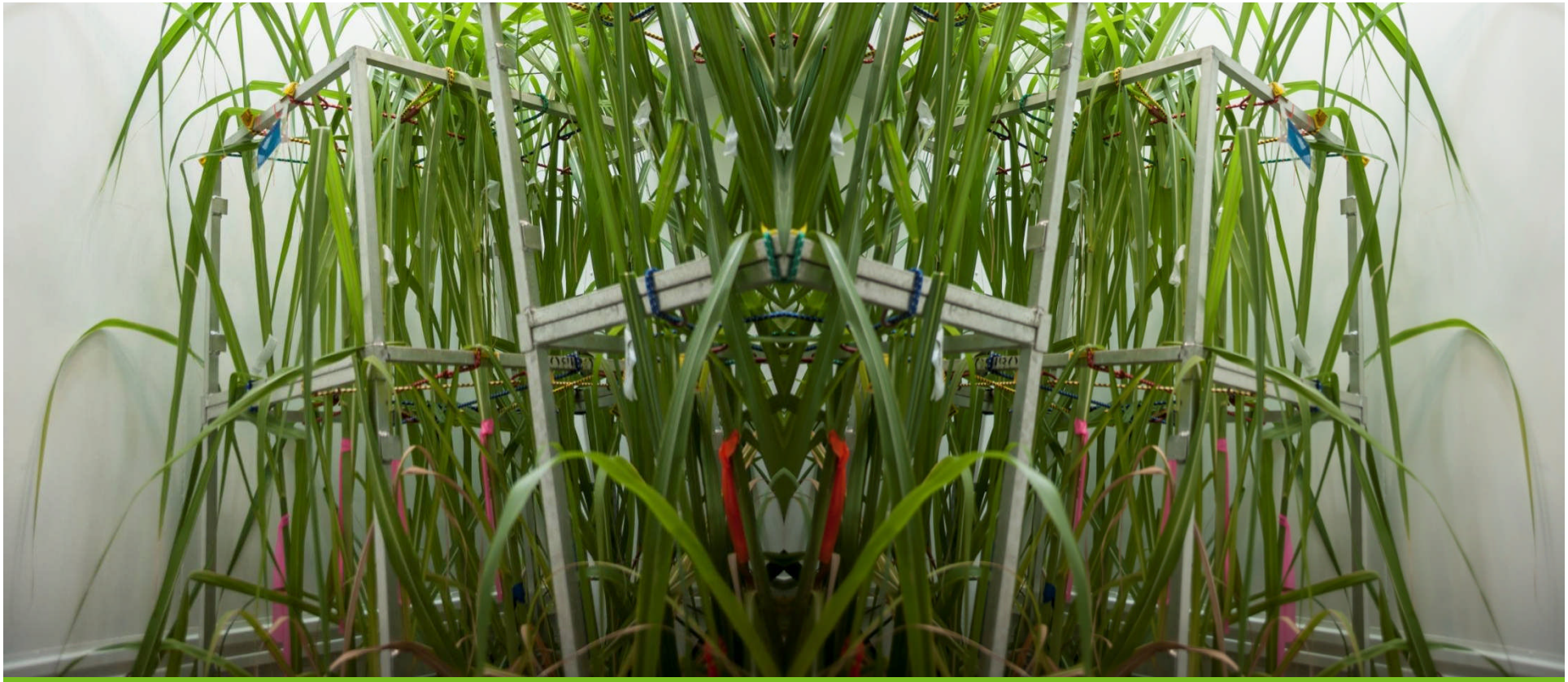


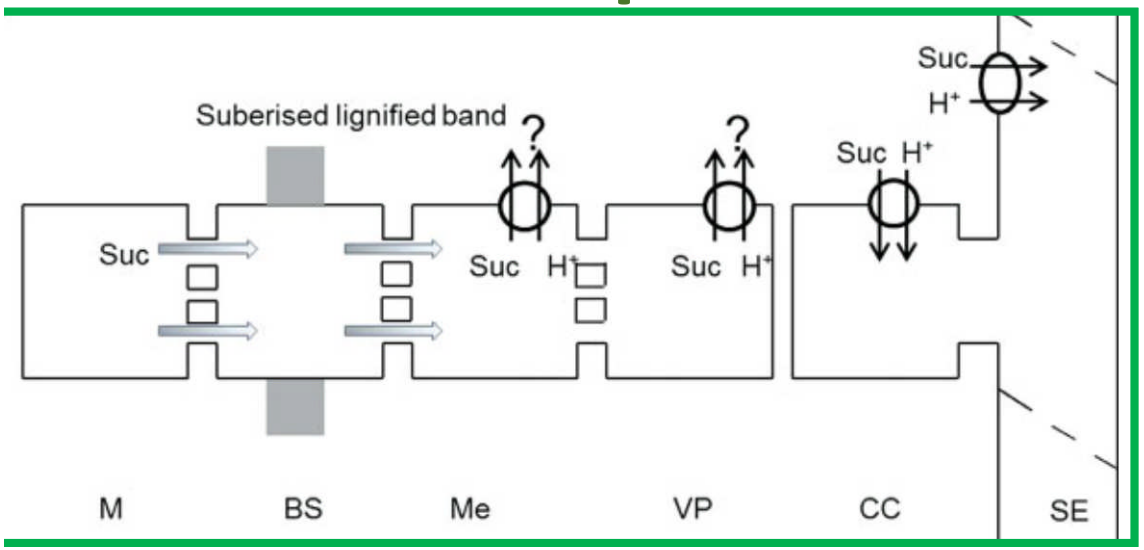
An analysis of ShSUT1 in transgenic sugarcane plants; changes to allele expression profiles

Donna Glassop & Anne Rae

June 2015

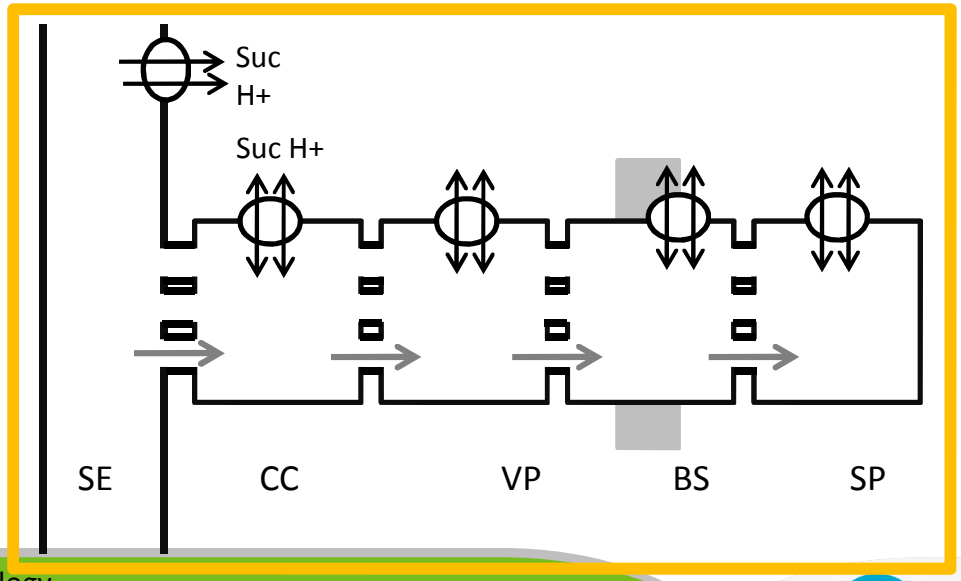
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Sucrose transport



