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STRUCTURAL ANALYSIS OF SECRETED SLIME FROM WHEAT AND COWPEA ROOTS

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Key Word Index—*Triticum aestivum*; Gramineae; *Vigna unguiculata*; Leguminosae; axenic root slime; polysaccharide composition.

Abstract—The water-soluble, high M_r components of slime secreted from the roots of wheat and cowpea are composed primarily of carbohydrate (95.5 and 97.5% w/w, respectively) and some protein (5 and 3% w/w, respectively). For wheat, arabinose, xylose, glucose and galactose are the major neutral monosaccharides whereas for cowpea, arabinose, galactose and glucose predominate. Fucose is a minor constituent of both wheat and cowpea root slimes. Cowpea root slime contains significantly more uronic acids than that from wheat roots (11.5 vs 4% w/w). Methylation analysis suggests the presence of a range of polymers in the root slimes. In general, their composition appears to reflect that characteristically found for cell wall preparations from dicotyledons and graminaceous monocotyledons. In addition, arabinogalactan-proteins are components of both root slimes.

INTRODUCTION

Attachment of micro-organisms to the root surface is a necessary pre-requisite for the successful establishment of many host–pathogen/symbiont interactions [1–3]. Adhesion of fungal propagules to root surfaces and to isolated slimes secreted by plant roots is dependant upon a number of factors, including the plant and fungal species and the type of fungal surface (e.g. hyphae, conidia) [4, 5]. In some instances, attachment involves specific saccharide residues on the root surface [6–8]. Thus, the composition of the slime secreted at the root surface may be important in determining the success or failure of microbial colonization. Knowledge of the slime composition will also lead to a better understanding of the role of secreted slimes in root function and rhizosphere composition [9, 10].

There is little detailed information available regarding the composition of root slimes collected under axenic conditions [9]. The monosaccharide composition of rice (*Oryza sativa*) root slime [11] and both the monosaccharide composition and linkage analysis of maize (*Zea mays*) root slime [12 and refs therein] have been published. A striking difference between the root slimes of these two graminaceous monocotyledons is their fucose content [maize (20–30%) and rice (5%)]. In this paper, we present monosaccharide and methylation analyses for root slimes from another graminaceous species, wheat (*Triticum aestivum*) and a dicotyledon, cowpea (*Vigna unguiculata*).

RESULTS

Composition of root slime preparations

The results are summarized in Table 1. Both wheat and cowpea are composed primarily of carbohydrate [95.5 and 97.5% (w/w), respectively] and a small amount of protein [5 and 3% (w/w), respectively]. The carbohydrate of cowpea contains considerably higher amounts of uronic acids [11.5% (w/w)] than that from wheat [4% (w/w)]. The uronic acids, identified as the per-*O*-trimeth-

Table 1. Composition of root slime preparations

	% w/w Root slime preparation			
Component	Cowpea*	Wheat*	Maize†	Rice‡
Protein	3	5	6	n.d.§
Carbohydrate				
Neutral Monosaccharide‖	86	91.5	91	n.d.
Rha	2	Tr¶	—	—
Fuc	9	3	21	5.2
Ara	31	31	16	13.7
Xyl	7	33	14	18.3
Man	6	1.5	2	4.6
Gal	28	16.5	33.5	20.3
Glc	18.5	15	13	37.9
Acidic Monosaccharide‖	11.5	4	3	n.d.
GlcA	Tr	24	100	
4-OMe GlcA	Tr	Tr	—	
GalA	100	76	Tr	

*Average of duplicate determinations on three separately collected batches of root slime.
†From Bacic *et al.* [12].
‡From Chaboud and Rougier [11]; not α-amylase digested.
§n.d.: not determined.
‖Slime preparations pre-treated with α-amylase.
¶Tr: trace.

ylsilyl methylglycosides, are primarly galacturonic acid for cowpea, whereas wheat contained a mixture of galacturonic and glucuronic acids. In contrast, for maize root slime, which contains similar amounts of uronic acids to wheat root slime [12], the uronic acid was identified as glucuronic acid.

