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Identification of mono-, oligo-, and polysaccharides secreted from soybean roots

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Abstract The mist culture system was conducted to study secreted polysaccharides from soybean (*Glycine max*) roots grown for 15 days. Roots were rinsed with distilled water (DW) for 15 min, then with 30 mM oxalic acid (OXA) for 15 min to remove ionically bound sugar. Released sugars were further fractionated into low (L) and high (H) molecular weight fractions with Sephadex G-10. DW rinsing released 190 μg neutral sugar (NS) and 62 μg uronic acid (UA) per plant, while 374 μg NS and 70 μg UA per plant were released by OXA rinsing. Acetylation analysis revealed that the L fraction by DW and OXA mainly consisted of glucose (Glc), pinitol, and UA, whereas the H fraction mainly consisted of arabinose (Ara), galactose (Gal), Glc, and UA. The presence of rhamnose (2%–6%) in both fractions suggests secretion of rhamnogalacturonans. Methylation analysis revealed that the H fraction by DW and OXA contained T-Ara, 3-, 6-, and 3,6-Gal, suggesting the presence of type II arabinogalactan and arabinogalactan proteins. HPLC analysis detected mono-, di-, and tri-GalA in the L fraction by DW and OXA. Substances corresponding to sucrose, kojibiose, cello- and laminari-oligosaccharides were also found in root exudates.

Key words Arabinogalactan · Pectin · Pinitol · Rhamnogalacturonan · Root exudates · Soybean (*Glycine max*)

Introduction

Soil with plant roots is a highly complex and dynamic ecosystem (Foster 1988; Smiles 1988). Plants release various organic matter into the soil. The amount of organic matter released into the rhizosphere varies over a wide range and its production may reach up 40% of total dry matter (Lynch and Whipps 1990). The rhizosphere is largely controlled by the plant root system through the secretion of substances into the surrounding soil. These substances, designated as root exudates (Waisel et al. 1996), are one of the most important factors responsible for rhizosphere maintenance and quality.

Plants could nutritionally benefit microorganisms through the secretion of a complex mixture of substances consisting of sugars, amino acids, and other compounds such as flavonoids, organic acids, enzymes, lectins, peptides, and other glycoproteins (Roschina and Roschina 1993; Fan et al. 1997). The most abundant root exudates are sugar and protein (Morre et al. 1967; Kirby et al. 1971; Jaeger et al. 1999). These substances directly or indirectly influence both quality and quantity of microorganisms in the rhizosphere. The presence of microorganisms has been suggested to enhance the amount of root exudates (Lynch and Whipps 1990; Meharg and Killham 1995).

The following possible functions of root exudates have been proposed: (1) protection of root surface from extremely dry conditions (Oades 1978), (2) adaptation to acid mineral soils (Horst et al. 1982), (3) adhesive substance (Bacic et al. 1986), and (4) a signal molecule in plant–microbe interaction, mainly in defense mechanisms (Lamb et al. 1989; Walton 1994).

There are three experimental approaches to root exudate study: (1) plants grown in a sterile hydroponic culture (Curl and Truelove 1986) or mist culture (Waisel et al. 1996), (2) plants grown in solid matrix such as soil, sand, or artificial soil (Curl and Truelove 1986), and (3) use of isotope tracers to detect labeled exudates in leachate (McDougall and Rovira 1970). Organic carbon of root exudates is classified into three groups, (1) high molecular weight such as mucilage, (2) low molecular weight organic solutes, and (3) sloughed-off cells or cell walls and their lysates (Warenbourg and Billes 1979).

High molecular weight of root exudates is composed of neutral and acid polysaccharides and primarily produced by the root cap known as mucilage (Bacic et al. 1986). Corn root mucilage consists of polysaccharides whose components are fucose (Fuc), arabinose (Ara), xylose (Xyl), man-

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nose (Man), galactose (Gal), and glucose (Glc) (Paull et al. 1975; Hinch and Clark 1980; Chaboud and Rougier 1984; Moody et al. 1988). Mucilage of wheat root secreted more Fuc component than uronic acid (UA). In contrast, cowpea root contained more UA (11.5%) than wheat root (4%) and arabinogalactan protein (AGP) was identified in the root slimes of both species (Moody et al. 1988). Analyses of mucilage from a number of species have been performed, but there was potential contamination by microbial polysaccharides (Emma et al. 1997).

Low molecular weight of root exudates predominantly contained oligomer and monomers of sugar such as Ara, Gal, Glc, and UA (Hinch and Clark 1980). Many investigators have worked with polymers and monomers of sugars, but the intermediate fraction, such as the oligosaccharides, secreted from plant roots is poorly understood.

In this study we have identified mono-, oligo-, and polysaccharides of root exudates from root surface of soybean. The sugar species of the root exudates may lead to an understanding of the function of secreted sugars on the growth and development of soil microorganisms.