Leaf

Stem

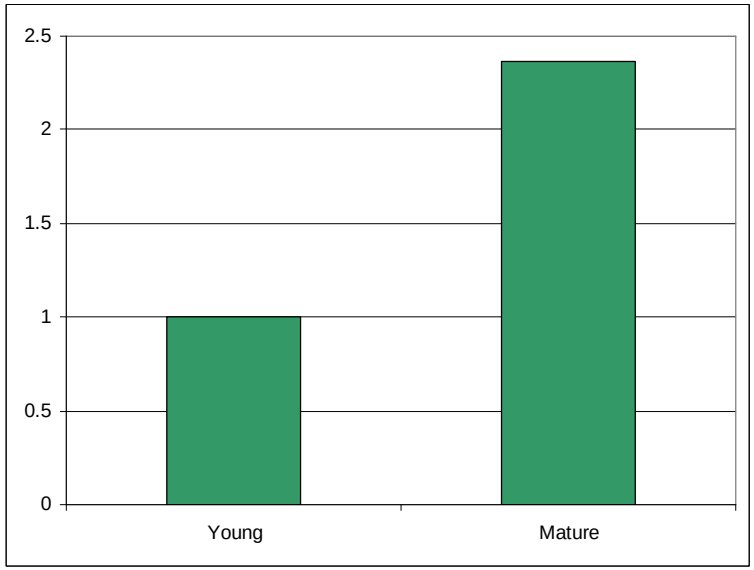


Grof et al 2013 Sugarcane: Physiology, Biochemistry and Functional Biology

Saccharum hybrid Sucrose Transporter1 – ShSUT1

- Microarray results with 3 cultivars
- Q117
- 74C42
- Q124

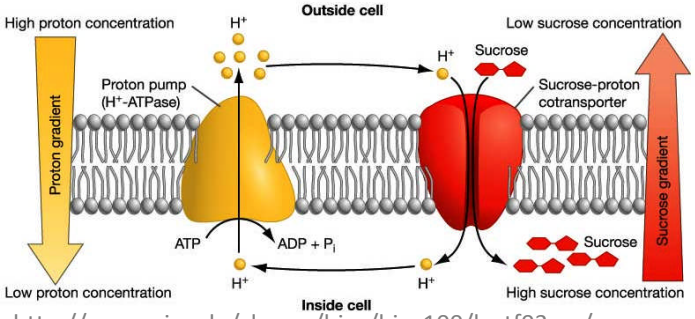
- Increase of more than 2 fold between young tissue and mature tissue



Casu et al 2003 Plant Molecular Biology 52:371-382

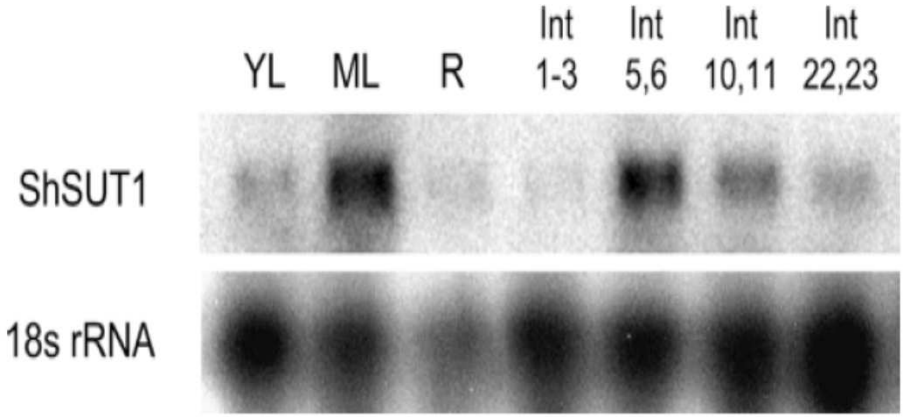
ShSUT1 – functional analysis

- Functional sucrose transporter – expression in yeast (SEY6210)



http://www.uic.edu/classes/bios/bios100/lectf03am/sucrosepump.jpg

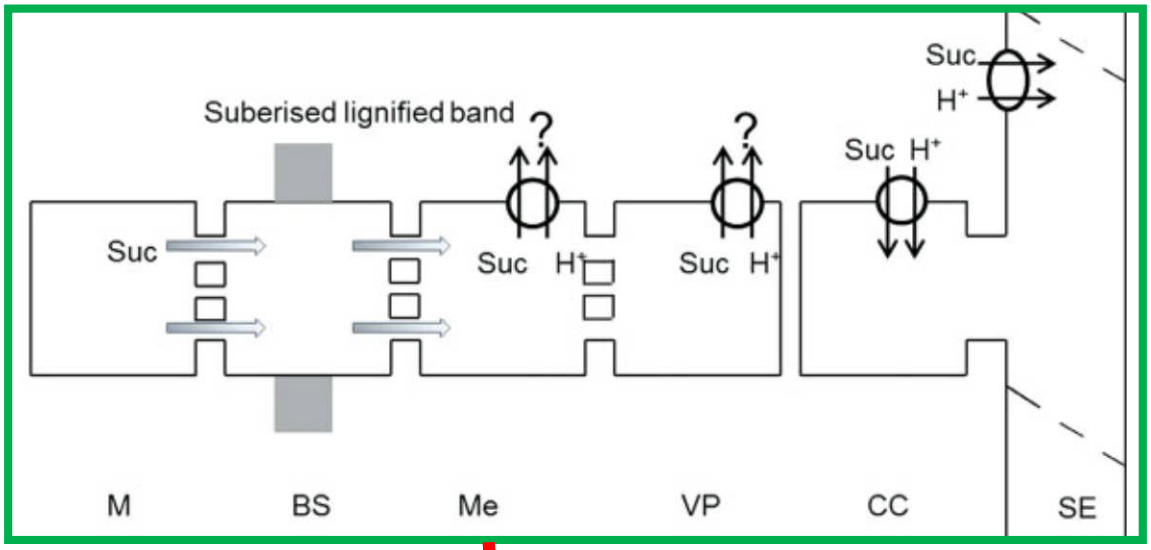
- *Km* 2 mM at pH 5.5
- Using two-electrode voltage clamping in Xenopus oocytes expressing ShSUT1.
- ShSUT1 is highly selective for sucrose with relatively low affinity for sucrose
- Expression in Q117

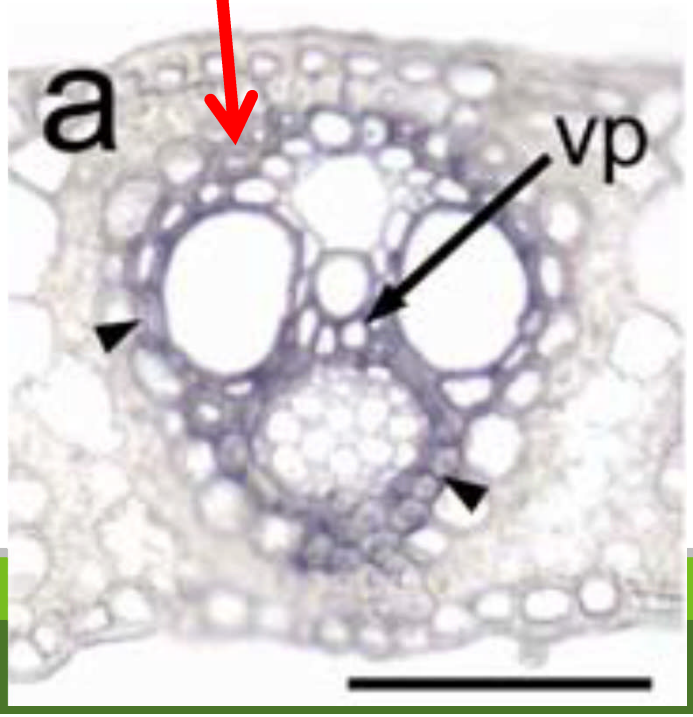


Rae et al. Planta (2005) 220:817-825.

Reinders et al. Plant, Cell and Environment (2006) 29:1871-1880.