Cowpea root slime contains arabinose, galactose and glucose as the major monosaccharides, with smaller but significant quantities of fucose, xylose, mannose and rhamnose. Wheat root slime contains arabinose, xylose, galactose and glucose as the major monosaccharides, with low amounts of fucose and mannose and traces of rhamnose.

Linkage composition

The glycosyl linkage composition, determined by methylation analysis of the α-amylase treated slime preparations, is shown in Table 2. Rhamnose is in the pyranose form and, for cowpea, is terminal and 2-linked. No rhamnosyl residues were detected in wheat slime. Fucose is present in the pyranose form as a terminal residue in both wheat and cowpea slimes.

Arabinose is present primarily in the furanose form, although traces of the pyranose form were detected in cowpea slime. For cowpea, arabinofuranosyl residues are primarly terminal, 3-, 5-, and 2,3,5-linked with small amounts of the 2- and 3,5-linked residues. In contrast, for wheat slime, the arabinofuranosyl residues are primarily terminal with only small amounts of the 2-, 3-, 5- and 2,3,5-linked residues.

Xylopyranosyl residues are primarily terminal, 2- and 4-, and 2,3,4-linked with small amounts of 2,4-linked for cowpea. However, wheat slime contains 2- and 4-, 3,4- and 2,3,4-linked residues as major components with small amounts of the terminal and 2,4-linked residues. The 2- and 4-linked xylopyranosyl residues are quantified as a single component as they co-chromatograph on both BP-75 and CPSil5 capillary columns. The ratio of 2-: 4-linked can be calculated by mass spectrometry from the ratio of the unique ions at *m/z* 117 and 118, respectively. Thus, for cowpea, the ratio is 1:2 and for wheat 1:4.7.

Mannose, in the pyranose form, was detected in cowpea slime as terminal, 2- and 2,3-linked residues, but was not detected in wheat slime preparations. Galactose is present in the pyranose form, primarily as 3-, 6- and 3,6-linked residues with small amounts of terminal, 4-, 3,4- and 3,4,6-linked residues and traces of 2-linked residues in cowpea slime. Wheat, in contrast, contains primarily 3,6-linked galactopyranosyl residues with small amounts of terminal, 3- and 6-linked residues.

Glucose is in the pyranose form and is predominantly terminal and 4-linked with a small amount of 4,6-linked in cowpea slime. In wheat, the glucopyranosyl residues are primarily terminal, 4- and 4,6-linked with small amounts of 3- and 6-linked residues also present.

The linkage positions of the uronosyl residues was deduced from the 6,6′-dideuterated portion of the corresponding per-*O*-methylated alditol acetate derived from methylation of the carboxyl-reduced polysaccharides. Galacturonopyranosyl residues in cowpea preparations are terminal and 4-linked in a ratio of 1:4. Insufficient wheat root slime was available to establish the linkage position(s) of the galacturonosyl and glucuronosyl residues.

Table 2. Methylation analyses of α-amylase treated root slime preparations

Monosaccharide	Deduced glycosidic linkage*	mol % Root slime preparation Cowpea†	Wheat†	Maize‡
Rha*p*	Terminal	0.5	—	—
	2-	0.5	—	—
Fuc*p*	Terminal	3	0.5	14.5
	2-		—	1.5
	3-	—	—	8.0
Ara*f*	Terminal	10	22	4
	2-	0.5	1	6
	3-	4	1	—
	5-	9	1.5	Tr§
	3,5-	1	—	—
	2,3,5-	5	2	—
Ara*p*	Terminal	Tr	—	1
Xyl*p*	Terminal	6	4.5	8
	2-and 4-‖	3	17	3
	2,4-	0.5	1	—
	3,4-	—	7	3
	2,3,4-	3	9	Tr
Man*p*	Terminal	0.5	—	—
	2-	1	—	—
	2,3-	0.5	—	3
Gal*p*	Terminal	3	3	4
	2-	Tr	—	8
	3-	10	3	3
	4-	2	—	—
	6-	6	2	Tr
	2,3-	—	—	6
	2,6-	—	—	Tr
	3,4-	0.5	—	—
	3,6-	14	11	7
	3,4,6-	1	—	—
Glc*p*	Terminal	6	3	Tr
	3-	—	1	3
	4-	7	6	5
	6-	—	1	—
	4,6-	2.5	3	7
	2,4,6-	—	—	2