Materials and methods

Plant materials and culture conditions

Seeds of soybean (*Glycine max* L.) were surface-sterilized using 3 mg Benlate-T (DuPont, Wilmington, DE, USA) per 1 g seed, then germinated on four sheets of tissue paper (JK Wiper 150-S, Kureshia, Tokyo, Japan) wetted by 60 ml distilled water (DW) in a sterilized plastic box (5 × 35 × 25 cm), and kept in a dark room for 2 days. Germinated seeds were transferred to a mist culture box (28 × 20 × 18 cm) to which mist of one-tenth strength of Hoagland solution containing 3 mg/l penicillin G (Sigma Chemical, St. Louis, MO, USA) was introduced through silicone tubing. The seeds were grown at 25°C in a growth chamber for 15 days with a 16:8 h light:dark cycle. Hoagland's mist was generated by an ultrasonic humidifier (model FC05-KXC; Matsushita Electric Industrial, Osaka, Japan).

Preparation of sugars of root exudates

Soybean roots of 29 plants (total weight 32.5 g) were extensively rinsed for 15 min with 300 ml DW. This solution was designated as the DW fraction. Roots were then rinsed for 15 min with 300 ml 30 mM oxalic acid (OXA). This solution was designated as the OXA fraction. The DW fraction was filtered through a glass filter (GF/C; Whatman, Maidstone, UK), then heated at 100°C for 10 min to inactivate any microorganisms and glycanases that might have been released from roots. The volumes of the DW and OXA fractions were reduced in vacuo by a rotary evaporator. A 10-ml sample of the DW or OXA fraction was applied to G-10 Sephadex columns (ϕ 2.0 × 25 cm). The column was eluted by DW. Low molecular weight (L) and high molecular weight (H) fractions were collected using a fraction collector (model FC203B; Gilson, Middleton, MI, USA). The fractions were pooled and lyophilized.

Sugar assay

The total sugar content was determined by the phenol sulfuric acid method (Dubois et al. 1956) and the acidic sugars by the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen 1973). To calculate the neutral sugar (NS) and acidic sugar content, the following equations were used (Sakurai and Nevins 1997):

$$\text{Neutral sugar } (\mu g/ml) = 92.2\ (A_{490} - 0.464 \times A_{520})$$
$$\text{Acidic sugar } (\mu g/ml) = 112.4\ (A_{520} - 0.0273 \times A_{490})$$

where A_{490} and A_{520} are the absorbance of the phenol sulfuric acid method and *m*-hydroxydiphenyl method, respectively.

Analysis of NS components

Acetylation analysis was performed by using the method of Albersheim et al. (1968) to determine the neutral monosaccharide composition of oligo- and polysaccharides. Lyophilized powder (1 mg) was dissolved in DW (1 ml) and placed in a screw-cap tube, and 1 ml 4 M trifluoroacetic acid containing *myo*-inositol (300 μg/ml) as an internal standard was added. Sugars in the tube were hydrolyzed at 121°C for 1 h. The hydrolyzed substances were dried by a stream of filtered air at 50°C, then reduced by 10 mg $NaBH_4$ in 0.5 ml 2 M NH_4OH for 1 h at room temperature. The excess amount of $NaBH_4$ was decomposed by 3–4 drops of glacial acetic acid. The reduced hydrolysates were then dried by a stream of filtered air at 50°C after which 1 ml methanol was added to the solution and dried. The same treatment was repeated five times to remove borate, which interferes the next acetylation reaction. To the dried hydrolysates, 200 μl acetic anhydride and 40 μl 1-methylimidazole were added (Chaplin 1986). The solution was agitated by ultrasonication for 10 min. To stop the acetylation, 1 ml DW was added and the alditol acetates thus obtained were extracted by chloroform. This step was repeated twice. The combined chloroform layer was washed by DW and dried with a stream of filtered air at 50°C. The alditol acetates were dissolved in 100–200 μl acetone and introduced into a gas-liquid chromatograph (GC-17 A; Shimadzu, Kyoto, Japan); the capillary column was SP-2340 (Supelco, Bellefonte, PA, USA). Oven temperature was raised from 220°C to 245°C at a rate of 2°C/min. Amounts of monosaccharides were determined by comparing the peak areas with that for *myo*-inositol to calculate the actual amount of monosaccharides.

Methylation analysis

Methylation analysis was used to determine the linkage of polysaccharides (Conrad 1972). The dried sample (ca. 1 mg) in a screw-cap tube was dissolved in 1 ml DMSO for 1 h at

room temperature with a stirrer in an N_2 atmosphere. Methylsulphinyl anion (ca. 2.3 mEq) was added to the tube and the tube was purged by N_2 gas, then capped and the solution was stirred for 4 h. To the solution, 0.1 ml CH_3I was added and the solution was then stirred at room temperature for 12 h. Permethylated substances were extracted by chloroform and hydrolyzed with 2 M trifluoroacetic acid for 1 h at 121°C, then reduced by $NaBH_4$. The substances then were acetylated by acetic anhydride as described above. Permethylated alditol acetates were dissolved in 100–200 µl acetone and introduced into a gas-liquid chromatograph (GC-17 A; Shimadzu, Kyoto, Japan). Oven temperature was raised from 160°C to 245°C at the rate of 2°C/min. Sugar linkages of polysaccharide were determined by comparing the retention times of peaks with those of standard oligo- and polysaccharides, i.e., cellobiose, larchwood arabinogalactan, xylan, and also identified by GC-MS (QP-5000; Shimadzu).

