

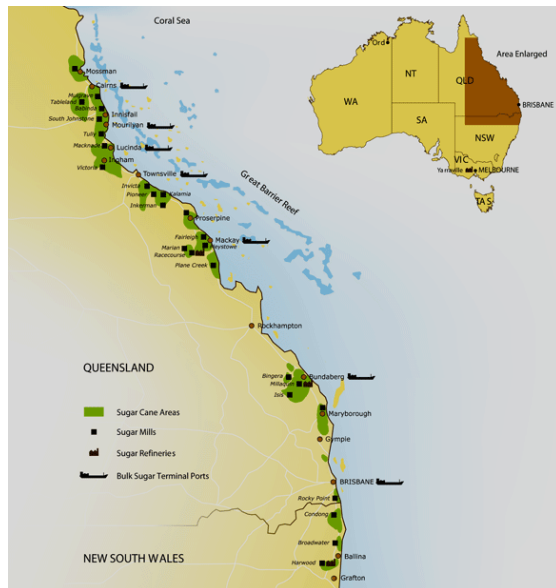
# Preferential co-expression of cellulose synthase subunits with specific COBRA-like proteins within the sugarcane stem

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**15 January 2012**

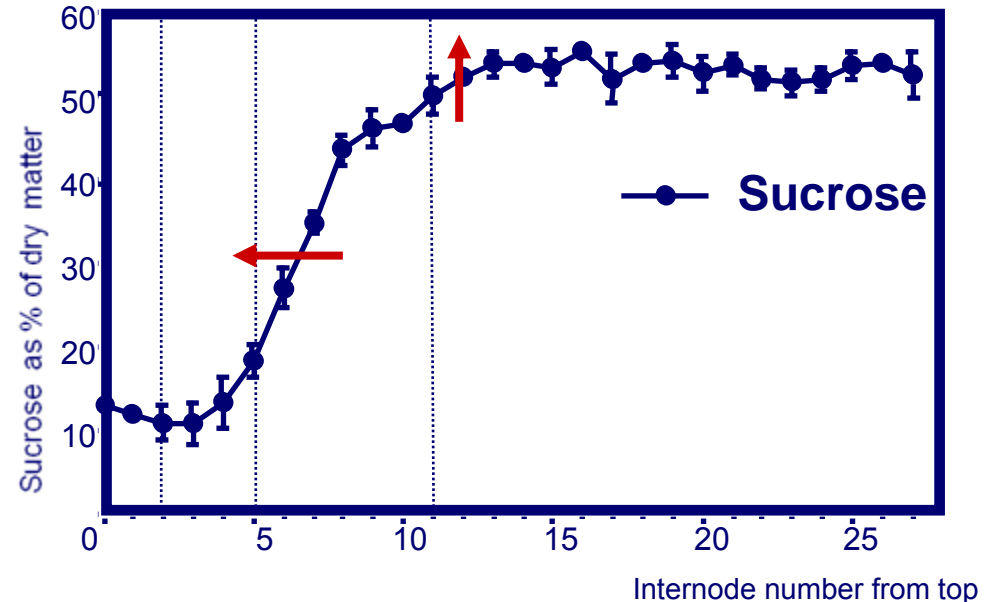


# Australian Sugar Industry

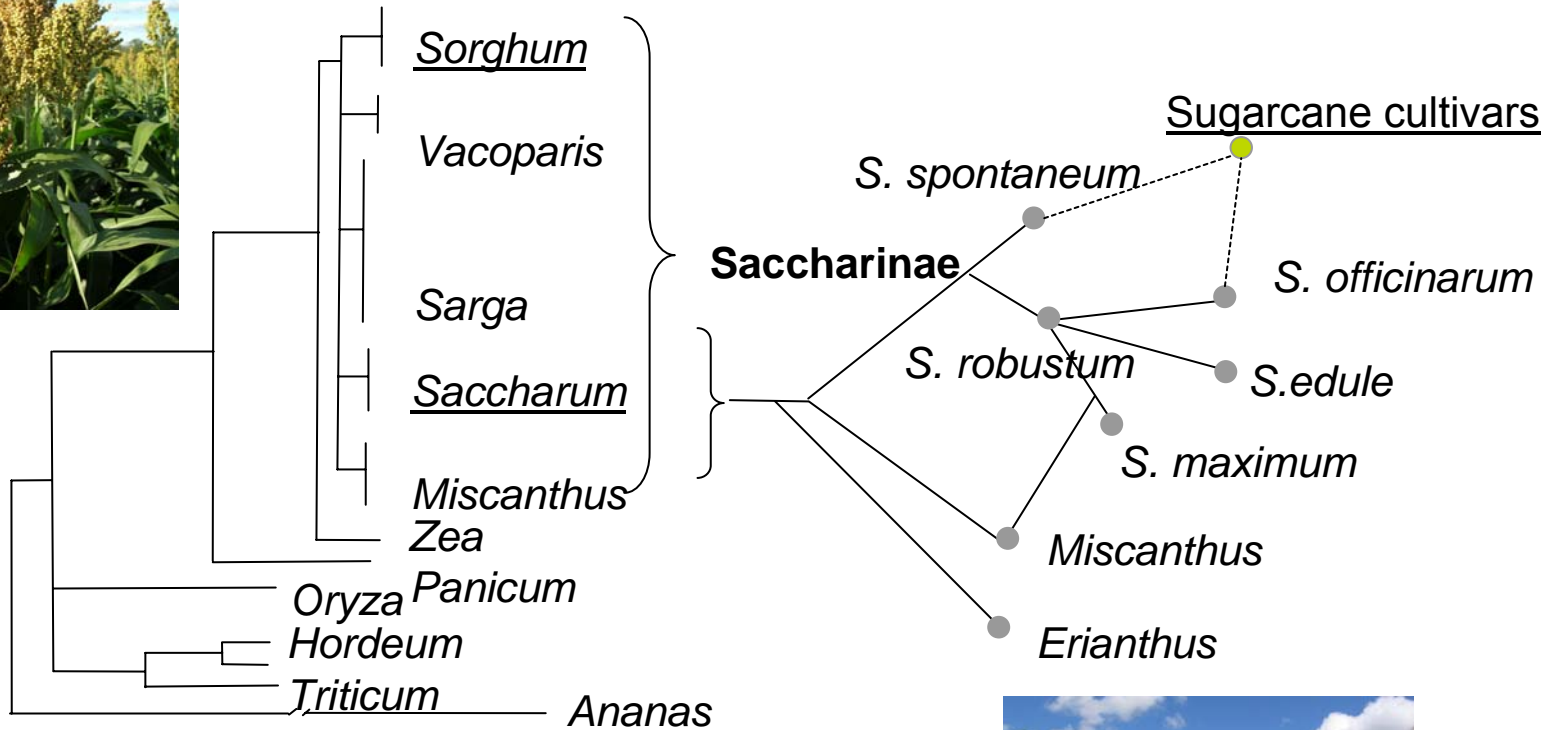
- 6,000 cane growers (4,500 cane farming business operations)
- 35 million tonnes sugarcane (4.75 million tonnes raw sugar)
- 80% exported e.g. Japan, Korea, Malaysia, Taiwan, Saudi Arabia, New Zealand, Canada and USA
- \$1.75 billion to the Australian economy



[Sucrose] increases exponentially down a sugarcane stem during growth and development



# C<sub>4</sub> biomass plants



Adapted from Paterson et al, Nature 2009 & Grivet et al 2006 Darwin's Harvest



# Cell walls

- **Type I cell wall**

- Cells of dicot and non-commelinoid monocot plants e.g. orchids and lilies
- Cellulose and the hemicellulosic polysaccharide, xyloglucan, exist in roughly equal amounts
- Cellulose-xyloglucan framework is embedded in a pectic gel

- **Type II cell wall**

- Cells of commelinoid monocots, including grasses
  - Rice
  - Maize
  - Sorghum
  - Sugarcane
- Glucoronoarabinoxylans or mixed link glucans are the major hemicellulosic polysaccharides
- Low pectin
- Extensive network of phenylpropanoids

# Transcripts of enzymes involved in polysaccharide synthesis and catalysis are relatively abundant in maturing stem

Metabolic class	# of matches in mature stem ESTs
Sucrose synthesis and catalysis	11
Central hexose, pentose and triose phosphate metabolism	76
Sugar transport	32
<b>Polysaccharide synthesis and catalysis</b>	<b>46</b>
Other carbohydrate metabolism pathways	14