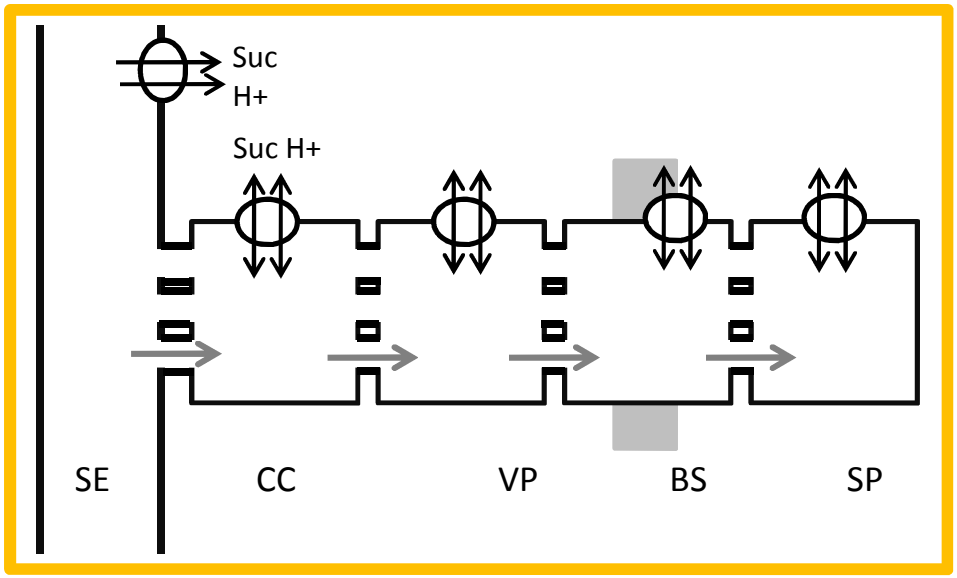
ShSUT1 localisation in the leaf

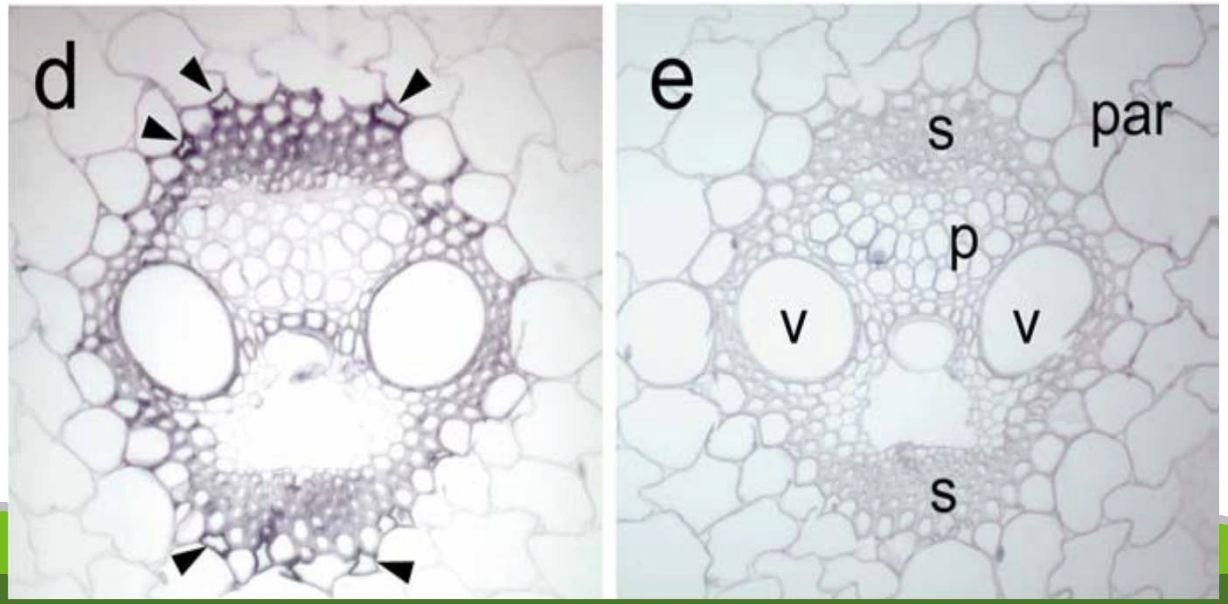




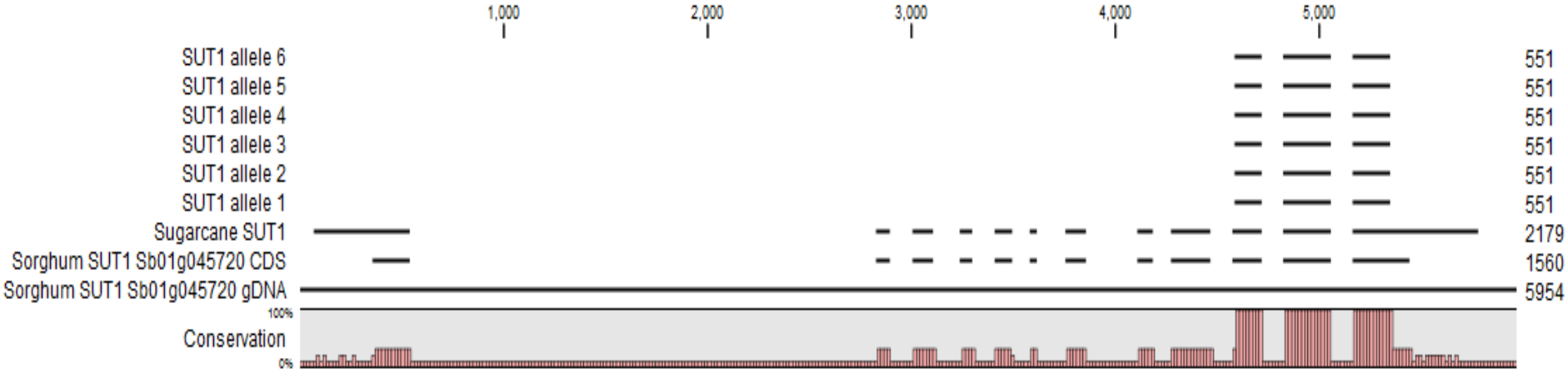
Rae et al 2005. Planta 220:817-825.
Grof et al 2013 Sugarcane: Physiology, Biochemistry and Functional Biology

