

LZT0570 – Qualidade e Conservação de Volumosos para Ruminantes

*Luiz Gustavo Nussio, Professor
Departamento de Zootecnia*





ESALQ

USP

Plant cell wall biosynthesis

nussio@usp.br

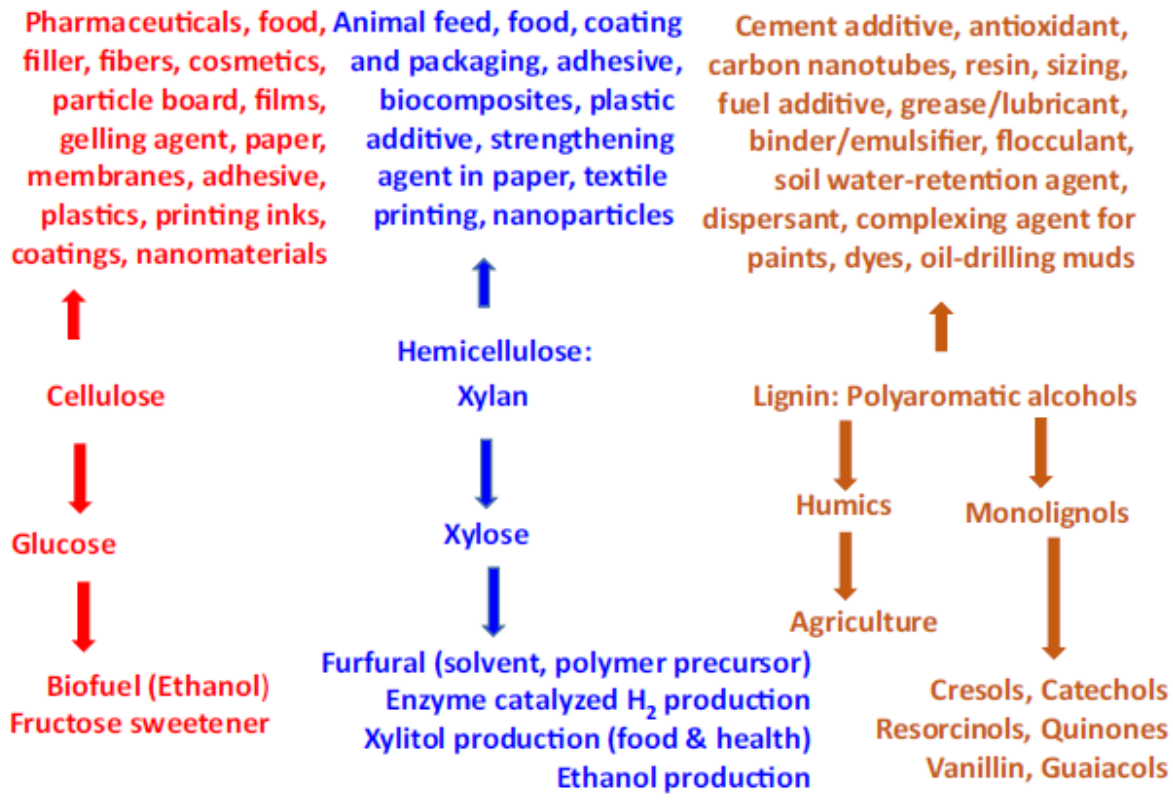
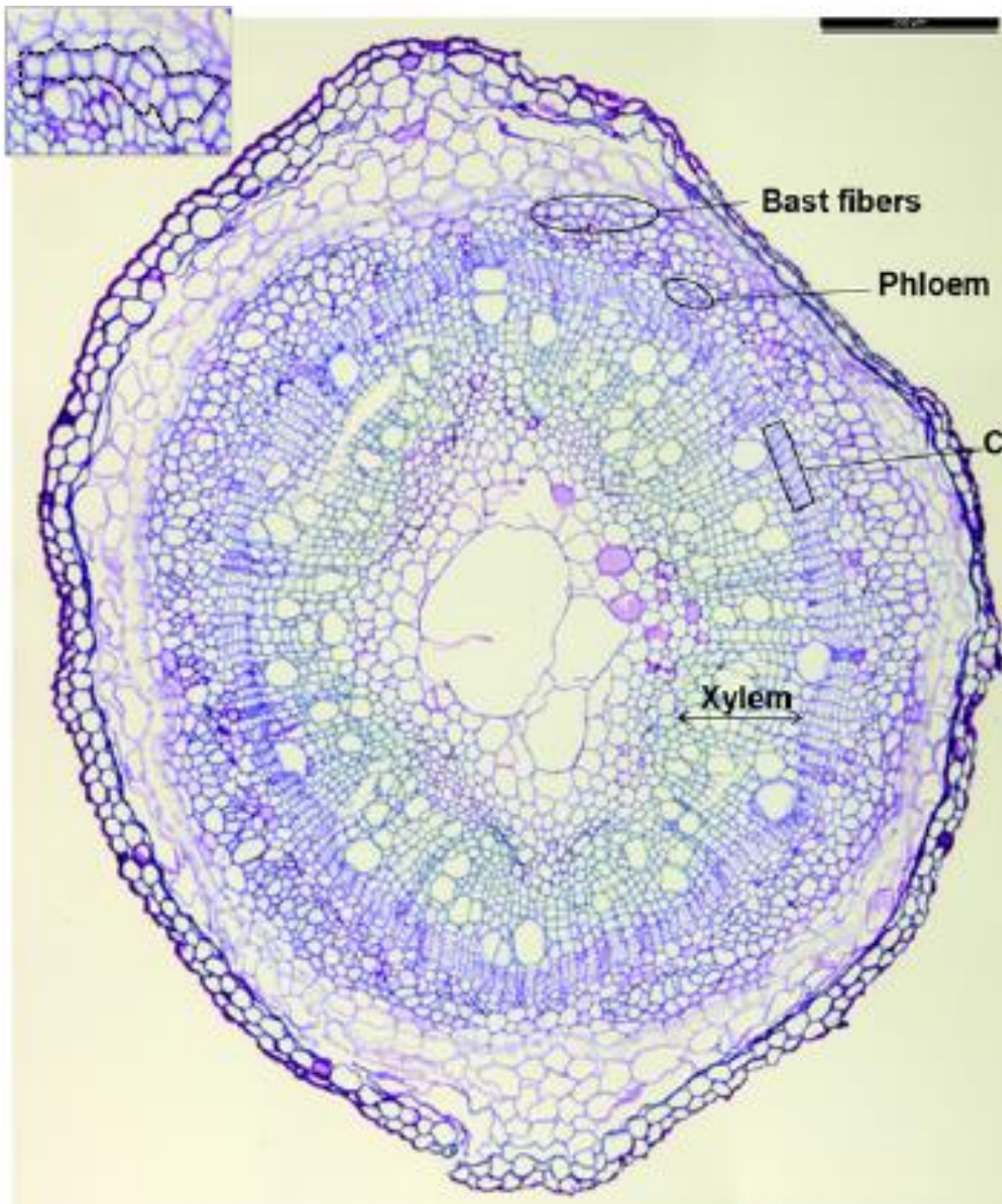
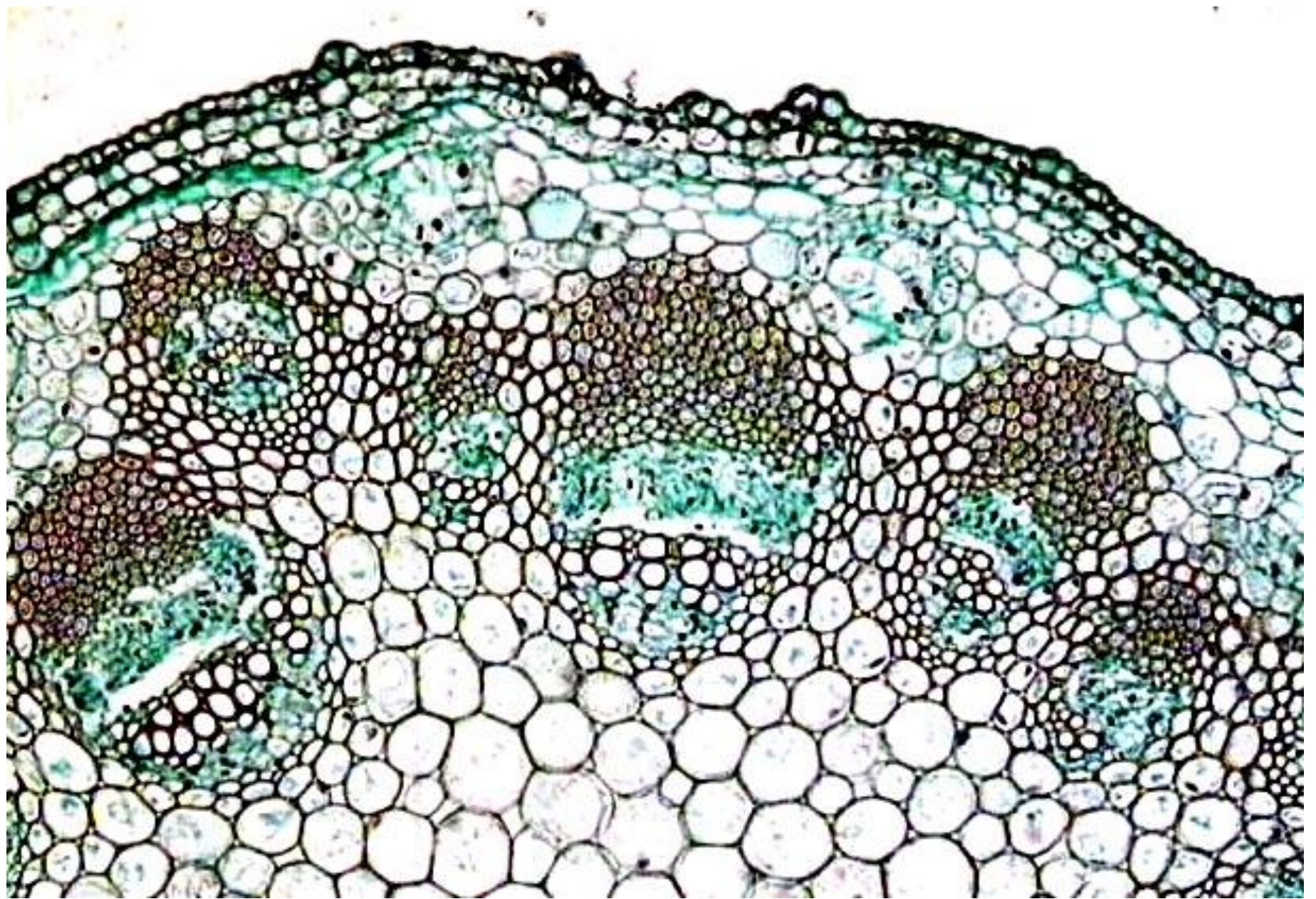
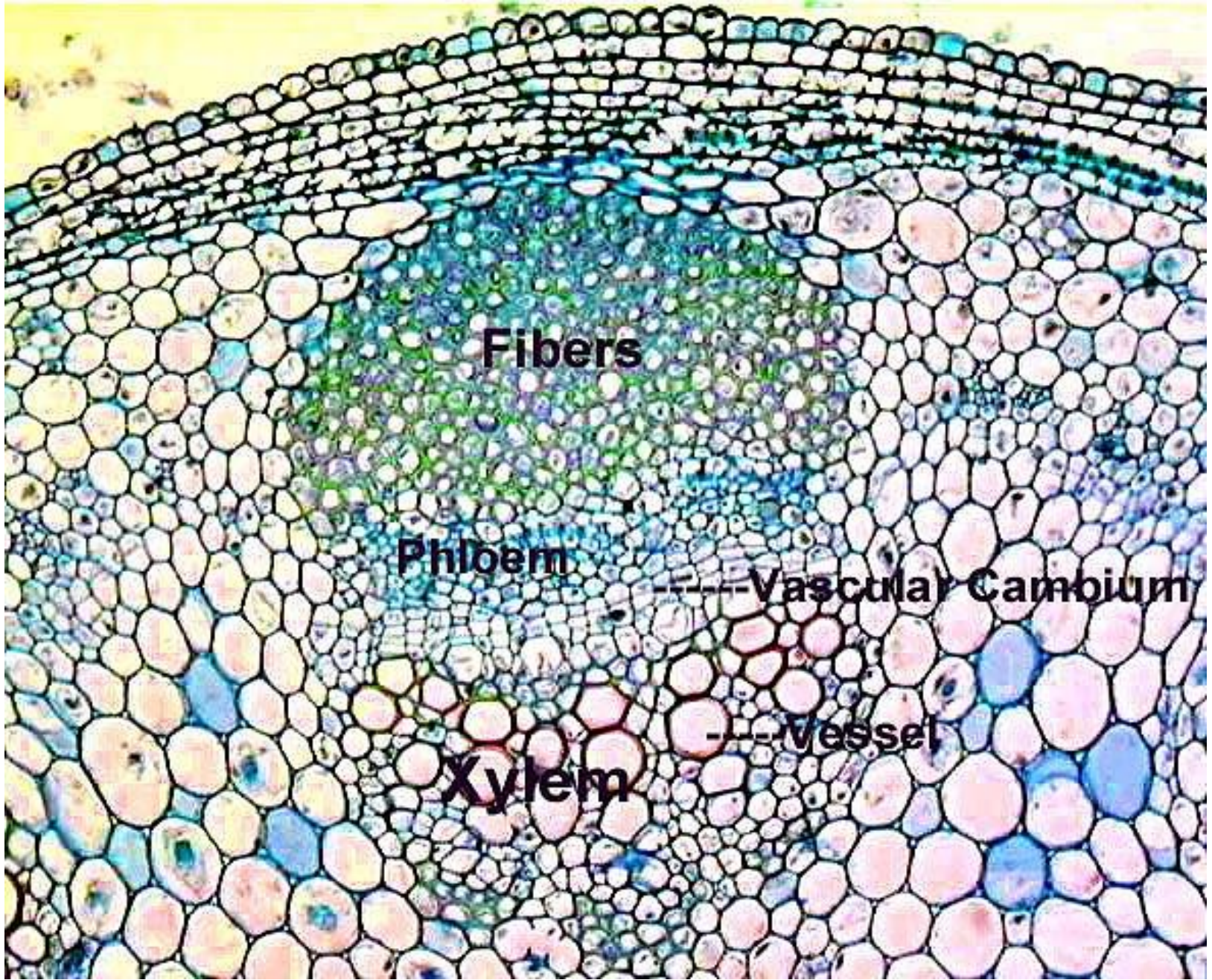


Figure 1. Applications of plant cell wall components and their degradation products. Red, cellulose; blue, xylan; and brown, lignin.