Analysis using HPLC-pulsed amperometric detector

Acidic oligosaccharides was analyzed using HPLC with a pulsed amperometric detector (PAD; Dionex, Sunnyvale, CA, USA) described by Hotchkiss and Hicks (1990) with some modification. The powdered sample (1 mg) was dissolved in 1 ml DW. CarboPac PA1 (Dionex) was used as a column. Oligogalacturonic acids were eluted by an OXA buffer (pH 6.0) as the mobile phase at a flow rate of 0.8 ml/min. A linear gradient of OXA buffer from 0–400 mM in 15 min and 400–500 mM in 5 min was used. Potassium hydroxide (500 mM) was added to the eluant via a mixing tree at a flow rate of 0.8 ml/min. The final flow rate was 1.6 ml/min. Triple pulse sequences used for amperometric detection were E1 = 0.1 V (480 ms), E2 = 0.6 V (300 ms), and E3 = −0.6 V (120 ms). Standards for different degrees of polymerization (DP) of galacturonic acids were galacturonic acid, digalacturonic acid, and trigalacturonic acid (Sigma Chemical, St. Louis, MO, USA) and those for DP of more than four were obtained from pectic acid (Katayama Chemicals, Osaka, Japan), partially hydrolyzed by 0.1 M trifluoroacetic acid for 30 min at 121°C. Neutral oligosaccharides were determined by HPLC-PAD with a different elution mode. The powdered sample (1 mg) was dissolved in 1 ml DW, and oligosaccharides were eluted by sodium acetate (0–600 mM) containing 160 mM NaOH as the mobile phase at flow rate 1.0 ml/min. The linear gradient of sodium acetate from 0 to 600 mM in 20 min was used. The triple pulse sequence condition was as described above. Standards for different DP of oligosaccharides were obtained from soluble starch (Katayama Chemicals) partially hydrolyzed by 0.1 M trifluoroacetic acid for 30 min at 121°C.

Results

Sugar content in root exudates

Two fractions of sugars from the soybean root surface were obtained. One was released from the root surface by simple rinsing with DW (loosely bound sugars), and the other by 30 mM OXA (ionically bound sugars). Both treatments released L and H sugars from the root surface grown in mist of one-tenth strength of Hoagland solution for 15 days. Figure 1 shows an example chromatograph of the OXA fraction on a Shepadex G-10 column. Released sugars were divided into H and L fractions. Changes in the amounts of H and L fractions during 15 days are shown in Fig. 2. The amount of total sugars of the L fraction by DW increased up to day 10 and slightly decreased on day 15. The amount of H fraction sugars released by DW changed little, around 60 µg/plant. The ratio of UA to total sugar was small on day 5 (17% for the L fraction, and 9% for the H fraction) but increased on days 10 and 15. OXA rinsing released a higher amount of L fraction than DW rinsing, and the amount increased as the root grew. The ratio of UA to total sugar also increased from 6% to 16%. The amount of the H fraction by OXA was comparable to that by DW, but increased from day 5 to 15 by three times.

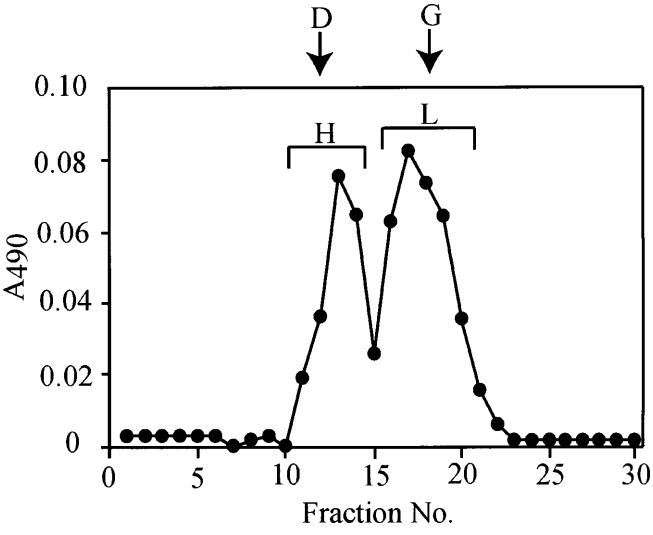

Fig. 1. Sephadex G-10 gel filtration pattern of polymer and oligomers of soybean root exudates by oxalic acid (OXA). Ten-milliliter samples of root exudates by OXA were applied to a Sephadex G-10 column, which was eluted with distilled water (DW). The fraction (5 ml) was collected by a fraction collector. Total sugar content in the eluate was determined by the phenol sulfuric acid method. Fractions (No. 11–14) were pooled as high molecular weight (*H*) fractions and fractions (No. 16–20) were pooled as low molecular weight fractions (*L*). OXA was eluted after glucose. Blue dextran (2000 kDa, *D*) was used as a standard of H sugars and glucose (*G*) was used as a standard of L sugars

Analysis of sugar components

Table 1 shows the sugar composition of soybean root exudates released by DW and OXA. The L fraction by DW yielded 33% Glc, 24% pinitol, and 20% UA. Conversely, the H fraction by DW mainly consisted of Ara, Gal, and UA, which accounted for 71% of the fraction, suggesting the existence of rhamnogalacturonan with arabinogalactan (AG) side chains. Glc, Fuc, and Xyl were also found in the H fraction. Major components of the L fraction by OXA

