

## Supplementary Table:

### Cell wall characteristics during sexual reproduction of *Mougeotia* sp. (Zygnematophyceae) revealed by electron microscopy, glycan microarrays and RAMAN spectroscopy

Protoplasma

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**Suppl. Table S1** Sampling dates and coordinates of field samples with zygospores or conjugating stages of *Mougeotia*

Sample Number	Sampling site	Sampling Date	Coordinates	Species
1	1	20.07.2019	N47°19'56" O11°09'84"	<i>Mougeotia parvula</i>
2	2	31.08.2019	N47°21'76" O11°03'77"	<i>Mougeotia disjuncta</i>
3	2	23.05.2020	N47°21'76" O11°03'77"	<i>Mougeotia disjuncta</i>
4	2	12.07.2020	N47°21'76" O11°03'77"	<i>Mougeotia disjuncta</i>
5	2	06.09.2020	N47°21'76" O11°03'77"	<i>Mougeotia disjuncta</i>

**Suppl. Table S2** Cell wall probes used in the present study

No.	Polymer class	Code	Epitope	Source	Reference
1	Pectin	2F4	Ca <sup>2+</sup> cross linked HG	PlantProbes	Liners et al. 1989
2		JIM5	Homogalactauronan with a low DE	PlantProbes	Willats et al. 2000
3		JIM7	Homogalactauronan with a high DE	PlantProbes	Willats et al. 2000
4		LM19	Methylesterified homogalactauronan (low DE)	PlantProbes	Verhertbruggen et al. 2009
5		LM20	Methylesterified homogalactauronan (high DE)	PlantProbes	Verhertbruggen et al. 2009
6		INRA-RU2	Backbone of rhamnogalacturonan I (4 units)	INRA	Ralet et al. 2010
7		INRA-RU1	Backbone of rhamnogalacturonan I (12 units)	INRA	Ralet et al. 2010
8		LM5	(1→4)-β-D-galactan	PlantProbes	Jones et al. 1997
9		LM6	(1→5)-α-L-arabinan	PlantProbes	Willats et al. 1998
10		LM13	Linearised (1→5)-α-L-arabinan	PlantProbes	Moller et al. 2008
11		LM16	(1→5)-α-L-arabinan, RG backbone	PlantProbes	Verhertbruggen et al. 2009
12	Xyloglucan	LM8	Xylogalacturonan	PlantProbes	Willats et al. 2004
13		LM15	Xyloglucan (XXXG motif)	PlantProbes	Marcus et al. 2008
14		LM24	Xyloglucan (XLLG motif, weak to XXLG)	PlantProbes	Pedersen et al. 2012
15	LM25	Xyloglucan (XXXG, XXLG, XLLG motif; also GGGGGG)	PlantProbes	Pedersen et al. 2012	
16	Xylan	LM10	(1→4)-β-D-xylan (low-substituted)	PlantProbes	McCartney et al. 2005
17		LM11	(1→4)-β-D-xylan (low-substituted)/arabinoxylan	PlantProbes	McCartney et al. 2005
18		LM23	(1→4)-β-D-xylan/xylogalacturonan	PlantProbes	Pedersen et al. 2012
19	β-glucans	BS-400-2	(1→3)-β-D-glucan	Biosupplies	Meikle et al. 1991