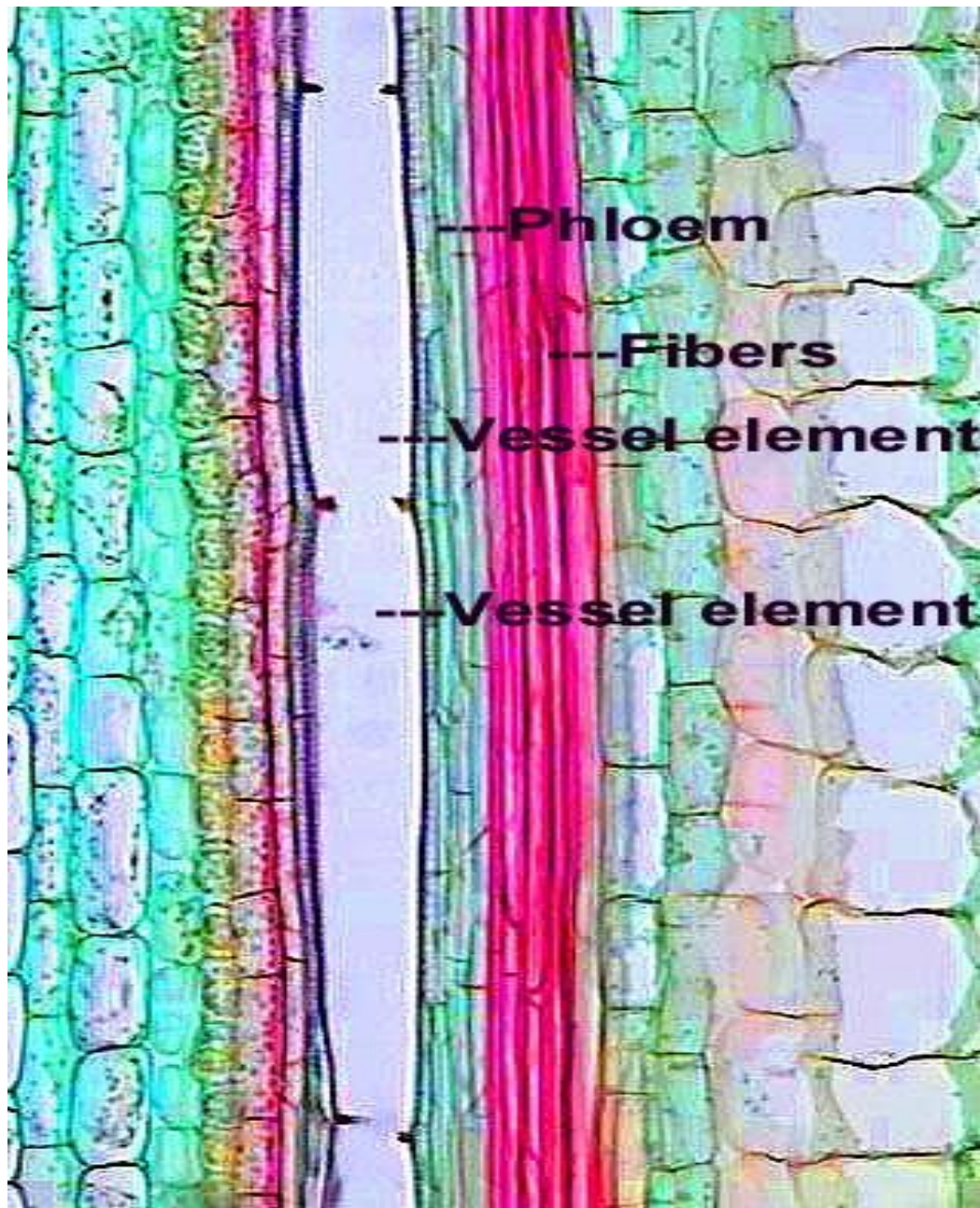
Fibers

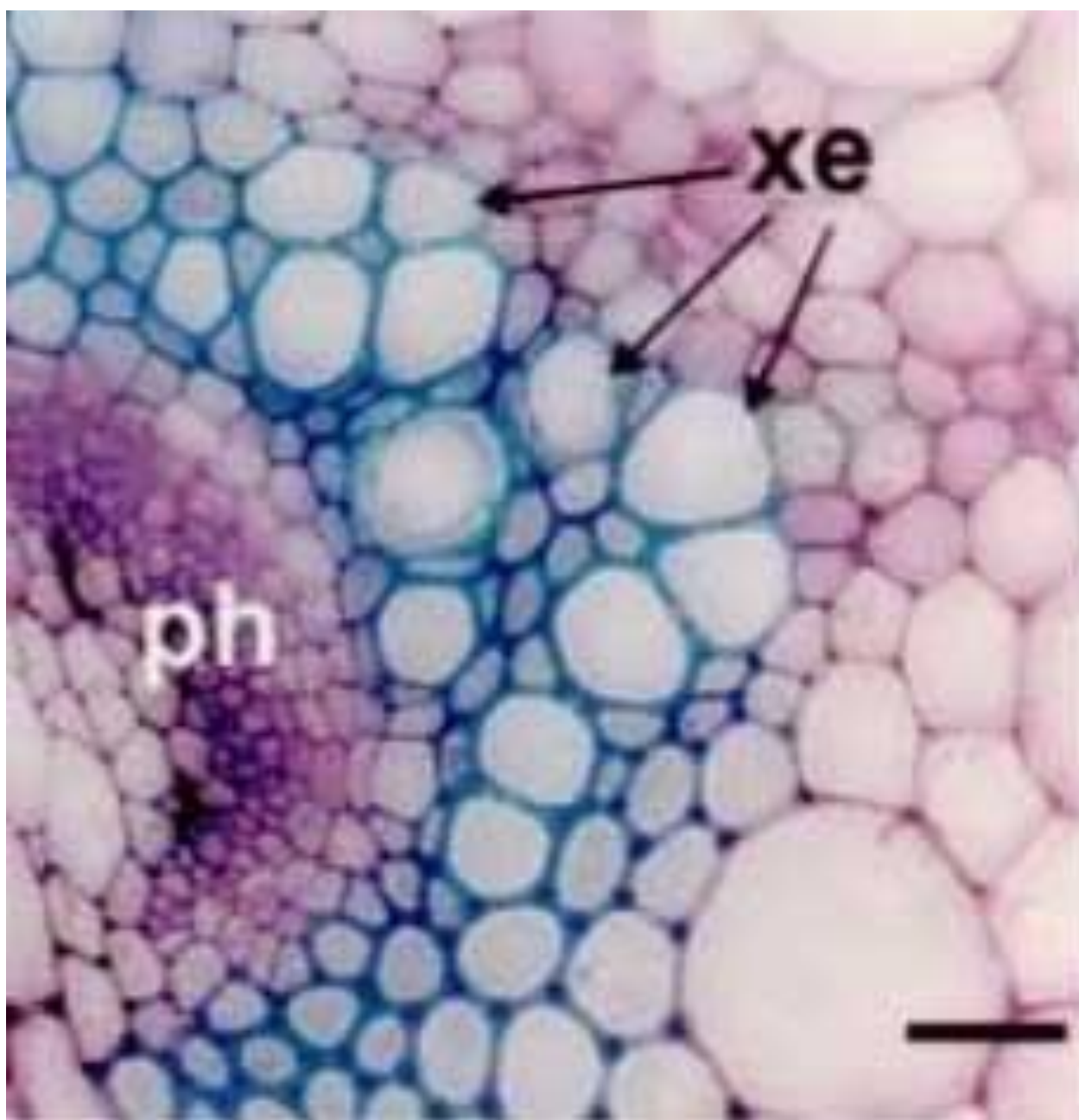
Phloem

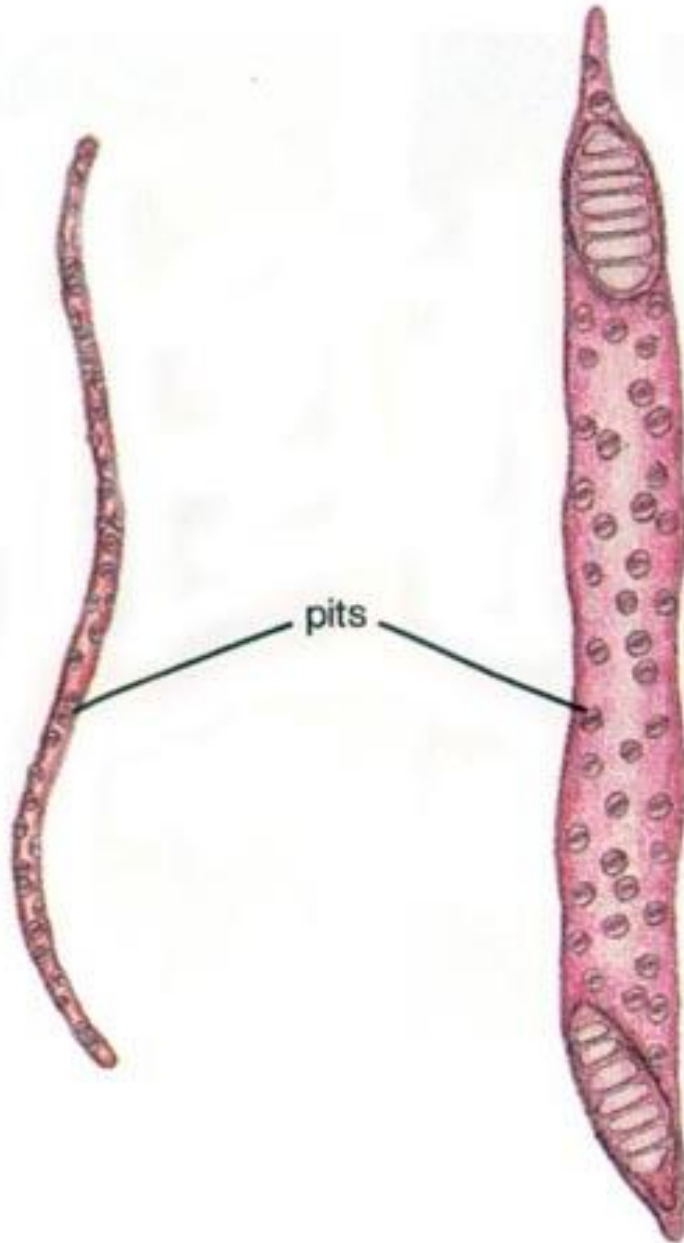
Vascular Cambium

Xylem

Vessel







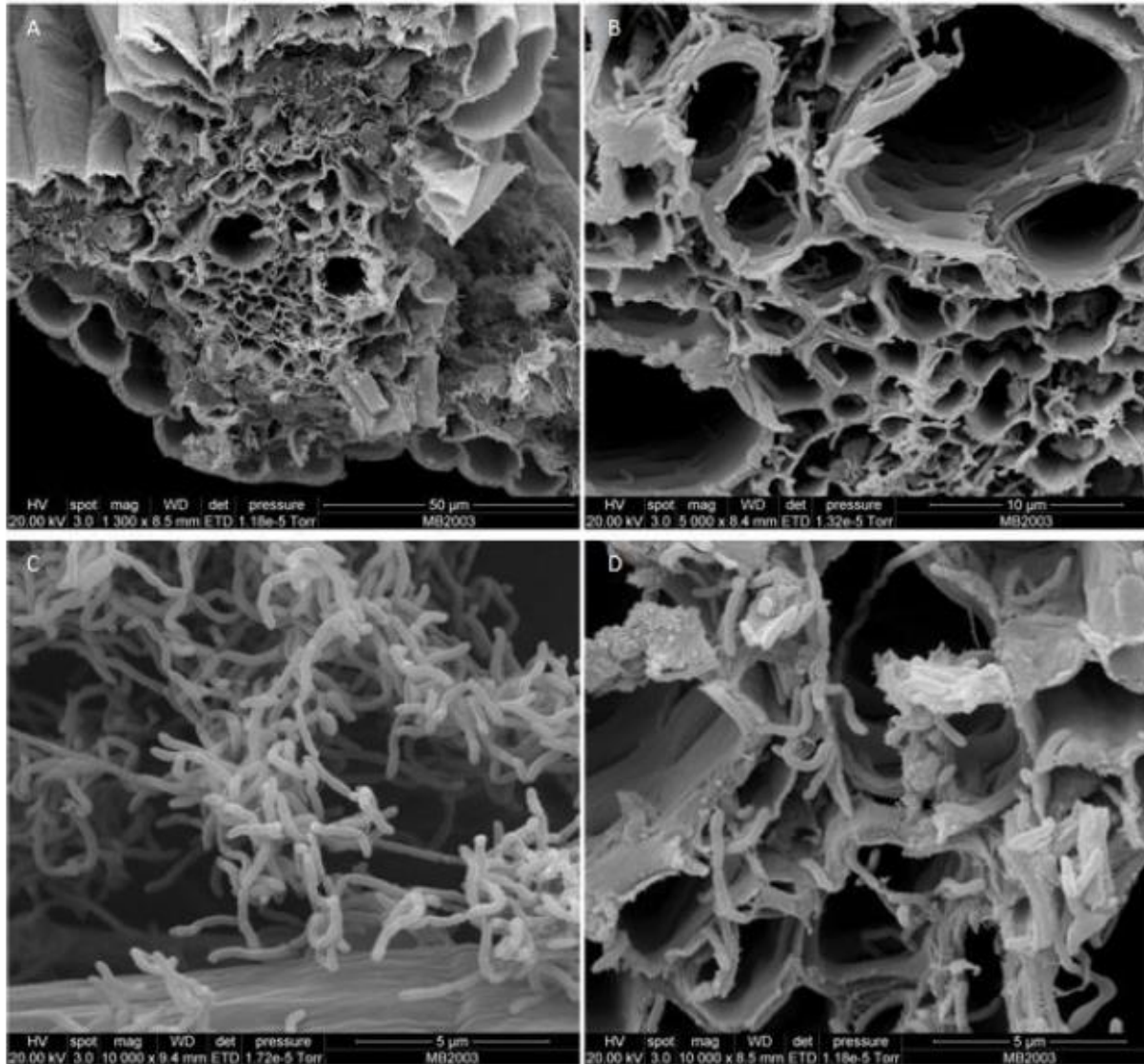
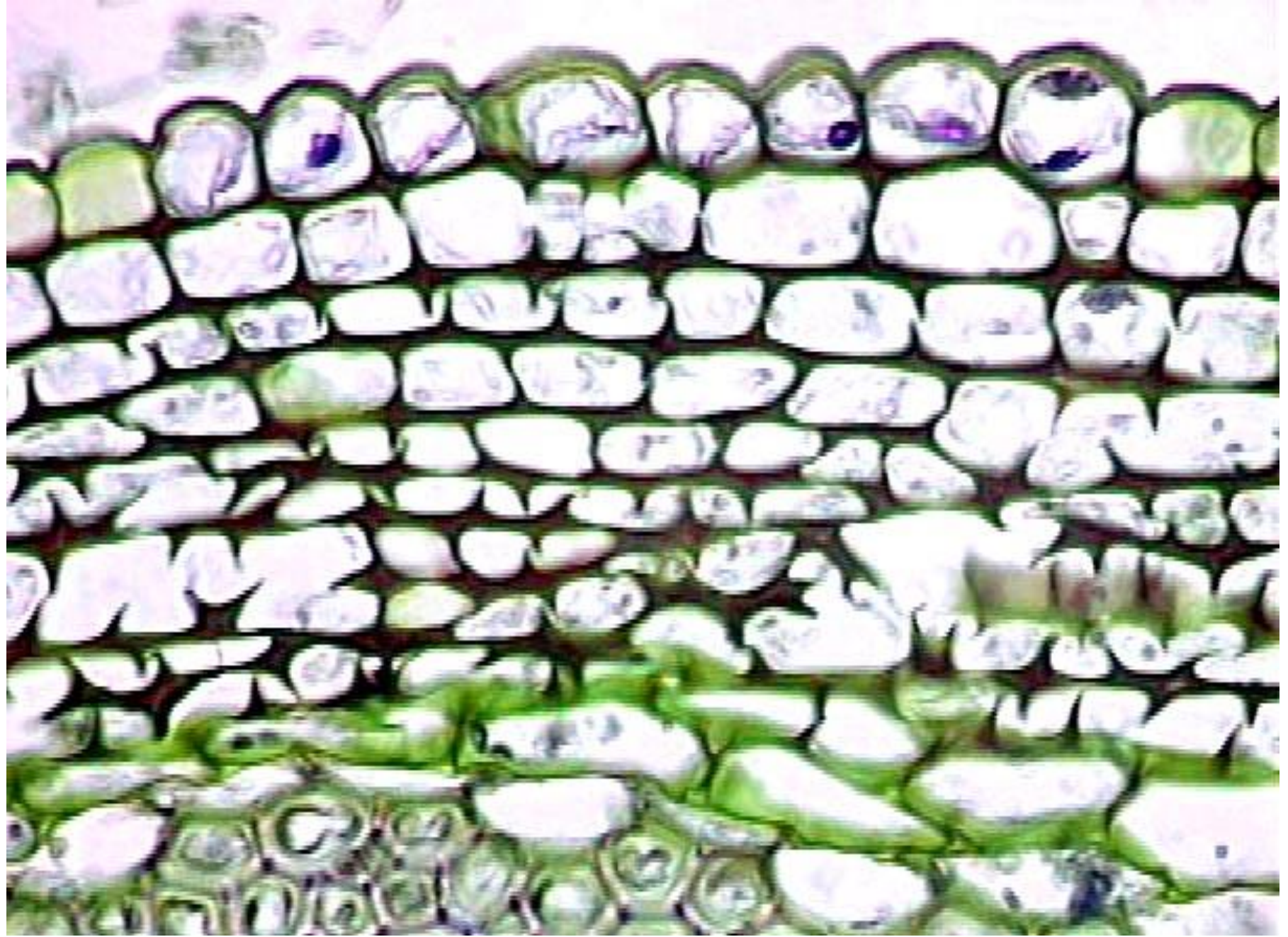
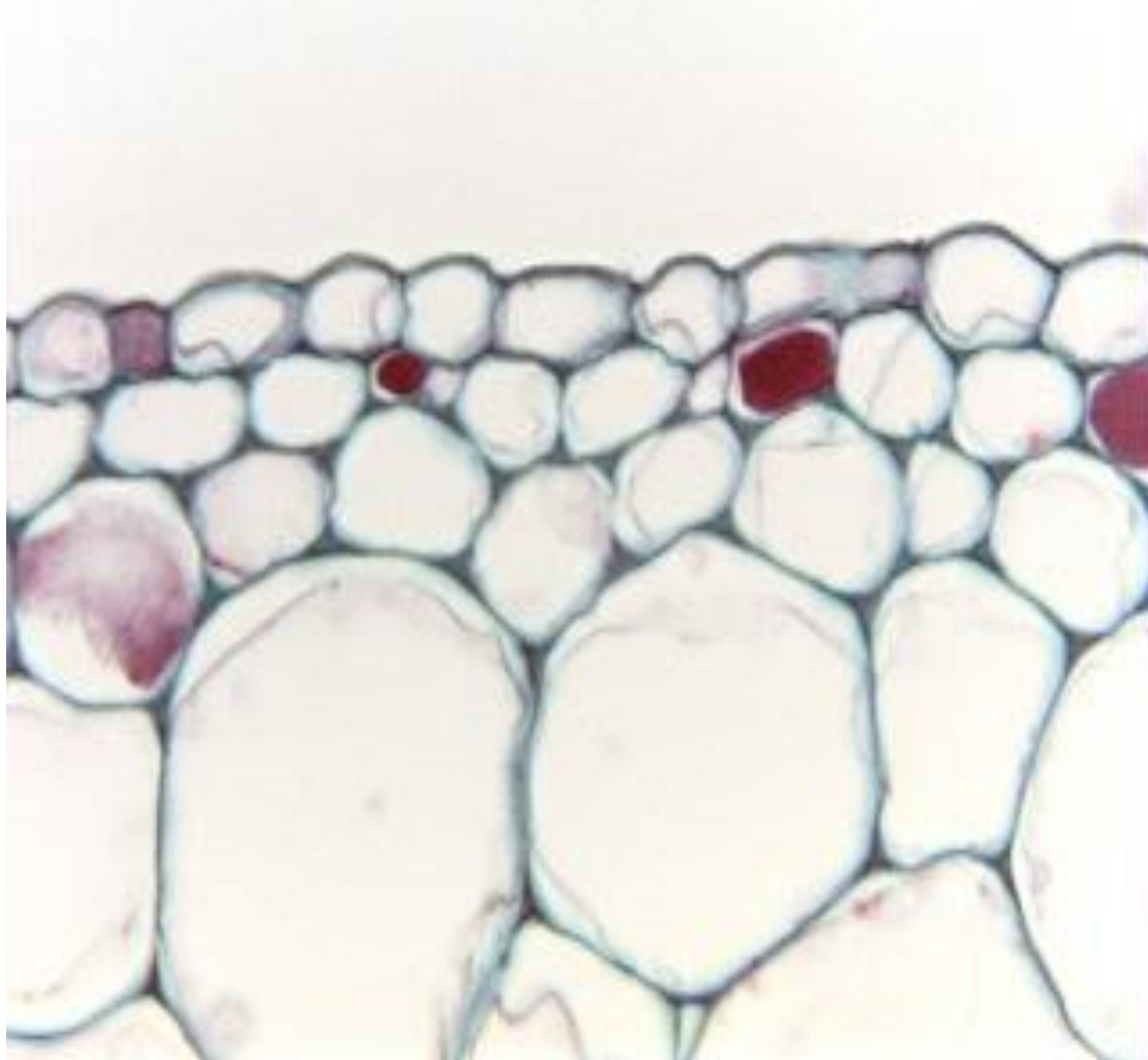
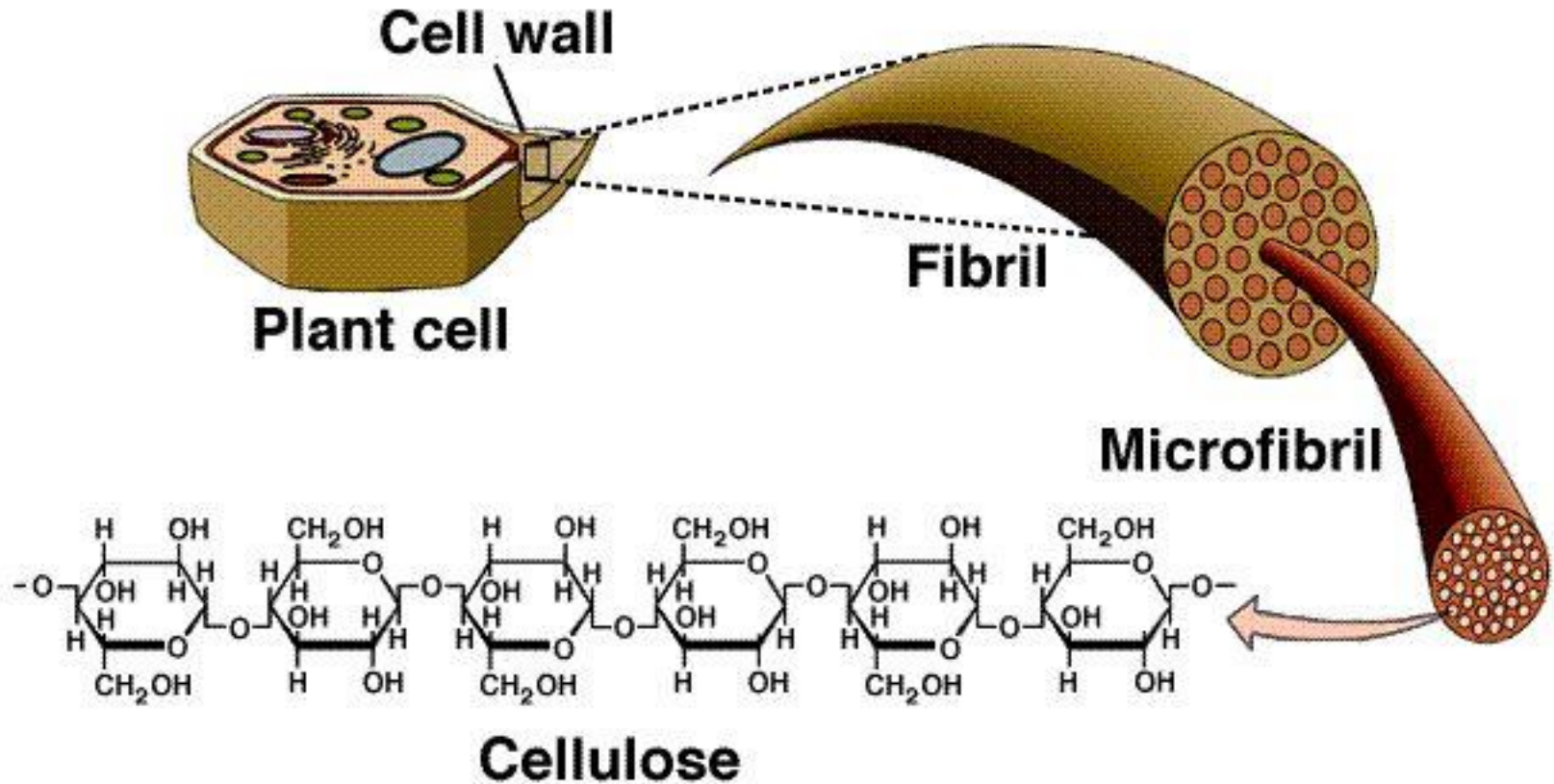


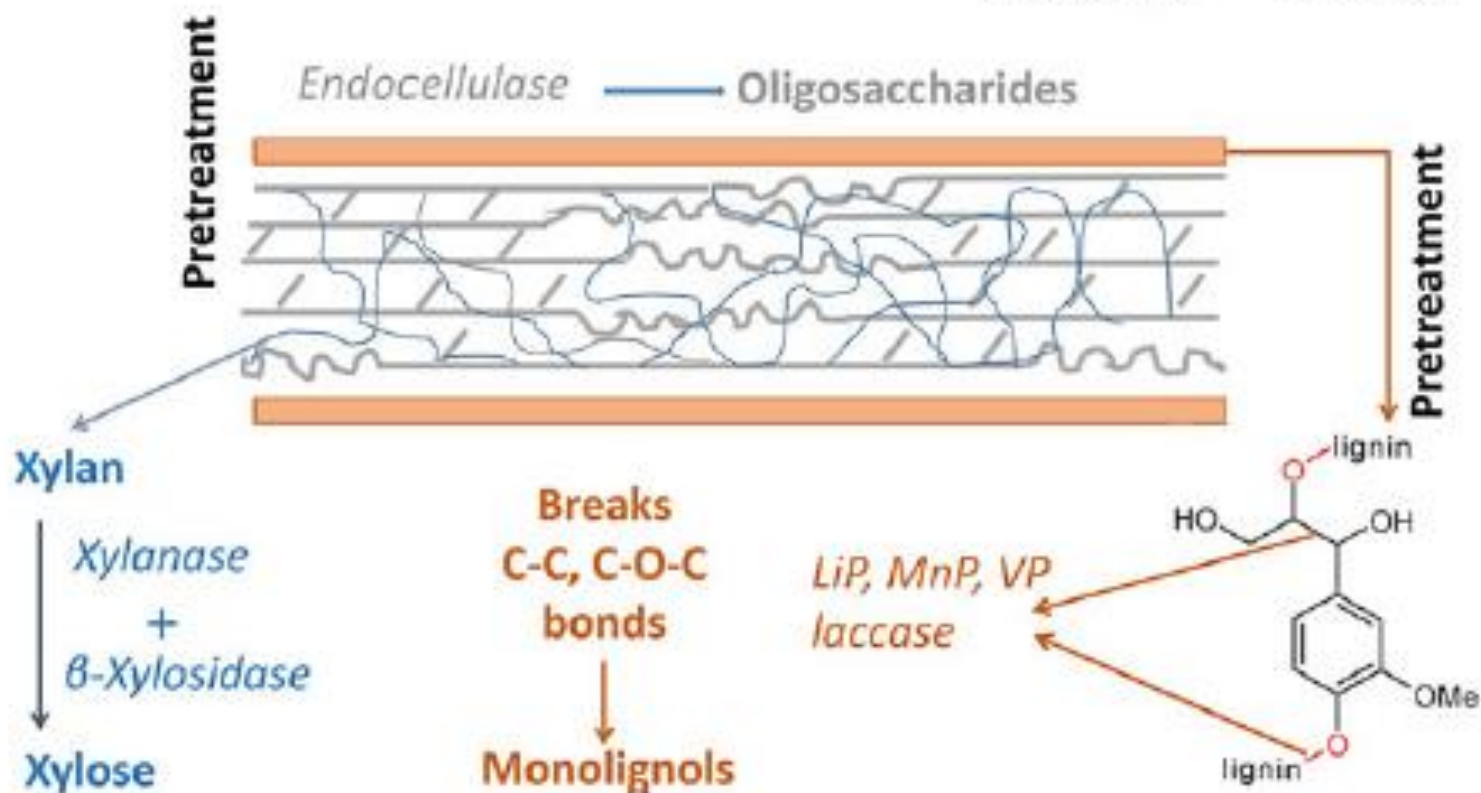
Figure 3.8. Scanning Electron Microscopy images of MB2003 cultures grown on BY media on NDF plant material. A, 1,300 \times magnification, B, 5,000 \times magnification, C and D, 10,000 \times magnification.