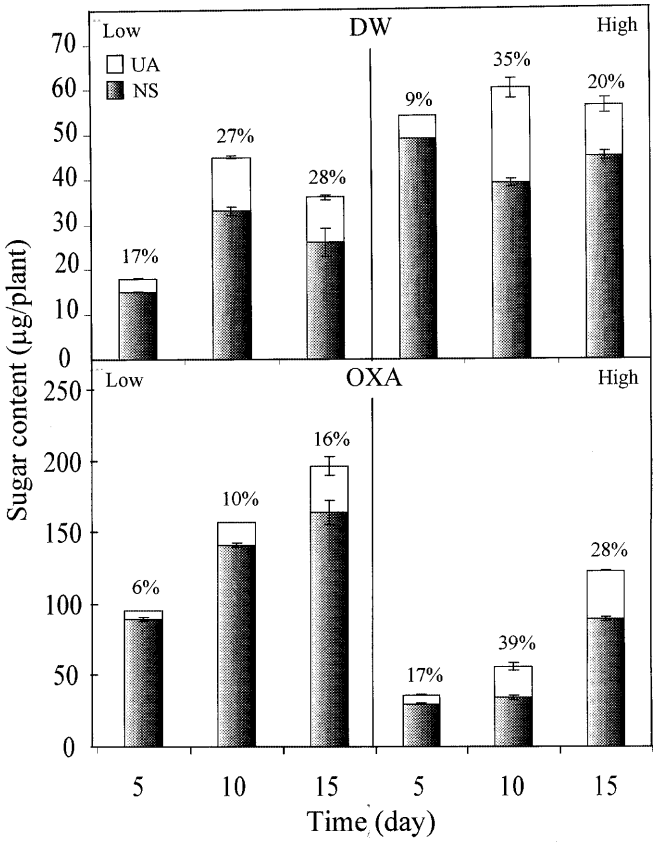

Fig. 2. Time course of neutral sugar and uronic acid (UA) content of soybean root exudates extracted by DW and OXA. Roots were rinsed with 300 ml DW, then 30 mM OXA for 15 min each. Of the DW and OXA fractions, 10 ml was applied to a Sephadex G-10 column. Neutral sugar (*NS*) was determined by the phenol sulfuric acid method and *UA* was determined by the *m*-hydroxydiphenyl method. The SDs shown in this figure were based on three replications

were Glc (38%) and pinitol (40%). UA content in the L fraction by OXA per plant was only 7%, less than the percentages shown in any other fractions (20% in the L fraction and 24% in the H fraction by DW, and 27% in the H fraction by OXA). Nevertheless, the actual amounts of UA were similar in all fractions (approximately 30 μg per plant). Sugar composition of the H fraction obtained by OXA was similar to that by DW. The ratios of components of Ara (12%) and Gal (14%) of the H fraction obtained by OXA were less than those by DW, respectively.

The presence of pinitol in soybean root exudates was identified by both gas liquid chromatography and mass-spectrometry (GC-MS). Pinitol was found only in the L fractions by DW and OXA.

Elution profiles of root exudates on HPLC-PAD

Monomers, oligomers, and polymers of galacturonic acid were separated by HPLC with an increasing concentration of OXA buffer (pH 6.0) to identify oligo- and polyuronides in the root exudates (Fig. 3). The UA content of the eluant was determined by the *m*-hydroxydiphenyl method. Monomeric UA was dominant in the L fraction by DW and OXA. The H fractions by DW and OXA also contained monomeric UA, suggesting the contamination of UA from the L fraction on the Sephadex G-10 column. Polyuronides were minor in both fractions.

Analysis of sugar linkage

Methylation analysis was conducted to identify the sugar linkage of root exudates in L and H fractions (Fig. 4). The H fraction by DW contained not only terminal sugars such as T-rhamnose (T-Rha), T-Man, T-Gal, and T-Glc, but also backbone chain sugars such as 3-, 4-Ara, 3-, 6-, 3,6-Gal, and 4-, and 6-Glc, clearly indicating the presence of polymerized

Table 1. Sugar composition of soybean root exudates

Sugar	Sugar content (μg/plant)				
	DW		OXA		Total
	L fraction	H fraction	L fraction	H fraction	
Rhamnose	4 (3)	5 (3)	10 (2)	8 (6)	27 (3)
Fucose	1 (1)	2 (1)	3 (1)	3 (2)	9 (1)
Arabinose	8 (6)	35 (22)	12 (3)	17 (12)	72 (8)
Xylose	3 (2)	5 (3)	12 (3)	5 (4)	25 (3)
Mannose	5 (4)	14 (8)	14 (3)	18 (13)	51 (6)
Galactose	8 (6)	39 (25)	13 (3)	20 (14)	80 (9)
Glucose	42 (33)	19 (12)	174 (38)	30 (22)	265 (30)
Pinitol	30 (24)	0 (0)	184 (40)	0 (0)	214 (25)
Uronic acid	25 (20)	37 (24)	33 (7)	37 (27)	132 (15)
Total	126 (100)	156 (100)	455 (100)	138 (100)	875 (100)