20		BS-400-3	(1→3) (1→4)-β-D-glucan	Biosupplies	Meikle et al. 1994
21	Mannans	BS-400-4	(1→4)-β-D-mannan	Biosupplies	Pettolino et al. 2001
22		LM21	(1→4)-β-D-mannan/galactomannan/glucomannan	PlantProbes	Marcus et al. 2010
23		LM22	(1→4)-β-D-mannan/glucomannan	PlantProbes	Marcus et al. 2010
24		AGPs	JIM8	AGP (Gal-rich)	PlantProbes
25	JIM13		β-GlcA-(1→3)-α-GalA-(1→2)-α-Rha)	PlantProbes	Knox et al. 1991
26	LM2		AGP (1→6)-β-Gal with terminal β-GlcA)	PlantProbes	Smallwood et al. 1996
27	LM14		AGP, enriched Ara, Gal and aldouronic acid	PlantProbes	Moller et al. 2008
28	MAC207		AGP (β-GlcA-(1→3)-α-GalA-(1→2)-α-Rha)	PlantProbes	Pennell. et al. 1989
29	Extensins	LM1	Extensin (hydroxyproline-rich motif THRGP)	PlantProbes	Smallwood et al. 1995
30		LM3	Extensin	PlantProbes	Feng et al. 2014
31		JIM11	Extensin	PlantProbes	Smallwood et al. 1994
32		JIM12	Extensin	PlantProbes	Smallwood et al. 1994
33		JIM19	Extensin	PlantProbes	Smallwood et al. 1994
34		JIM20	Extensin	PlantProbes	Smallwood et al. 1994
35	Cellulose	CBM3a	Crystalline cellulose	PlantProbes	Blake et al. 2006
36		CBM2a	Crystalline cellulose	PlantProbes	McLean et al. 2000
37	Lectins	B-1005	Concanavalin A; α-linked mannose	Vector Laboratories	Yue et al. 2009
38		B-1015	Soybean agglutinin; α- or β-linked GalNAc	Vector Laboratories	Yue et al. 2009
39		B-1025	Wheat germ agglutinin; dimers and trimers of GlcNAc	Vector Laboratories	Yue et al. 2009
40		B-1085	Ricinus communis agglutinin; Gal or GalNAc residues	Vector Laboratories	Yue et al. 2009
41		B-1285	Bauhinia purpurea lectin; Gal-α-(1→3) GalNAc, terminal GalNAc	Vector Laboratories	Yue et al. 2009
42		B-1305	Sambucus nigra lectin; sialic acid attached to term. Gal, GalNAc	Vector Laboratories	Yue et al. 2009
43		B-1335	Euonymus europaeus lectin; Gal-α-(1→3)-Fuc-α-(1→2)-Gal	Vector Laboratories	Opitz et al. 2007
44		B-1365	Psophocarpus tetragonolobus lectin; GalNAc	Vector Laboratories	McCarter et al. 2013
45		B-1405	Psophocarpus tetragonolobus lectin II; Gal and GalNAc	Vector Laboratories	Barkhordari et al. 2004

## Supporting references

Liners F, Letesson JJ, Didembourg C, et al. (1989) Monoclonal antibodies against pectin: recognition of a conformation induced by calcium. *Plant Physiol* 91:1419-1424.

Barkhordari A, Stoddart RW, McClure SF, et al. (2004) Lectin histochemistry of normal human lung. *J Mol Histol* 35:147-156.

Blake AW, McCartney L, Flint JE, et al. (2006) Understanding the biological rationale for the diversity of cellulose-directed carbohydrate-binding modules in prokaryotic enzymes. *J Biol Chem* 281:29321-29329.

Feng T, Nyffenegger C, Højrup P, et al. (2014) Characterization of an extensin-modifying metalloprotease: N-terminal processing and substrate cleavage pattern of *Pectobacterium carotovorum* Prt1. *Appl Microbiol Biotechnol* 98:10077-10089.

Jones L, Seymour GB, Knox JP (1997) Localization of pectic galactan in tomato cell walls using a monoclonal antibody specific to (1 → 4)-[β]-D-galactan. *Plant Physiol* 113:1405-1412.

Knox JP, Linstead PJ, Cooper JPC, et al. (1991) Developmentally regulated epitopes of cell surface arabinogalactan proteins and their relation to root tissue pattern formation. *Plant J* 1:317-326.

Marcus SE, Blake AW, Benians TA, et al. (2010) Restricted access of proteins to mannan polysaccharides in intact plant cell walls. *Plant J* 64:191-203.

Marcus SE, Verhertbruggen Y, Hervé C, et al. (2008) Pectic homogalacturonan masks abundant sets of xyloglucan epitopes in plant cell walls. *BMC Plant Biol* 8:1-12.

McCabe PF, Valentine TA, Forsberg LS, et al. (1997) Soluble signals from cells identified at the cell wall establish a developmental pathway in carrot. *Plant Cell* 9:2225-2241.

McCarter C, Kletter D, Tang H, et al. (2013) Prediction of glycan motifs using quantitative analysis of multi-lectin binding: Motifs on MUC 1 produced by cultured pancreatic cancer cells. *Proteomics Clin Appl* 7:632-641.