Casu et al (2003) Plant Mol Biol 52:371-386

# Enzymes involved in polysaccharide synthesis

Putative Functional Identity (EC #)	# of matches in mature stem ESTs
4-alpha-glucanotransferase (EC 2.4.1.25)	1
beta-amylase (EC 3.2.1.2)	1
<b>beta-glucanase (EC 3.2.1.4)</b>	<b>15</b>
beta-glucosidase (EC 3.2.1.21)	6
<b>cellulose synthase (EC 2.4.1.29)</b>	<b>14</b>
starch branching enzyme (EC 2.4.1.18)	1
starch synthase (EC 2.4.1.21)	1
UDP-glucose dehydrogenase (EC 1.1.1.22)	6
UDP-glucuronyltransferase (EC 2.4.1.17)	1

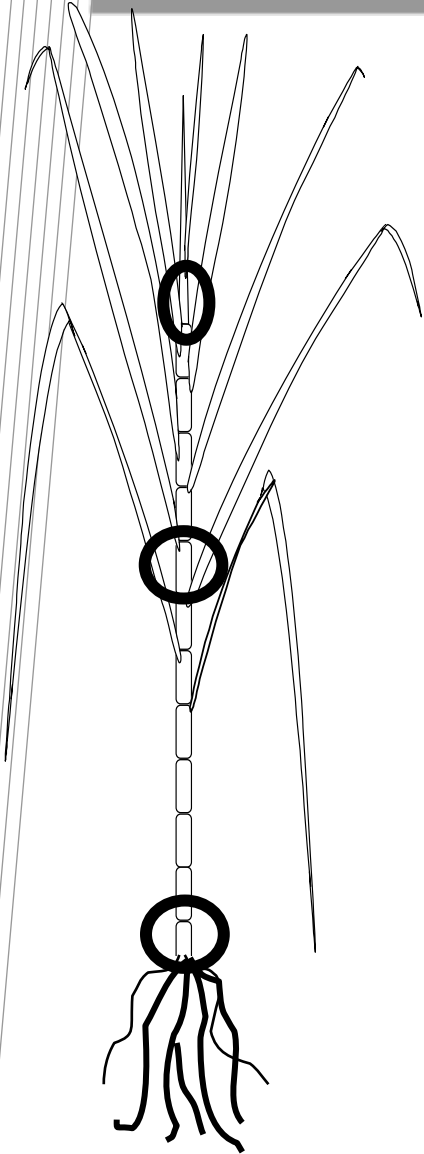


# Carbohydrate metabolism transcripts are differentially expressed in the stem

Examples

Examples

Examples



# CesA and Csl transcripts are differentially expressed between young, maturing and mature sugarcane stem

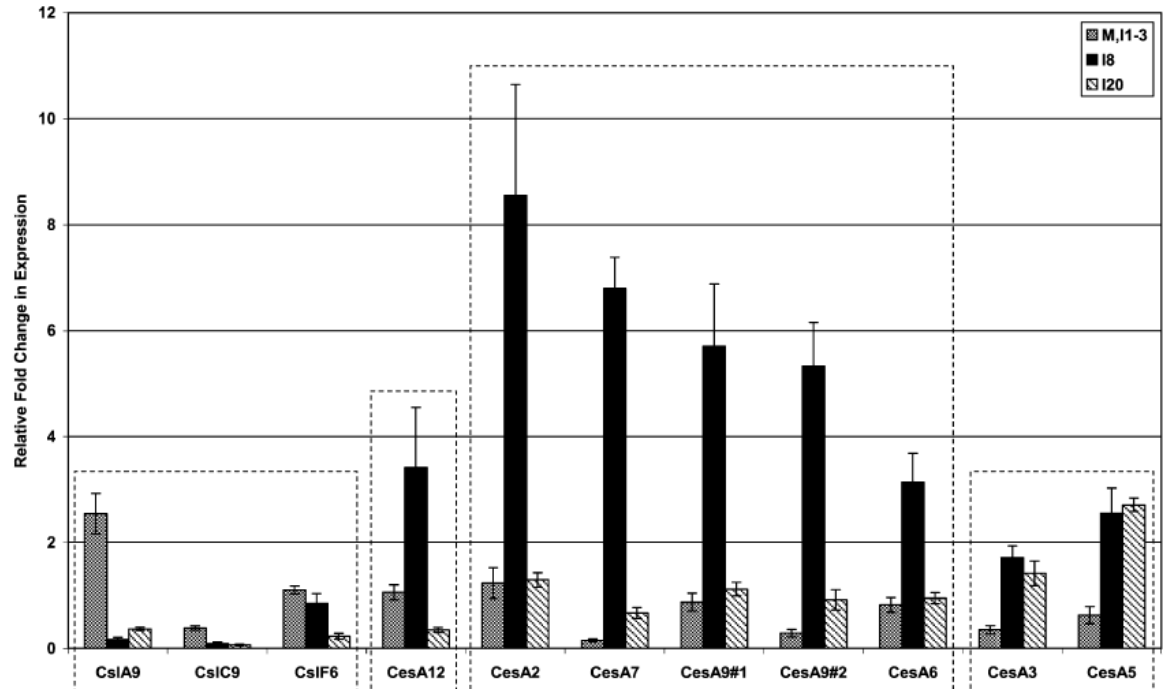
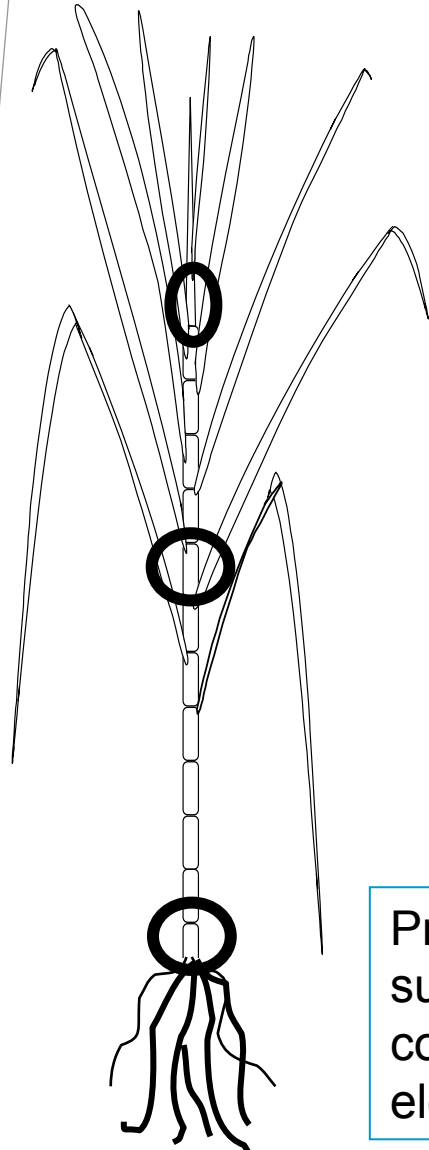


Fig. 3 Relative fold changes in the expression of CesA and Csl transcripts when standardised to that of ShCesA2 in young stem (replicate 1). Error bars Standard error of the mean. Dotted lines indicate groups of transcripts with similar expression patterns. M, I1–3 Young stem, I8: maturing stem, I20 mature stem

Casu et al. 2007, Funct Integr Genom 7:153-167

Primary and secondary cell wall synthesis in the sugarcane stem appears to be a dynamic process that continues throughout the stem even after cell elongation would appear to be finished

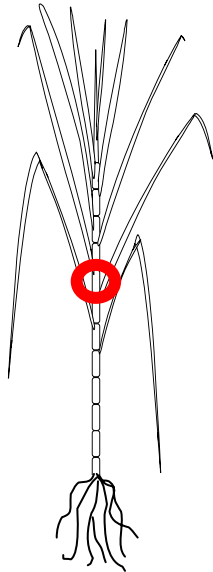


# Aim

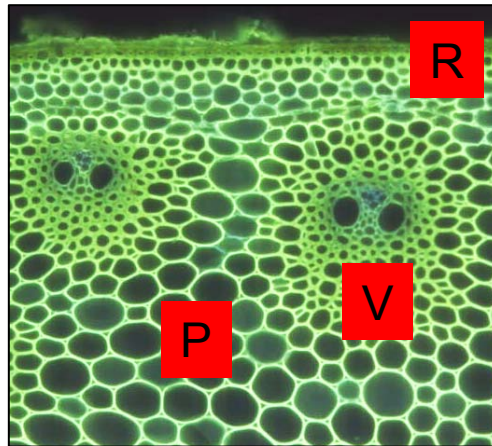
- To identify transcripts that are differentially expressed between the storage parenchyma, vascular bundles and rind of maturing sugarcane stem using large-scale expression profiling
- To gain an increased understanding of the processes undertaken within the maturing sugarcane stem, particularly with regard to sugar and fibre metabolism