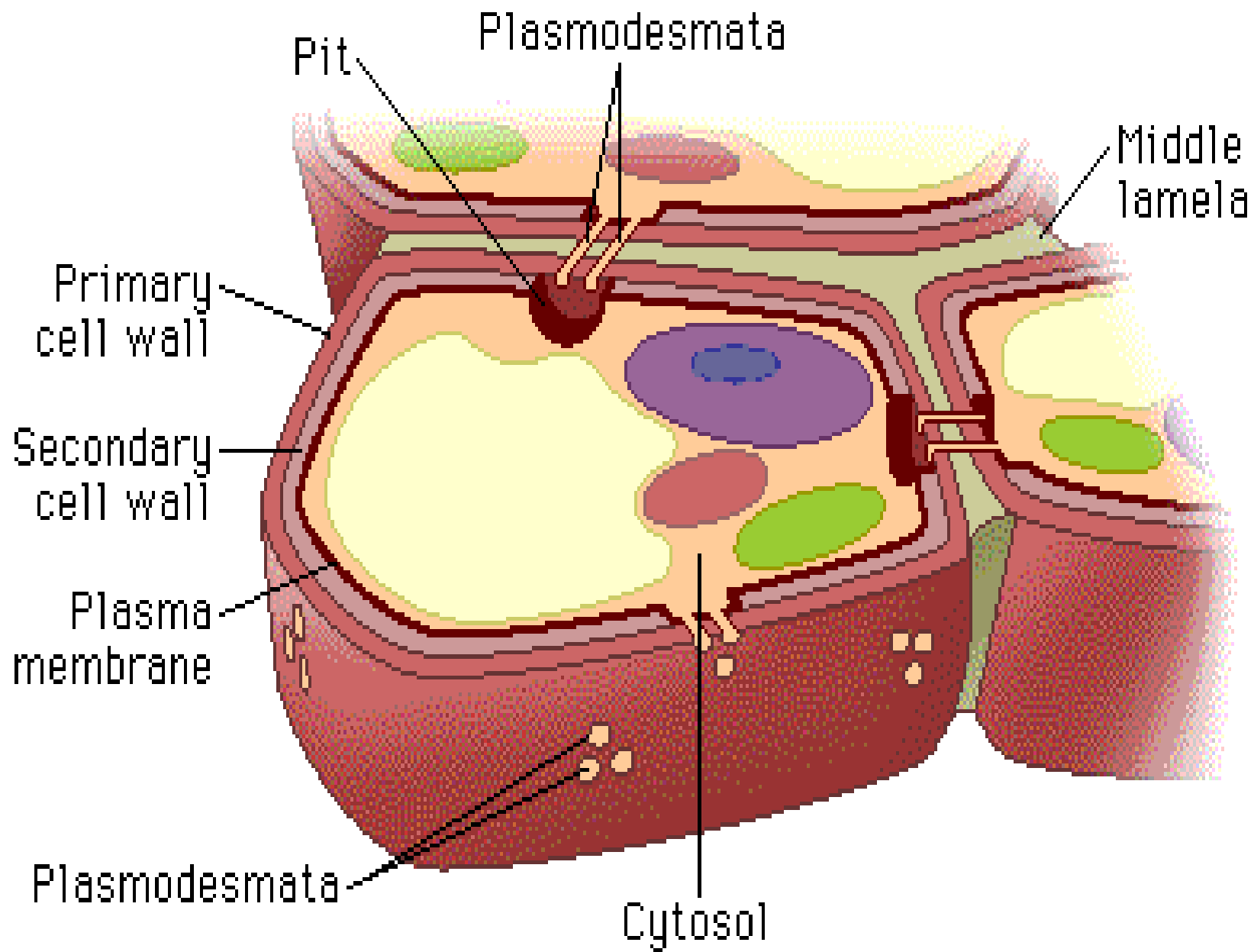


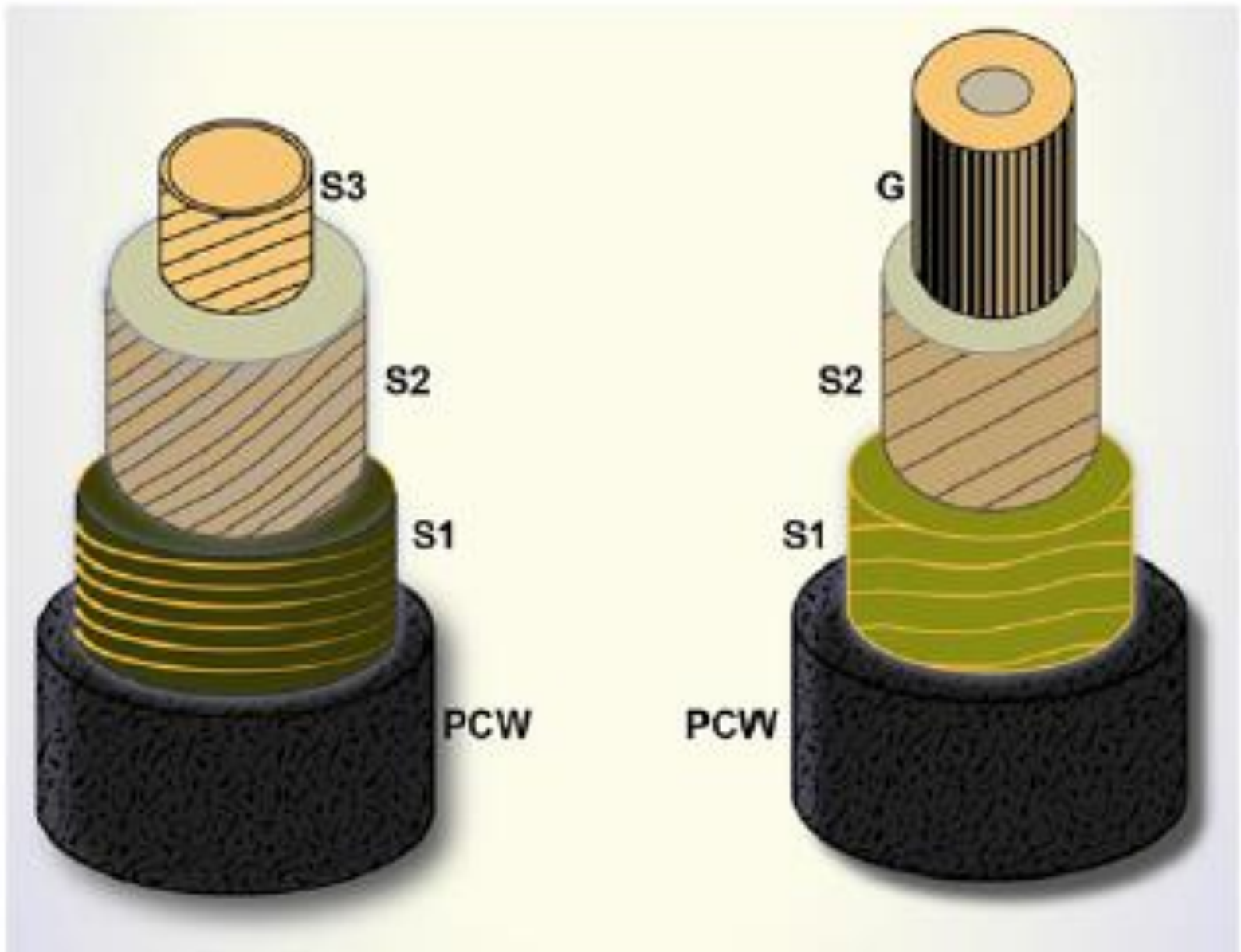


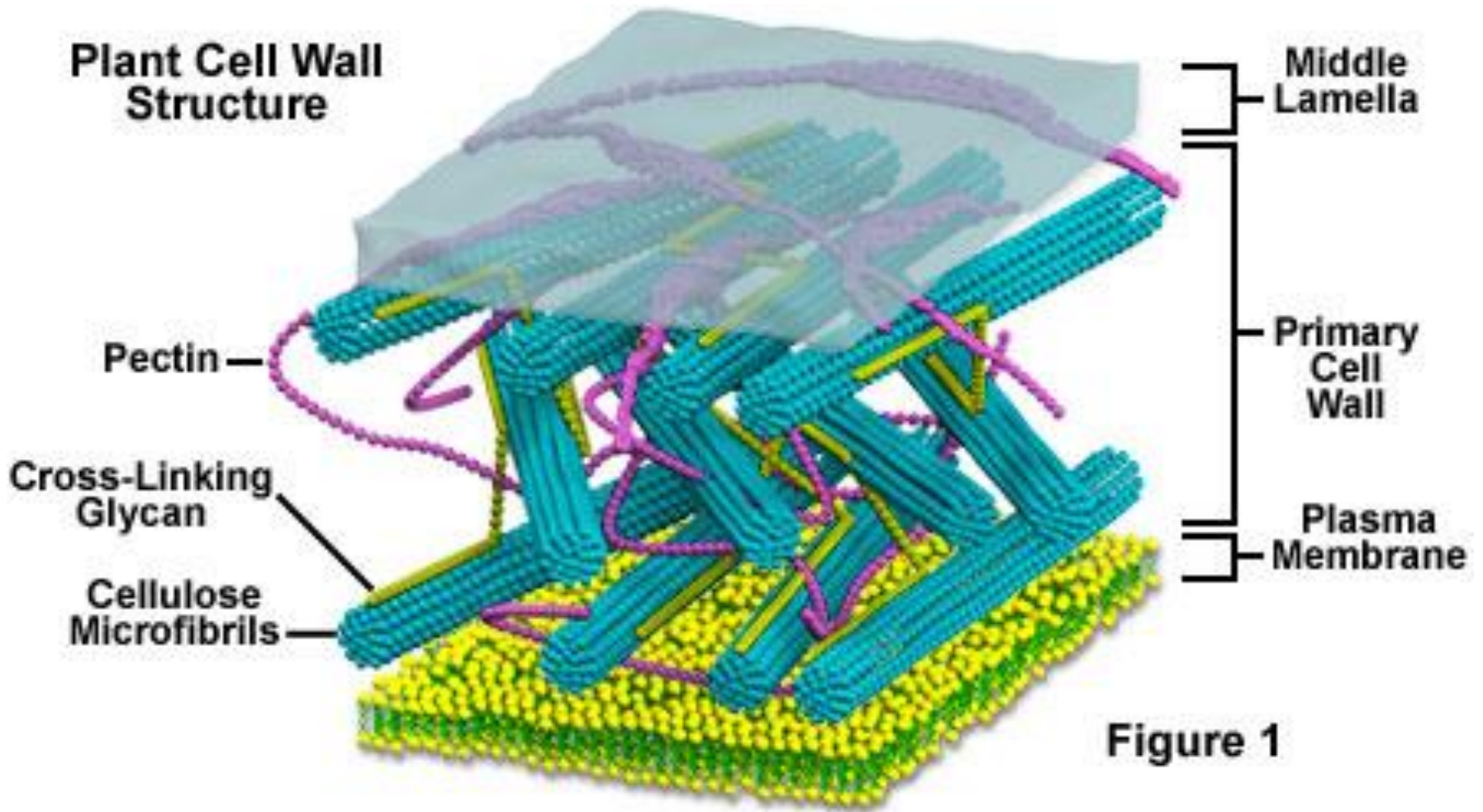
Arrangement of Fibrils, Microfibrils, and Cellulose in Cell Walls



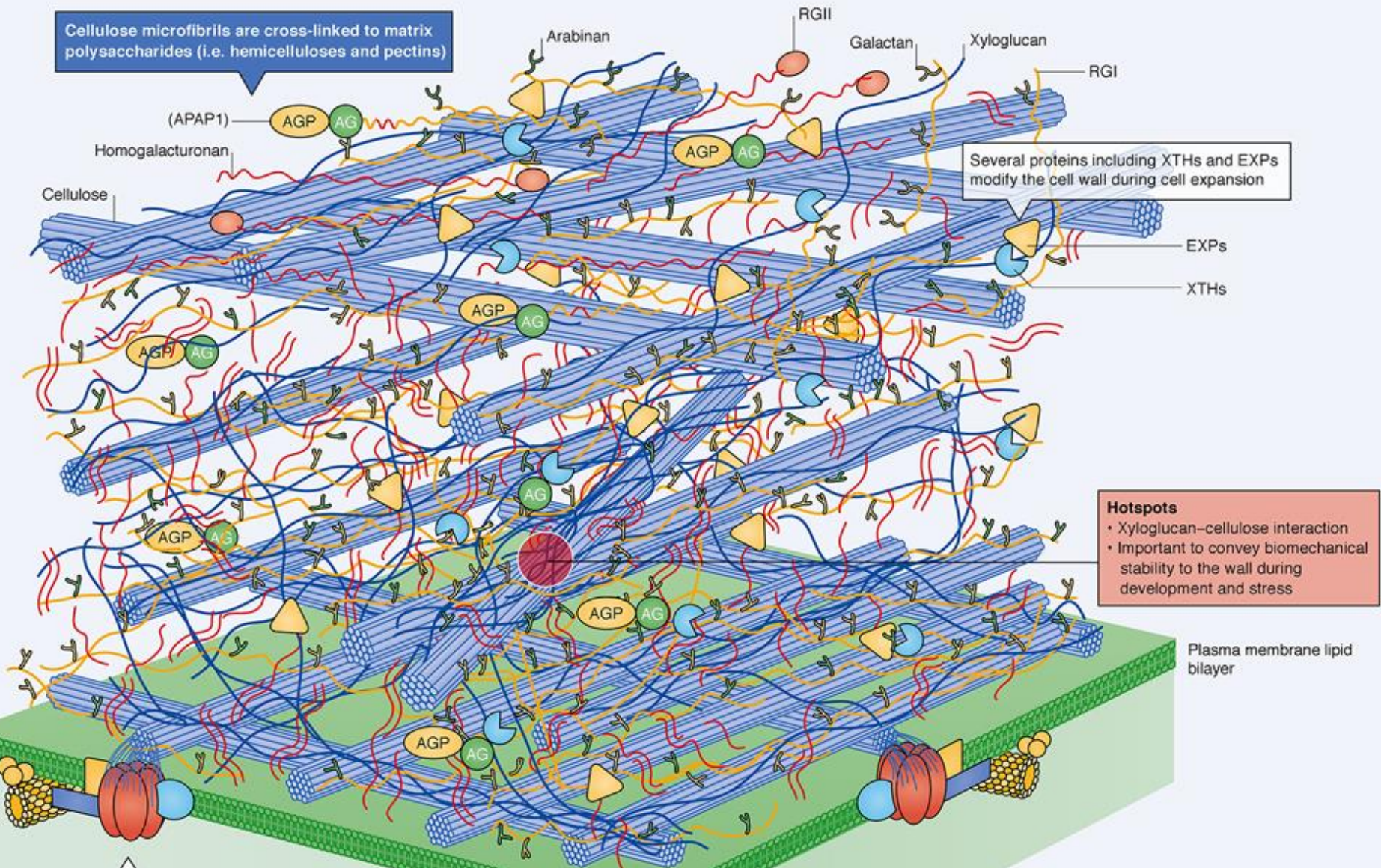








Cellulose microfibrils are cross-linked to matrix polysaccharides (i.e. hemicelluloses and pectins)



Several proteins including XTHs and EXPs modify the cell wall during cell expansion

Hotspots

- Xyloglucan–cellulose interaction
- Important to convey biomechanical stability to the wall during development and stress

Plasma membrane lipid bilayer

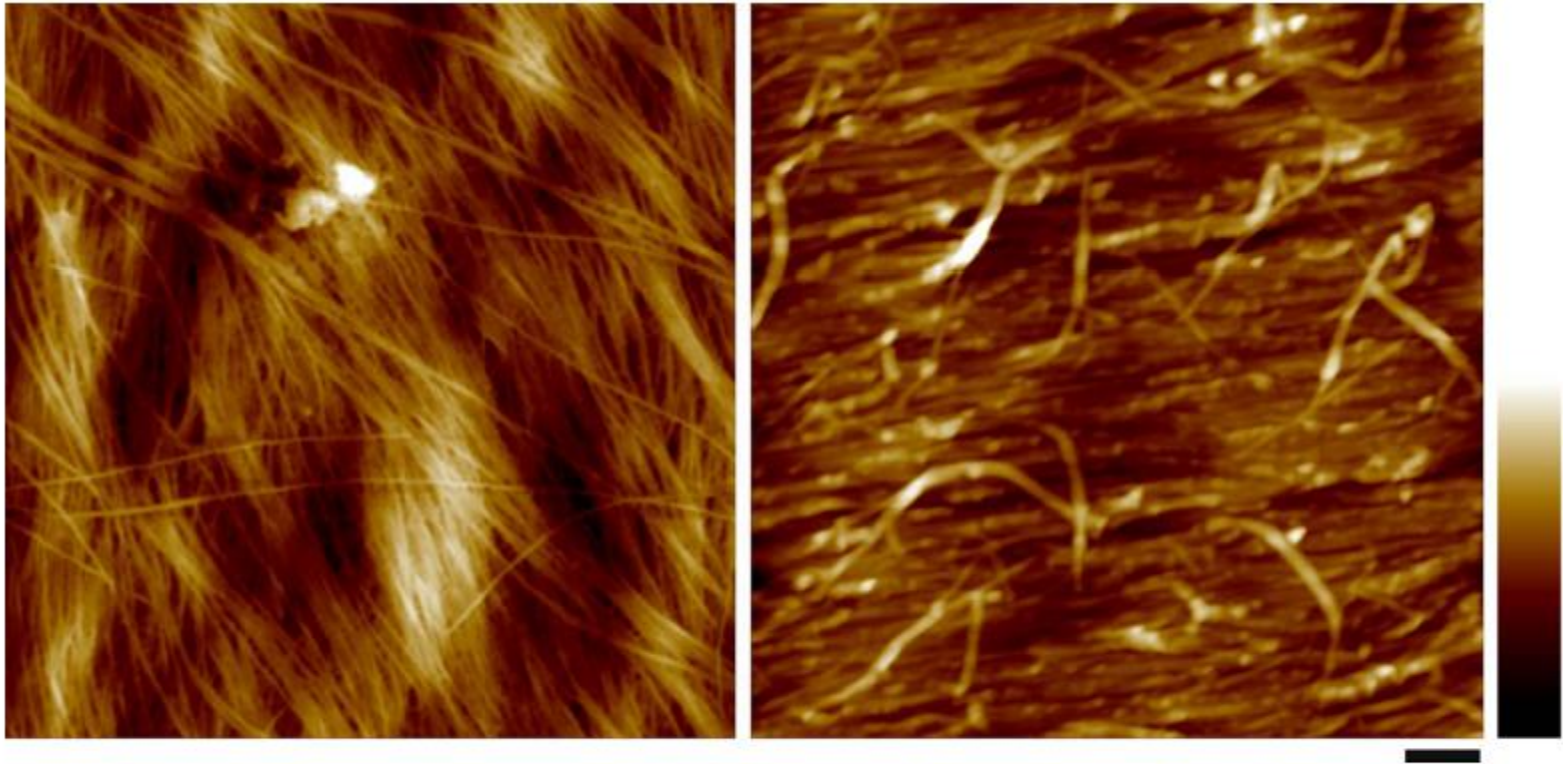


Fig. 1 Atomic force micrograph of primary and secondary cell wall structure from maize. Left, cellulose microfibrils form varying sizes of bundles in PW. Right, cellulose microfibrils are heavily coated by matrix polymers in the S2 layer of SW. The image was taken from the cutting face of a vascular fiber cell from maize (reprinted from [10] with permission). Scale bar = 100 nm, color bar = 30 nm

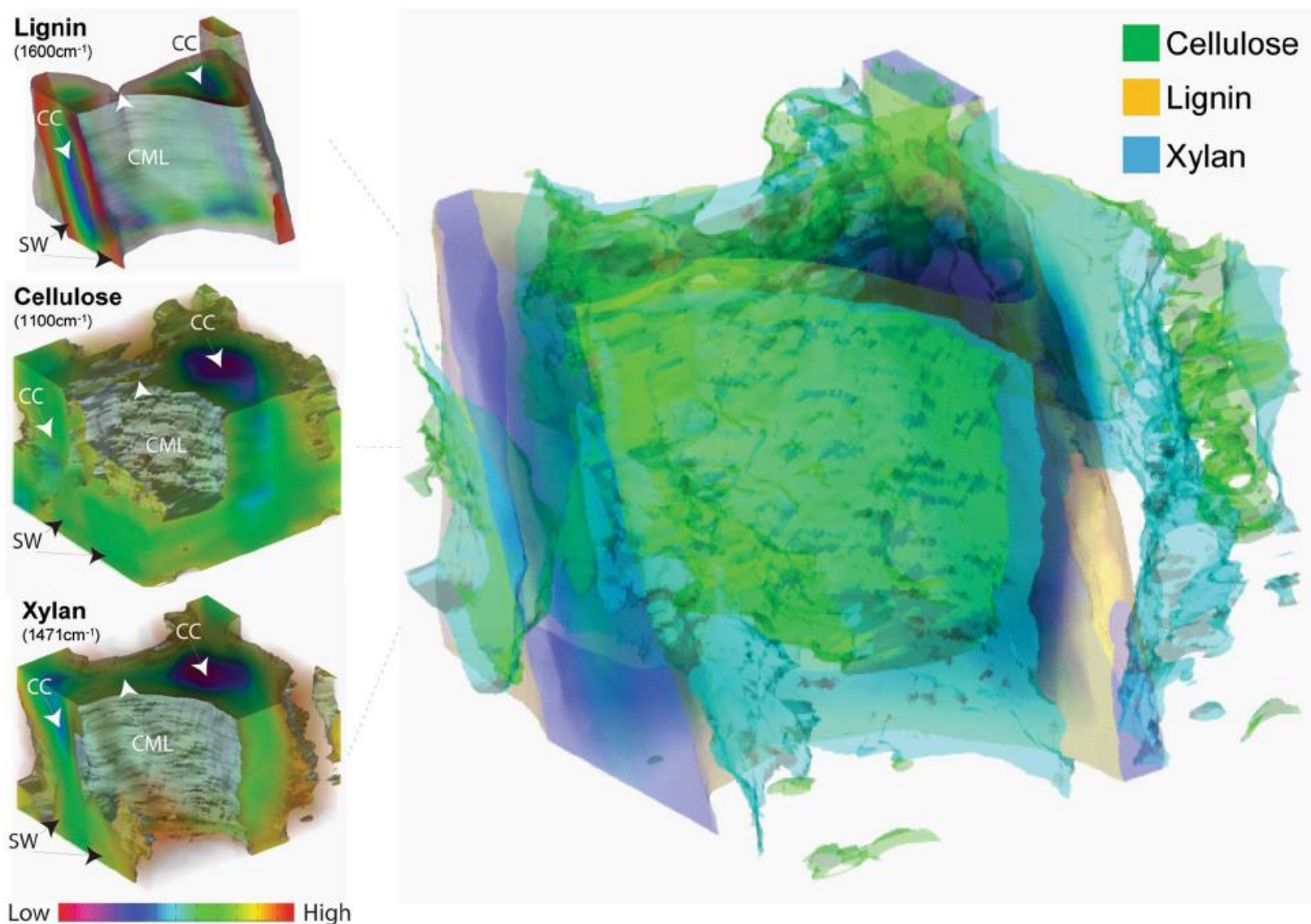


Fig. 3 Lignin, cellulose, xylan in corn stover cell wall shown in individual 3D concentration distribution (left) and overlay of their isosurfaces (right) by reconstruction of stimulated Raman scattering microscopy sectioning scans. Lignin is more concentrated at cell corner and compound middle lamella, while cellulose and xylan are more abundant in secondary wall. Raman frequencies used for stimulated Raman scattering microscopy: lignin—1600 cm⁻¹, cellulose—1100 cm⁻¹, and xylan—1471 cm⁻¹. CC cell corner; CML compound middle lamellae; SW secondary wall

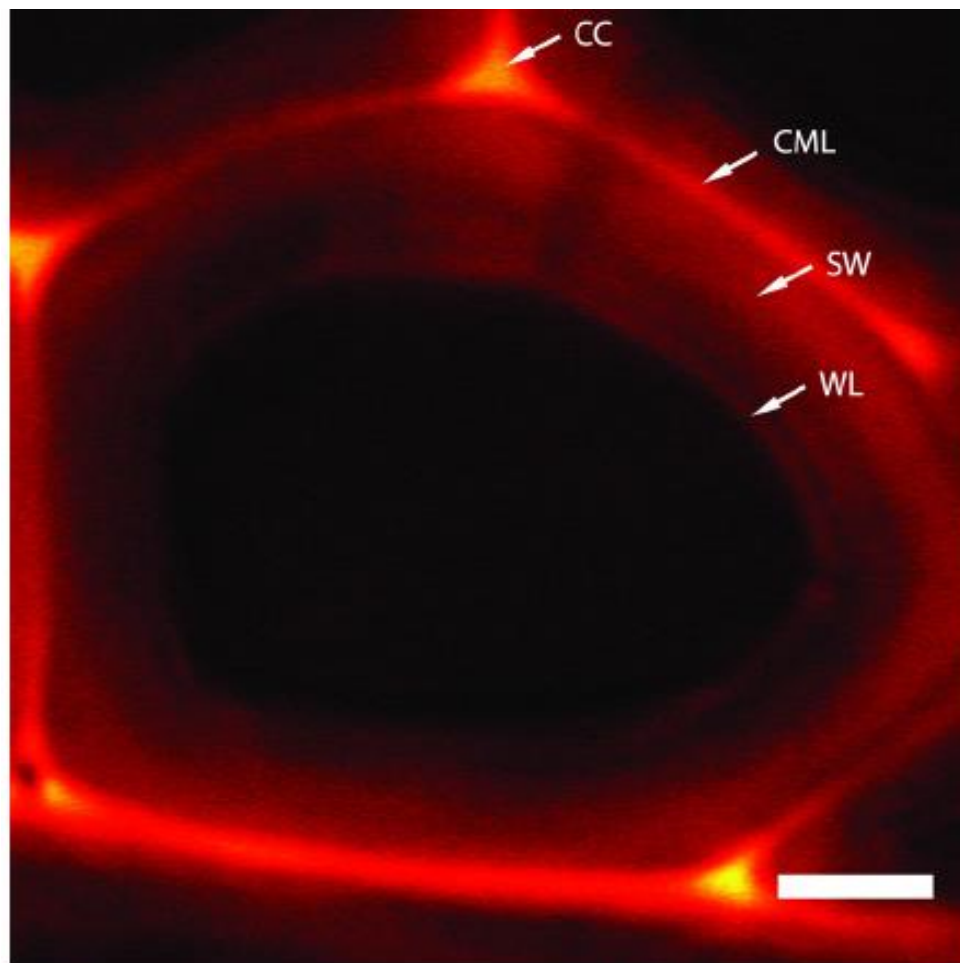
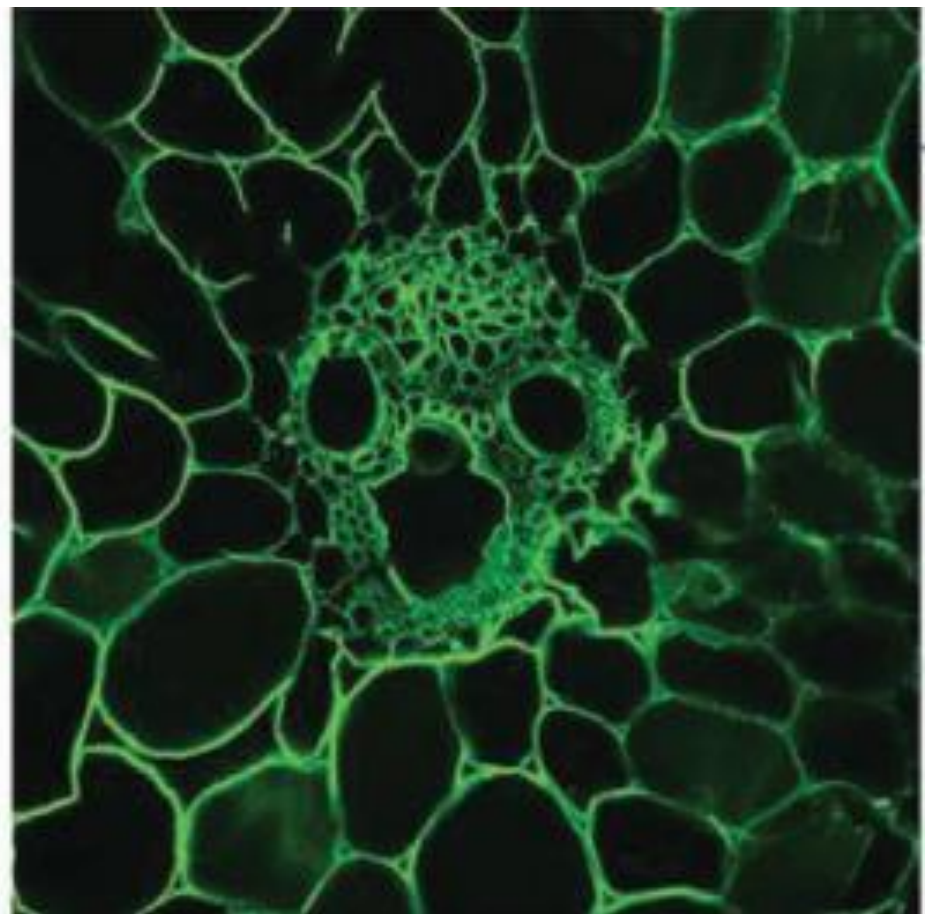
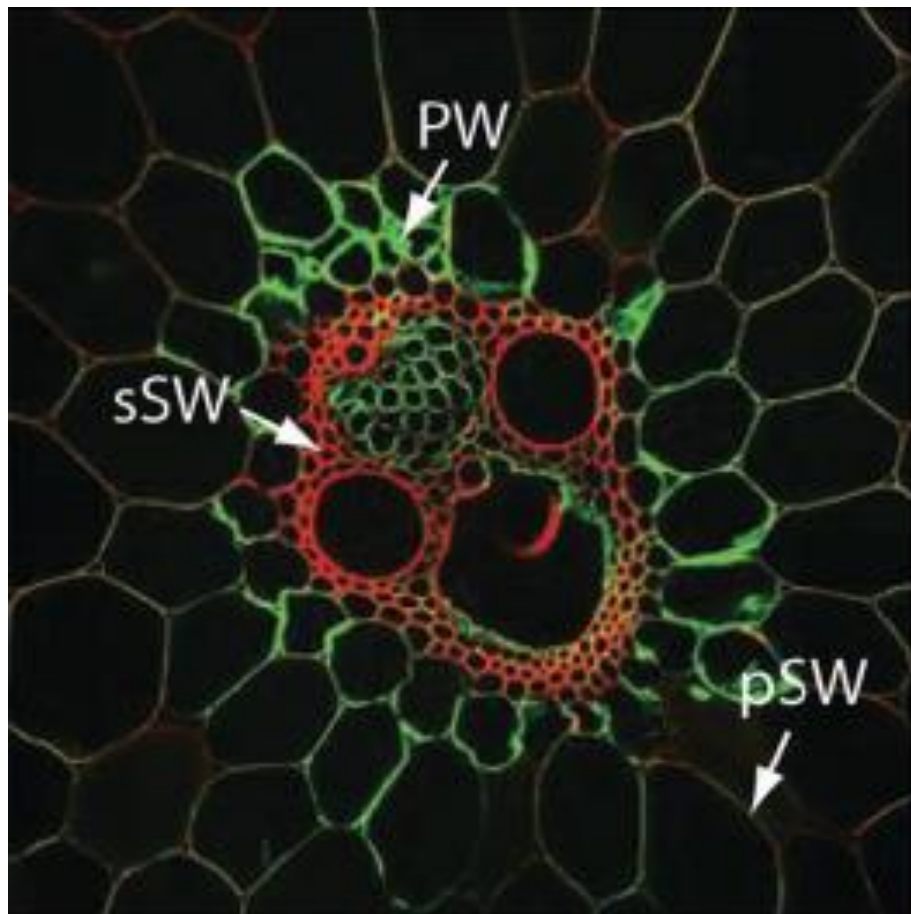