ShSUT1 localisation in Internode 5

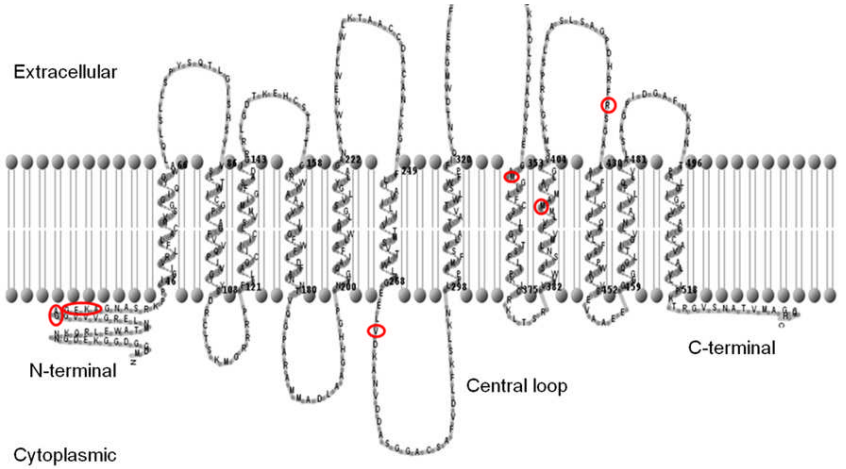




Sequence and alleles observed
-ShSUT1 = allele 2

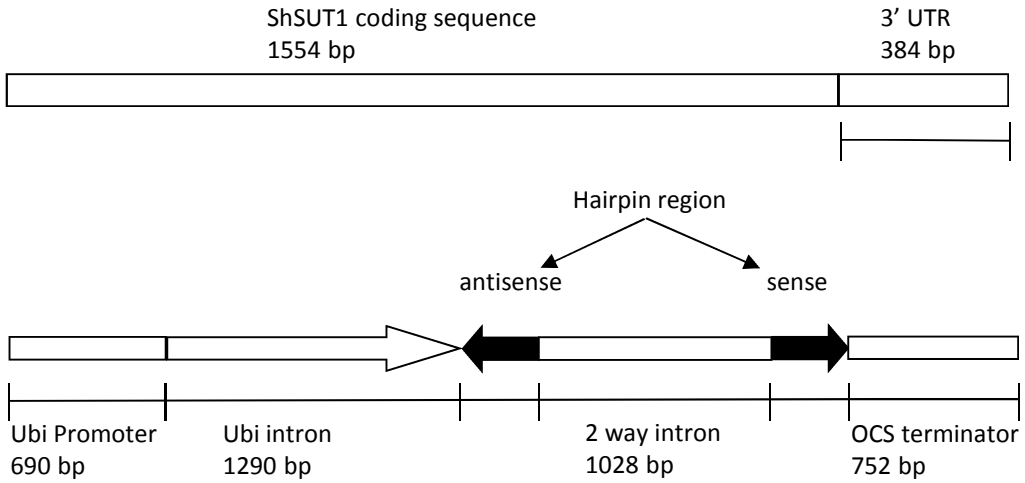


	66	97	170	250	307	355	400
1	C	C		C			
2			T				
3					G	T	
4						T	
5							A
6	G	T	A	G	A	A	G



http://journal.frontiersin.org/article/10.3389/fpls.2013.00223/full

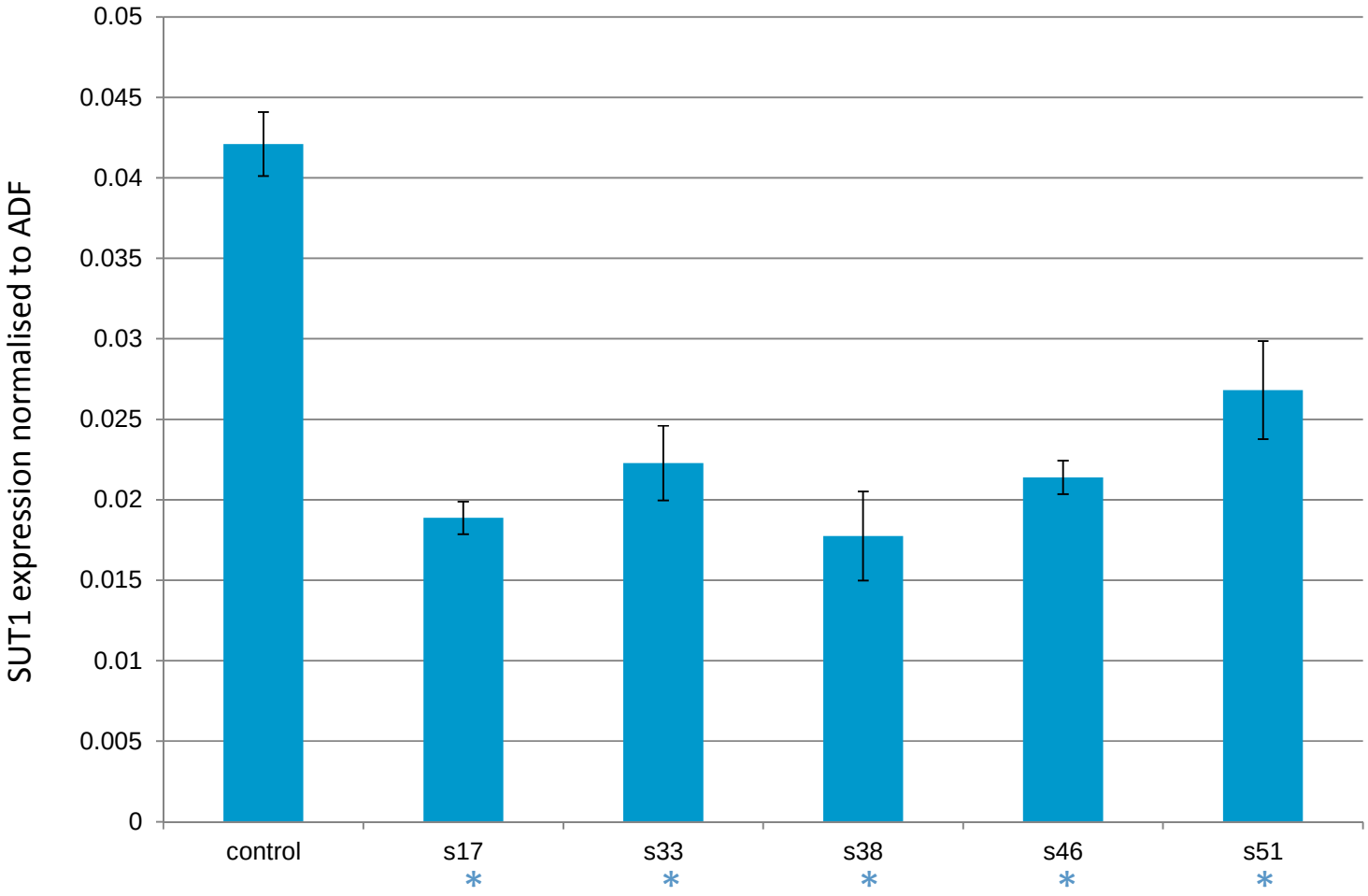
RNAi – transgenic strategy



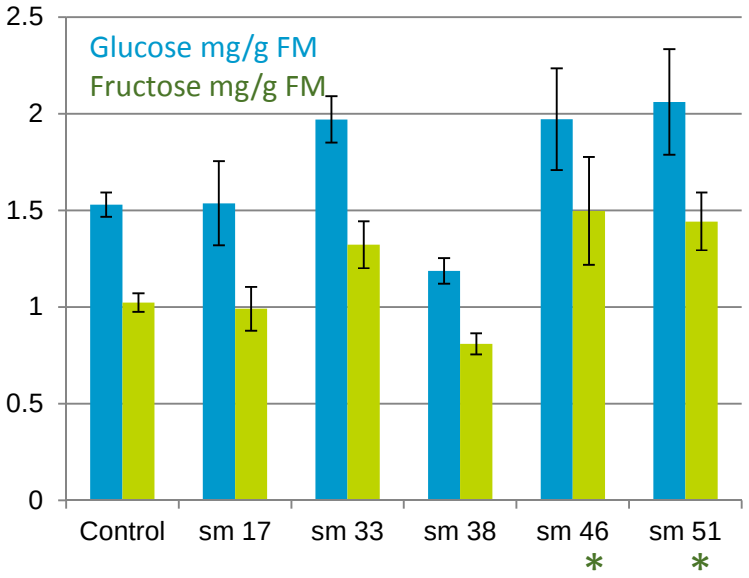
mi RNA was detected in 5 transgenic lines, via Northern blot
no detection in 12 tissue culture control lines.

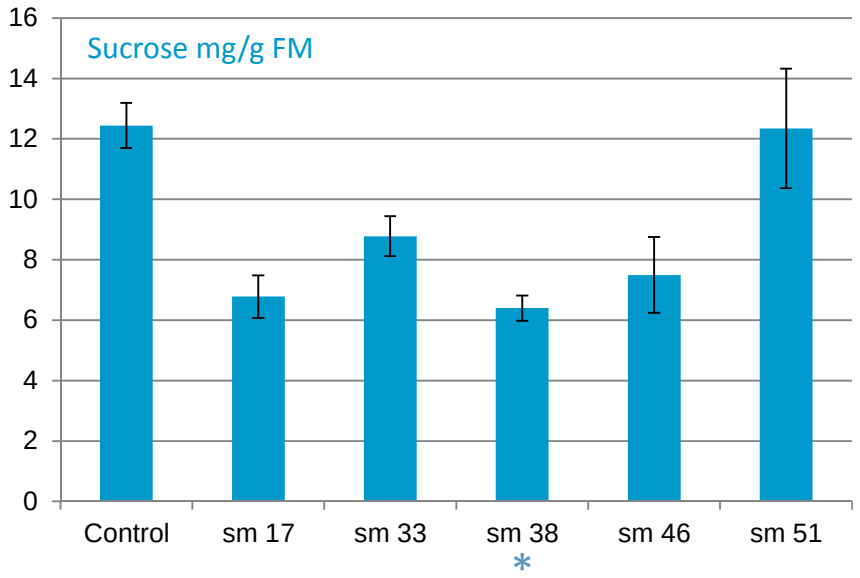
All plants looked normal. T0 plants were vegetatively propagated (T1).

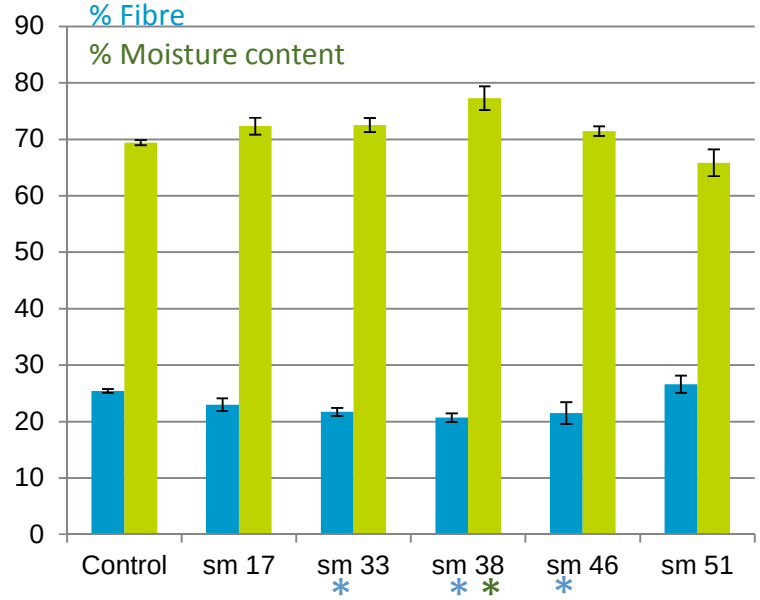
SUT1 expression in Mature leaf of transgenic plants