Soybean plants were grown for 15 days. Neutral sugar components were determined by gas-liquid chromatography and uronic acid (UA) by the *m*-hydroxydiphenyl method. Figures in parentheses are percent of total sugar content.
L, low molecular weight; H, high molecular weight; DW, distilled water; OXA, oxalic acid (30 mM)

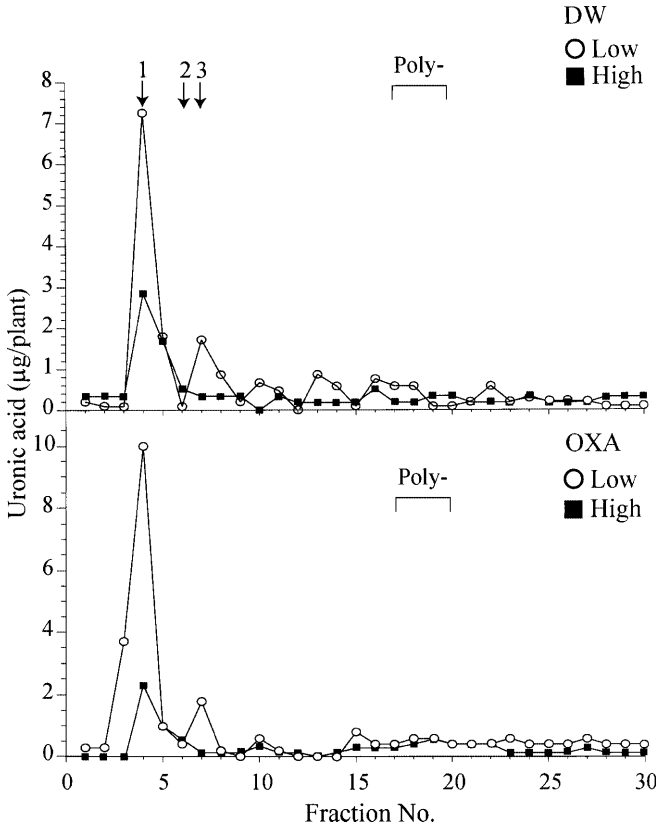

Fig. 3. Elution profiles of HPLC-PAD of H and L fractions of soybean root exudates by DW and OXA. Lyophilized powder of L and H fractions was dissolved in 1 ml DW and an aliquot (500 μl) was introduced to HPLC-PAD with a CarboPac PA1-column. The sample was eluted by a linear gradient of 0–500 mM OXA buffer (pH 6.0) as the mobile phase at a flow rate of 0.8 ml/min. The eluate was collected with a fraction collector at 1 min intervals. UA content of L (○) and H (■) fractions in the eluate was determined by the *m*-hydroxydiphenyl method. Numbers *1*, *2* and *3*, correspond to DP1, 2 and 3 of galacturonic acid (GalA)

carbohydrates. The L fraction by DW predominantly contained T-, and 6-Glc, T-Rha, T-Ara, T-Man, and T-Gal, indicating mainly monomeric sugars, except 6-Glc. The major constituents of the H fraction by OXA were T-Rha, T-Ara, T-Man, and T-Glc with an appreciable amount of backbone sugars, such as 2,4-Xyl, 2-, 4-, and 2,4,6-Man, 2-Gal, 3,6-Gal, 2,4,6-Gal, and 3-Glc. The L fraction predominantly consisted of T-Glc, accounting for 61% of total neutral sugar of the fraction, with minor amounts of T-Rha, T-Fru*f*, T-Ara*f*, T-Man, T-Gal, and 6-Glc.

The presence of several kinds of L fraction by DW as mentioned above was confirmed by the HPLC-PAD chromatograph for NS (Fig. 5 *upper panel*). The L fraction by DW was separated by HPLC with a gradient of 0.6 M sodium acetate to elute different DP of neutral oligosaccharides. It showed that the main peaks corresponded to pinitol, monomeric hexose, sucrose or gentibiose, kojibiose, cello- and laminari-oligosaccharides, and galacturonic acid (GalA). The other peaks eluted after 15 min might be uronide molecules. The L fraction by OXA mainly contained monomeric hexose and pinitol (Fig. 5 *lower panel*). The other peaks were minor and corresponded to sucrose or gentibiose, kojibiose, cello-, and laminari-oligosaccharides, galacturonic acid, and probably uronides.

Discussion

The present study indicates that soybean root secretes L and H carbohydrates (Fig. 1). In dicot plants, there are four developmental zones that can be distinguished in a root: the root cap, the meristimatic zone, the elongation zone, and the maturation zone (Taiz and Zeiger 1998). All the zones are commonly surrounded by carbohydrates (collectively known as mucigel). Secretion of mucigel from plant roots primarily occurs from the cap cells at the root tip, although epidermal cells in the elongation and maturation zones may also contribute (Curl and Truelove 1986). Further analysis of carbohydrates secreted in different regions along the root is necessary to understand the function of root exudates.