Fig. 2 Lignin distribution in poplar tracheid cell wall imaged by stimulated Raman scattering microscopy by lignin's aryl ring stretch at 1600 cm^{-1} (reprinted from [11] with permission). Lignin is unevenly distributed in cell wall layers. Highest lignin content is shown in the cell corner (CC), compound middle lamella (CML), and the warty layer (WL). Secondary wall (SW) has a lignin distribution gradient from outside (high) to inner side (low). Scale bar = $5\text{ }\mu\text{m}$













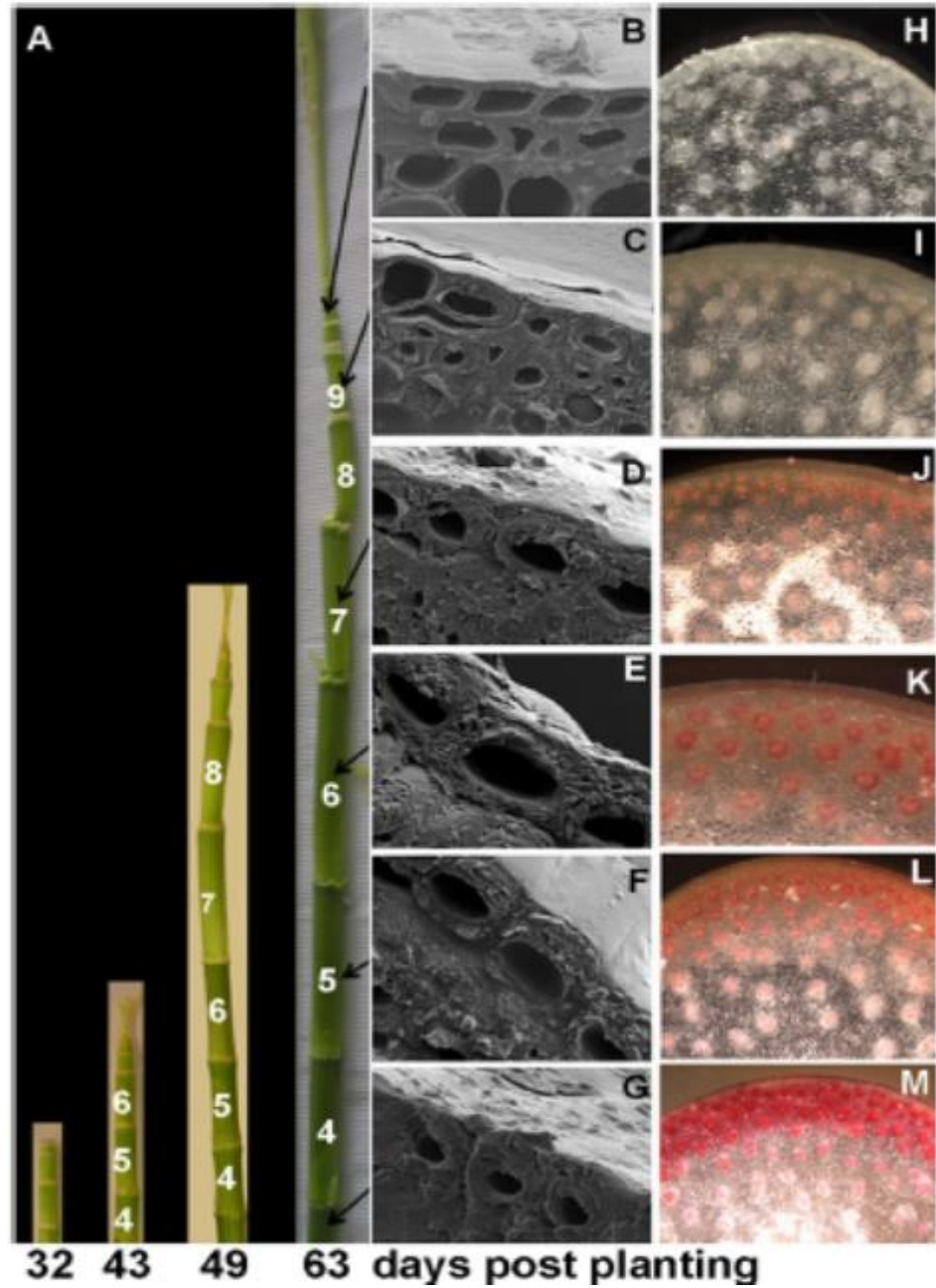
RESEARCH ARTICLE

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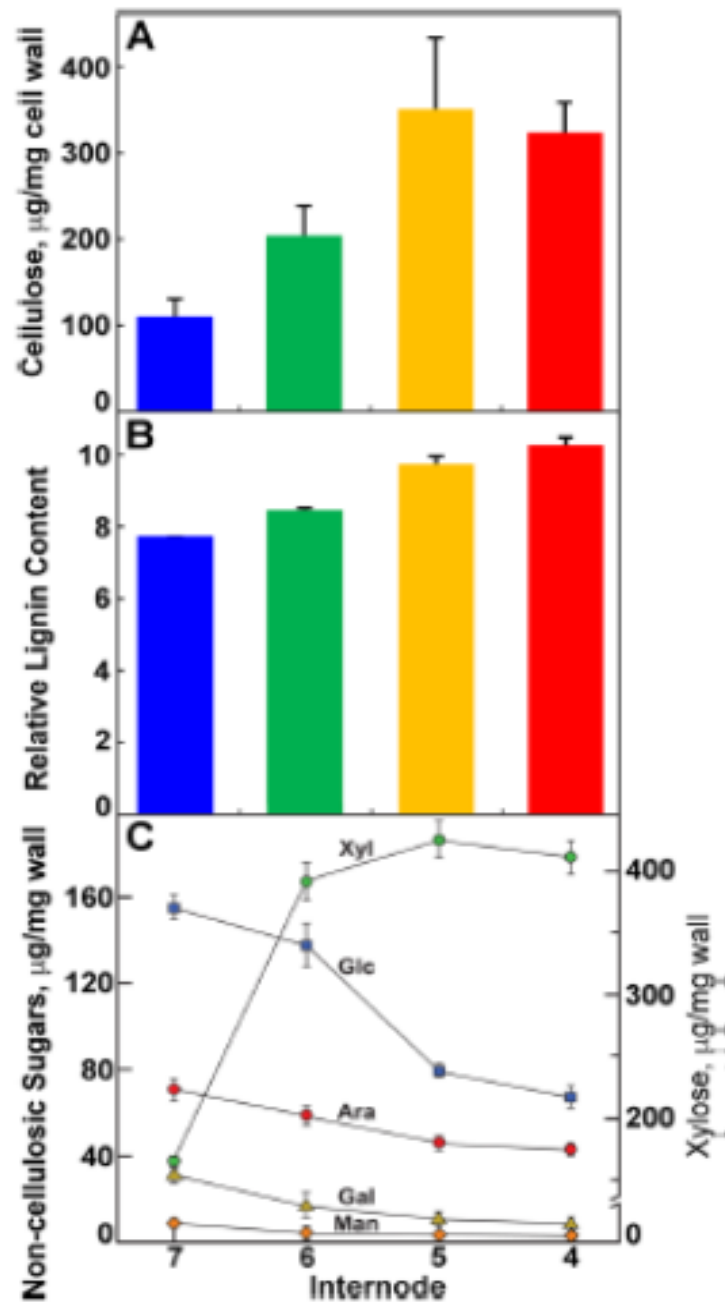
Expression profiles of cell-wall related genes vary broadly between two common maize inbreds during stem development



Bryan W. Penning^{1,2,3} , Tânia M. Shiga^{1,4} , John F. Klimek¹ , Philip J. SanMiguel⁵ , Jacob Shreve^{6,7} ,
Jyothi Thimmapuram^{4,6} , Robert W. Sykes^{8,9} , Mark F. Davis⁸ , Maureen C. McCann^{2,10}  and
Nicholas C. Carpita^{1,2,10*} 



32 43 49 63 days post planting



REVIEW

WILEY



Food and Energy Security

Open Access

Suberin and hemicellulose in sugarcane cell wall architecture and crop digestibility: A biotechnological perspective

Raquel Figueiredo¹ | Pedro Araújo² | Juan Pablo P. Llerena¹ | Paulo Mazzafera^{1,3} 

TABLE 1 Hemicellulose and suberin wax composition of several species of *Saccharum* spp. and other grasses

	Hemicelluloses monomers (%)			Suberin waxes (g/100 g rind DW ^a)	References
	Glucose	Xylose	Arabinose		
Sugarcane					
<i>Saccharum officinarum</i>	47.6	48.7	2.4	3.50 ^b	Portilla Llerena (2016)
<i>Saccharum barberi</i>	46.4	49.2	3.9	—	Portilla Llerena (2016)
<i>Saccharum robustum</i>	50.1	46.4	2.9	—	Portilla Llerena (2016)
<i>Saccharum spontaneum</i>	50.7	45.8	2.8	8.00 ^b	Portilla Llerena (2016)
<i>Saccharum</i> cv.	59.80	33.50	2.40	0.93–1.65	de Souza et al. (2013), Asikin et al. (2012)
Miscanthus					
<i>Miscanthus sinensis</i>	52.84	30.94	3.26	0.69	Van der Weijde et al. (2017), Attard et al. (2016)
<i>Miscanthus sacchariflorus</i>	70.6	24.2	5.3	1.81	Visser and Pignatelli (2001)
<i>M. × giganteus</i>	37.39–42.9	18.26–22.0	1.45	0.52	Wang et al. (2017), Zhang, Wyman, Jakob, and Yang (2012), Attard et al. (2016)
Switchgrass					
<i>Panicum virgatum</i>	43.7–46.1	22.8–24.6	2.1–2.3	0.45	Hu et al. (2010), Tulloch and Hoffman (1980)
Maize					
<i>Zea mays</i>	36.8	22.2	5.5	0.61	Galbe and Zacchi (2007), Zhao et al. (2007)
Rice					
<i>Oryza sativa</i>	38.66	22.93	3.94	0.82 ^c	Wu et al. (2018), Zhao et al. (2007)
Wheat					
<i>Triticum aestivum</i>	34.9–37.8	22.8	2.37	0.57 ^c	Zhang et al. (2012), Tozluoglu, Özyürek, Çöpür, and Özdemir (2015), Zhao et al. (2007)

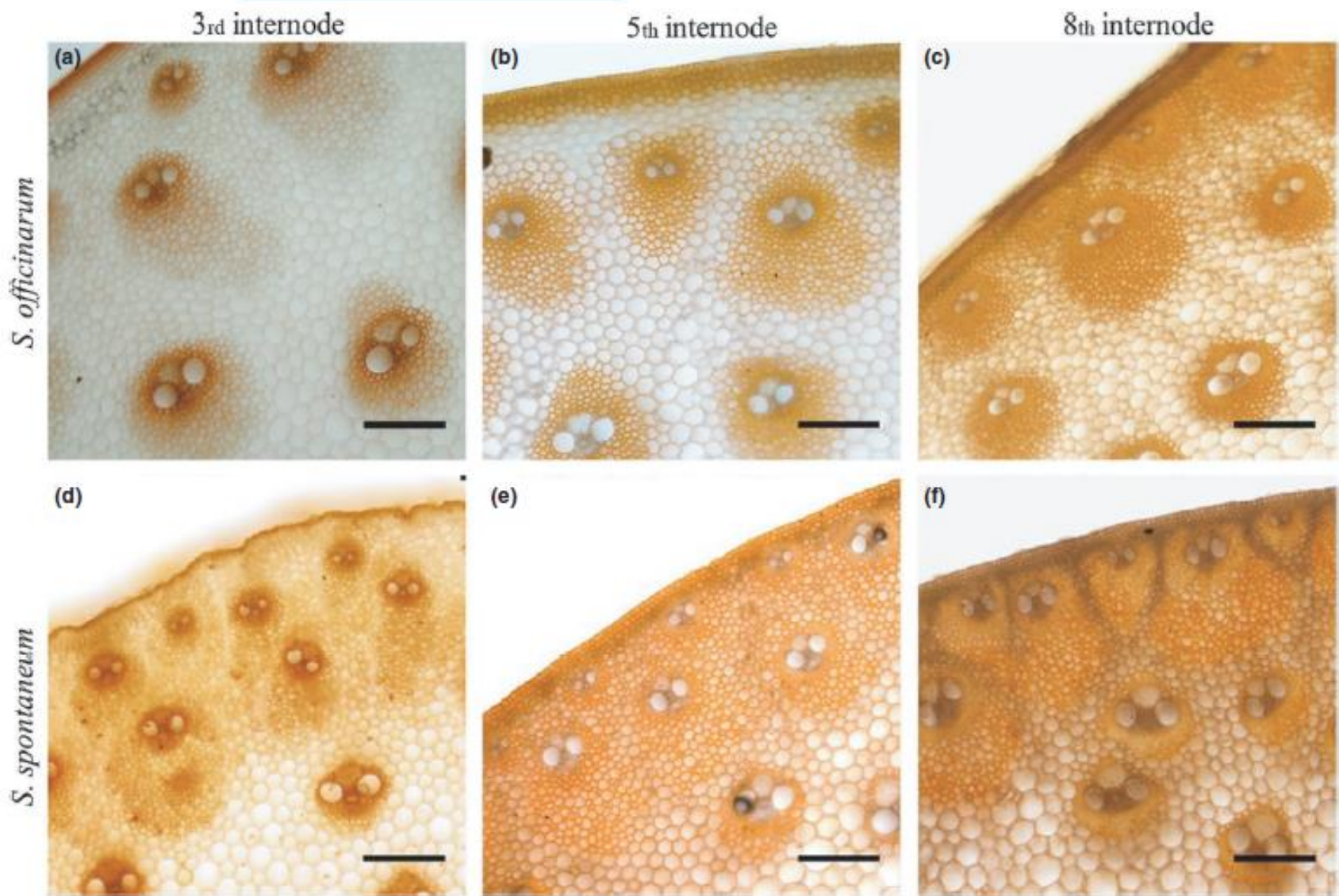


FIGURE 3 Suberin staining with Sudan IV of internode transverse sections of two sugarcane species. (a) *Saccharum officinarum* 3rd internode (young). (b) *Saccharum officinarum* 5th internode. (c) *Saccharum officinarum* 8th internode (mature). (d) *Saccharum spontaneum* 3rd internode (young). (e) *Saccharum spontaneum* 5th internode. (f) *Saccharum spontaneum* 8th internode (mature). Scale bar = 100 μ m



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Research review paper

Genetic modification of plant cell walls to enhance biomass yield and biofuel production in bioenergy crops

Yanting Wang^{a,b,c,1}, Chunfen Fan^{a,b,c,1}, Huizhen Hu^{a,b,c}, Ying Li^{a,b,c}, Dan Sun^{a,c,d},
Youmei Wang^{a,b,c}, Liangcai Peng^{a,b,c,*}

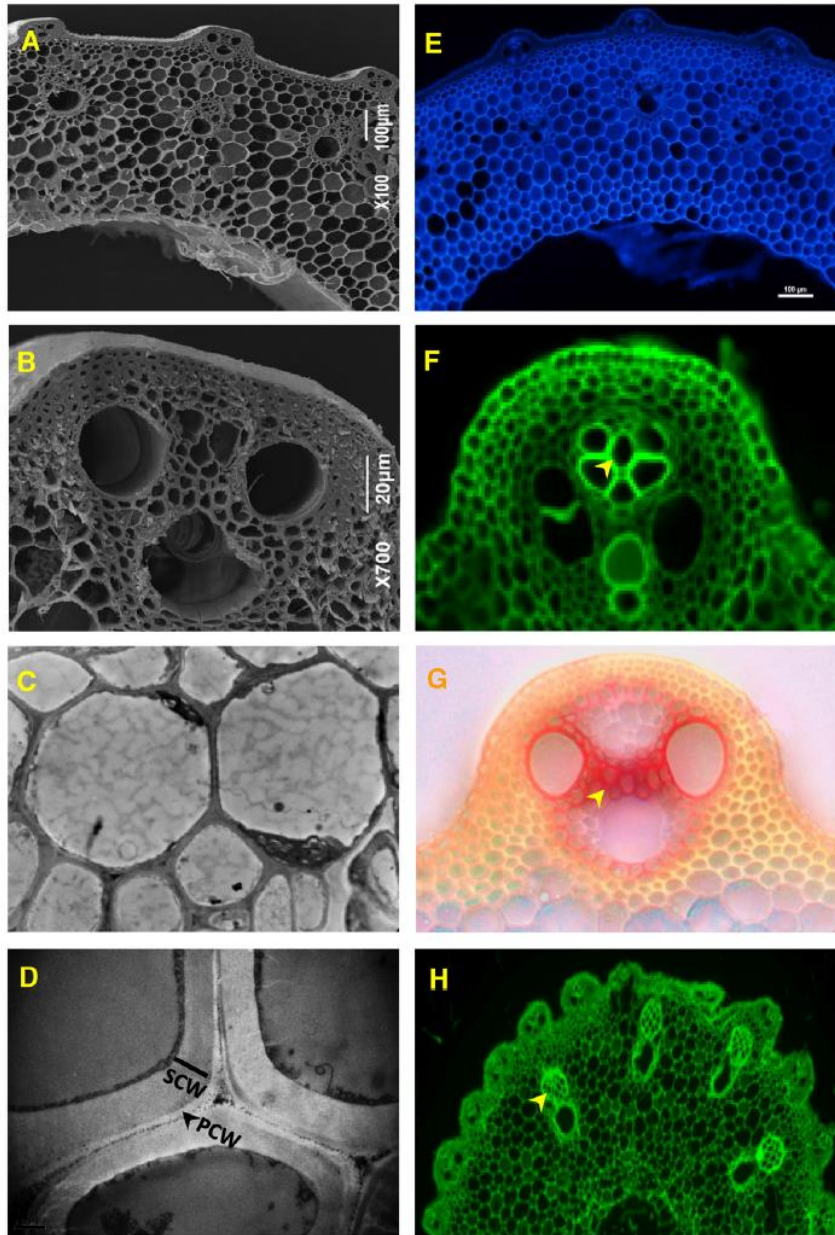
^a Biomass and Bioenergy Research Centre, Huazhong Agricultural University, Wuhan 430070, China

^b National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China

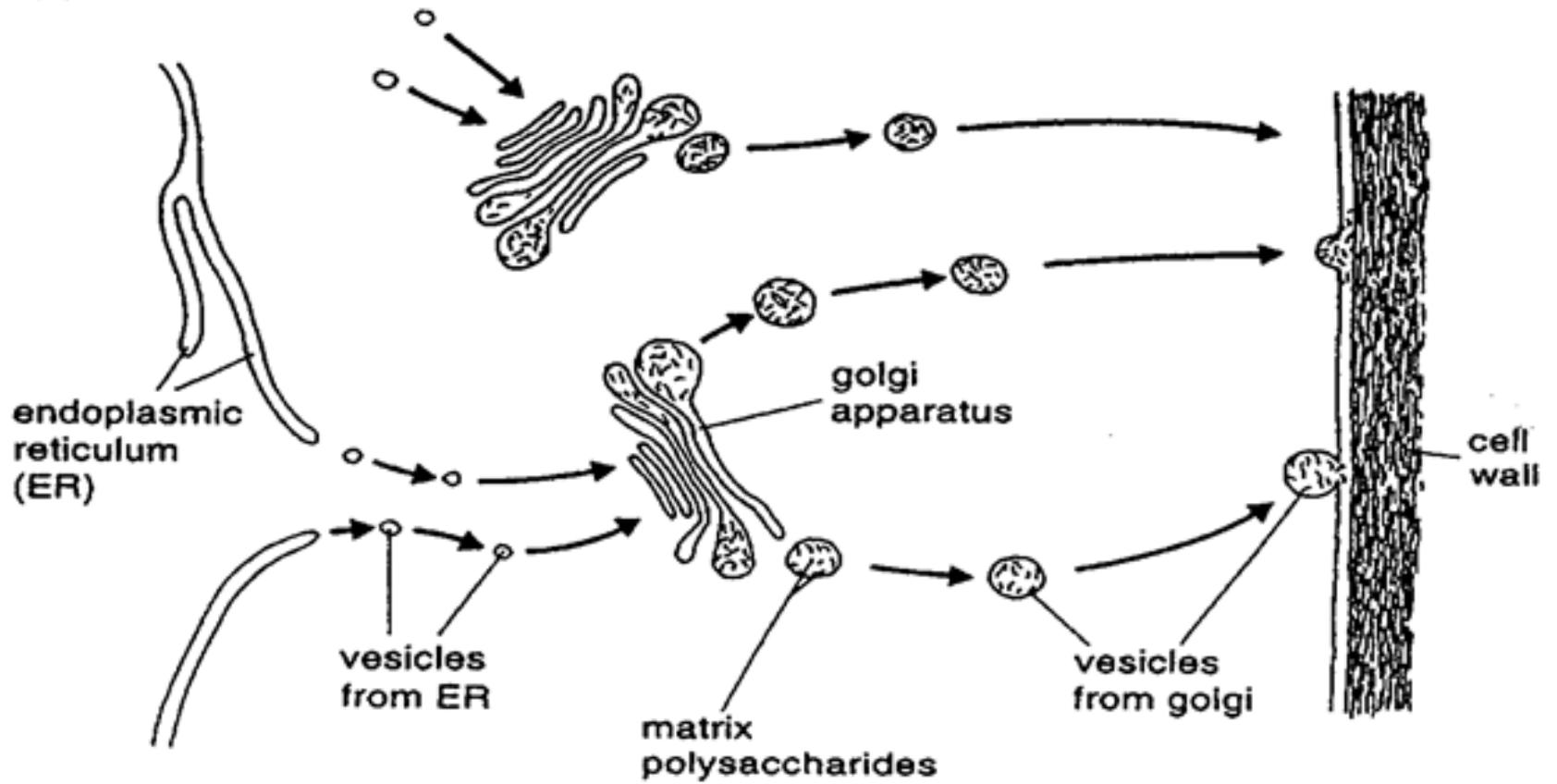
^c College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