*2-Rha*p* is deduced from 1,2,5-tri-*O*-acetyl-6-deoxy-3,4-di-*O*-methylhexitol etc.

†Average of duplicate determinations on three separately collected batches of root slime.

‡From Bacic *et al.* [12].

§Tr: Trace.

‖2- and 4-Xyl*p* coelute on BP-75 and CPSil-5.

Amino acid composition of root slime preparations

The amino acid composition of cowpea and wheat root slime preparations which have not been digested with α-amylase is given in Table 3. Asparagine/aspartate, glutamine/glutamate and glycine are the major amino acids in cowpea with significant quantities of serine, alanine and proline. For wheat there was significant amounts of glycine, alanine, leucine, valine, proline, glutamate/glutamine, serine, threonine, lysine and aspartate/asparagine. Both slime preparations contain low

Table 3. Amino acid composition of root slime preparations

Amino acid	mol % Root slime preparation Cowpea	Wheat	Maize*
Lys	2.0	5.6	1.8
His	0.9	1.9	1.5
Arg	2.0	3.6	2.6
Trp	ND†	ND	ND
CysA	4.6	0.1	6.4
Asx	14.7	9.4	10.1
Thr	5.1	7.3	5.6
Ser	7.4	8.6	6.1
Glx	15.1	6.9	14.1
Pro	5.9	6.2	8.2
Gly	13.1	10.1	13.8
Ala	7.2	9.3	8.1
1/2-Cys	0.4	1.1	ND
Val	5.2	7.0	4.6
Met‡	0.8	1.8	1.0
Ile	3.4	4.4	2.7
Leu	5.1	7.7	4.4
Tyr	1.6	3.0	1.4
Phe	2.9	3.9	2.3
Hyp	Tr§	0.7	0.7
GlcNAc	2.6	1.7	1.1
GalNAc	—	—	Tr
Unknown	—	—	3.5

*From Bacic *et al.* [12].
†ND: not detected.
‡Met + Met (O).
§Tr: Trace.

amounts of the imino acid, hydroxyproline, and *N*-acetylglucosamine.

Gel diffusion

Both root slime preparations gave precipitin bands in double diffusion tests with the β-glucosyl Yariv reagent (Biosupplies Australia Pty Ltd, Melbourne, Australia) and the IgA mouse myeloma, J539 (a kind gift from Dr M. Potter, National Institute of Health, Bethesda, U.S.A.).

DISCUSSION

Plant roots secrete and release a range of products which have diverse functions in plant growth and rhizosphere composition [for reviews see refs 9, 10]. Secretion from plant roots occurs primarily from the cap cells at the root tip, although epidermal cells in the zone of elongation also contribute [9]. Assessment of the contribution plants make to the high M_r mucilages in the rhizosphere has been hampered by the lack of analytical information on root slimes collected under axenic conditions. To date, such analyses on the water-soluble, high M_r components of root slimes have been available only for the cereals, maize [12–14] and rice [11]. These analyses have raised several questions regarding their composition, for example, is the high fucose content (20–30%) of maize slime unique and is fucose a characteristic of cereal root slimes? [11]

The present data suggests that the high fucose content of maize root slime is indeed unique, although fucose is a common minor constituent, of all root slimes analysed (see Table 1). Fucose is present only as terminal residues, in all the slime preparations examined, except for maize slime in which it is also 2- and 3-linked (see Table 2). There is also indirect evidence for the presence of terminal fucosyl residues on the surface of roots of the dicot *Lepidium sativum* [8]. In three separate host–pathogen systems, adhesion of fungal propagules to the root surface involves fucosyl residues on the root surface [6,8, Ralton and Clarke, unpublished observations]. These studies indicate that fucosyl residues of root slime are contact recognition determinants in these systems. The graminaceous root slimes also contain *ca* equal proportions of arabinose and xylose, whereas, cowpea slime contains considerably lower quantities of xylose (see Table 1) probably reflecting a low amount of secreted heteroxylans. A low heteroxylan content is a feature of dicotyledon cell walls and secretions [15, 16].