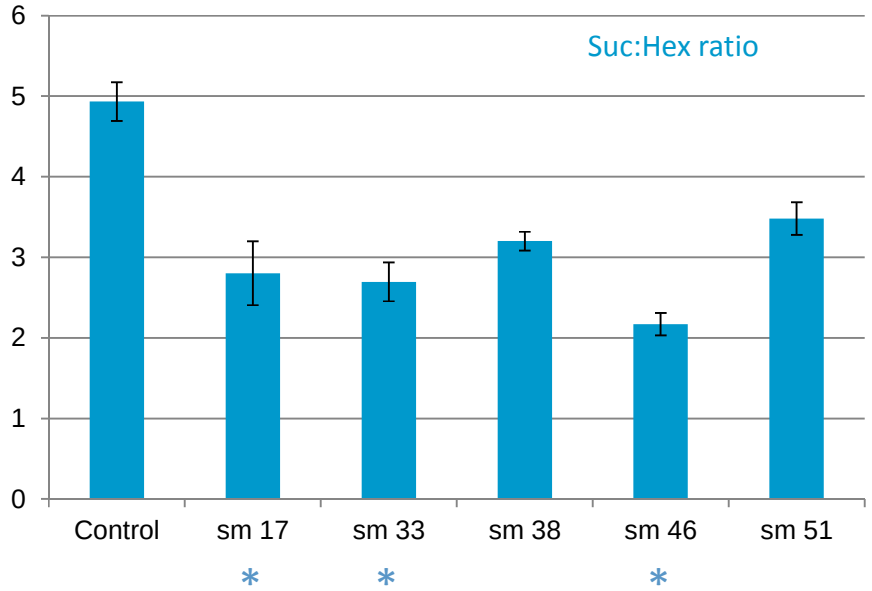


Expression was reduced by 36 – 58%.