# Parenchyma, Vascular bundles and Rind (PVR) expression profiling

Field-grown Q117 plants (11 months old, 3 biological replicates)



Tissue dissected from internode 8



Amplified RNA derived from three biological replicates



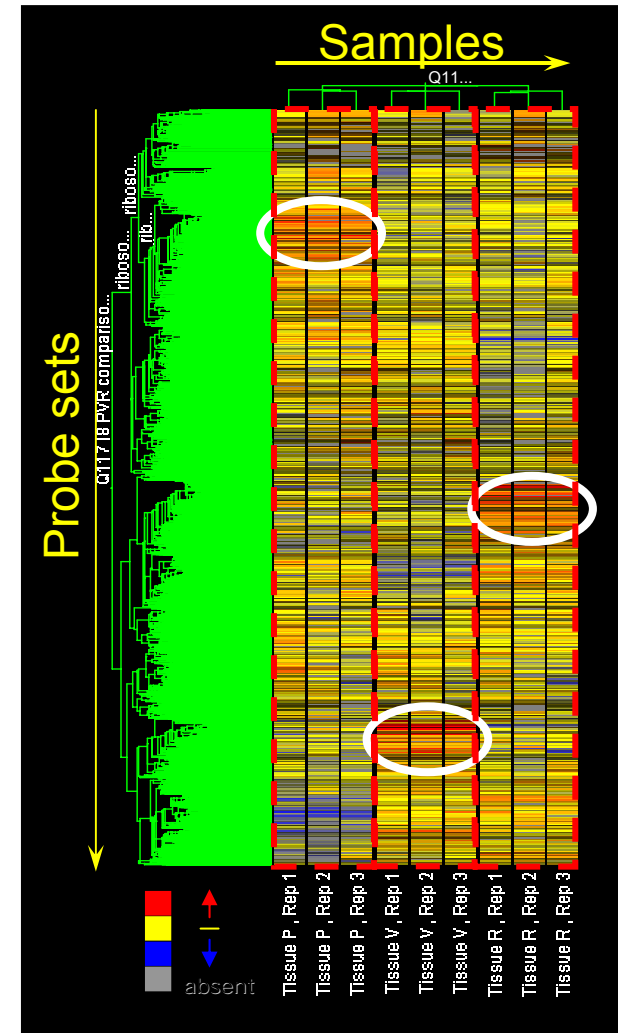
Hybridization to Affymetrix GeneChip® Sugar Cane Genome Arrays (8,236 sugarcane probe sets, 7,505 DNA sequences, ~ 6,500 contigs)



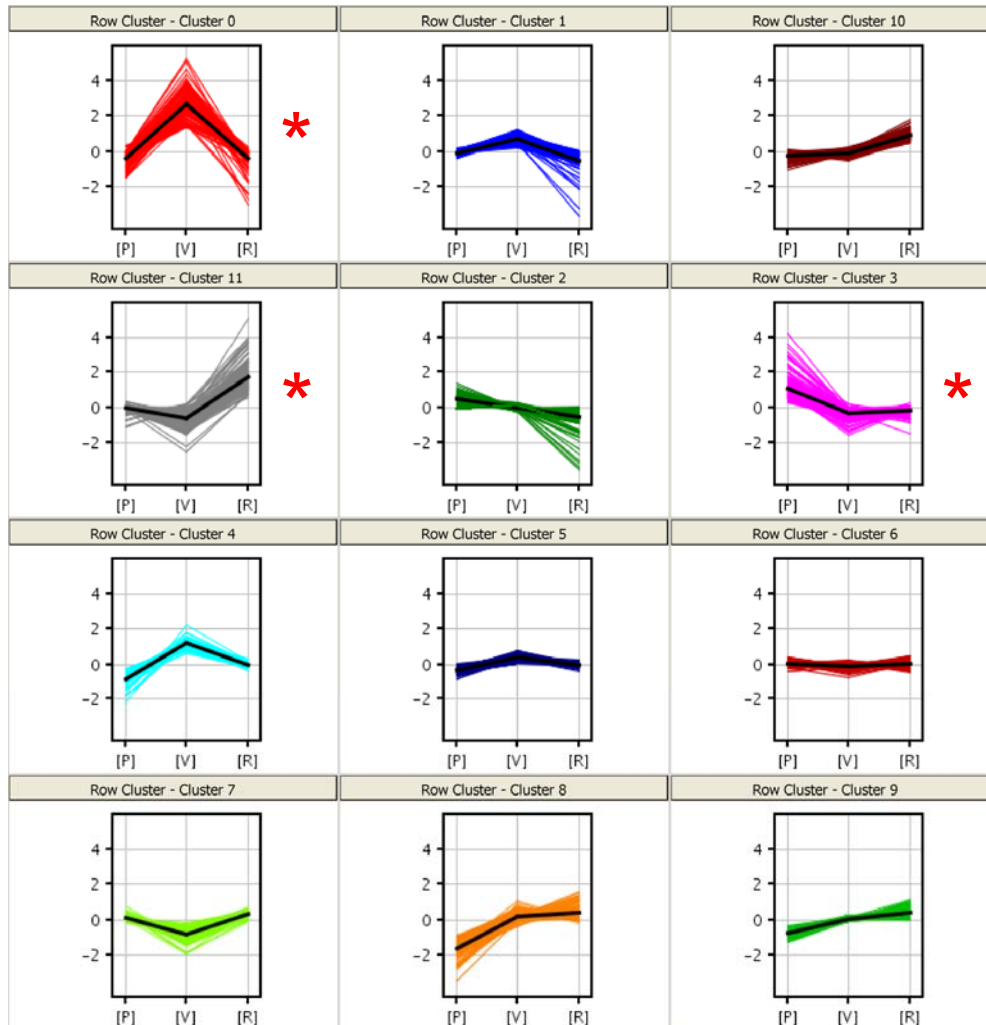
Signal detection

# Analysis of GeneChip Sugarcane Genome Array data

- QA of data
  - Hierarchical clustering of all probe sets to determine data integrity
    - 8,387 probe sets
  - Data filtration
    - 1,904 probe sets
  - Annotations updated
    - Sugarcane Gene Index 3.0 TCs
    - Blast2GO
    - MapMan
- Statistical and other analyses
  - One-way ANOVA unequal variance test
    - 1,296 probe sets were identified as being significantly differentially regulated between P, V and R
  - Identification of significant groups of differentially expressed transcripts
    - Cluster analysis using SOM
    - GO term enrichment
    - Network analysis



# Significantly differentially regulated probe sets between P, V and R can be separated easily by 3x4 SOM



Selected for further analysis:  
**Cluster 0:** 80 probe sets  
**Cluster 3:** 133 probe sets  
**Cluster 11:** 119 probe sets

# Major metabolic categories are differentially expressed across P, V and R

- **SOM cluster 3 – high in P**
  - Amino acid metabolism
  - **Cell wall**
  - Protein synthesis
  - RNA metabolism
  - Sugar transport
  - TCA cycle
- **SOM cluster 0 – high in V**
  - No major functional categories apparent
  - Probe sets corresponding to proteins that had no assigned function or an unknown function accounted for 62 of the 80 probe sets in this cluster
  - One probe set corresponding to sugar transport noted
- **SOM cluster 11 – high in R**
  - Photosynthesis (> 33% of probe sets)
  - **Cell wall**
  - Lipid metabolism
  - Protein synthesis