From methylation and other analyses, it was deduced that the polymeric components of maize slime included arabinogalactan-proteins (AGPs), xyloglucans, heteroxylans and glucans [12]. Wheat root slime analyses are consistent with a similar range of heteropolymers, in different proportions. For example, there is a higher content of heteroxylans, based on the relative proportions of 4-linked xylopyranosyl residues, and also a lower concentration of the fucose-containing polymer(s). However, wheat root slime probably contains, in addition, small amounts of a neutral pectic arabinan. This is deduced from the presence of 2,3,5-, 5- and 3-linked arabinofuranosyl residues [15]. Cowpea root slime differs in composition from the slime of graminaceous roots in containing higher contents of neutral (as arabinan) and acidic (as rhamnogalacturonan) pectic polysaccharides as well as different types of glucans. The presence of a rhamnogalacturonan is deduced from 2-linked rhamnopyranosyl and 4-linked galacturonosyl residues. Glucuronic acid is the major acidic monosaccharide of maize root slime and accounts for *ca* 24% of the wheat root slime. This may be associated with heteroxylans (glucuronoarabinoxylans) and/or AGPs [16, 17]. Wheat slime does contain *ca* 76% of its acidic monosaccharides as galacturonic acid. It may either be associated with AGPs [17] or very low amounts of acidic pectic polysaccharides [15]. Thus, the root slimes contain polysaccharides analogous to those identified in cell wall preparations. Cell walls of dicotyledons are characteristically rich in pectic polysaccharides, whereas, graminaceous monocotyledon cell walls contain low amounts of pectic polysaccharides but characteristically contain glucuronoarabinoxylans [16].

The absence of 3-linked glucopyranosyl residues in cowpea root slimes indicate that both 3-linked glucans and 3,4-linked glucans are absent. In contrast, wheat and maize root slimes contain both 3- and 4-linked glucopyranosyl residues indicating the presence of 3-linked glucan, 4-linked glucan and/or 3,4-linked glucan. We can make no prediction about the presence of these polysaccharides from the methylation data. The 3,4-linked glucans are characteristic components of graminaceous cell walls [16]. Both the dicotyledon and graminaceous root slimes probably contain xyloglucan as deduced from the presence of 4,6-linked glucopyranosyl residues [15].

AGPs are common constituents of plant secretions

[17] and are present in root slimes. This is demonstrated by the analyses of both wheat and cowpea root slimes which contain 3-, 6- and 3,6-linked galactopyranosyl residues, interact with the β-glucosyl Yariv reagent and the IgA myeloma J539, and contain hydroxyproline. The β-glucosyl Yariv reagent specifically binds to and precipitates many AGPs [17], and the J539 myeloma is specific for β-(1→6)-linked galacto-oligosaccharides [18]. The 3-, 6-, and 3,6-linked galactopyranosyl residues are restricted to the Type II AGs and/or AGPs in plant polysaccharides [15, 19]. The function of AGPs remains unknown, however, their high water binding capacity and their ability to form gels may reflect a physiological role as anti-desiccants and gelling agents [17]. The ability of root slimes to act as lubricants and anti-desiccants is likely to be related to their ability to form gels. One mechanism for gel formation was proposed by Wright and Northcote [20]. They envisaged a central cellulosic polymer encased in a hydrophilic uronic-acid-containing pectic-like material. It is also possible that other polymers within the root slimes can interact non-covalently, and perhaps covalently, to form a continuous network that immobilises water and gels. The organisation of these polymers within such a network would also determine the capacity for specific contact recognition between saccharides on the root surface and receptors of micro-organisms.