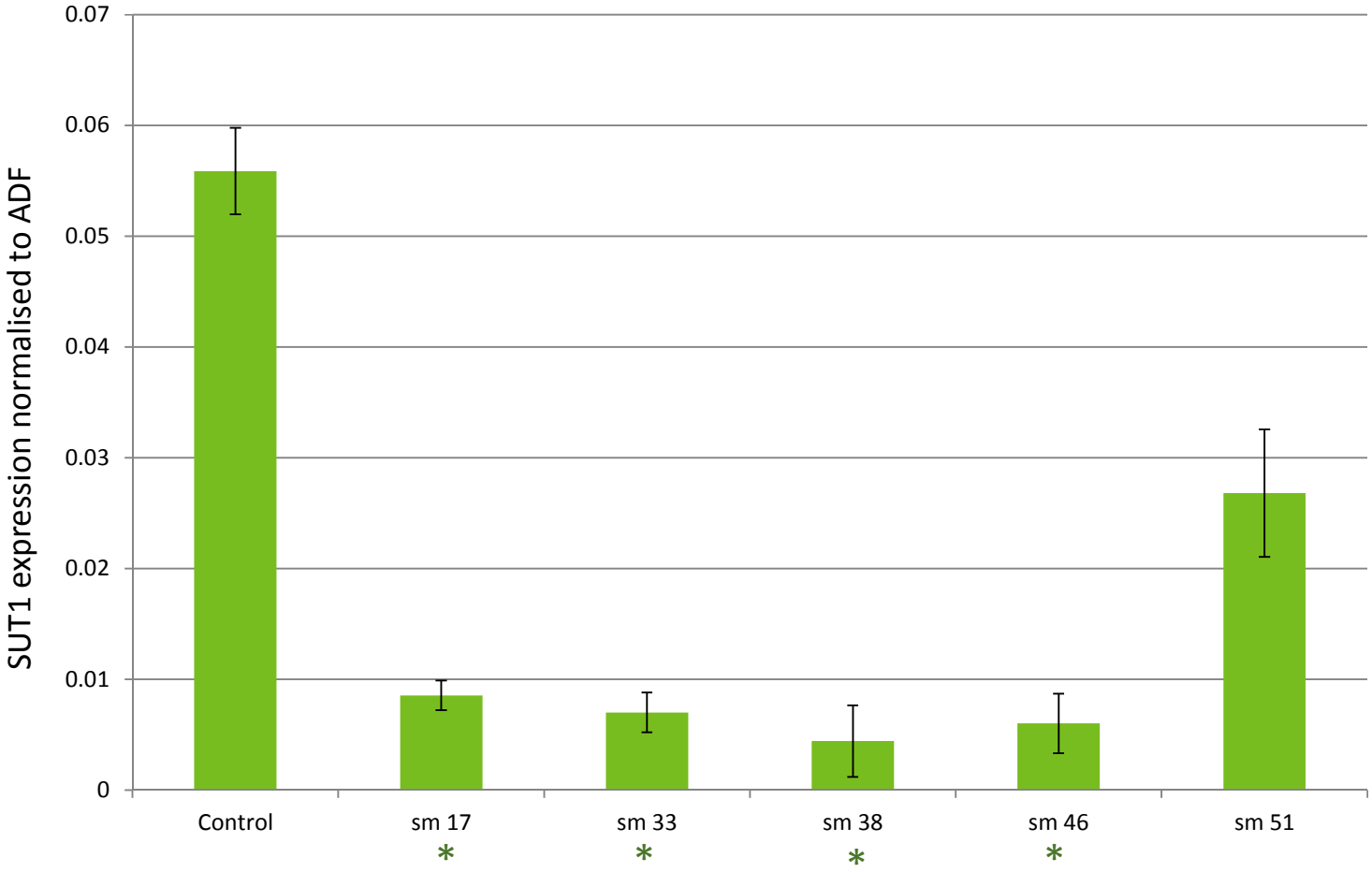




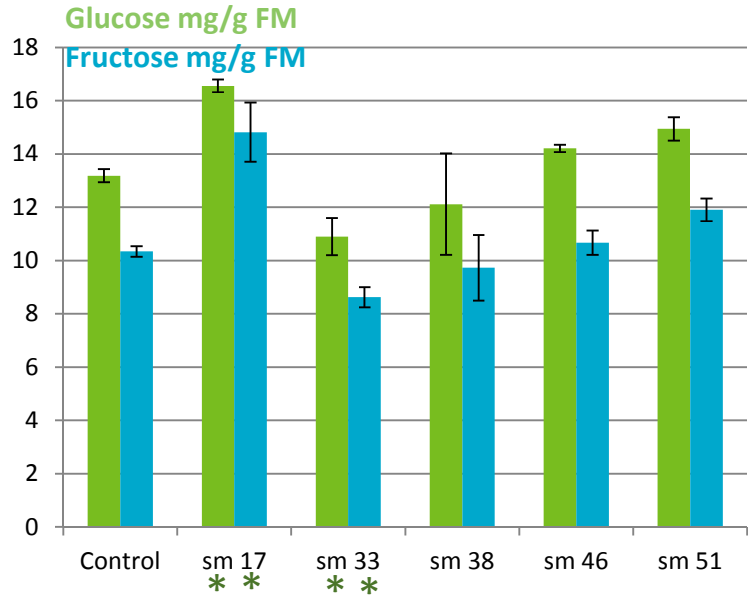


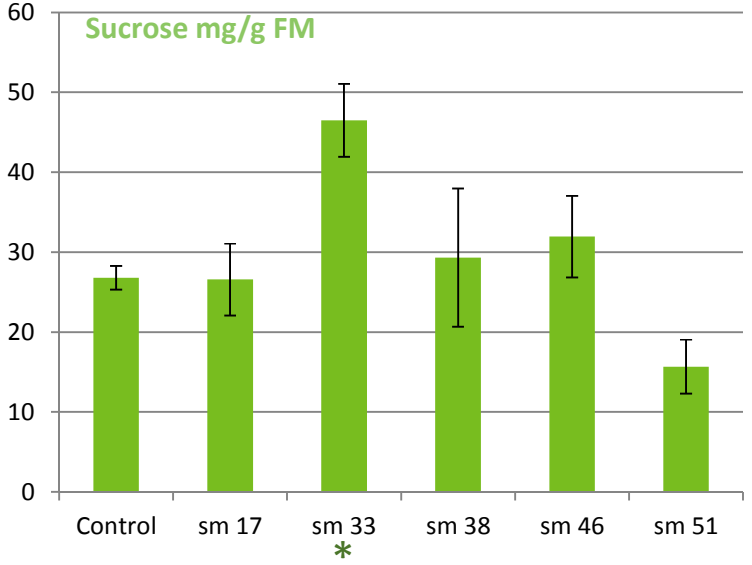
Phenotypes in mature leaf

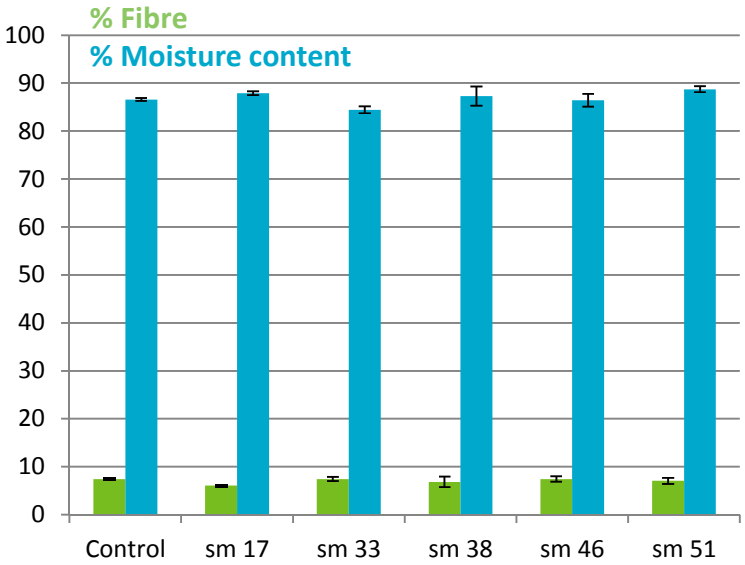
SUT1 expression in Internode 7of transgenic plants

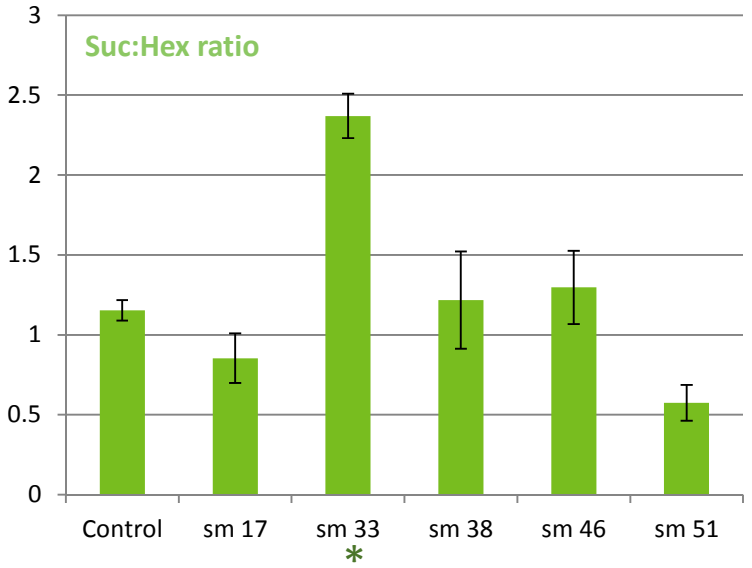


Expression was reduced by 52 - 92%.

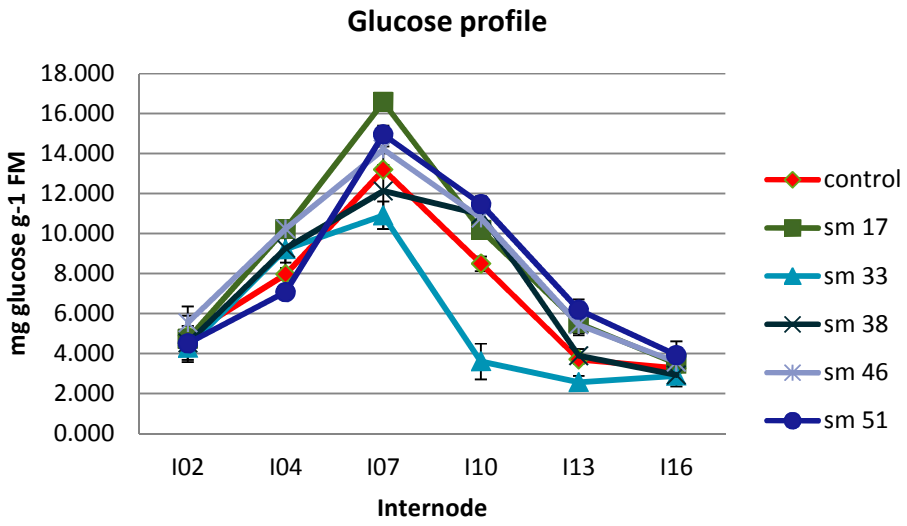


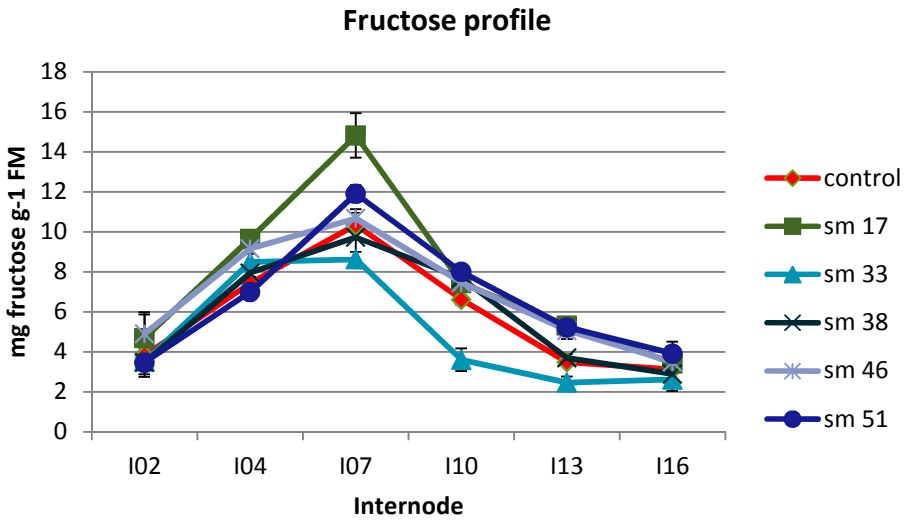


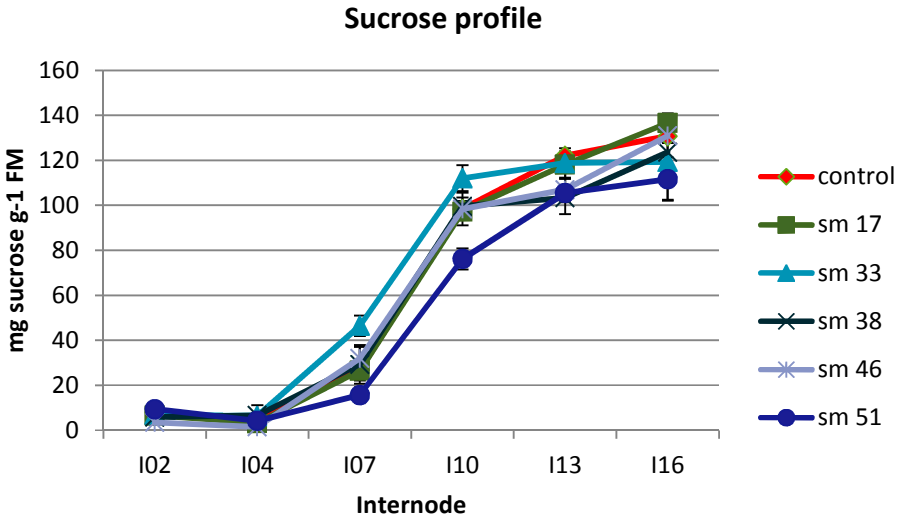




Phenotypes in internode 7


Glucose profile
mg glucose g-1 FM
18.000
16.000
14.000
12.000
10.000
8.000
6.000
4.000
2.000
0.000
I02
I04
I07
I10
I13
I16
Internode
control
sm 17
sm 33
sm 38
sm 46
sm 51


Fructose profile
mg fructose g-1 FM
18
16
14
12
10
8
6
4
2
0
I02
I04
I07
I10
I13
I16
Internode
control
sm 17
sm 33
sm 38
sm 46
sm 51


Sucrose profile
mg sucrose g-1 FM
160
140
120
100
80
60
40
20
0
I02
I04
I07
I10
I13
I16
Internode
control
sm 17
sm 33
sm 38
sm 46
sm 51

