thermo Scientific).

Results and discussion

Gel-filtration on Biogel P-2 sorbent is known to be effective for fractionation of low molecular weight compounds. Standard curve calibration has shown that Blue Dextran output takes place on 6-12 minute, maltoheptaose – on 12-18 minute, sucrose – on 18–25 minute. Two samples of extra-cellular liquid were fractionated by size-exclusion chromatography.

Retention time of oligosaccharide's peak of sample S6 was 13-18 minutes with the maximum on 16th minute, which corresponds to the retention time of maltoheptaose. Consequently, oligosaccharides molecular mass in sample S6 is around of 1153 Da, and the quantity of monosaccharides is approximately 7 monosaccharide residues.

Retention time of oligosaccharide's peak of sample S7 was 13-18 minutes with the maximum on 14th minute, which corresponds to the retention time of maltoheptaose (Figure 1). Consequently, oligosaccharides molecular mass in sample S7 was around of 1153 Da and higher, and the quantity of monosaccharides is approximately 7-10 monosaccharide residues.

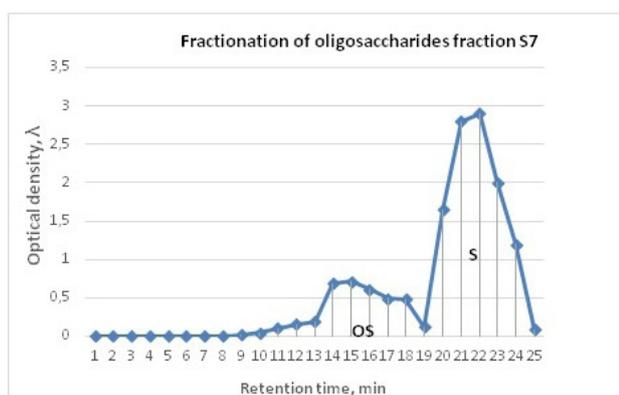


Figure 1 – Size-exclusion chromatography of S7-OS fraction; indicated OS-oligosaccharides, S – sucrose

Thus, two fractions of oligosaccharides have been obtained from extracellular liquid of wheat cell culture. These two fractions contain 7-10 monosaccharide residues. Study of these two oligosaccharide

fractions on monosaccharide composition revealed almost similar characteristics. It was determined that in sample S6 oligosaccharide fraction is rich for glucose (50%) and contains the following monosaccharides: xylose (25%), rhamnose (18%), fructose (4%) and galactose (3%). Nevertheless, residues of arabinose, mannose, fucose, galacturonic and glucouronic acids were not identified in S6 fraction. Fraction S7 contains 50% glucose, 30% xylose, 11% fructose, 4% mannose and a small amount of arabinose (2%), galactose (2%), differed from the previous sample by the presence of arabinose, galactose and mannose, and a small amount of rhamnose (Table 1, Figure 2).

Table 1 – Monosaccharide composition of oligosaccharides from extracellular liquid of wheat cell culture, in %

Monosaccharide	S6 – fraction	S7 – fraction	% in both fractions
Arabinose	0	2	0-2%
Galactose	3	2	2-3%
Rhamnose	18	1	9,5%
Glucose	50	50	50%.
Xylose	25	30	27%
Mannose	0	4	0-4%
Fucose	0	0	0
Fructose	4	11	7,5%
Galacturonic acid	0	0	0
Glucorinic acid	0	0	0

Thus, results of high performance liquid chromatography of oligosaccharide fractions revealed, that in fraction S6 there are peaks of glucose, xylose, rhamnose, galactose and fructose. This S6 fraction, presumably, contain xyloglucan and ramnogalacturonan oligosaccharides with approximate 7 residues. S7 fraction consists of glucose, xylose, fructose, mannose and galactose with arabinose. Consequently, S7 fraction, could be represented by xyloglucan, glucomannan, arabinogalactan oligosaccharides, consists of approximate 7-10 monosaccharide residues.

On the basis of obtained results it can be presumed that oligosaccharides, obtained from wheat cell culture in the presence of 2,4 –D in medium, are mainly presented by xyloglucans, ramnogalactans, glucomannans, arabinogalactans, etc. and consist of 7-10 monomers. Further analysis of extracellular oligosaccharides will be targeted to study of their functional activity and structural study of physiologically active fractions.

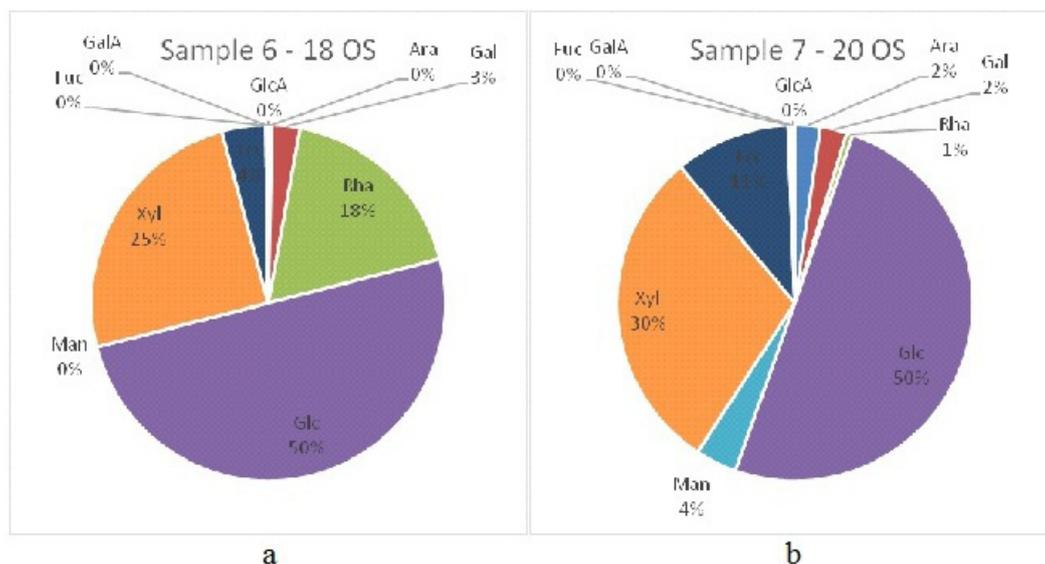


Figure 2 – Monosaccharide composition of oligosaccharide fractions, %; indicated samples 6 (a) and 7 (b)

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