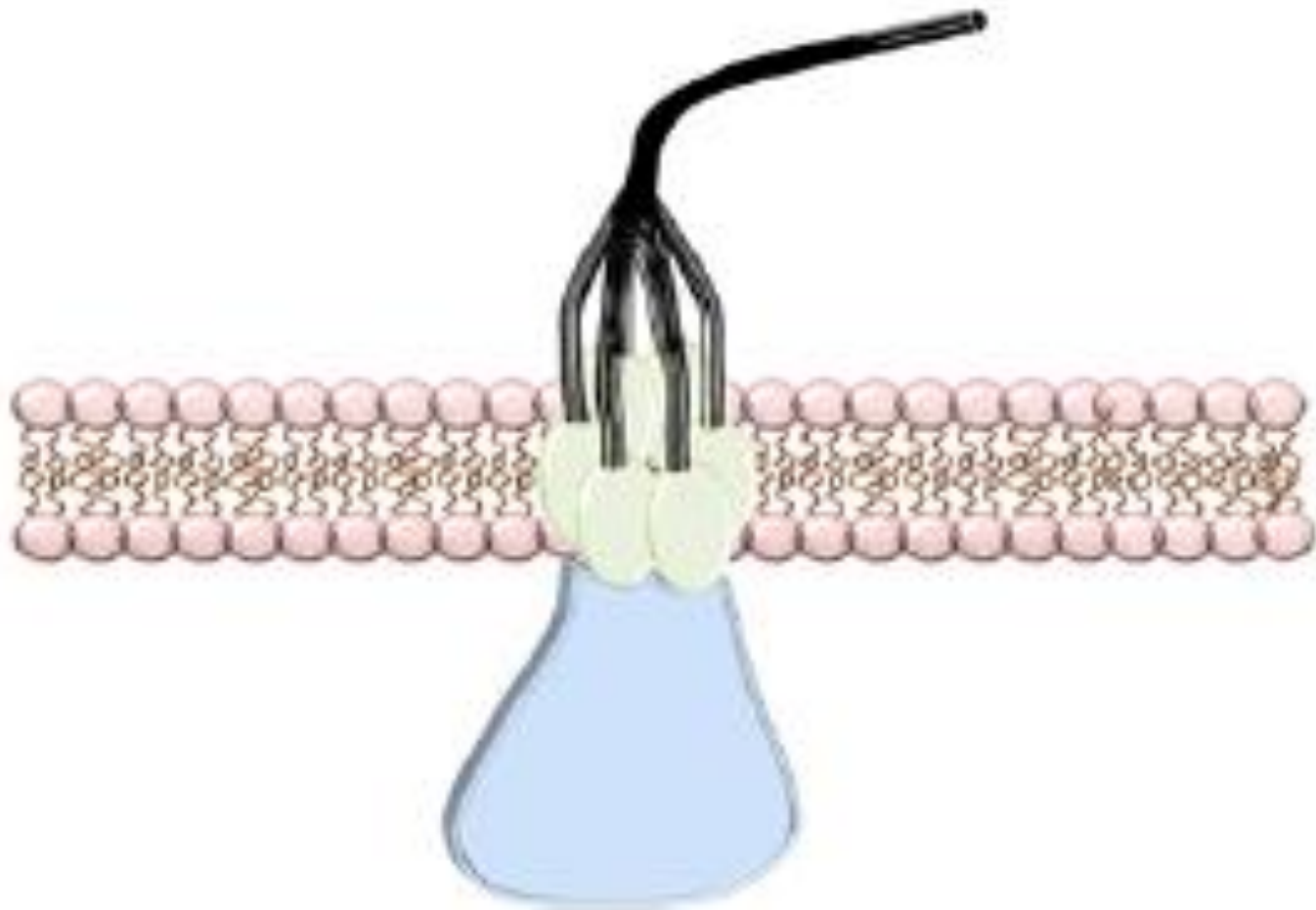
^d College of Chemistry and Chemical Engineering, Hubei University of Technology, Wuhan, Hubei 430068, China

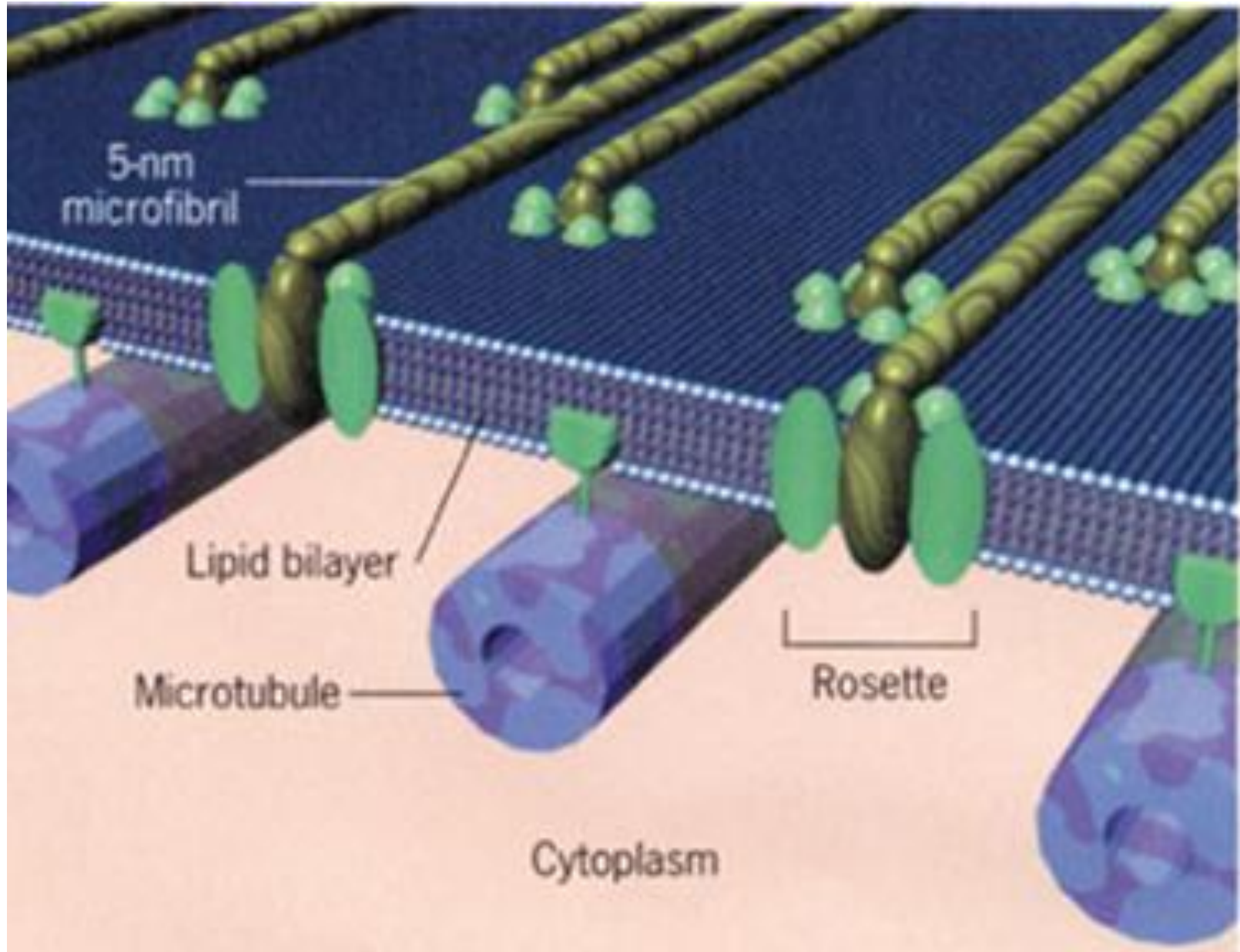


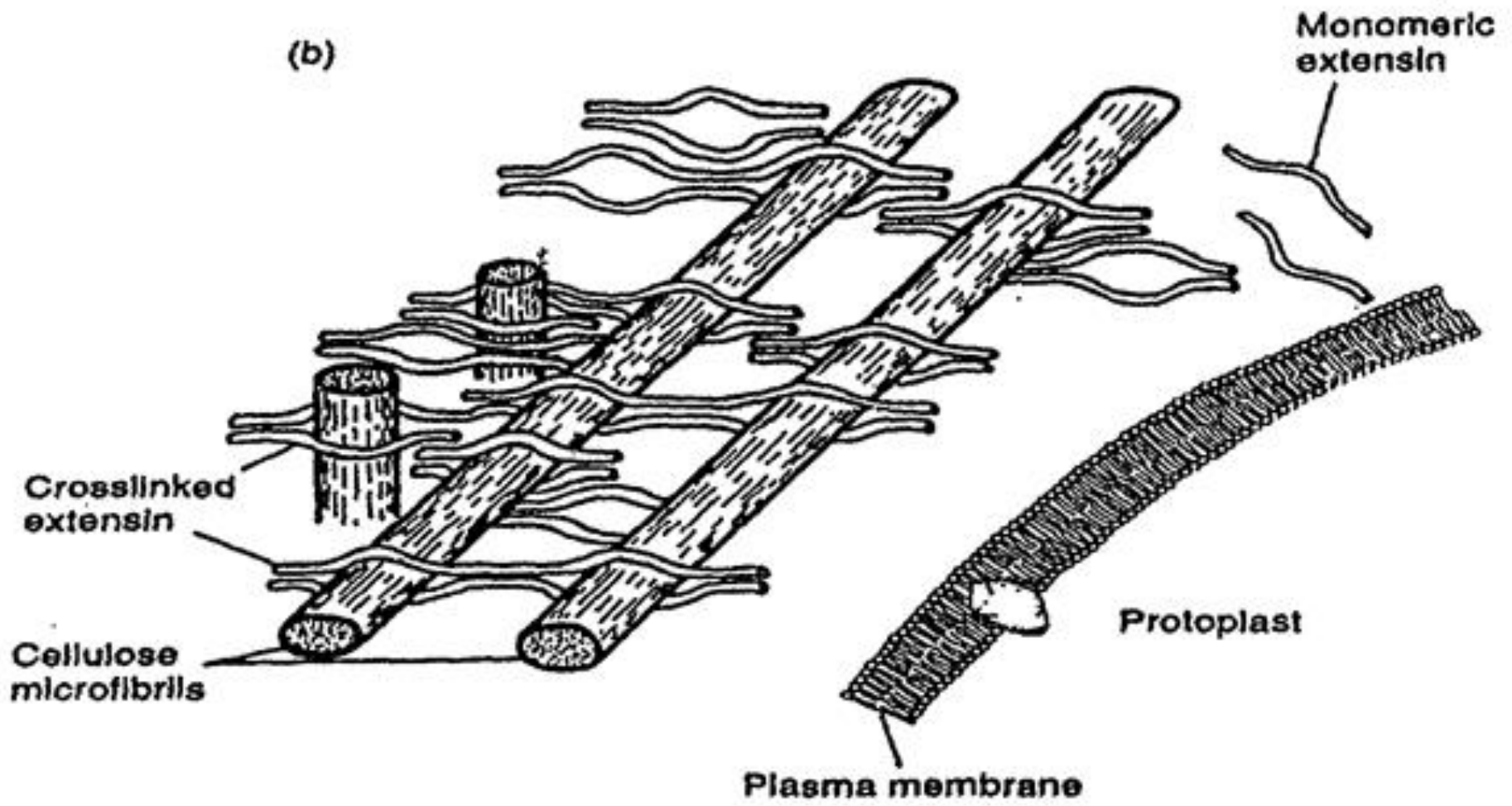


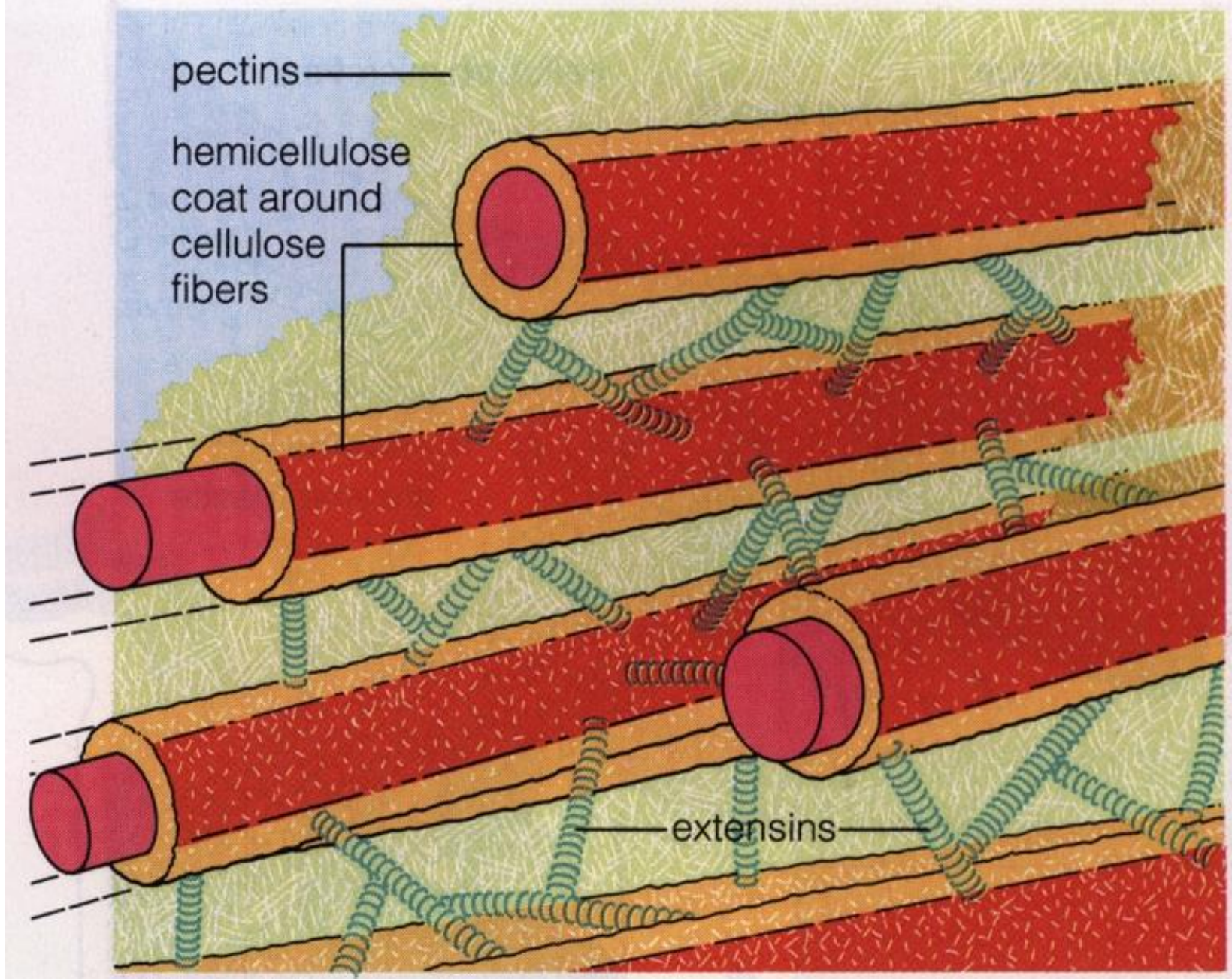
(b)

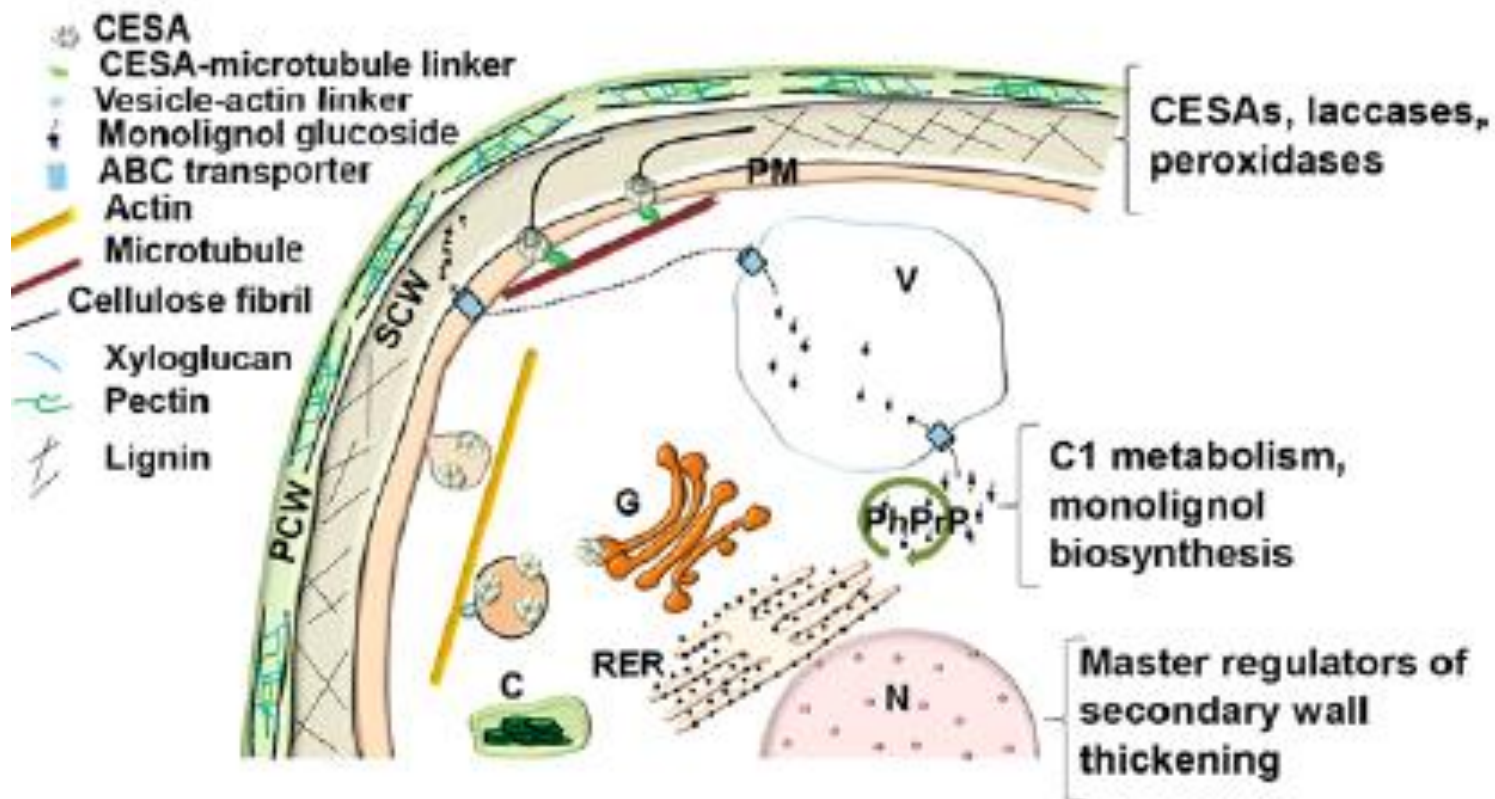












CELL SCIENCE AT A GLANCE

SPECIAL ISSUE: PLANT CELL BIOLOGY

Building a plant cell wall at a glance

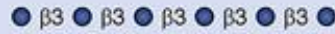
Edwin R. Lampugnani*, Ghazanfar Abbas Khan*, Marc Somssich* and Staffan Persson[‡]

Structures of common cell wall polysaccharides

Cellulose



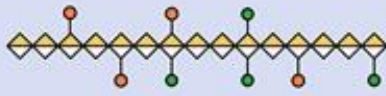
Callose



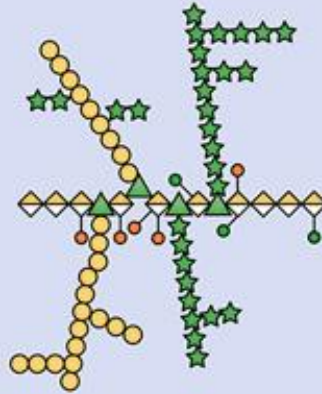
Xyloglucan



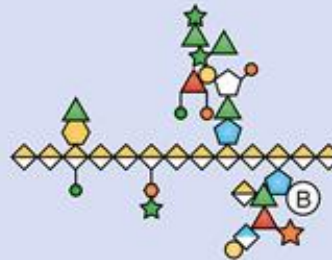
Homogalacturonan



Rhamnogalacturonan I (RGI)



Rhamnogalacturonan II (RGI)



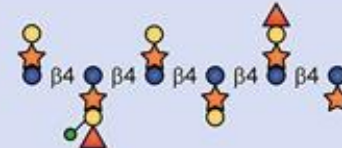
Xylogalacturonan



Arabinogalactoxyloglucan



Fucogalactoxyloglucan



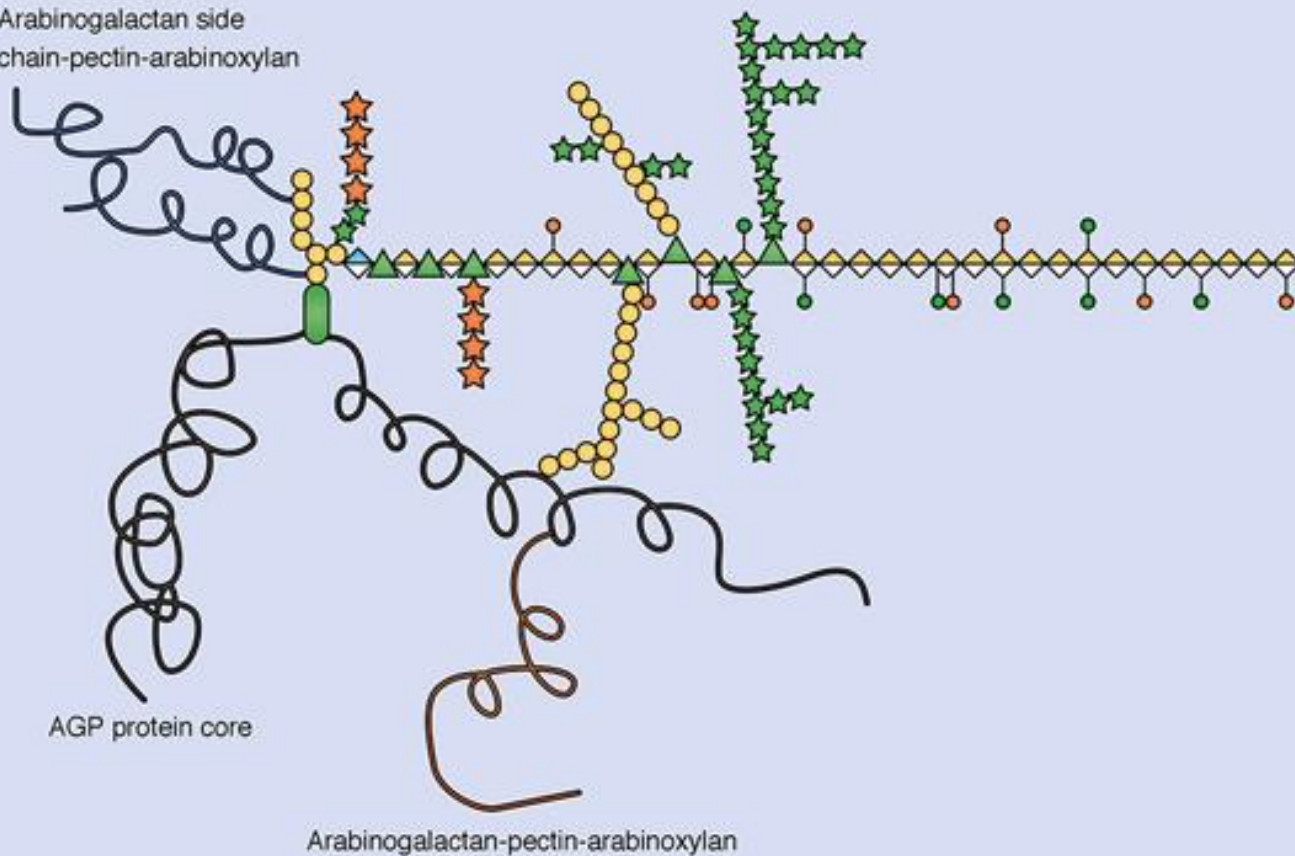
Key

- ◇ GalA
- Gal
- △ Rha
- ★ Ara
- Dha
- △ Fuc
- Kdo
- ◇ GlcA
- ◇ Ace
- ◇ Apio
- ★ Xyl
- Glc
- Hyp
- Ⓟ Borate
- O-Acetyl
- O-Methyl