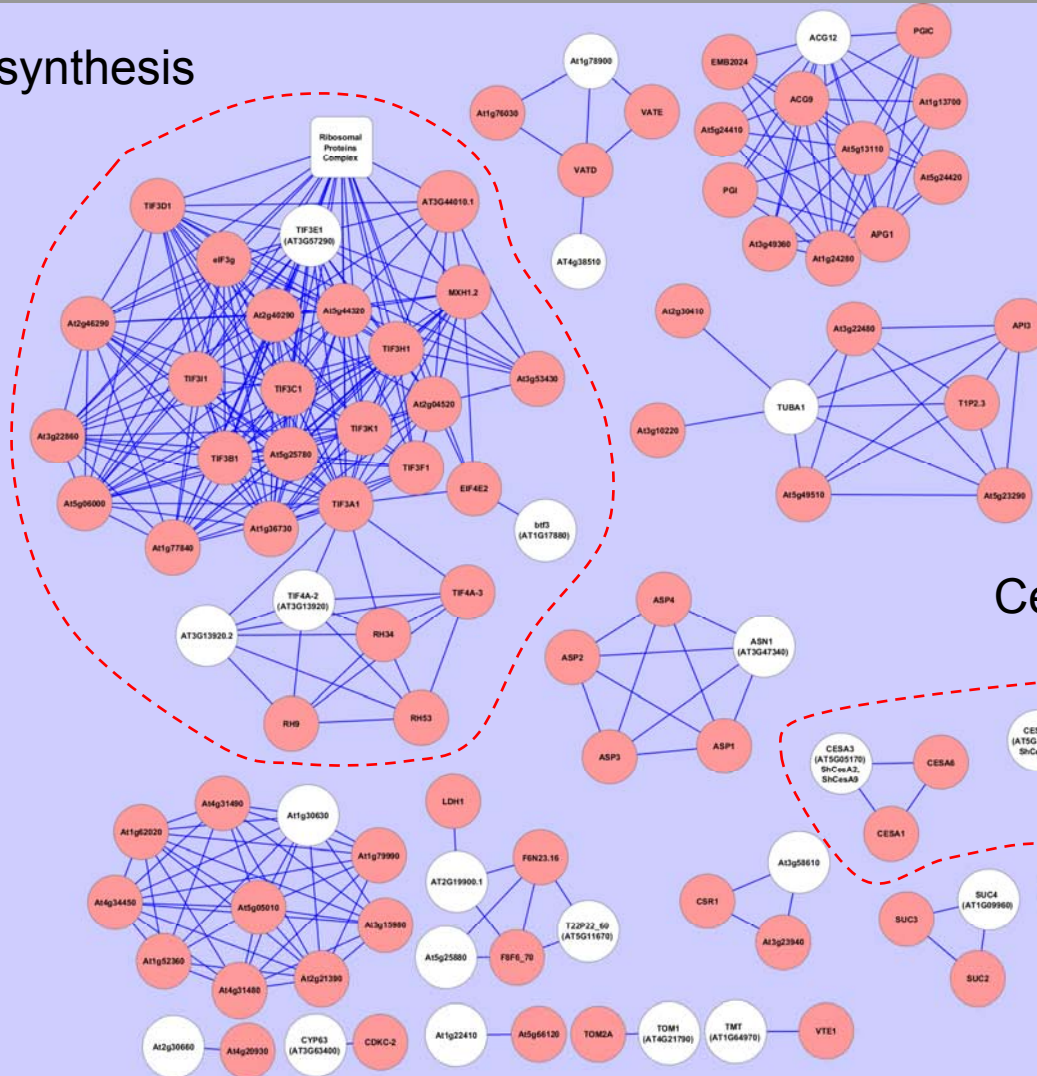
# GO terms related to protein synthesis and cell wall metabolism are enriched in P, and photosynthesis in R

Test-Set TCs (cluster)	GO Term	Name	Type
3	GO:0042254	<b>ribosome biogenesis</b>	P
	GO:0003735	<b>structural constituent of ribosome</b>	F
	GO:0006412	<b>translation</b>	P
	GO:0004473	malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) activity	F
	GO:0007047	<b>cellular cell wall organization</b>	P
	GO:0030244	<b>cellulose biosynthetic process</b>	P
	GO:0006108	malate metabolic process	P
	GO:0015935	small ribosomal subunit	C
	GO:0006011	<b>UDP-glucose metabolic process</b>	P
	GO:0016760	<b>cellulose synthase (UDP-forming) activity</b>	F
0	GO:0016671	oxidoreductase activity, acting on sulfur group of donors, disulfide as acceptor	F
	GO:0010188	response to microbial phytotoxin	P
11	GO:0009535	<b>chloroplast thylakoid membrane</b>	C
	GO:0016021	integral to membrane	C
	GO:0009539	<b>photosystem II reaction center</b>	C
	GO:0016168	<b>chlorophyll binding</b>	F
	GO:0018298	<b>protein-chromophore linkage</b>	P
	GO:0048038	<b>quinone binding</b>	F
	GO:0030076	<b>light-harvesting complex</b>	C
	GO:0006744	ubiquinone biosynthetic process	P
	GO:0008137	NADH dehydrogenase (ubiquinone) activity	F
	GO:0006120	mitochondrial electron transport, NADH to ubiquinone	P
	GO:0006814	sodium ion transport	P
	GO:0019253	reductive pentose-phosphate cycle	P
	GO:0009654	oxygen evolving complex	C
	GO:0045156	<b>electron transporter, transferring electrons within the cyclic electron transport pathway of photosynthesis activity</b>	F