RNAi conclusions

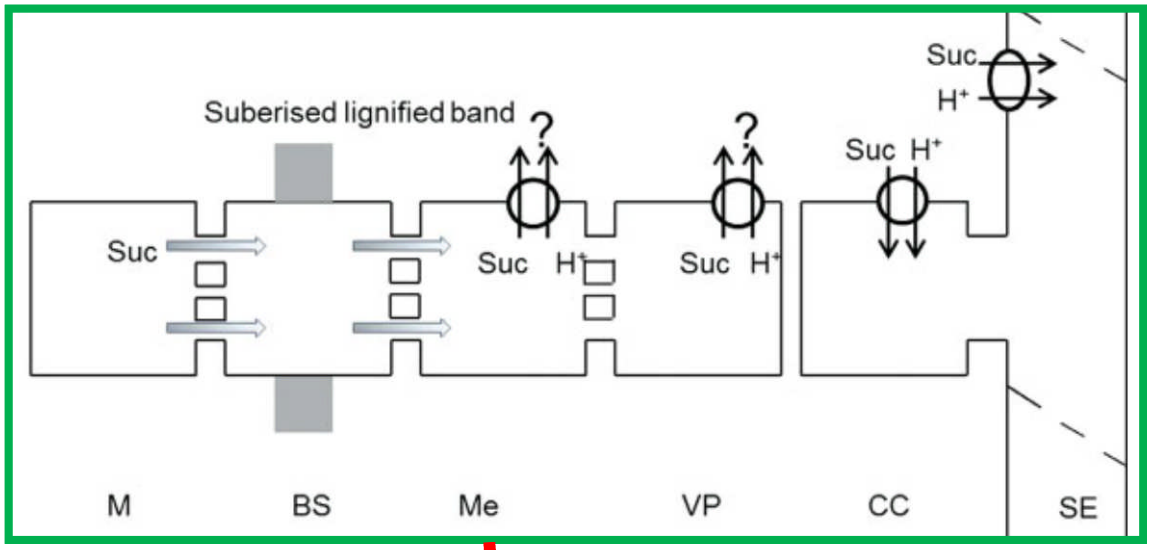
- Expression was reduced by 36 – 58% in mature leaves

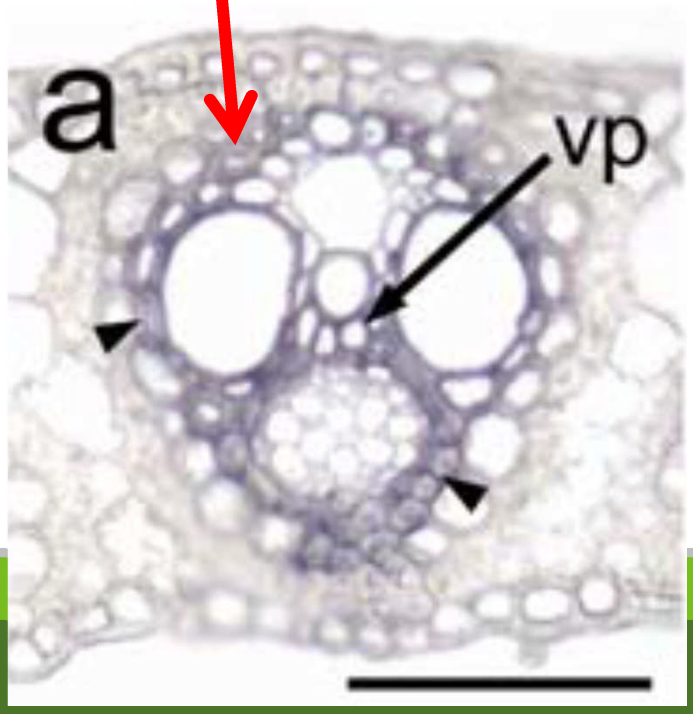
	S17	S33	S38	S46	S51
Fibre/MC		↓ /	↓ /↑	↓ /	
Glu/Fru			/↑	/ ↑	
Suc			↓		
Suc:Hex	↓	↓		↓	

- Expression was reduced by 52 - 92% in internode 7

	S17	S33	S38	S46	S51
Fibre/MC					
Glu/Fru	↑ / ↑	↓ /↓			
Suc		↑			
Suc:Hex		↑			

Impact of reduced ShSUT1 in the leaf



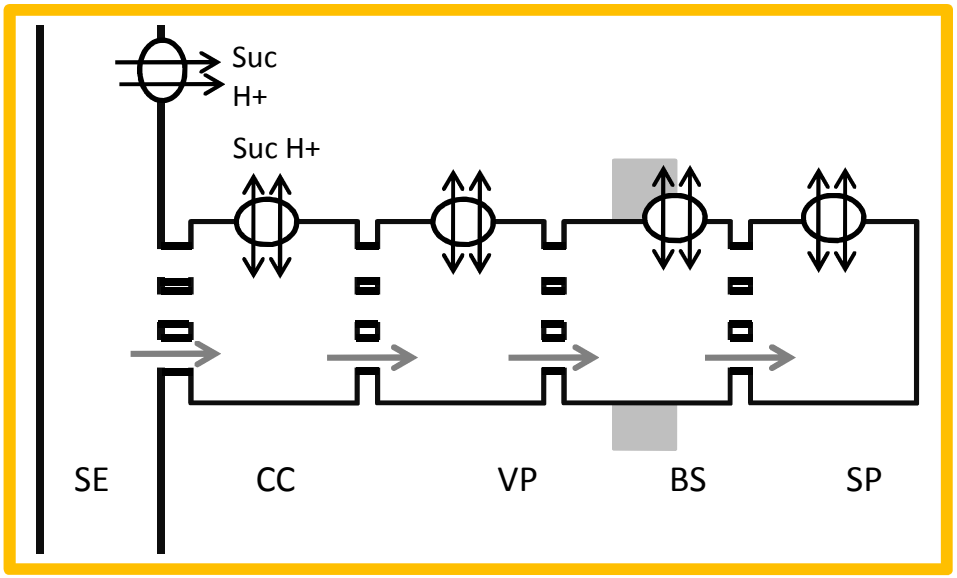


Predicted role:

- Export of sucrose from leaves
- Possibly moving sucrose out of the cells for apoplastic uptake
- Important for leaf carbohydrate metabolism

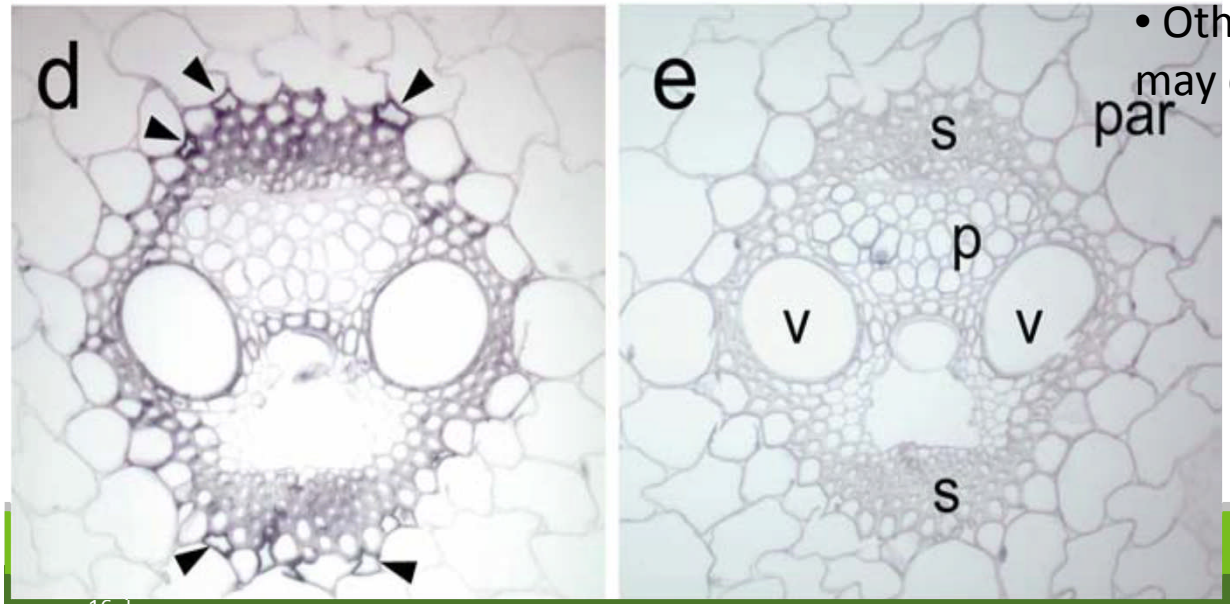
Rae et al 2005. Planta 220:817-825.
Grof et al 2013 Sugarcane: Physiology, Biochemistry and Functional Biology