NS of soybean root exudates predominantly consist of Glc, Gal, Ara, and a small amount of Fuc (Table 1). The presence of Fuc (3%–21%) was reported in root mucilage of maize (Bacic et al. 1986), cowpea, wheat (Moody et al. 1988), and rice (Chaboud and Rougier 1984). Because mist culture allows plant roots to grow without physical contact with soil, the present experimental conditions may reduce the secretion of Fuc-containing carbohydrates such as xyloglucan. Indeed, T-Xyl and 4,6-Glc were minor, less than 0.5 μg/plant, suggesting that the root exudates have a low xyloglucan content. Previous studies showed that the Xyl level of cell walls in the soybean root was about 12% (Mort and Grover 1988), and in Alaska pea 14% (Tanimoto 1988), while our results showed that the content of Xyl was only about 2%–4%. It can be concluded that root exudates collected in our experiment were not simply derived from the cell walls of dead root cells.

Methylation analysis showed that the L fraction was predominantly composed of monomeric sugars such as T-Glc, T-Ara, T-Rha, T-Man, and T-Gal, with the exception of 6-Glc (Fig. 4). It shows that the Sephadex G-10 clearly separated L and H carbohydrates from root exudates. The fact that pinitol was found only in the L fraction by both DW and OXA (Table 1) also supports the validity of using the Sephadex G-10. The trace amount of fructose (Fru) and the appreciable amounts of 2-, 3-, 4-, and 6-Glc in the L fraction might be related to the presence of kojibiose, sucrose, gentibiose, laminari-, malto-, and cello-oligosaccharides. This result was supported by HPLC-PAD (Fig. 5). Fru may be derived from sucrose, and 4-Glc from starch or cellulose that were degraded into malto- or cello-oligosaccharides during root growth. We could not identify whether 4-Glc was derived from starch or cellulose. The presence of 6-Glc suggests microbial contamination on the root surface, since dextran that contains 6-Glc is known as a product of microbes. The ratio of 2-Glc to 6-Glc in common dextran is only 0.003 (Matheson and McCleary 1985), but the ratio in root exudates was 0.33. Therefore, most of the 2-Glc is not derived from dextran. 2-Glc is probably derived from kojibiose.