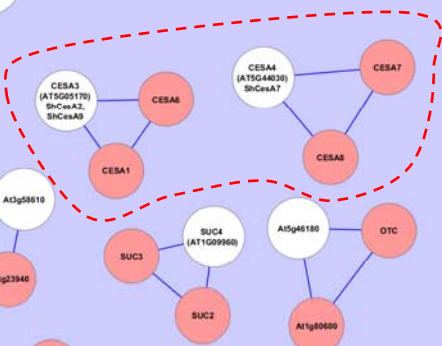


# P – STRING network analysis

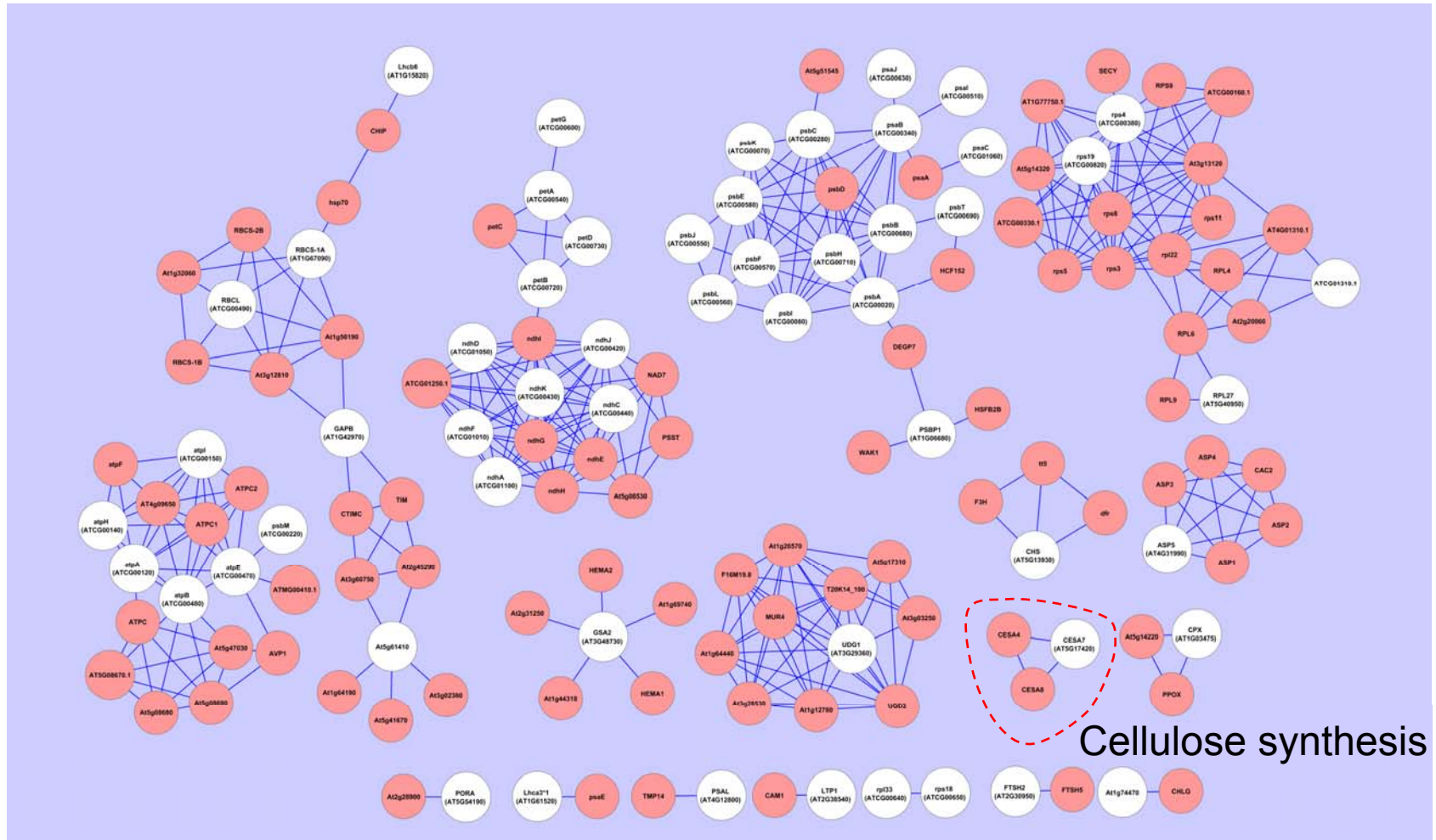
## Protein synthesis



## Cellulose synthesis



# R – STRING network analysis



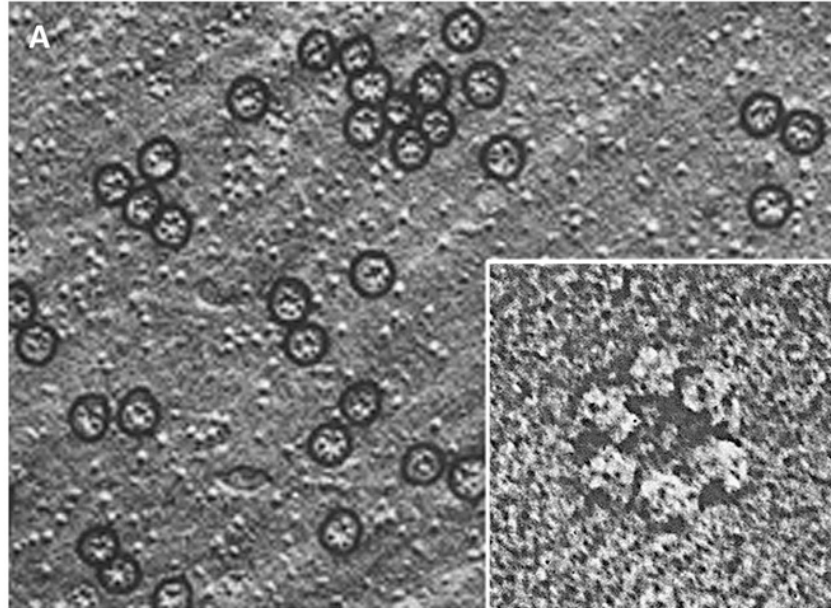
# Cell wall-related proteins are preferentially co-expressed in storage parenchyma and rind

- 'Cell wall' (MapMan bin name or Blast2GO GO term)
  - 18 TCs were up-regulated in storage parenchyma
    - cellulose synthase subunits ShCesA2 ShCesA7 and ShCesA9
    - cellulose synthase-like protein ShCsIF6
    - COBRA-like protein Shbk2I3
    - endo-1,4-beta-D-glucanase
    - Ketol-acid reductoisomerase
    - purple acid phosphatase
    - alpha-glucan-protein synthase
  - 15 TCs were up-regulated in rind
    - cellulose synthase subunit ShCesA12
    - cellulose synthase-like protein ShCsID2
    - COBRA-like protein Shbk-2
    - xyloglucan endo-beta-1,4-glucanase
    - UDP-glucose 6-dehydrogenase
    - dTDP-glucose 4,6-dehydratase
    - catalase

# Cellulose synthesis

- Cellulose is a para-crystalline array of about two to three dozen  $\beta$ -(1,4)-D-glucan chains
- The microfibrils are synthesized at the plasma membrane by terminal complexes of six-membered “particle rosettes” that produce a single microfibril
- Each of the six components of the particle rosette is expected to synthesize four to six of the glucan chains, and 24 to 36 chains are then assembled into a functional microfibril
- The cellulose synthase (CesA) genes are believed to encode the catalytic subunits of the rosette or terminal complex that is located at the plasma membrane





## Particle rosette structures associated with cellulose synthesis in angiosperms

A, Freeze-etch images of the P-face of the plasma membrane showing clusters of rosettes associated with the developing of secondary wall spiral thickenings of a *Lepidium* tracheary element (from Herth, 1985).

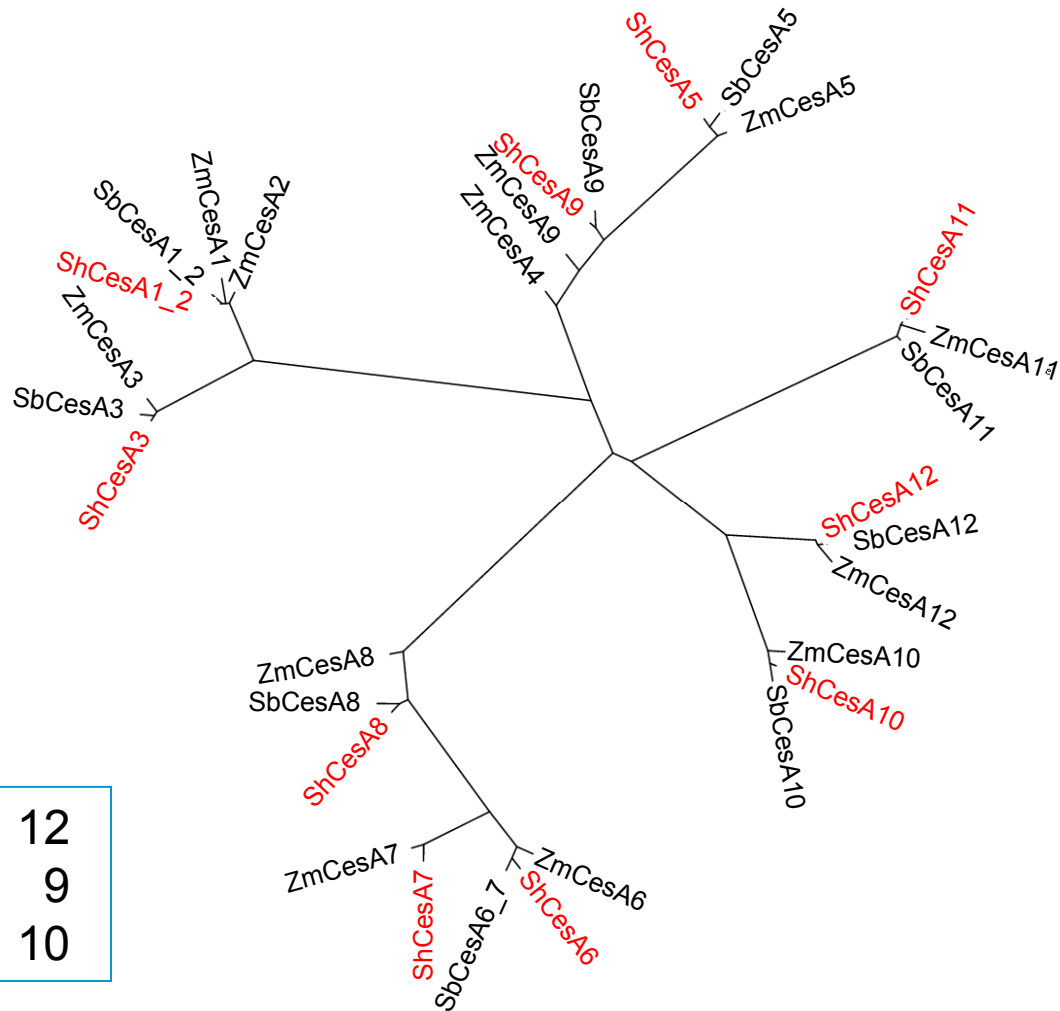
The inset shows the 6-fold symmetry of a single particle rosette from a *Zinnia* tracheary element enveloping in vitro (from C. Haigler, unpublished data, as seen in Delmer, 1999) (from Carpita NC (2011) *Plant Physiol* 155:171-184)