ARABINOXYLAN PECTIN ARABINOGALACTAN PROTEIN1 (APAP1)

Proposed structural model

Arabinogalactan side chain-pectin-arabinoxylan

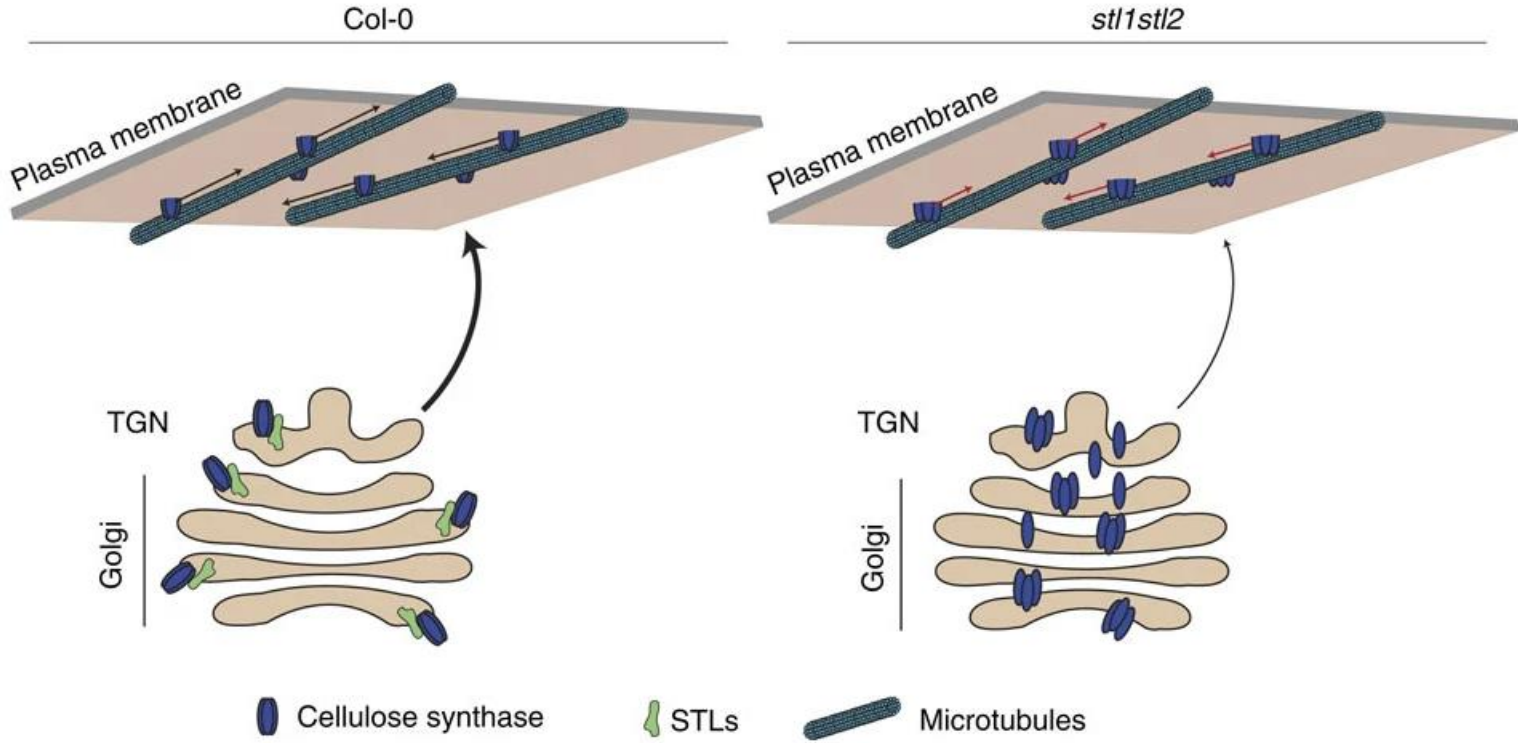


Key

GalA	Gal	Rha	Ara	Dha	Fuc	Kdo	GlcA
Ace	Apio	Xyl	Glc	Hyp	Borate	O-Acetyl	O-Methyl

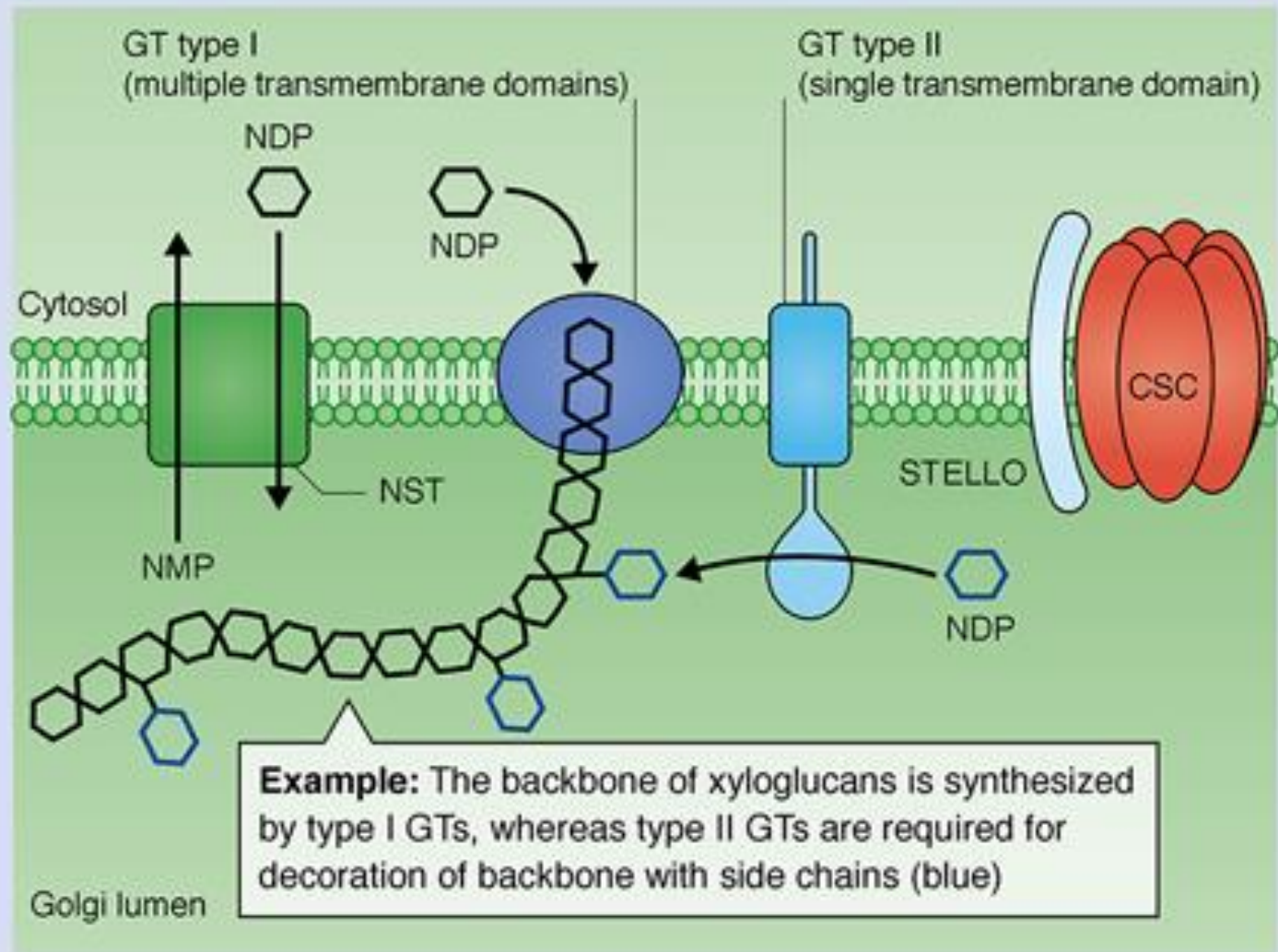
Figure 9: Schematic model of how the STL proteins function in cellulose production.

From: Golgi-localized STELLO proteins regulate the assembly and trafficking of cellulose synthase complexes in *Arabidopsis*



Assembly of polysaccharides in the Golgi

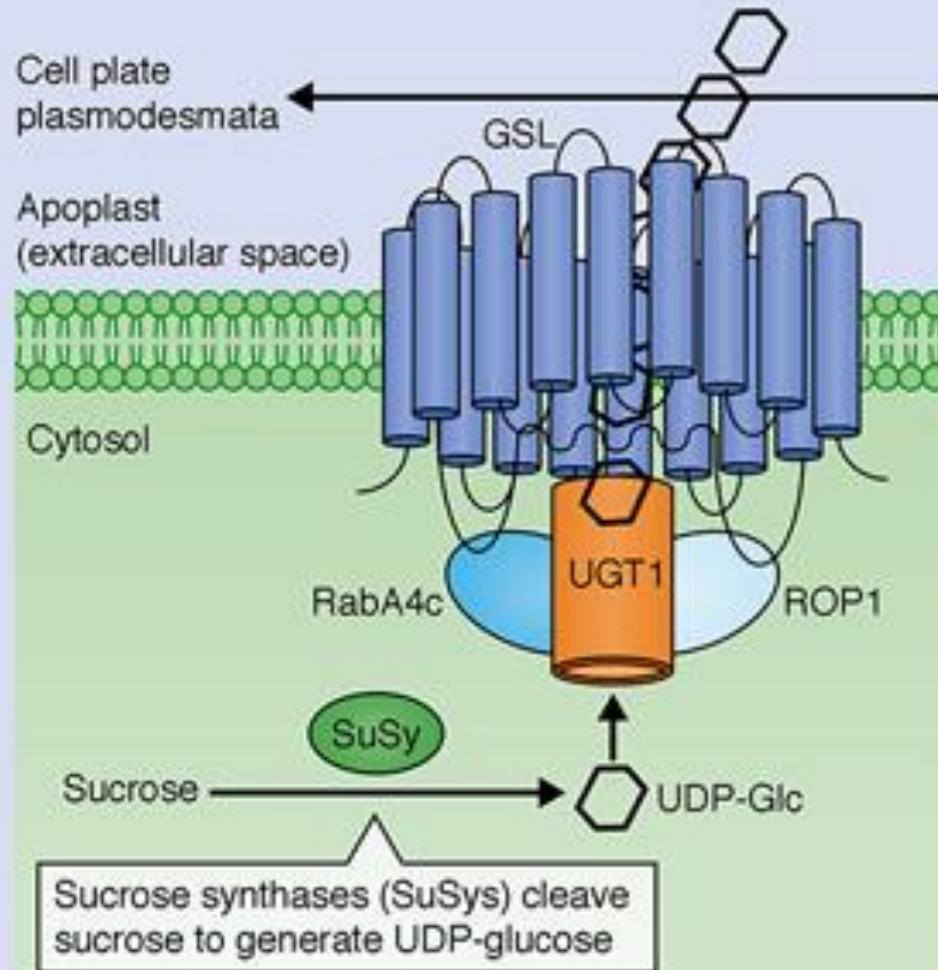
NSTs transport NDP-sugars from the cytosol into the Golgi lumen. Type I and type II GTs produce complex polysaccharides.



Synthesis of callose

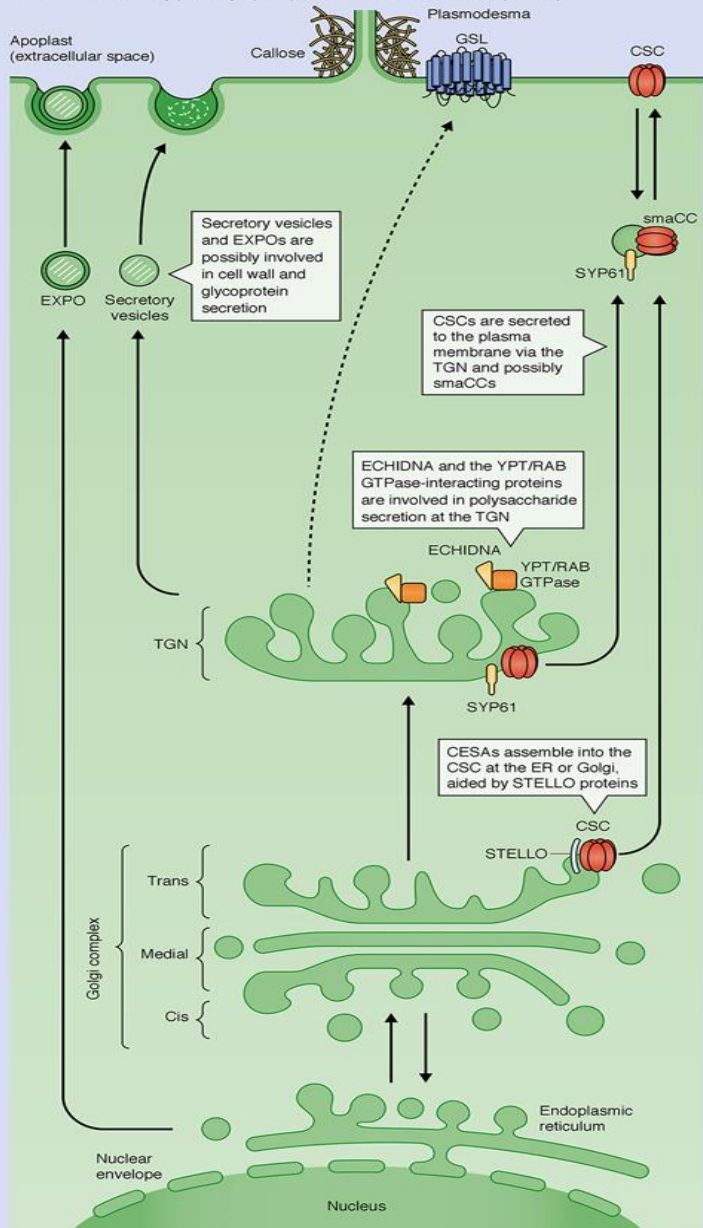
Callose is produced in specialized locations, including during cell plate formation and at plasmodesmata, and in response to environmental stress.

GSLs utilize UDP-glucose as substrate to synthesize callose. Several ancillary proteins, such as Rho-of-plant-1, the GTPase RabA4c and Sucrose synthase, support transport and activity of GSLs.

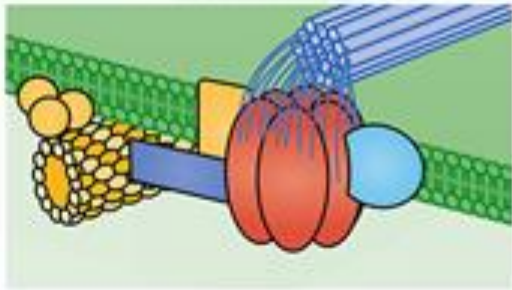


Trafficking of cell wall polysaccharides and proteins

Golgi synthesis of polysaccharides and secretion to the apoplast. This either occurs through conventional secretory pathways (TGN) or so-called unconventional pathways.



Key



CMU proteins



CELLULOSE SYNTHASE-
INTERACTING 1



COMPANIONS OF
CELLULOSE SYNTHASE



KORRIGAN

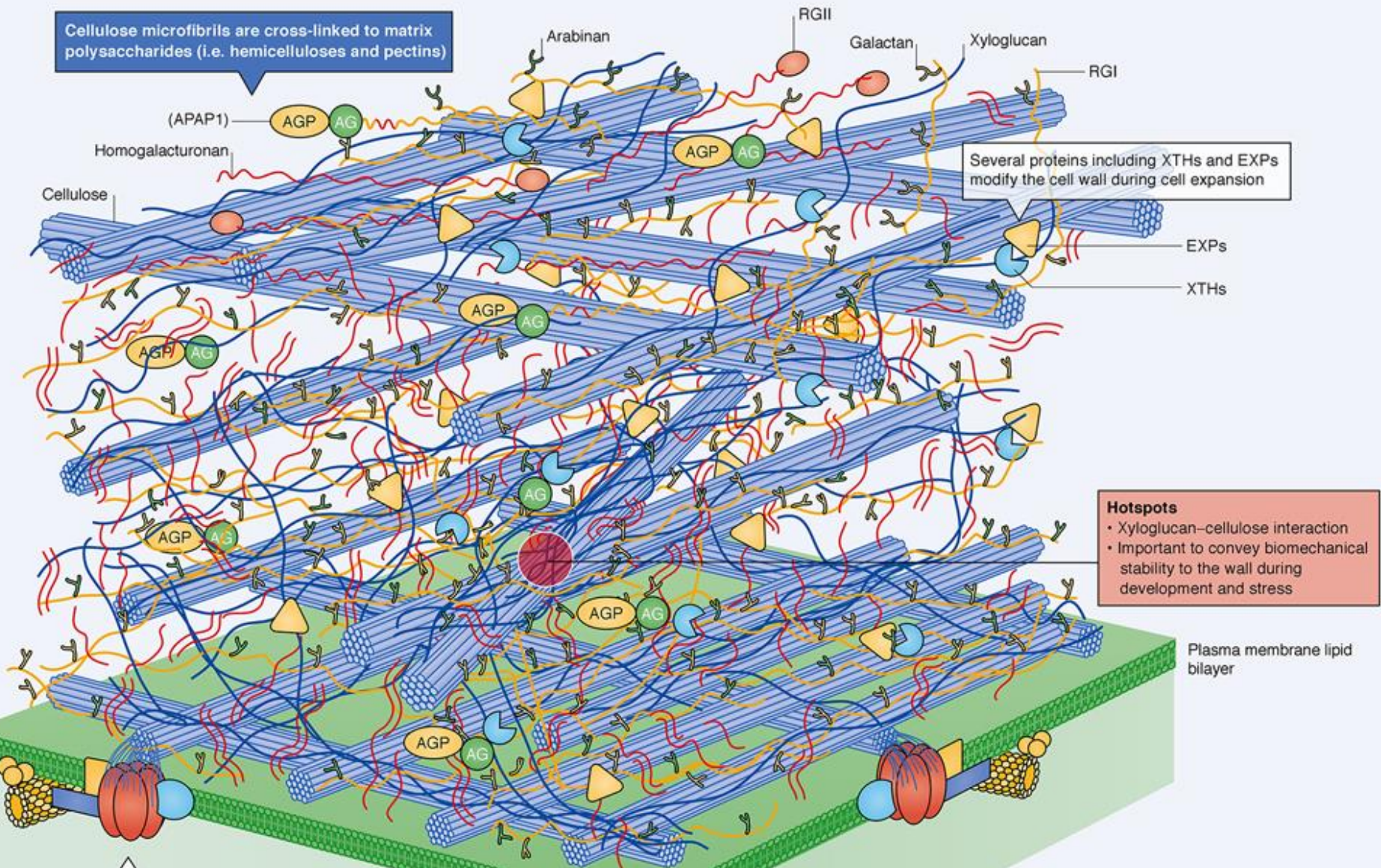


Microtubule



CSC

Cellulose microfibrils are cross-linked to matrix polysaccharides (i.e. hemicelluloses and pectins)

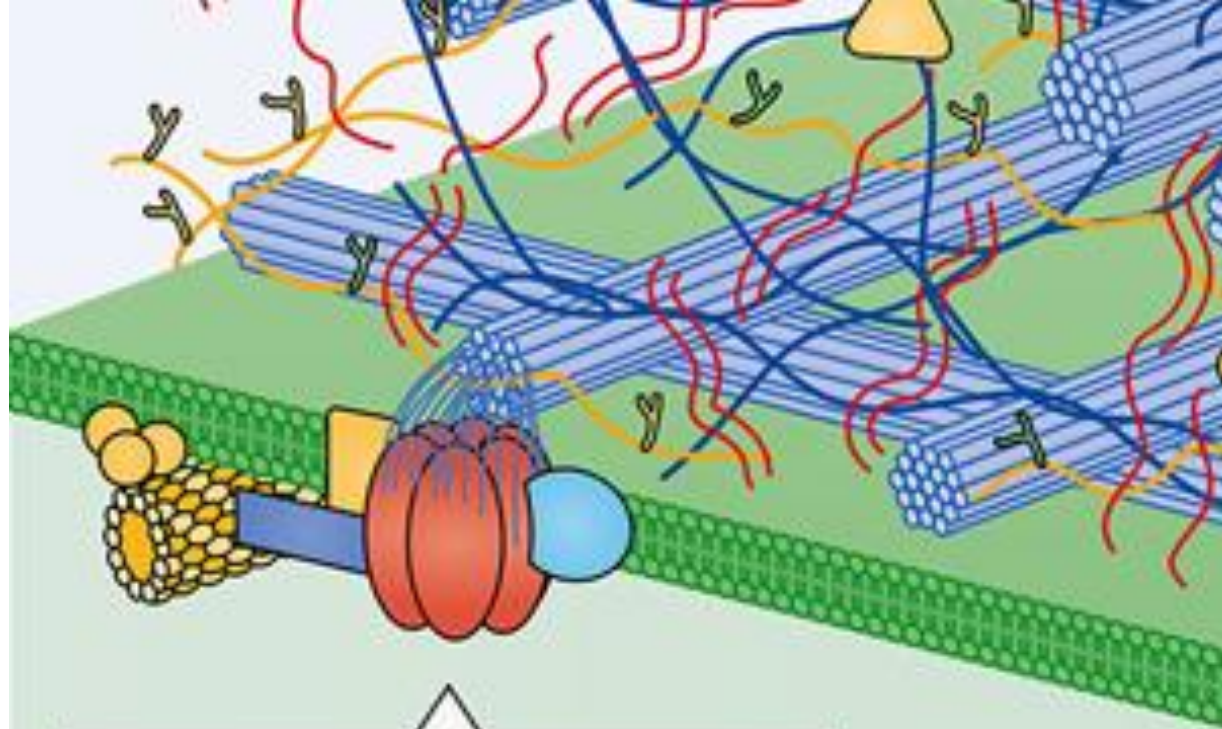


Several proteins including XTHs and EXPs modify the cell wall during cell expansion

Hotspots

- Xyloglucan-cellulose interaction
- Important to convey biomechanical stability to the wall during development and stress

Plasma membrane lipid bilayer



Cellulose is synthesized at the plasma membrane by the cellulose synthase complex (CSC). It becomes activated and tracks along cortical microtubules. CSI1, the CC and the CMU proteins are involved in connecting the CSC to the microtubules, and KORRIGAN may act on cellulose chains



ESALQ



USP



Fiber potential degradation

Article

Consequences of Increases in Ambient Temperature and Effect of Climate Type on Digestibility of Forages by Ruminants: A Meta-Analysis in Relation to Global Warming

Mehluli Moyo  and Ignatius Nsahlai * 

Animals **2021**, *11*, 172. <https://doi.org/10.3390/ani11010172>

<https://www.mdpi.com/journal/animals>

Table 1. Descriptive statistics of diet, feed and climatic factors affecting degradation of feeds in the rumen.