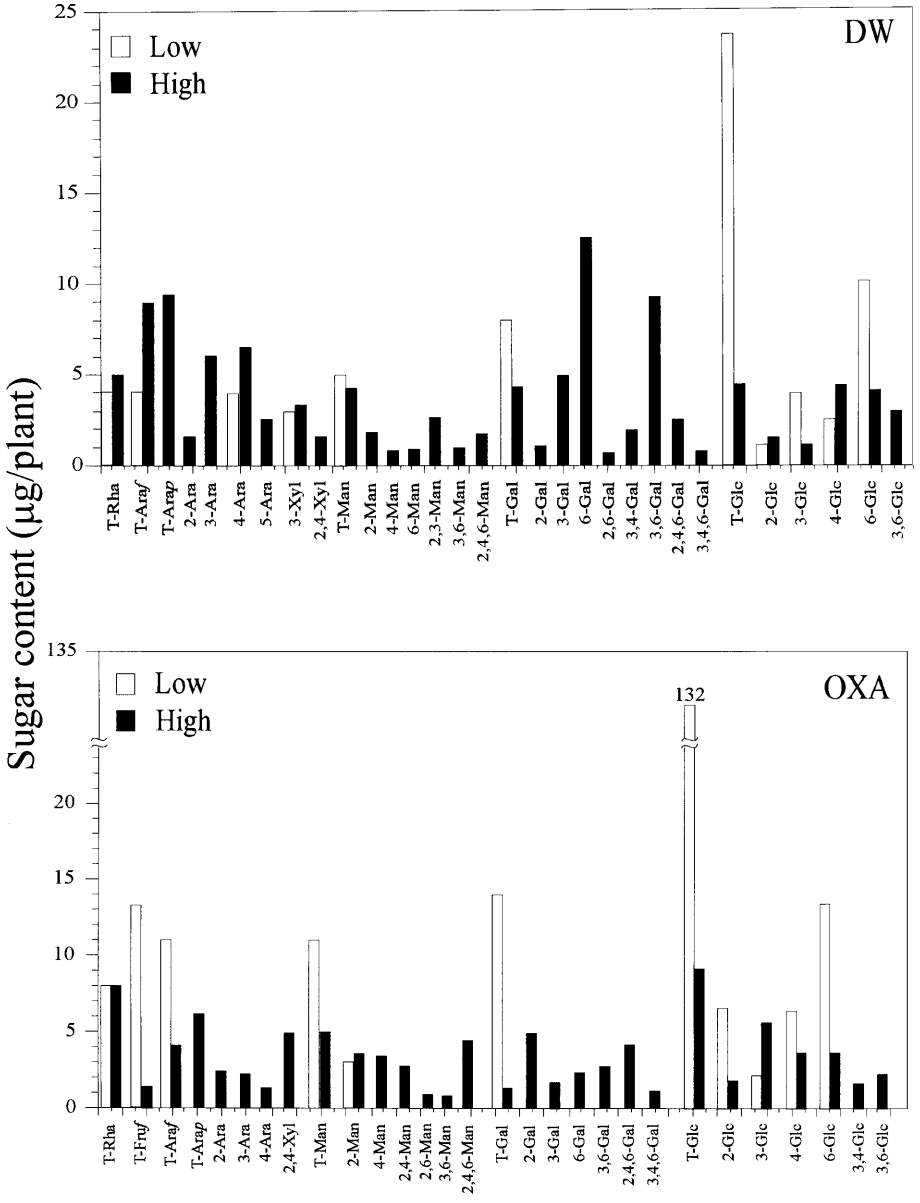

Fig. 4. Methylation analysis of L and H fractions of soybean root exudates by DW and OXA. One milligram dried sample was dissolved in 1 ml DMSO and methylated. Permethylated polysaccharides were hydrolyzed and acetylated and sugar derivatives were analyzed by GLC. The linkage analyses were performed by GC-MS. Some permethylated polysaccharides that contained less than 0.5 μg sugar content/plant are not shown. *Ara*, arabinose; *Fru*, fructose; *Gal*, galactose; *Glc*, glucose; *Man*, mannose; *Rha*, rhamnose; *Xyl*, xylose

Soybean root exudates contained small amounts of 3-Glc, suggesting that they contain callose and/or fungal cell walls and glucanases, such as endo-1,3-β-glucanase. Cline and Albersheim (1981) reported that the cell walls of soybean contained β-glucosidase that can degrade glucans of pathogen origin. We do not deny the possibility of microbe contamination of our experimental conditions, but if any, the degree of contribution of sugars of microbe origin to the total root exudates could be small, because the 1,3-β-glucan content was less than 2% of the total sugar exudates.

The results of this study clearly show that the soybean plant secretes a high amount of UAs in the root exudates (7%–27%; Table 1). The UAs might be derived from pectic polysaccharides in the cell walls. Moody et al. (1988) also reported that root slime of cowpea contained a high level of UA. The fact that the H fraction by DW and OXA predominantly consisted of Rha, Ara, Gal, and UA also support its presence, since pectin is generally rich in Rha, Ara, Gal, and GalA (Brett and Waldron 1997). Methylation analysis of the H fraction also showed that the root exudates probably contain pectin component type II AG or AGPs, since T-, 2-, and 3-Ara, and T-, 3-, 6-, 3,6-Gal were found. Trace amounts of 3,4-Gal in the DW fractions suggests the presence of type I AG. The type II AG consists of β-1,3- and short chains of 1,6-linked Gal unit (Brett and Waldron 1997; Youl et al. 1998). Type II AG or AGPs are water-soluble and therefore likely to be extracted together with the pectic type I AG (Fincher et al. 1983; Stephen 1983). Type II AG is usually found in AGPs. AGPs are ubiquitous in most plant tissues and secreted by plant cells in suspension culture (Clarke et al. 1979; Fincher et al. 1983; Sholwater 1993; Youl et al. 1998). AGPs are not covalently binding to any cell wall component and can migrate through cell walls to the outside. AGPs are not regarded as wall