# Cellulose synthesis

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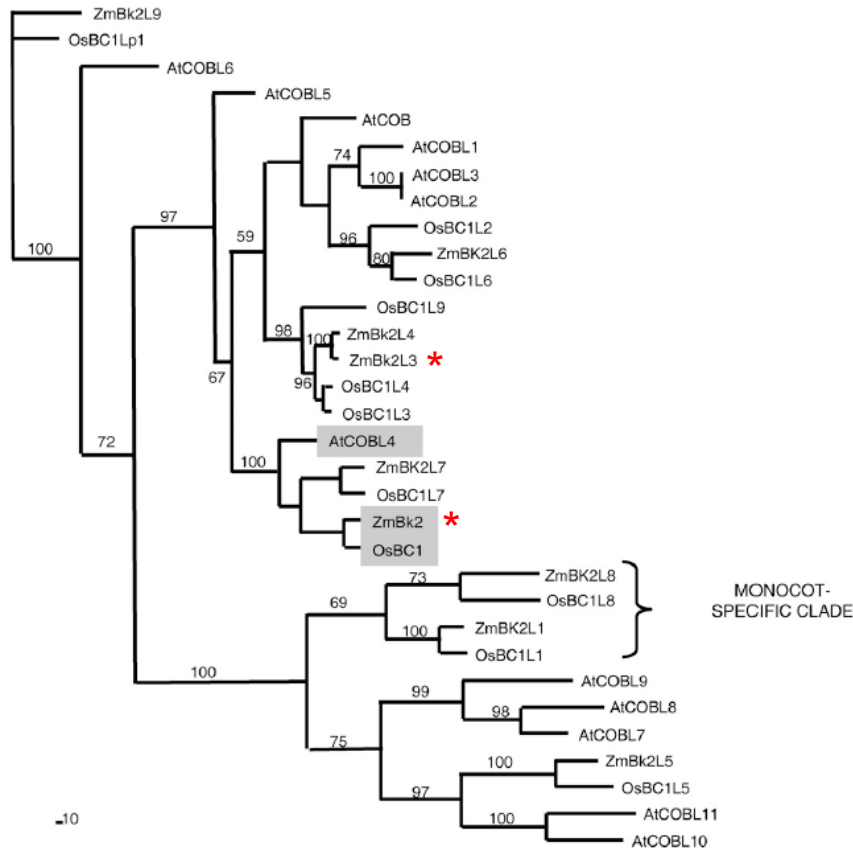


# Grass C4 CesA family – how many CesA genes are present in sugarcane?



ZmCesA:	12
SbCesA:	9
ShCesA:	10

# Identification of COBRA transcripts for RT-qPCR validation

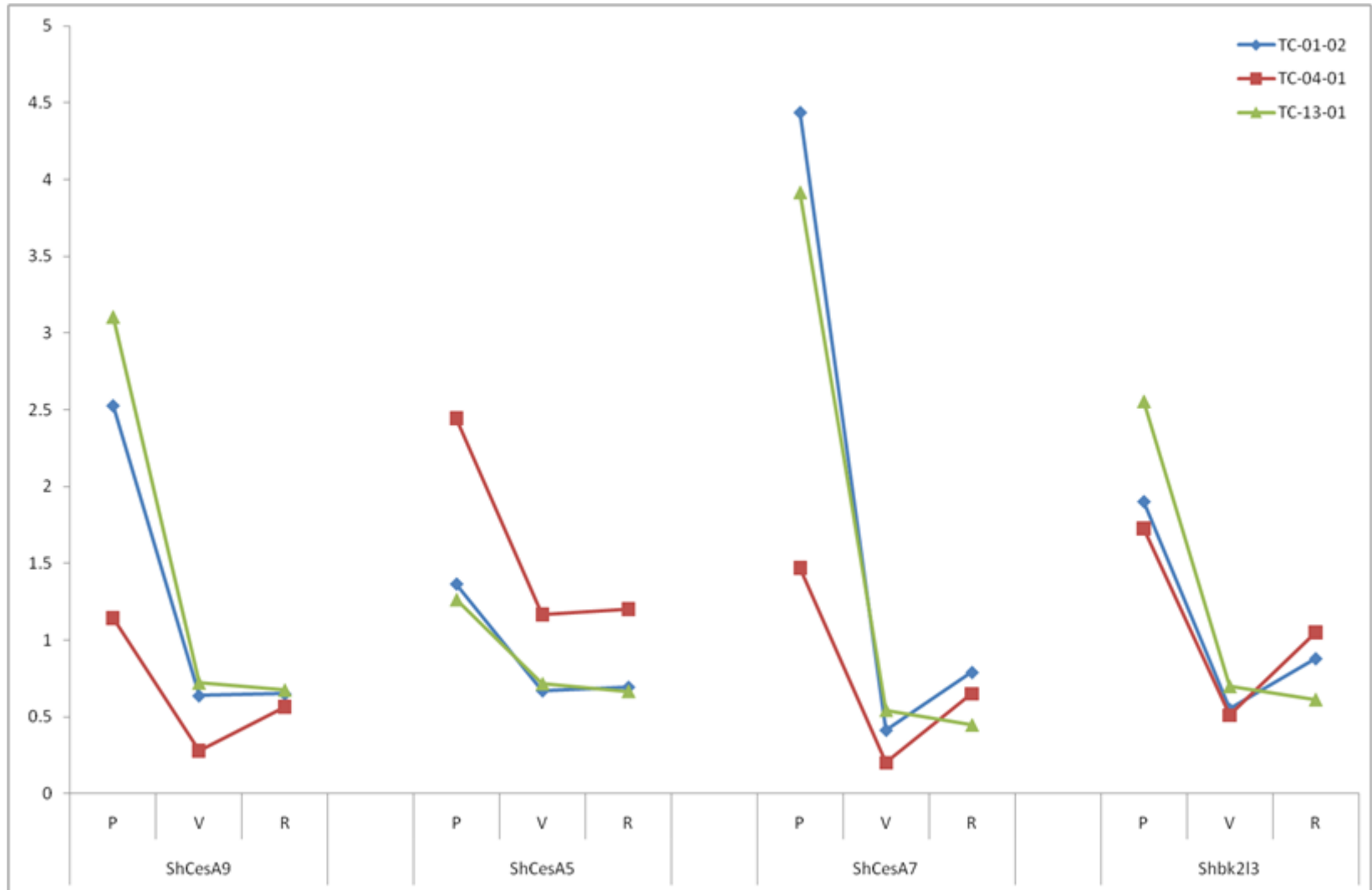


**Figure 2.** The *COB* gene family phylogeny. Unrooted cladogram of the encoded proteins of the *COB* gene superfamily in Arabidopsis, rice, and maize determined by the heuristic algorithm. Bootstrap values are indicated for only those branches where they exceeded 50%. *AtCOB* represents the Arabidopsis *COB* family members. *OsBC* represents the rice *COB* family members. *ZmBk2* represents the maize *COB* family members. The monocot-specific clade is marked with a bracket. *AtCOBL4* and its probable functional orthologs *ZmBk2* and *OsBC1* are indicated in shading.