Diet	N	Max	Min	Mean \pm SD	SEM	CV (%)
Crude protein (g/kg)	1006	311	20	124 \pm 44.5	1.41	35.98
Neutral detergent fibre (g/kg)	1006	913	129	565 \pm 124.4	3.92	22.04
Protein-free cell contents (g/kg)	1006	740	48	311 \pm 109.5	3.45	35.24
Feed sample						
Dry matter (g/kg)	1015	992	70	713 \pm 302.8	9.51	42.47
Crude protein (g/kg)	1009	519	19	119 \pm 75.6	2.38	63.67
Neutral detergent fibre (g/kg)	1006	919	69.3	558 \pm 193.7	6.14	35.01
Acid detergent fibre (g/kg)	1006	715	29	357 \pm 136.2	4.31	38.48
Hemicellulose (g/kg)	1006	524	5.8	202 \pm 100.4	3.17	50.04
Ash (g/kg)	1009	330	11	87 \pm 39.9	1.25	45.91
Particle size (mm)	1015	100	0.5	3.3 \pm 6.72	0.21	206.4
Soluble fraction (g/kg)	945	751	27	214 \pm 119.3	3.89	55.81
Slowly degradable fraction (g/kg)	947	984	64	502 \pm 149.9	4.87	29.86
Rate of degradation (per h)	997	2.148	0.007	0.050 \pm 0.085	0.003	170.5
Potential degradability (g/kg)	974	1000	31	711 \pm 151.1	4.84	21.22
Lag (h)	375	17.90	0.00	2.24 \pm 2.763	0.143	123.4
Climate						
Ambient temperature ($^{\circ}$ C)	1015	28.2	-5.9	17.8 \pm 8.14	0.26	45.66
Experimental factors						
Incubation time (h)	1015	336	36	117 \pm 79.3	2.49	67.58
No. of replicates used	977	12	1	3.7 \pm 1.7	-	46.28

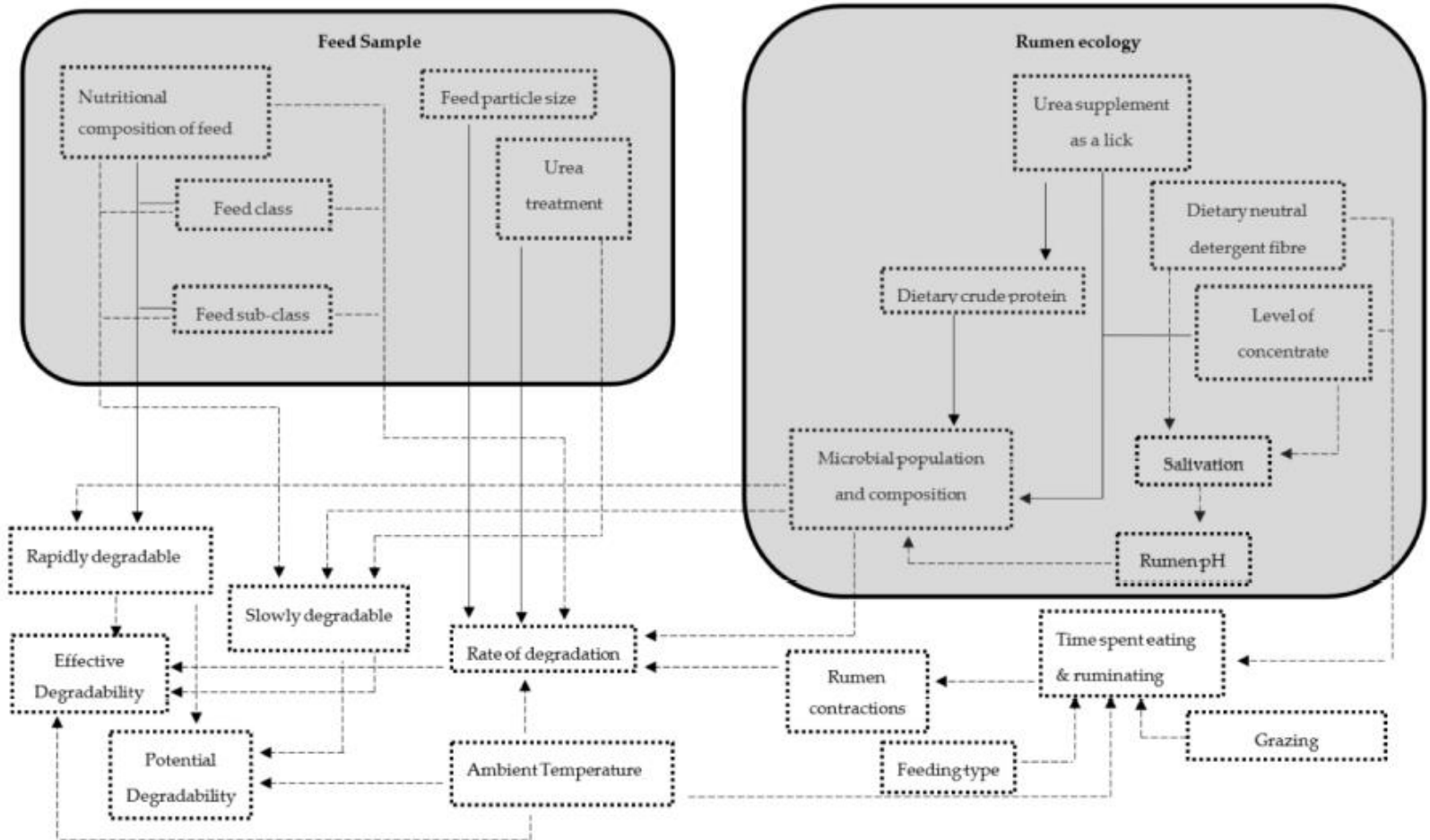
CV, coefficient of variation; SEM, standard error of the mean; SD, standard deviation.

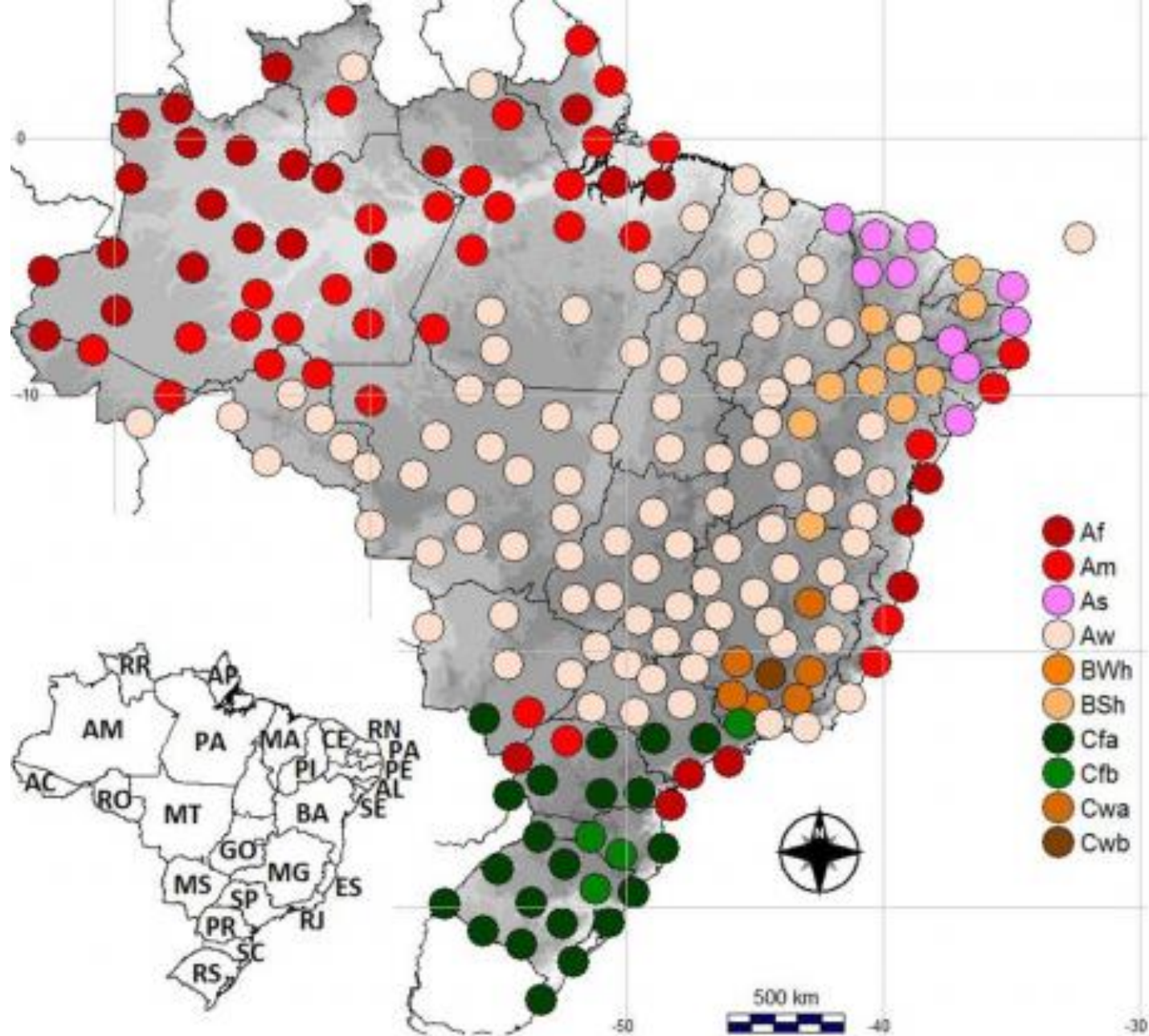
Article
Consequences of Increases in Ambient Temperature and Effect of Climate Type on Digestibility of Forages by Ruminants: A Meta-Analysis in Relation to Global Warming

Mehluli Moyo and Ignatius Nsahlai *

Animals 2021, 11, 172. <https://doi.org/10.3390/ani11010172>

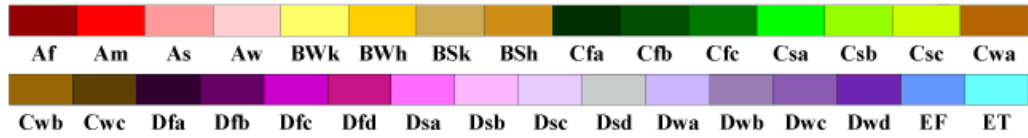
<https://www.mdpi.com/journal/animals>





World Map of Köppen–Geiger Climate Classification

updated with CRU TS 2.1 temperature and VASclimO v1.1 precipitation data 1951 to 2000



Main climates

- A: equatorial
- B: arid
- C: warm temperate
- D: snow
- E: polar

Precipitation

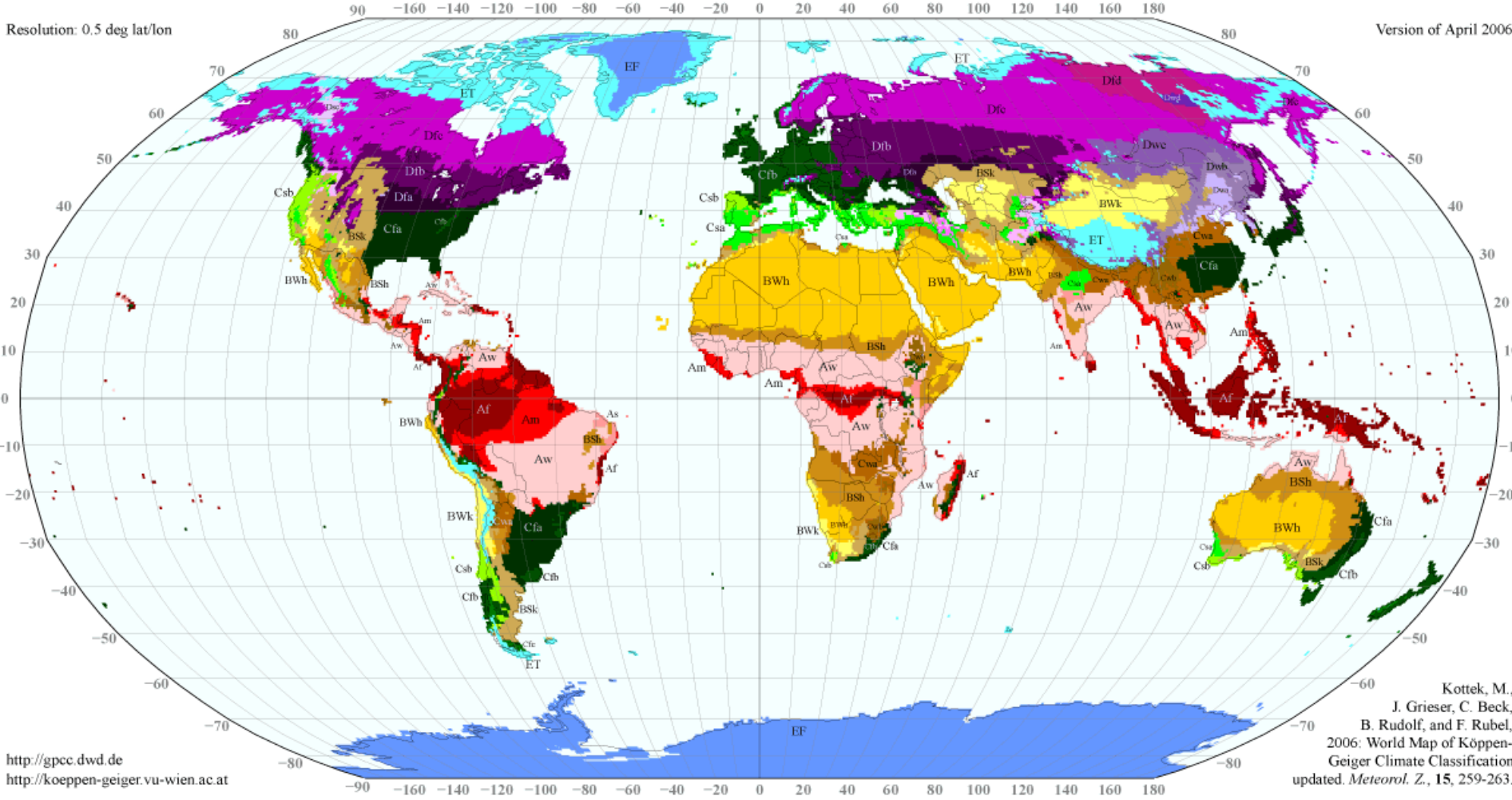
- W: desert
- S: steppe
- f: fully humid
- s: summer dry
- w: winter dry
- m: monsoonal

Temperature

- h: hot arid
- k: cold arid
- a: hot summer
- b: warm summer
- c: cool summer
- d: extremely continental
- F: polar frost
- T: polar tundra

Resolution: 0.5 deg lat/lon

Version of April 2006



<http://gpcc.dwd.de>
<http://koeppen-geiger.vu-wien.ac.at>

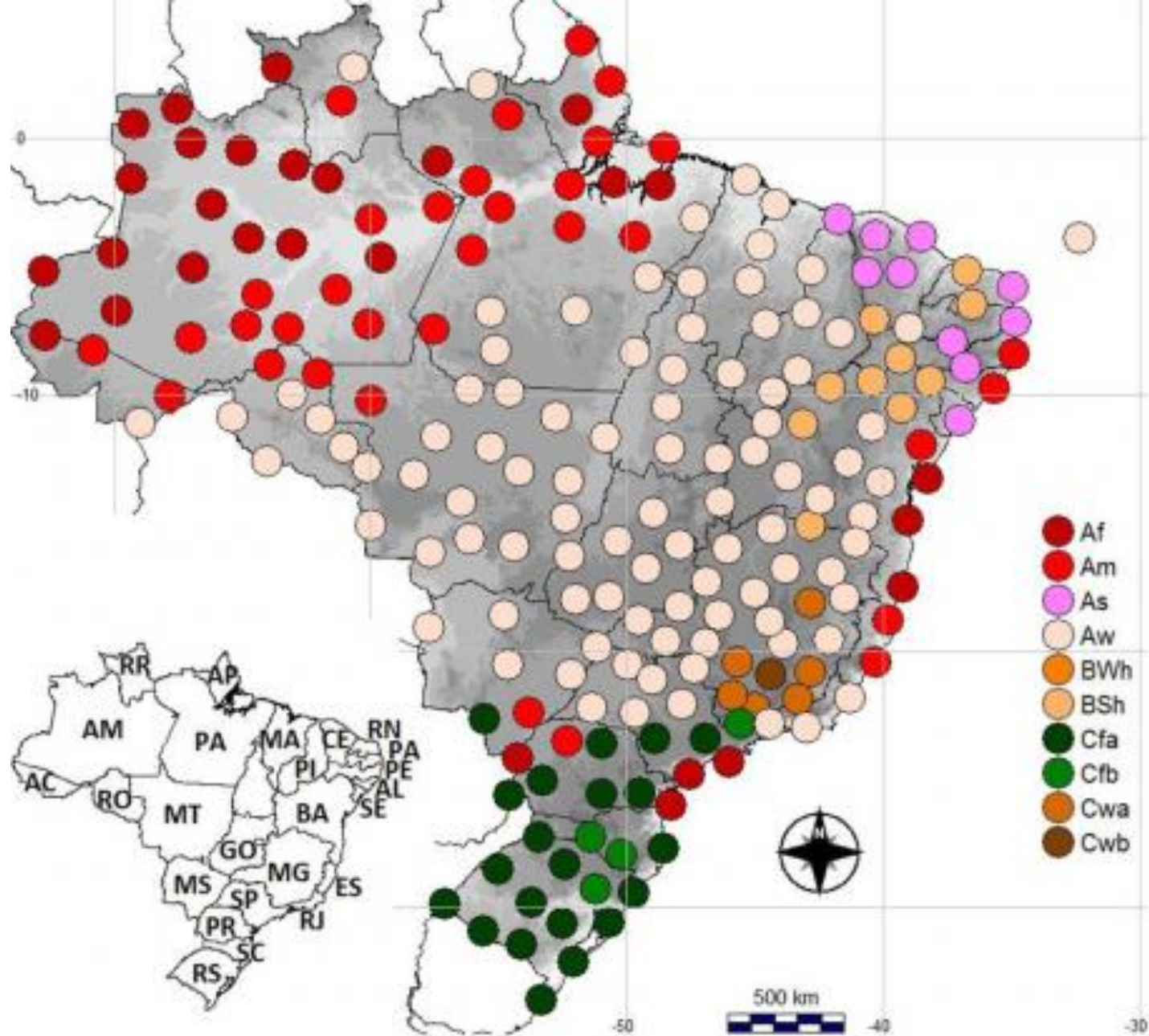
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 B. Rudolf, and F. Rubel,
 2006: World Map of Köppen–
 Geiger Climate Classification
 updated. *Meteorol. Z.*, 15, 259-263.

Table 3. Effects of feed sample and diet properties, ambient temperature, ruminant type, feeding type, and climatic region on rumen degradation of feeds.

Test of Fixed Effects	Degradation Parameter Estimates (Mean ± SE)				
	a (g/kg)	b (g/kg)	c (per h)	PD (g/kg)	Lag (h)
Effect of Feed Type					
Roughages	212 ± 53.68	491 ± 99.24	0.046 ± 0.0072	697 ± 56.9	2.78 ± 4.311
Concentrates	237 ± 60.57	538 ± 99.84	0.080 ± 0.0088	780 ± 60.4	0.96 ± 4.411
Mixed diets	199 ± 103.76	634 ± 190.80	0.051 ± 0.0154	833 ± 103.2	0.00 ± 6.62
Significance	***	***	*	**	NS
Effect of climatic region					
Tropical climates					
Af	92 ± 326.81	619 ± 731.79	0.029 ± 0.0281	643 ± 128.8	6.07 ± 6.098
Aw	200 ± 90.55	466 ± 195.93	0.039 ± 0.0161	666 ± 87.1	1.54 ± 5.57
Arid climates					
BSh	176 ± 96.07	404 ± 201.34	0.037 ± 0.0180	581 ± 102.2	-
BSk	160 ± 95.27	537 ± 196.73	0.068 ± 0.0210	697 ± 93.7	5.13 ± 5.593
BWh	151 ± 484.22	358 ± 718.25	0.052 ± 0.0444	606 ± 203.4	-
Temperate climates					
Cfa	266 ± 92.72	476 ± 223.75	0.051 ± 0.0154	697 ± 87.5	0.62 ± 5.253
Cfb	258 ± 70.82	535 ± 162.21	0.052 ± 0.0143	792 ± 77.9	2.79 ± 6.03
Csa	288 ± 89.65	437 ± 176.15	0.055 ± 0.0189	725 ± 94.1	2.60 ± 5.723
Csb	231 ± 135.18	491 ± 274.70	0.056 ± 0.0319	722 ± 121.6	-
Cwa	247 ± 103.03	476 ± 240.21	0.064 ± 0.0207	722 ± 117.8	0.58 ± 5.828
Cwb	185 ± 82.56	533 ± 159.05	0.036 ± 0.0134	715 ± 77.7	2.71 ± 5.60
Cold climates					
Dfa	241 ± 183.70	447 ± 266.03	0.080 ± 0.0271	688 ± 112.7	1.31 ± 5.956
Dfb	120 ± 141.29	615 ± 478.79	0.234 ± 0.0511	735 ± 115.0	2.03 ± 5.840
Dfc	98 ± 333.95	624 ± 609.91	0.027 ± 0.0426	722 ± 210.8	-
Dsb	213 ± 100.76	478 ± 233.82	0.032 ± 0.0274	691 ± 150.8	-
Significance	NS	NS	NS	NS	***
Effect of climate type					
Tropical	193 ± 63.90	476 ± 147.93	0.038 ± 0.0113	664 ± 54.105	2.62 ± 1.828
Arid	166 ± 55.52	439 ± 133.61	0.050 ± 0.0128	621 ± 55.482	3.14 ± 3.200
Temperate	237 ± 27.98	515 ± 69.991	0.048 ± 0.0054	745 ± 25.097	2.19 ± 0.684
Cold	152 ± 64.35	562 ± 164.32	0.103 ± 0.0167	715 ± 62.092	1.85 ± 1.914
Significance	NS	NS	NS	***	NS

Table 8. Effects of feed type, climatic region and ambient temperature on chemical composition of feed samples incubated in the rumen.