EXPERIMENTAL

Plant material. Caryopses of *T. aestivum* L (cv Condor) were kindly supplied by Dr W. Williams (Horsham Research Institute, Department of Agriculture, Victoria, Australia). Seeds of *V. unguiculata* (L) Walp. (cv Caloona) were purchased from Wright and Stephenson, Ermington, N.S.W., Australia.

Sterilization and germination. Wheat caryopses and cowpea seeds were germinated and grown under axenic conditions. Microbial contamination was excluded by autoclaving (120°, 106 kPa, 30 min) all solns and equipment, and carrying out all transfers in a laminar flow cabinet.

Wheat caryopses were surface sterilized by sequential soaking in 70% EtOH (1–2 min), 0.75% $AgNO_3$ (30 min; freshly prepd) and 9% NaOCl. Each treatment was followed by several washes in sterile dist H_2O. Cowpea seeds were surface sterilized as previously described for maize caryopses [12] except that the concn of $HgCl_2$ was lowered to 0.01% (w/v). Wheat caryopses and cowpea seeds were then imbibed in dist H_2O containing antibiotics [12] and following their removal germinated on moist glass fibre filter discs in the dark for 96 and 48 hr respectively, i.e. until roots were 1–2 cm long.

Collection of root slime. Secreted slime was collected from roots suspended in sterile dist H_2O for 24 hr in the dark [12]. Slime collected in this manner was processed as described in ref. [12] except that, for cowpea, insol. PVP (0.1%) was included during the concn steps to prevent browning. The resulting high M_r slime prepn was stored at −20° prior to analysis.

Analytical methods. All carbohydrate structural analyses were performed on high M_r root slime prepns that had been pretreated with porcine pancreatic α-amylase (Sigma). Conditions were as described in ref. [12].

Neutral monosaccharides were identified and quantified as their alditol acetates by GC and GC/MS following hydrolysis with 2.5 M TFA [12]. Acidic monosaccharides were determined by GC and GC/MS as their per-*O*-TMSi Me glycosides following methanolysis (1 M methanolic–HCl, 16 hr, 80°; [21]). Conditions were as described in ref. [22].

Glycosyl linkage composition was established by methylation using the procedure of ref. [23]. To determine the linkage position of uronosyl residues, polysaccharides were treated with methanolic HCl (0.08 M, 24 hr, room temp [24]) and the resulting Me esterified uronosyl residues reduced with $NaBD_4$ (0.25 M, 24 hr, 4°). The carboxyl-reduced polysaccharides were then methylated as described above. The per-*O*-methylated alditol acetates were identified and quantified by GC/MS as described in ref. [12]. Chromatography was routinely performed on two separate columns with stationary phases of different polarities to aid in identification and quantification. The conditions of chromatography on BP75 (S.G.E.) were as described in ref. [25] and on CP Sil5 (Chrompack) the conditions and elution characteristics were the same as those described for SP2100 [26].

Amino acid analyses on root slime prepns, not treated with α-amylase, (1–2 mg; 6 M HCl, 110°, 24 hr) were performed on an amino acid analyser by Mr A. Inglis (Division of Protein Chemistry, CSIRO, Parkville, Australia).

General methods. Microbial contamination of roots and collected slime was evaluated by plating out the slime, or scraping the root surface, onto 1% glucose–peptone agar plates which were incubated for 24–72 hr at 37°. None was detected. Total protein was determined as BSA by the Bio-Rad Bradford microassay procedure [27]. Total carbohydrate was determined colorimetrically by the phenol–H_2SO_4 method [28], using glucose as std. Uronic acids were estimated colorimetrically using galacturonic acid as std [29]. Gel diffusion was performed in 1% agarose containing 1% NaCl and 0.02% NaN_3 [30].

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