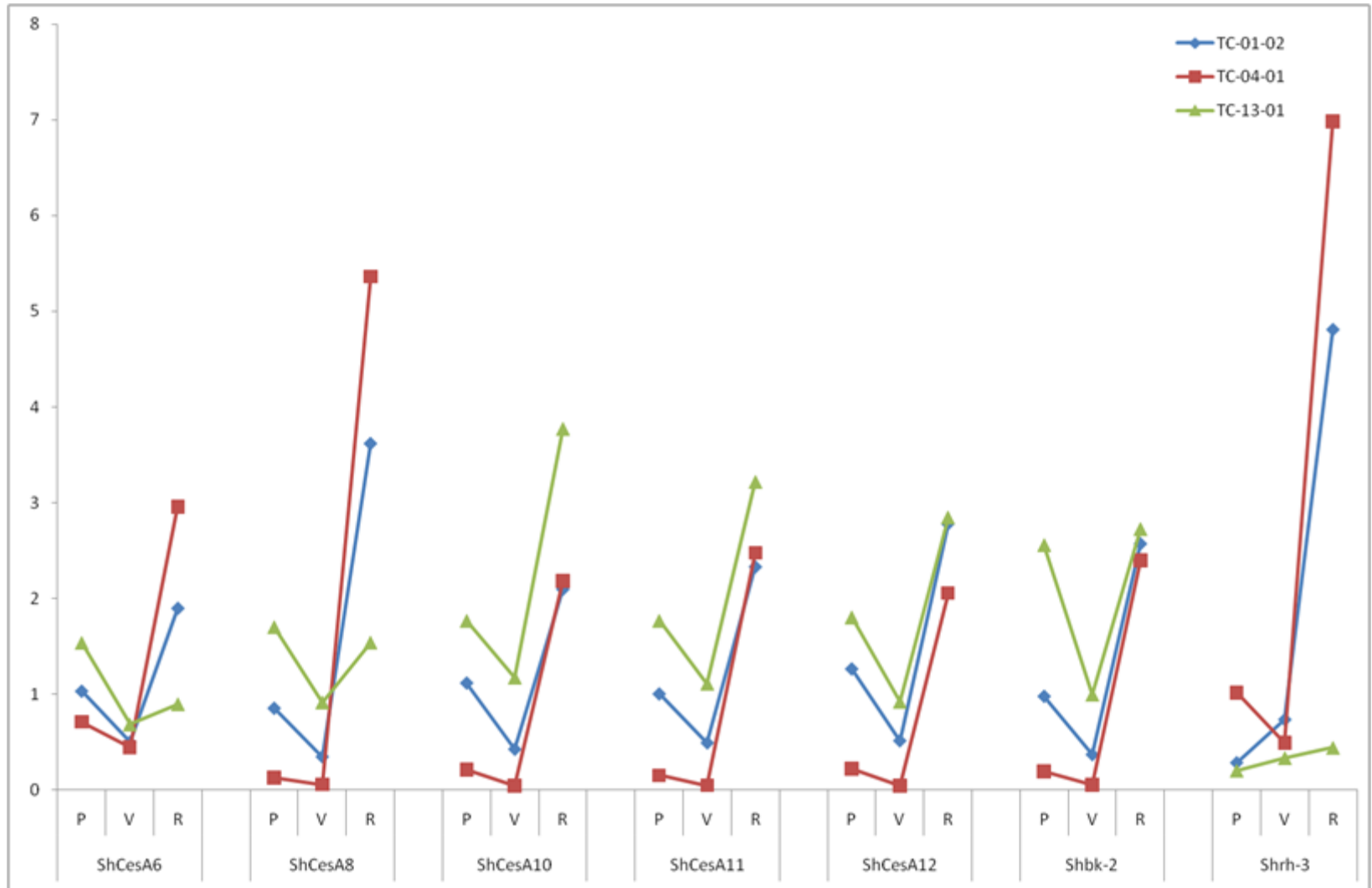
Brady SM et al (2007) Plant Physiol 143:172-187

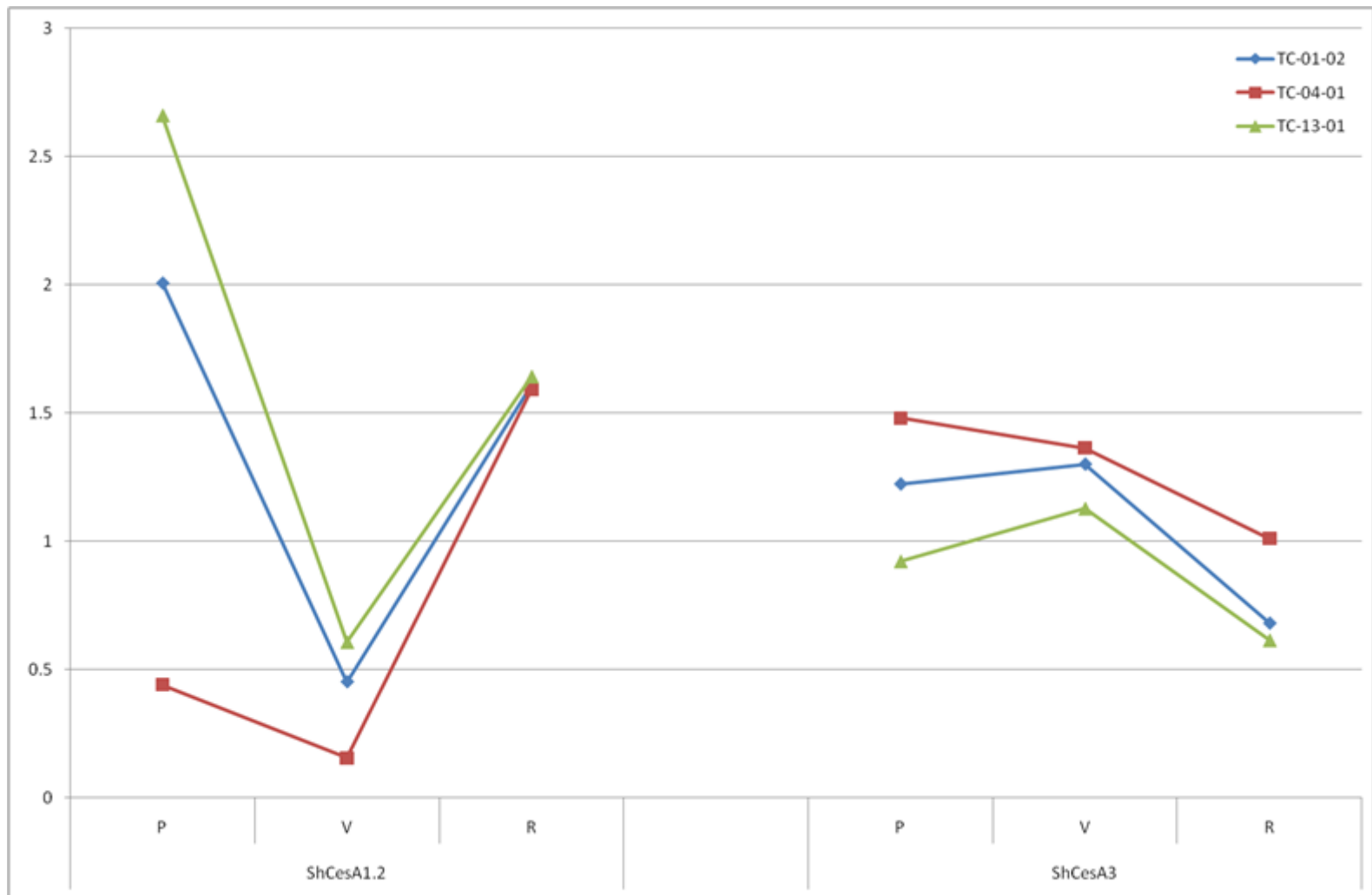
- COBRA is a GPI-anchored protein of unknown function with a possible role in controlling microfibril orientation in a microtubule-dependent manner
- Mutants display various phenotypes
  - Severe inhibition of root elongation and progressive radial swelling of cortical cells away from the root tip
  - Leaf and stem brittleness
- Three reliable sequences for COBRA or COBRA-like proteins also identified
  - Shbk2I3
  - Shbk-2
  - Shrh-3