Impact of reduced ShSUT1 in the stalk



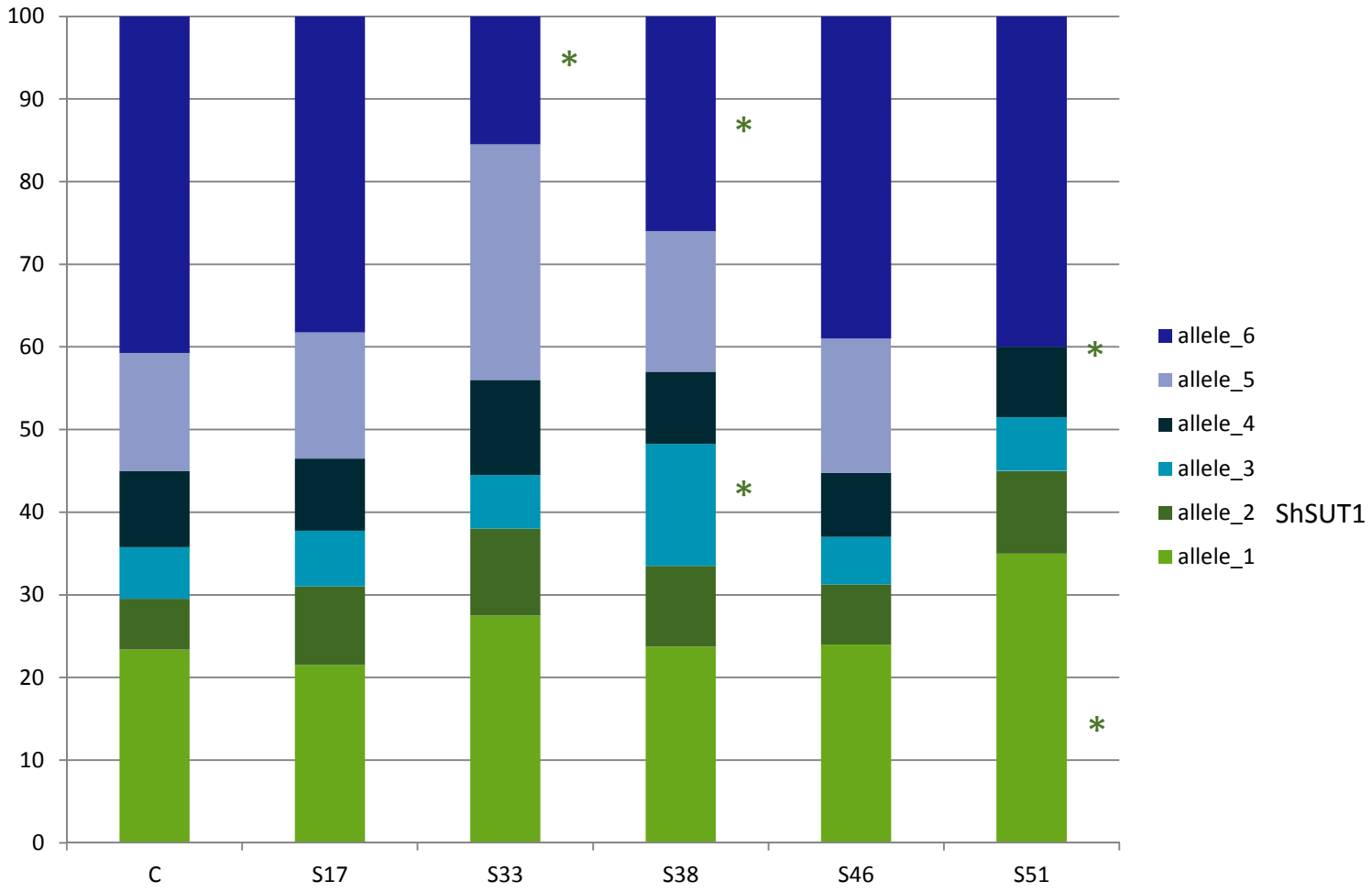
Predicted role:

- Partitioning from the vascular tissue into the storage tissue
- 2 transgenic plant lines showed changes in sugar concentrations with no impact on final stalk sucrose
- Other transporter mechanisms may compensate

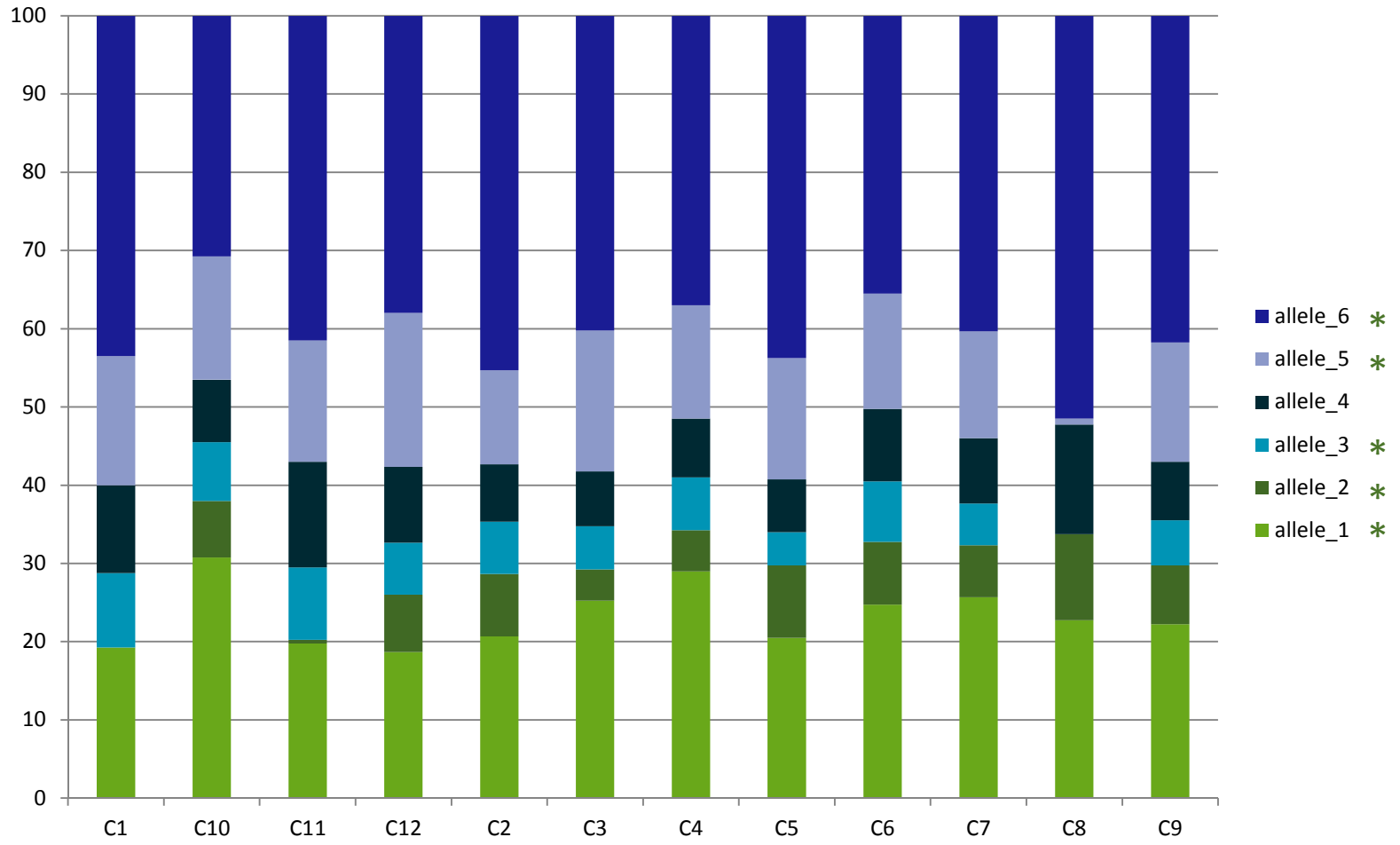


Allele expression internode 7

Expression was reduced by 52 - 92%.



Allele expression internode 7, Control lines



Summary:
Investigation of the role of SUT1

- RNAi can be used to reduce expression of a sucrose transporter
- Analysis of transgenic plants shows that the activity/role of SUT1 is more important in the leaf than the stalk.
- Variations in the proportion of alleles expressed in controls and transgenic lines were observed in internode 7.

Thank you

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CSIRO