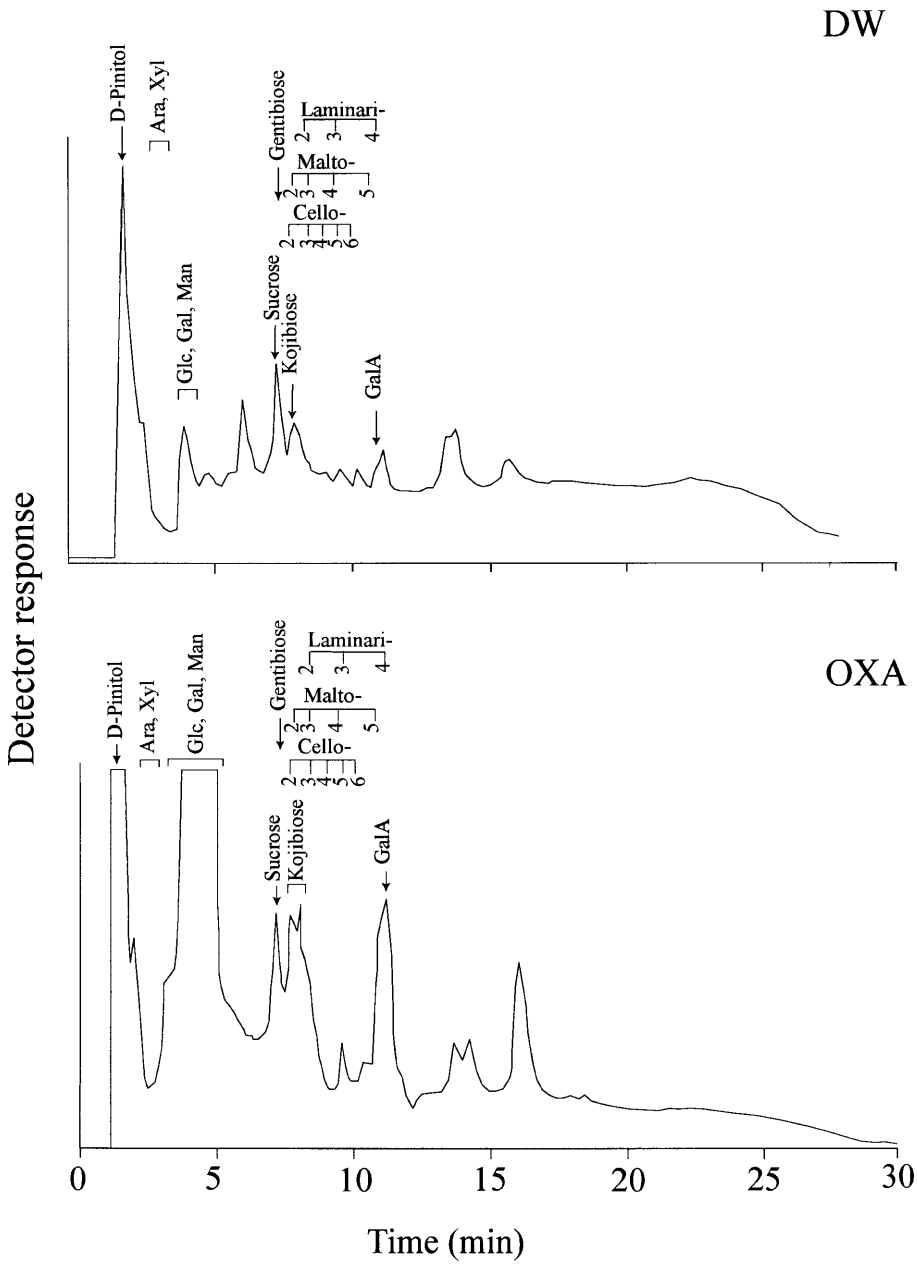

Fig. 5. HPLC-PAD separation of L fraction of soybean root exudates by DW and OXA. Lyophilized powder of the L fraction by DW of soybean root exudates was dissolved in 1 ml DW and an aliquot (500 µl) was introduced to HPLC-PAD with a CarboPac PA1-column. The sample was eluted by a linear gradient 0–600 mM sodium acetate containing 160 mM of NaOH as the mobile phase at a flow rate 1.0 ml/min. A linear gradient of sodium acetate 0–600 mM in 20 min was used. The markers of D-pinitol, Ara, Xyl, Glc, Gal, Man, GalA, sucrose, kojibiose, gentibiose, and laminari-, malto-, and cello-oligomer are based on the standard retention times. *Cello-2*, cellobiose; *Cello-3*, cellotriose; *Cello-4*, cellotetraose; *Cello-5*, cellopentaose; *Cello-6*, cellohexaose; *Laminari-2*, laminaribiose; *Laminari-3*, laminaritriose; *Laminari-4*, laminaritetraose; *Malto-2*, maltose; *Malto-3*, maltotriose; *Malto-4*, maltotetraose; *Malto-5*, maltopentaose

components but as constituents of extra cellular space or at the cell surface (Fincher et al. 1983; Samson et al. 1983; Norman et al. 1990; Komalavilas et al. 1991). Some evidence shows that AGPs are involved in cell fate (Schindler et al. 1995), cell proliferation (Sherpe and Nothnagel 1994), and cell expansion (Knox 1995). High water binding capacity and ability to form AGP gel may function as an antidesiccant, gelling agent, and lubricant in root slimes (Moody et al. 1988). Recent works showed that AGPs were involved in the control of root epidermal cell expansion (Ding and Zhu 1997).

UAs in soybean root exudates predominantly consist of the monomeric form (Fig. 3). The reason monomeric UA is secreted from roots is poorly understood. Apparently, the presence of monomeric UA in root exudates results from the activity of galacturonase. One possible reason the plant secreted the monomer or oligomer form of UA could be related to plant defense or signaling. Endogenous elicitors obtained from soybean cell walls are rich in GalA with small amounts of Rha and Xyl (Hahn et al. 1981). Jin and West (1984) reported that the GalA oligomer (DP $>$ 8) secreted from castor bean seedlings could act as an elicitor. Therefore, small oligouronides (DP $<$ 8) are not involved in defense mechanisms; rather, they may act as a carbon source for microbes (Lamb et al. 1989; Walton 1994; Jaeger et al. 1999).

Carbohydrates in the L fraction by OXA are supposed to be ionically bound to the root surface. Why OXA can release a high amount of monomeric sugars such as Glc and pinitol is still unknown. There is a possibility that monomeric Glc and pinitol are surrounded by pectic substances, and only microorganisms capable of digesting pectic sub-

stances can utilize them. Harley and Smith (1983) reported that pectic substance can be used for growth of certain ectomycorrhizal fungi.

Soybean root exudates contained a high amount of pinitol. Pinitol is known to be a major component of soybean plants (Ford 1984; Guo and Oosterhuis 1995). Present results demonstrate that pinitol is secreted to the root surface of soybeans. In clover root nodules (Davis and Nordin 1983), the amount of pinitol was higher than sucrose. The amount of pinitol in the soybean root surface was comparable to monomeric Glc. Therefore, pinitol is possibly utilized by soil microbes as well as Glc. The effect of pinitol, as well as the combination of other sugars, on rhizobium culture is under investigation. An appropriate composition of carbohydrates in root exudates may establish the best flora of soil microorganisms for plants.

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