# ShCesA5, A7 and A9 are co-expressed with Shbk2I3



# ShCesA10, A11 and A12 are co-expressed with Shbk-2

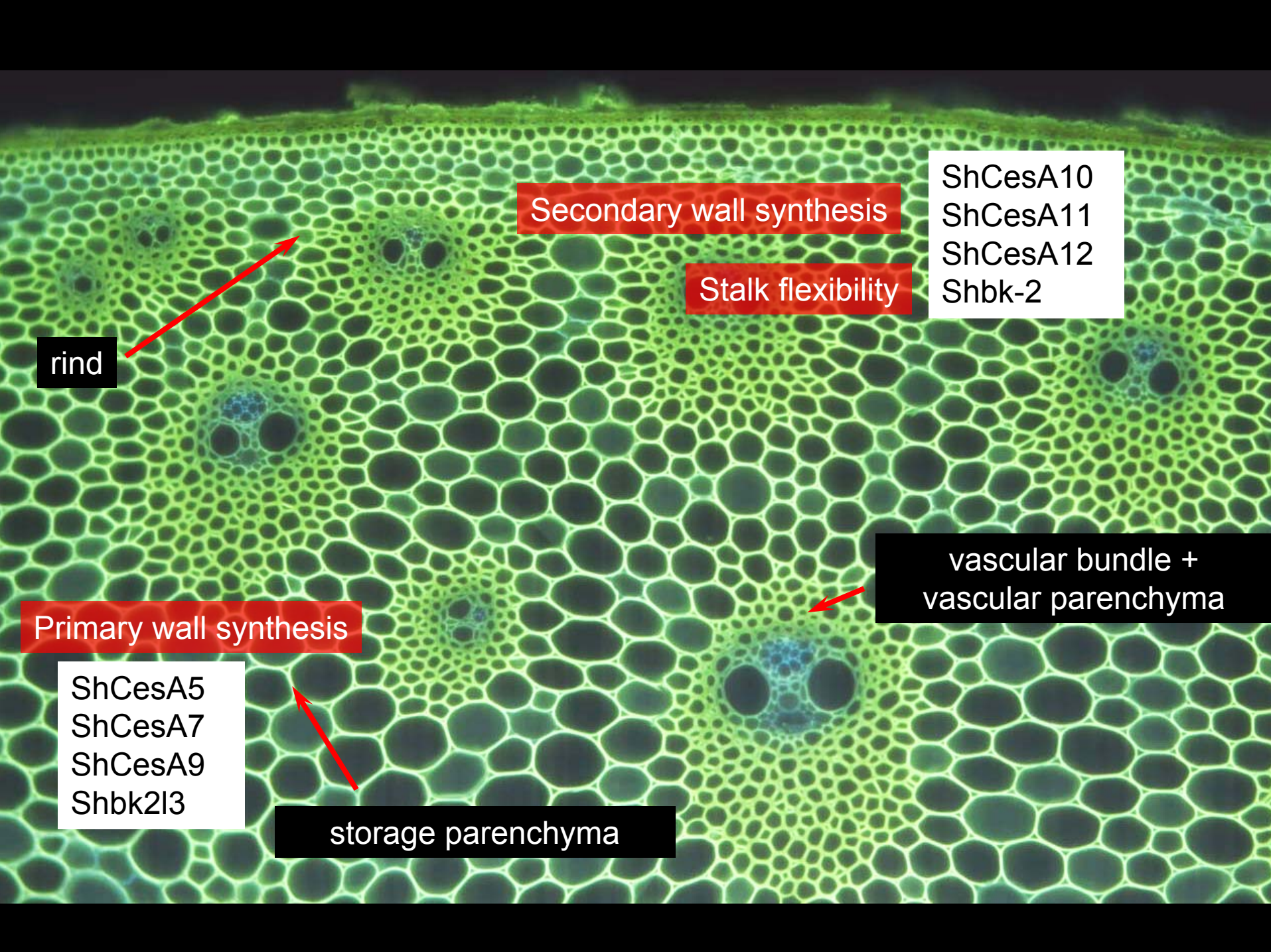




# Pearson correlation of RT-qPCR results

	ShCesA1	ShCesA3	ShCesA5	ShCesA6	ShCesA7	ShCesA8	ShCesA9	ShCesA10	ShCesA11	ShCesA12	Shbk2l3	Shbk-2	Shrh-3
ShCesA1													
ShCesA3						-0.724		-0.776	-0.780	-0.764		-0.785	
ShCesA5													
ShCesA6	0.783												
ShCesA7													
ShCesA8	0.861			0.870									
ShCesA9					0.968								
ShCesA10	0.900					0.932							
ShCesA11	0.889			0.723		0.955		0.994					
ShCesA12	0.920			0.733		0.943		0.991	0.991				
Shbk2l3					0.950		0.891						
Shbk-2	0.925			0.777		0.960		0.987	0.990	0.987			
Shrh-3													





rind

Secondary wall synthesis

Stalk flexibility

ShCesA10  
ShCesA11  
ShCesA12  
Shbk-2

Primary wall synthesis

ShCesA5  
ShCesA7  
ShCesA9  
Shbk2I3

vascular bundle +  
vascular parenchyma

storage parenchyma

# Summary

- Storage parenchyma is a dynamic tissue with a varied cohort of transcripts being highly expressed, including numerous transcripts involved in cell wall synthesis and protein synthesis
  - ShCesA5, A7 and A9 are co-expressed with Shbk2I3
  - Significant primary cell wall synthesis appears to be still occurring
- Vascular bundles had only one major named transcript involved in either sucrose transport or cell wall synthesis highly expressed with no other particular categories showing high expression
  - CesA subunits are expressed here but not up-regulated
- A major activity of rind is photosynthesis, but cellulose synthesis is heavily skewed towards different CesA and COBRA proteins to those in parenchyma
  - ShCesA10, A11 and A12 are co-expressed with Shbk-2
  - Secondary cell wall synthesis is predominant in the rind, coupled with protein synthesis implicated in wall flexibility
  - Is Shbk-2 a candidate gene for lodging resistance?

# Acknowledgements

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[www.csiro.au](http://www.csiro.au)

# Thank you

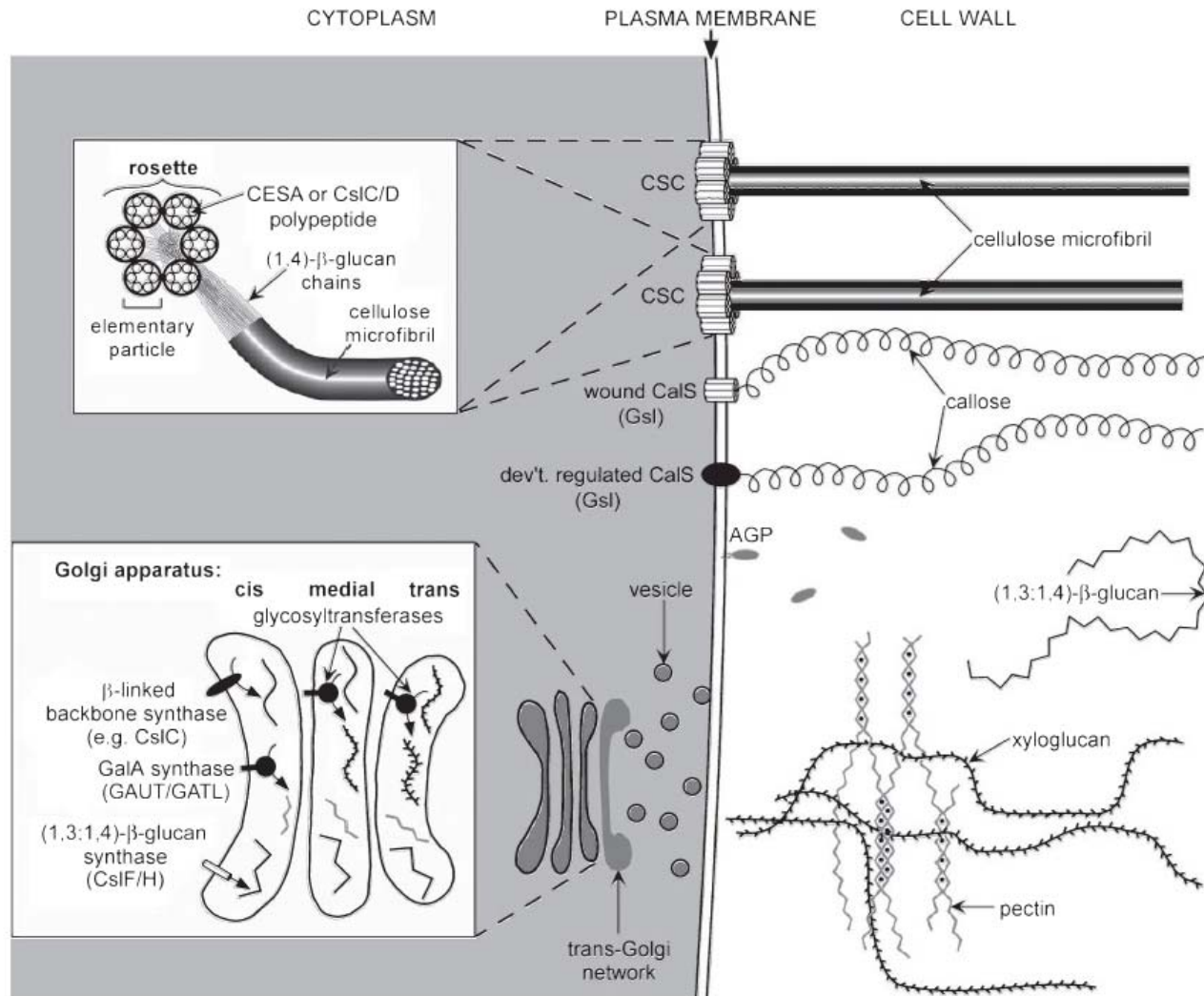
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Doblin et al. 2010