Test of Fixed Effects	Chemical Composition Estimates (g/kg DM) (Mean ± SE)					
Effect of Feed Type	DM	CP	NDF	ADF	HEM	Ash
Roughages	822.5 ± 31.30	109.6 ± 8.42	606.5 ± 21.19	395 ± 14.23	211 ± 11.94	90.5 ± 4.58
Concentrates	878.8 ± 38.39	166.7 ± 11.04	355.7 ± 26.32	209 ± 18.15	147 ± 15.06	68.0 ± 5.88
Mixed diets	803.0 ± 63.28	131.9 ± 19.42	590.7 ± 43.67	367 ± 30.96	222 ± 25.39	95.1 ± 10.16
Significance	NS	***	***	***	***	***
Effect of climatic region						
Tropical climates						
Af	806 ± 159.02	83.3 ± 42.56	609 ± 107.82	365 ± 72.17	241 ± 60.67	122.9 ± 23.20
Aw	565 ± 70.91	133.2 ± 19.50	466.0 ± 48.31	297 ± 32.68	168 ± 27.35	88.5 ± 10.54
Arid climates						
BSh	895.5 ± 93.97	112.5 ± 25.69	560.1 ± 63.86	406 ± 43.11	154 ± 36.11	73.4 ± 13.91
BSk	888.4 ± 84.04	85.8 ± 23.87	660.9 ± 57.39	416 ± 39.39	232 ± 32.76	95.9 ± 12.78
BWh	865 ± 129.37	145.1 ± 35.01	481.2 ± 87.81	374 ± 59.04	107 ± 49.53	61.8 ± 19.01
Temperate climates						
Cfa	669.9 ± 72.04	188.1 ± 19.88	517.5 ± 48.96	310 ± 33.19	208 ± 27.75	99.3 ± 10.73
Cfb	781.3 ± 66.44	136.0 ± 18.27	491.6 ± 45.10	300 ± 30.55	193 ± 25.55	68.9 ± 9.87
Csa	772.2 ± 86.29	135.6 ± 23.56	419.7 ± 58.62	275 ± 39.56	144 ± 33.14	91.9 ± 12.76
Csb	493 ± 131.79	153.0 ± 36.01	400.4 ± 90.22	222 ± 60.67	177 ± 50.91	101.7 ± 19.55
Cwa	921 ± 91.90	123.7 ± 24.97	531.0 ± 62.41	315 ± 42.02	215 ± 35.23	70.8 ± 13.54
Cwb	835.3 ± 62.35	126.3 ± 17.13	509.6 ± 42.48	307 ± 28.73	206 ± 24.04	85.4 ± 9.26
Cold climates						
Dfa	884 ± 137.52	201.4 ± 37.25	420.8 ± 93.28	247 ± 62.76	176 ± 52.64	72.4 ± 20.22
Dfb	913 ± 137.48	187.5 ± 37.09	507.0 ± 93.19	273 ± 62.60	235 ± 52.54	48.9 ± 20.16
Dfc	890 ± 232.82	89.7 ± 62.30	657.0 ± 157.7	428 ± 105.6	231 ± 88.77	109.6 ± 33.96
Dsb	928 ± 158.12	139.8 ± 42.50	533 ± 107.19	321 ± 71.88	214 ± 60.38	76.7 ± 23.13
Significance	**	NS	NS	NS	NS	NS
Effect of covariates						
Feed sample						
Silage	***	NS	*	NS	***	*
Urea treatment	NS	NS	NS	*	*	NS
Environmental factors						
Ambient temperature	*	NS	NS	NS	NS	NS
Test of random effects						
Study × incubation time	***	***	***	***	***	***



Test of Fixed Effects	Degradation Parameter Estimates (Mean ± SE)				
	a (g/kg)	b (g/kg)	c (per h)	PD (g/kg)	Lag (h)
Effect of feeding type					
Grazers	310 ± 57.92	506 ± 107.95	0.050 ± 0.0084	711 ± 38.1	2.34 ± 2.112
Intermediate feeders	209 ± 67.14	413 ± 113.16	0.045 ± 0.0084	723 ± 104.0	0.65 ± 9.708
Significance	NS	NS	***	NS	NS
Effect of ruminant type					
Buffalo	140 ± 163.43	519 ± 275.95	0.050 ± 0.0175	649 ± 95.64	3.33 ± 2.80
Cattle	140 ± 65.50	520 ± 124.65	0.052 ± 0.0093	726 ± 43.36	1.86 ± 2.487
Goats	307 ± 75.71	414 ± 137.50	0.046 ± 0.0098	722 ± 105.98	0.65 ± 9.789
Sheep	218 ± 68.19	492 ± 133.48	0.048 ± 0.0098	703 ± 48.64	2.67 ± 2.472
Significance	NS	NS	***	NS	*

SUMMARY

- 1°C decreased *in situ* PD fraction by : 0,39% roughage, 0,76% concentrate and 2,41% mixed diets
- 1°C increased *in situ* “b” fraction by 0,1% roughage, 1,1% concentrate and 2,27% mixed diets
- 1°C increased NDF fraction by 0,4%
- Greater impact over concentrate
- Higher temperature increased NDF and decreased PD, *a* and *b* fractions

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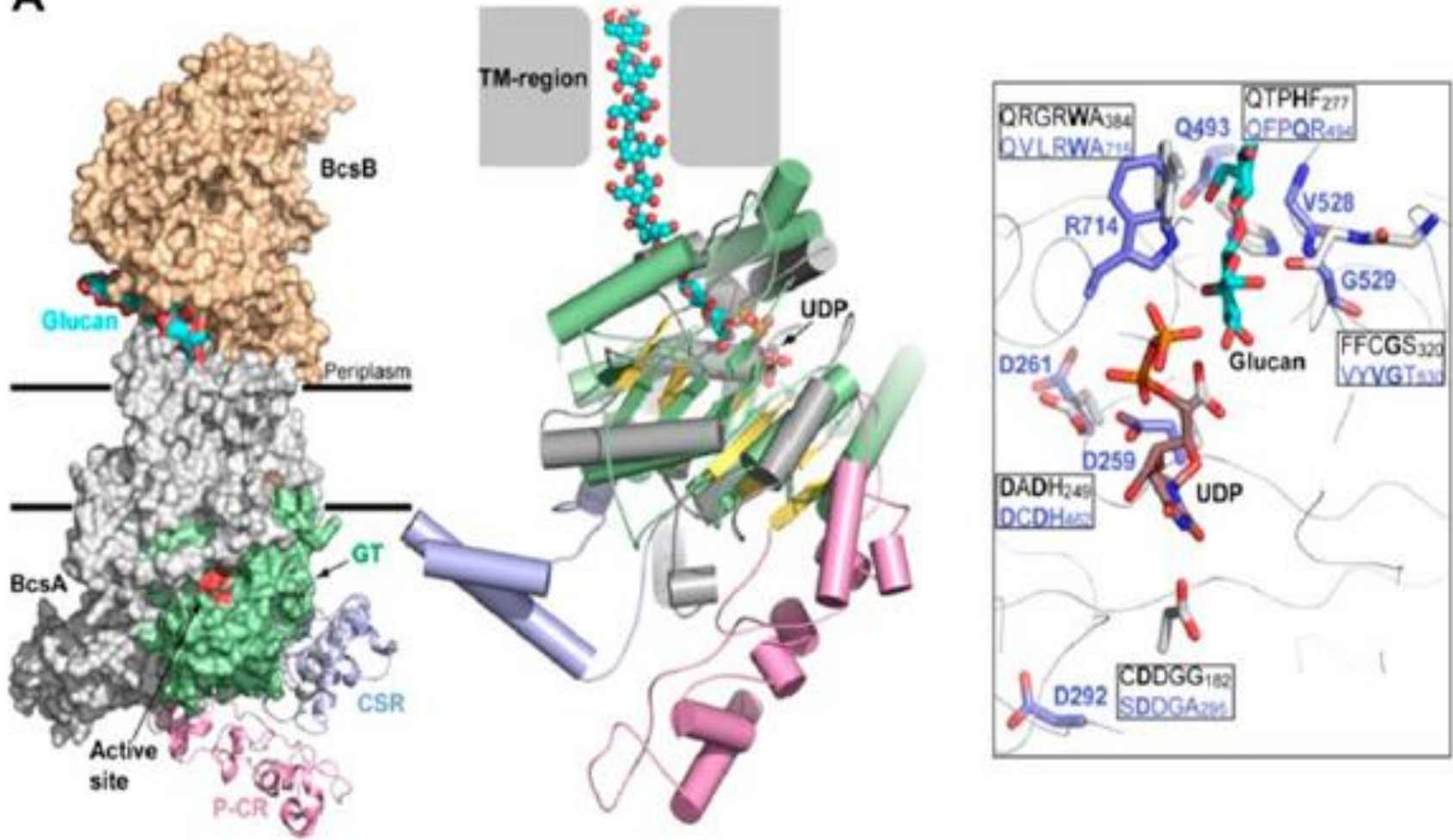
Haluk Ertan^{4,5}

Khawar Sohail Siddiqui⁶

Review

Lignocellulosic biomass: Biosynthesis, degradation, and industrial utilization

A



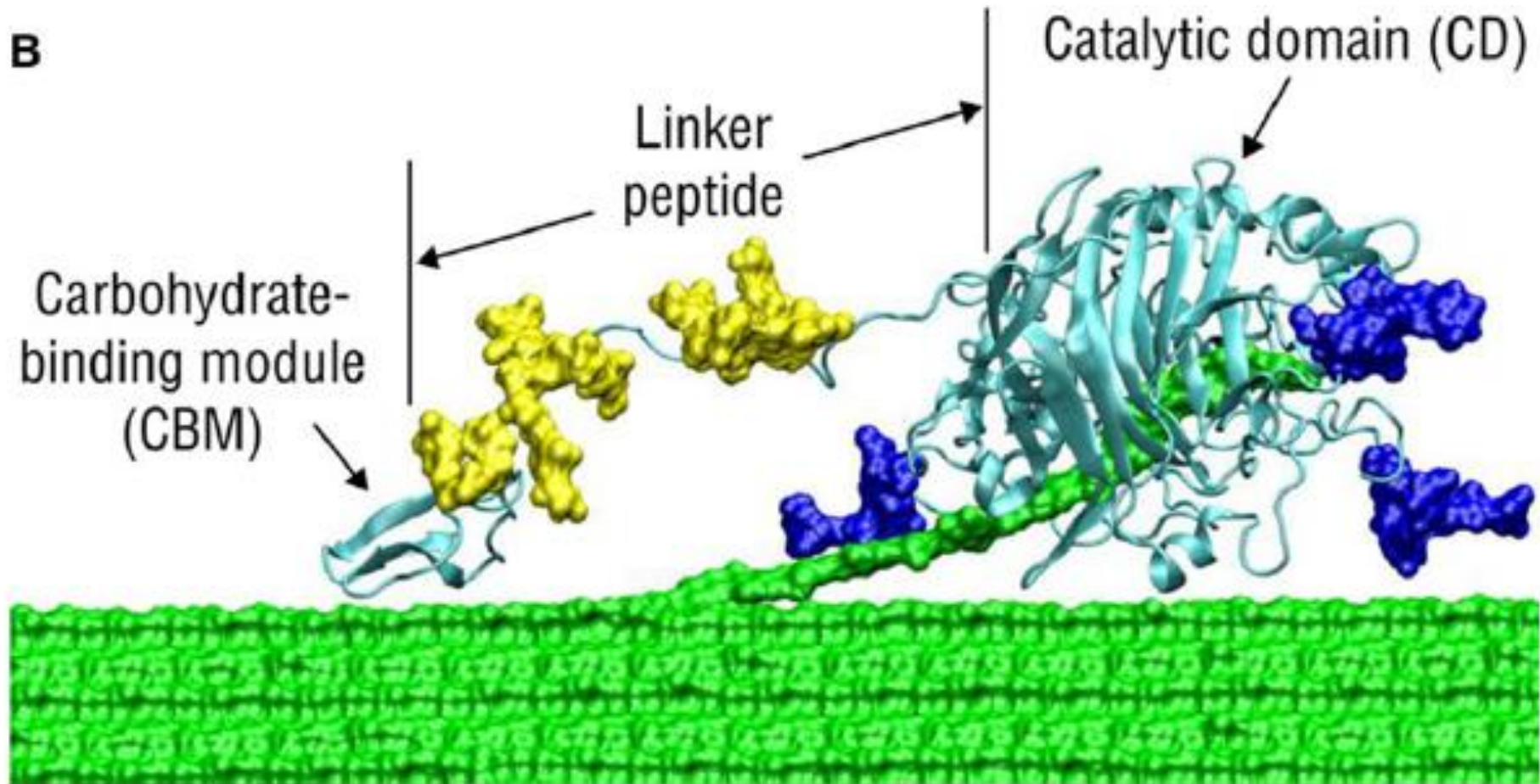


Table 1. Engineering improvements in the catalytic properties of enzymes involved in lignocellulose degradation.

Enzyme: Organism	Modifications	Catalytic properties	Remarks/causes	Reference
Exo-cellulase:				
<i>T. reesei</i> (Cel6A, Cel7A)	All R to E and S to T mutated in the linker region.	Increased hydrolytic activity on lignocellulosic substrate.	Reduced binding of the deglycosylated linker to lignin.	[118]
<i>T. reesei</i> (Cel6A)	Y103, L136, S186, G365, R410 to other residues.	Enhanced cellulose hydrolysis.	2 to 7-folds reduction in glucose inhibition.	[119]
<i>P. funiculosus</i>	A196S	Productivity of cellulose hydrolysis doubled from 12% to 25% after 120 h at 38°C. T_m (67.5→66.5°C)	Additional glycosylation slightly reduced stability but increased activity.	[120]
Endo-cellulase:				
<i>C. thermocellum</i> (Ct)	S329G/S269P/H194G	T_{opt} (75→85°C) $t_{1/2}$ (6→60 min: 86°C) Activity (16→19U mg ⁻¹ : 60°C)	Additional seven electrostatic interactions on the surface of active-site cleft.	[121]
<i>Cryptococcus sp</i>	CBD from <i>T. reesei</i> exo-cellulase fused to the C-terminal end of endo-cellulase from <i>Cryptococcus</i>	Activity: Sigmacell (0→2.3 U mg ⁻¹) Activity: Avicel (0→0.5 U mg ⁻¹)	Activity on insoluble cellulosic substrates was dramatically enhanced due to increased binding of EG to the substrate.	[122]
<i>B. amyloliquefaciens</i>	E289V	Activity (0.7→5.6 U mg ⁻¹ : 50°C)	Eight-folds activity increased with a single amino acid change in the catalytic domain.	[123]
Cel5A from metagenomic library (vermicompost)	Error-prone PCR mutagenesis +	$t_{1/2}$ (1→seven-fold increase: 65°C) Activity, FP (0.4→2.9 U μmol ⁻¹)	The GM endo-cellulase fused with CBM had both seven-fold higher thermostability and activity on crystalline cellulose	[124]
CBD: <i>S. degradans</i>	Fusion of Cel5A with CBD	Activity, Avicel (0.2→1.4 U μmol ⁻¹)		
Engineered cellulase mixture: Endo-cellulase (Cel5A) +	Surface + core mutations that retained 100% activity and imparted thermostability by a combination of chimera-formation, foldX, consensus, α-helical dipole, backbone stability and improved core packing mechanisms.	Opt-Cel5A: T_{opt} (64→81°C) Activity on Avicel (1.5-fold higher) 3C6P-Cel6A: T_{opt} (65→75°C)	Individually engineered endo- and exo-cellulases showed higher activity and stability.	[116]
Exo-cellulases (Cel6A) +		Activity on Avicel (1.6-fold higher) TS8-Cel7A: T_{opt} (55→65°C) Activity on Avicel (1.5-fold excess)	T-PRIMED, showed three-folds higher productivity on crystalline cellulose relative to wild-type mix at 70°C due to synergistic activation between endo-and exo-cellulases.	
Exo-cellulase (Cel7A) (<i>T. reesei</i>)		T-PRIMED (mix of three engineered cellulases) showed 2.5 to 3-fold higher productivity on avicel and rice-straw, respectively, at 70°C.		
β-glucosidase: <i>T. fusca</i> and <i>P. polymyxa</i>	Combination of gene shuffling from <i>T. fusca</i> and <i>P. polymyxa</i> , site-saturation and site-directed mutagenesis. L444Y/G447S/A433V	Both activity and stability were enhanced simultaneously. $t_{1/2}$ (12→1732 min: 61°C) k_{cat} (2.8→5.4 s ⁻¹ : 37°C)	L444Y/G447S confers extra H-bonding. A433V results in an increased sidechain volume, increased hydrophobicity and packing efficiency. Reduced distance (5.84-5.29 Å) between V433 and catalytic Glu388.	[117]

(Continued)

Effects of addition of different sources and doses of sugars on *in vitro* digestibilities of dry matter, fibre and cell wall monosaccharides of corn silage in ruminants


F. P. Campos^{1†} , L. G. Nussio², P. Sarmiento¹, J. L. P. Daniel³ and C. G. Lima⁴

Table 1 The influence of sugar dose and source on the in vitro digestibility DM (IVDDM), neutral detergent fibre (NDFD) and acid detergent fibre (ADFD) and pH after DM digestion of corn silage and of the substrate (mix)

Sugar source	Sugar dose (g/kg DM)					SE	P-value	
	0	100	200	300	400		L	Q
IVDDM (g/g)¹								
Gluc	0.770 ^a	0.770 ^a	0.757 ^a	0.754 ^a	0.718 ^a	0.01	<0.0001	0.033
Fruc	0.769 ^a	0.758 ^a	0.737 ^{bc}	0.709 ^b	0.643 ^c	0.01	<0.0001	0.0004
Sucr	0.767 ^a	0.735 ^b	0.719 ^b	0.674 ^c	0.644 ^c	0.01	<0.0001	0.581
Arab	0.762 ^a	0.736 ^b	0.741 ^{ab}	0.681 ^c	0.681 ^b	0.01	<0.0001	0.685
Xyl	0.763 ^a	0.731 ^b	0.719 ^c	0.626 ^d	0.610 ^d	0.01	<0.0001	0.216
	0.766	0.746	0.735	0.688	0.659	–	–	–
NDFD (g/g)¹								
Gluc	0.497 ^a	0.498 ^a	0.471 ^a	0.462 ^a	0.385 ^a	0.02	<0.0001	0.069
Fruc	0.499 ^a	0.475 ^{ab}	0.426 ^{ab}	0.367 ^b	0.254 ^{bc}	0.02	<0.0001	0.040
Sucr	0.514 ^a	0.424 ^c	0.390 ^b	0.291 ^c	0.223 ^{bc}	0.02	<0.0001	0.725
Arab	0.455 ^a	0.427 ^{bc}	0.435 ^{ab}	0.347 ^b	0.308 ^b	0.02	<0.0001	0.149
Xyl	0.505 ^a	0.417 ^c	0.389 ^b	0.198 ^d	0.195 ^c	0.02	<0.0001	0.542
	0.494	0.448	0.422	0.333	0.273	–	–	–
ADFD (g/g)²								
Gluc	0.311 ^a	0.299 ^a	0.281 ^a	0.287 ^a	0.223 ^a	0.03	0.0014	0.145
Fruc	0.326 ^a	0.299 ^a	0.286 ^a	0.213 ^b	0.163 ^b	0.03	<0.0001	0.017
Sucr	0.283 ^a	0.262 ^a	0.227 ^b	0.221 ^b	0.156 ^b	0.03	<0.0001	0.099
Arab	0.313 ^a	0.301 ^a	0.284 ^a	0.262 ^a	0.195 ^a	0.03	<0.0001	0.039
Xyl	0.279 ^a	0.291 ^a	0.257 ^{ab}	0.160 ^c	0.139 ^b	0.03	<0.0001	0.006
	0.302	0.290	0.267	0.229	0.175	–	–	–
pH³								
Gluc	6.13 ^a	6.05 ^a	5.93 ^a	5.87 ^{ab}	5.85 ^b	0.001	<0.0001	0.070
Fruc	6.21 ^a	6.06 ^a	5.95 ^{ab}	5.90 ^{bc}	5.91 ^c	0.001	<0.0001	0.0003
Sucr	6.13 ^a	6.05 ^a	6.01 ^{bc}	5.91 ^{bc}	5.86 ^{bc}	0.001	<0.0001	0.943
Arab	6.20 ^a	6.08 ^a	5.92 ^a	5.83 ^a	5.73 ^a	0.001	<0.0001	0.391
Xyl	6.14 ^a	6.08 ^a	6.03 ^c	5.95 ^c	5.91 ^c	0.001	<0.0001	0.713
	6.16	6.06	5.97	5.89	5.85	–	–	–

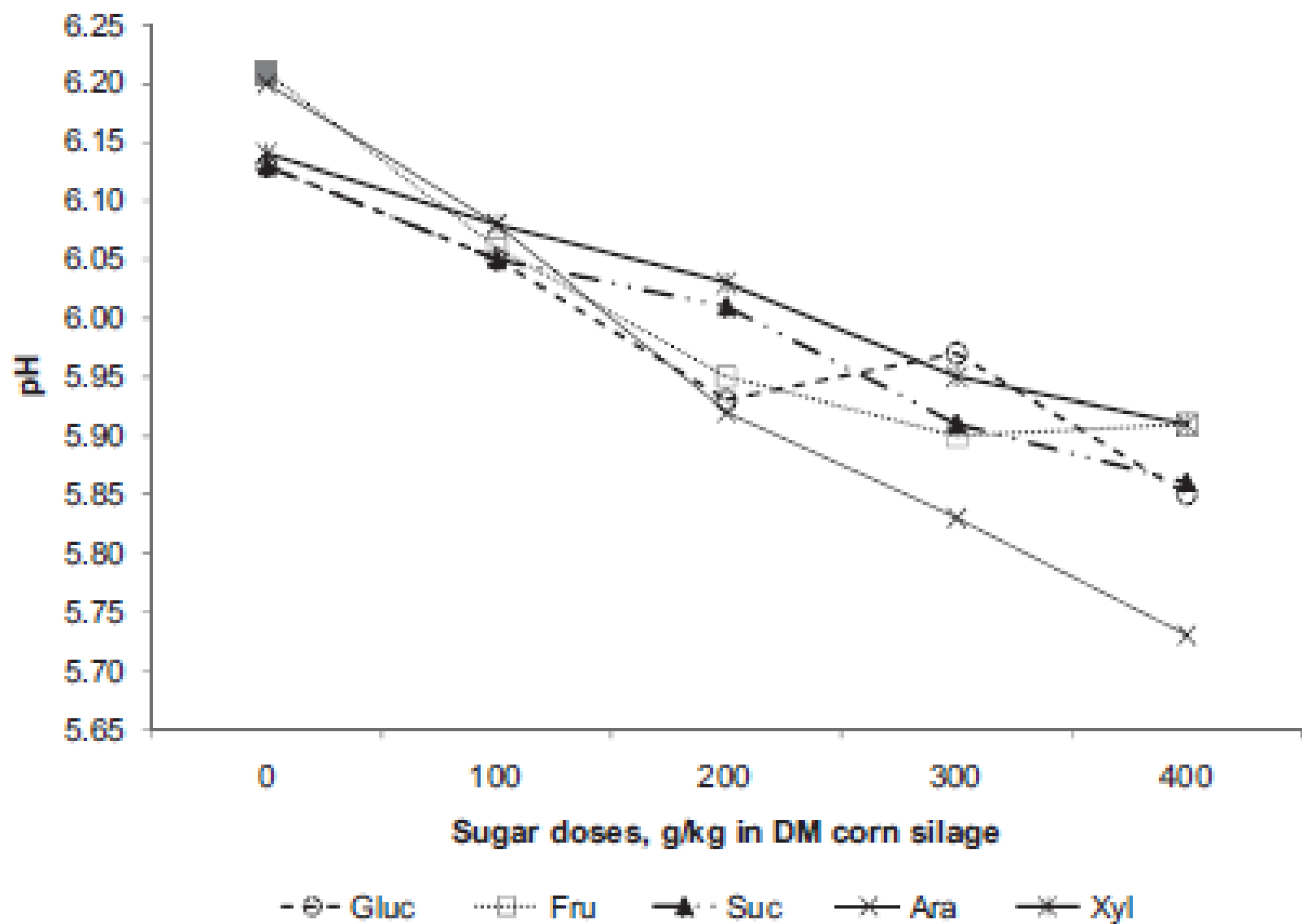


Table 2 The influence of sugar dose and source on the in vitro digestibility cell wall neutral monosaccharides in corn silage and substrate (mix)

Sugar source	Sugar dose (g/kg DM)					SE	P-value	
	0	100	200	300	400		L	Q
Darab^{#1}								
Gluc	0.509	0.318	0.288	0.296	0.231	0.03	–	–
Fruc	0.489	0.331	0.446	0.285	0.203	0.03	–	–
Sucr	0.506	0.308	0.391	0.188	0.184	0.03	–	–
Arab	0.449	0.314	0.302	0.277	0.248	0.03	–	–
Xyl	0.474	0.351	0.348	0.193	0.168	0.03	–	–
	0.485	0.324	0.355	0.248	0.207	–	<0.0001	0.195
Dxyl^{#2}								
Gluc	0.658 ^a	0.662 ^a	0.586 ^a	0.595 ^a	0.486 ^a	0.03	0.0018	0.332
Fruc	0.683 ^a	0.577 ^{ab}	0.531 ^a	0.435 ^{bc}	0.304 ^b	0.03	<0.0001	0.495
Sucr	0.679 ^a	0.522 ^b	0.516 ^a	0.361 ^{cd}	0.287 ^b	0.03	<0.0001	0.913
Arab	0.674 ^a	0.516 ^b	0.540 ^a	0.458 ^b	0.446 ^a	0.03	0.0002	0.213
Xyl	0.666 ^a	0.578 ^{ab}	0.550 ^a	0.280 ^d	0.265 ^b	0.03	<0.0001	0.515
	0.672	0.571	0.545	0.426	0.358	–	–	–
Dgluc^{#3}								
Gluc	0.393 ^a	0.408 ^a	0.413 ^a	0.391 ^a	0.332 ^a	0.02	0.0600	0.037
Fruc	0.429 ^a	0.422 ^a	0.358 ^{bc}	0.330 ^b	0.227 ^b	0.02	<0.0001	0.062
Sucr	0.387 ^a	0.370 ^{ab}	0.309 ^{cd}	0.255 ^c	0.186 ^{bc}	0.02	<0.0001	0.238
Arab	0.365 ^a	0.379 ^a	0.379 ^{ab}	0.282 ^{bc}	0.197 ^{bc}	0.02	<0.0001	0.001
Xyl	0.437 ^a	0.320 ^b	0.290 ^d	0.147 ^d	0.153 ^c	0.02	<0.0001	0.100
	0.402	0.380	0.350	0.281	0.219	–	–	–