McCartney L, Marcus SE, Knox JP (2005) Monoclonal antibodies to plant cell wall xylans and arabinoxylans. *J Histochem Cytochem* 53:543-546.

- McLean BW, Bray MR, Boraston AB, et al. (2000) Analysis of binding of the family 2a carbohydrate-binding module from *Cellulomonas fimi* xylanase 10A to cellulose: specificity and identification of functionally important amino acid residues. *Protein Eng* 13:801-809.
- Meikle PJ, Bonig I, Hoogenraad NJ, Clarke AE et al. (1991) The location of (1→ 3)- $\beta$ -glucans in the walls of pollen tubes of *Nicotiana glauca* using a (1→ 3)- $\beta$ -glucan-specific monoclonal antibody. *Planta* 185:1-8.
- Meikle PJ, Hoogenraad NJ, Bonig I et al. (1994) A (1→ 3, 1→ 4)- $\beta$ -glucan-specific monoclonal antibody and its use in the quantitation and immunocytochemical location of (1→ 3, 1→ 4)- $\beta$ -glucans. *Plant J* 5:1-9.
- Moller I, Marcus SE, Haeger A, et al. (2008) High-throughput screening of monoclonal antibodies against plant cell wall glycans by hierarchical clustering of their carbohydrate microarray binding profiles. *Glycoconj J* 25:37-48.
- Opitz L, Salaklang J, Büttner H, et al. (2007) Lectin-affinity chromatography for downstream processing of MDCK cell culture derived human influenza A viruses. *Vaccine* 25:939-947.
- Pedersen HL, Fangel JU, McCleary B, et al. (2012) Versatile high resolution oligosaccharide microarrays for plant glycobiology and cell wall research. *J Biol Chem* 287:39429-38.
- Pennell RI, Knox JP, Scofield GN et al. (1989) A family of abundant plasma membrane-associated glycoproteins related to the arabinogalactan proteins is unique to flowering plants. *J Cell Biol* 108:1967-1977.
- Pettolino FA, Hoogenraad NJ, Ferguson C, et al. (2001) A (1→ 4)- $\beta$ -mannan-specific monoclonal antibody and its use in the immunocytochemical location of galactomannans. *Planta*, 214:235-242.
- Ralet MC, Tranquet O, Poulain D, et al. (2010) Monoclonal antibodies to rhamnogalacturonan I backbone. *Planta* 231:1373-1383.
- Smallwood M, Beven A, Donovan N, et al. (1994) Localization of cell wall proteins in relation to the developmental anatomy of the carrot root apex. *Plant J* 5:237-246.
- Smallwood M, Martin H, Knox JP (1995) An epitope of rice threonine- and hydroxyproline-rich glycoprotein is common to cell wall and hydrophobic plasma-membrane glycoproteins. *Planta* 196:510-522.
- Smallwood M, Yates EA, Willats WG, et al. (1996) Immunochemical comparison of membrane-associated and secreted arabinogalactan-proteins in rice and carrot. *Planta* 198:452-459.
- Verhertbruggen Y, Marcus SE, Haeger A, et al. (2009) An extended set of monoclonal antibodies to pectic homogalacturonan. *Carbohydr Res* 344:1858-1862.
- Verhertbruggen Y, Marcus SE, Haeger A, et al. (2009) Developmental complexity of arabinan polysaccharides and their processing in plant cell walls. *Plant J* 59:413-425.
- Willats WGT, Limberg G, Buchholt HC, et al. (2000) Analysis of pectic epitopes recognised by hybridoma and phage display monoclonal antibodies using defined oligosaccharides, polysaccharides, and enzymatic degradation. *Carbohydr Res* 327:309-320.
- Willats WGT, Marcus SE, Knox JP (1998) Generation of a monoclonal antibody specific to (1→ 5)- $\alpha$ -L-arabinan. *Carbohydr Res* 308:149-152.
- Willats WGT, McCartney L, Steele-King CG, et al. (2004) A xylogalacturonan epitope is specifically associated with plant cell detachment. *Planta* 218:673-681.
- Yue T, Goldstein IJ, Hollingsworth MA, et al. (2009) The prevalence and nature of glycan alterations on specific proteins in pancreatic cancer patients revealed using antibody-lectin sandwich arrays. *Mol Cell Proteomics* 8:1697-1707.