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Supplementary Material

An analysis of the role of the ShSUT1 sucrose transporter in sugarcane using RNAi suppression

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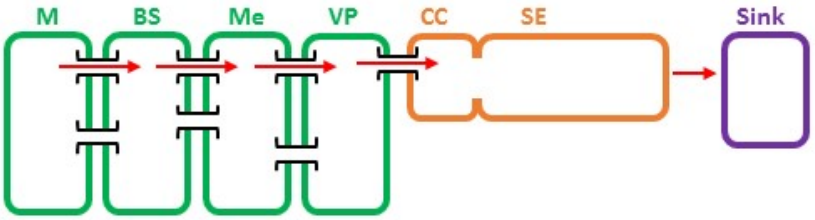
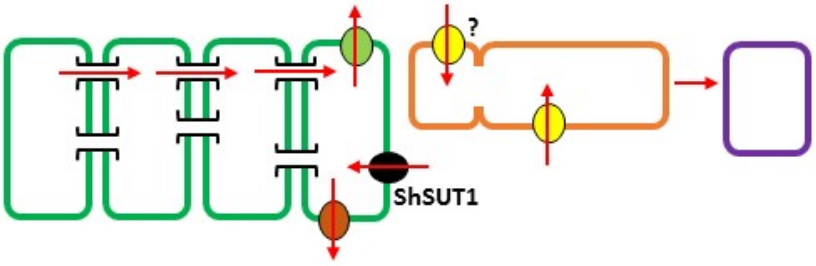
(a) Symplastic loading



(b) Apoplastic loading



(c) Unloading

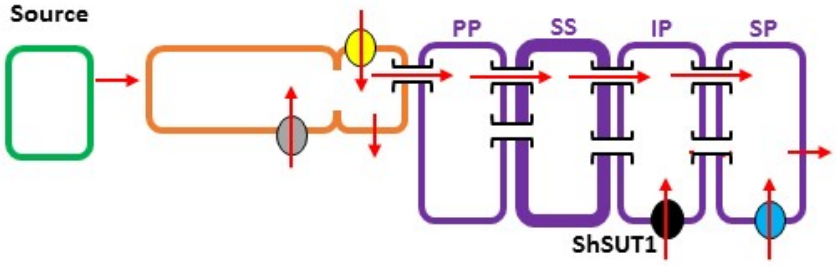


Fig. S1. A simplified schematic of sucrose loading and unloading. (a) Symplastic loading. Carbon is fixed from the atmosphere and converted to sucrose in the source leaf (green cells). The sucrose is then moved from the mesophyll through the bundle sheath, mestome sheath and vascular parenchyma cells to the sieve elements with or without companion cells that comprise the phloem (orange cells) where it is transferred to the sink tissues (purple cells), with the pathway of movement indicated by the red arrows. While sucrose transporters (coloured ovals with arrows indicating direction of sucrose transport) are present within symplastic loading plants, the major route of sucrose transport is through plasmodesmata, which provide cytoplasmic continuity to the phloem. Within the phloem of **some** symplastic loading plants, some of the sucrose is converted to larger polymers that may not be able to pass back through the plasmodesmata efficiently (e.g. verbascose, raffinose, stachyose). (b) Apoplastic loading. In plants with apoplastic loading the sucrose moves in a similar way to symplastic loading plants via plasmodesmata from the mesophyll cells through the bundle sheath, mestome sheath and vascular parenchyma cells. However, in these plants, the phloem cells do not have **abundant** plasmodesmatal connections with the vascular parenchyma and sucrose transporters (oval symbols) load sucrose that has been deposited in the apoplast. Sucrose is probably released into the apoplast surrounding the phloem cells by export transporters located in the vascular parenchyma, known as SWEETS (Chen *et al.* 2010). In this model there may also be a role for import transporters that return sucrose to the symplast, possibly including ShSUT1. (c) Unloading. In the sink tissue, unloading of sucrose from the phloem can utilise similar symplastic or apoplastic pathways as seen in the source tissues as well as a combination of both. In sugarcane a physical barrier of sclerenchyma cells forms a sheath around the phloem and phloem parenchyma, restricting any apoplastic flow of sucrose between the storage parenchyma cells and the phloem. It is likely that sucrose moves from sieve elements and companion cells via plasmodesmata to the phloem parenchyma cells, sclerenchyma sheath cells and finally into the storage parenchyma cells. Sucrose is also found in the apoplast surrounding the storage parenchyma cells and may be retrieved by transporters such as ShSUT1 back into the symplast. (Grof *et al.* 2013). M: mesophyll, BS: bundle sheath, Me: mestome, VP: vascular parenchyma, CC: companion cell, SE: **thin** walled sieve element, PP: phloem parenchyma, SS: scelerenchyma sheath, IP: intermediate parenchyma, SP: storage parenchyma.

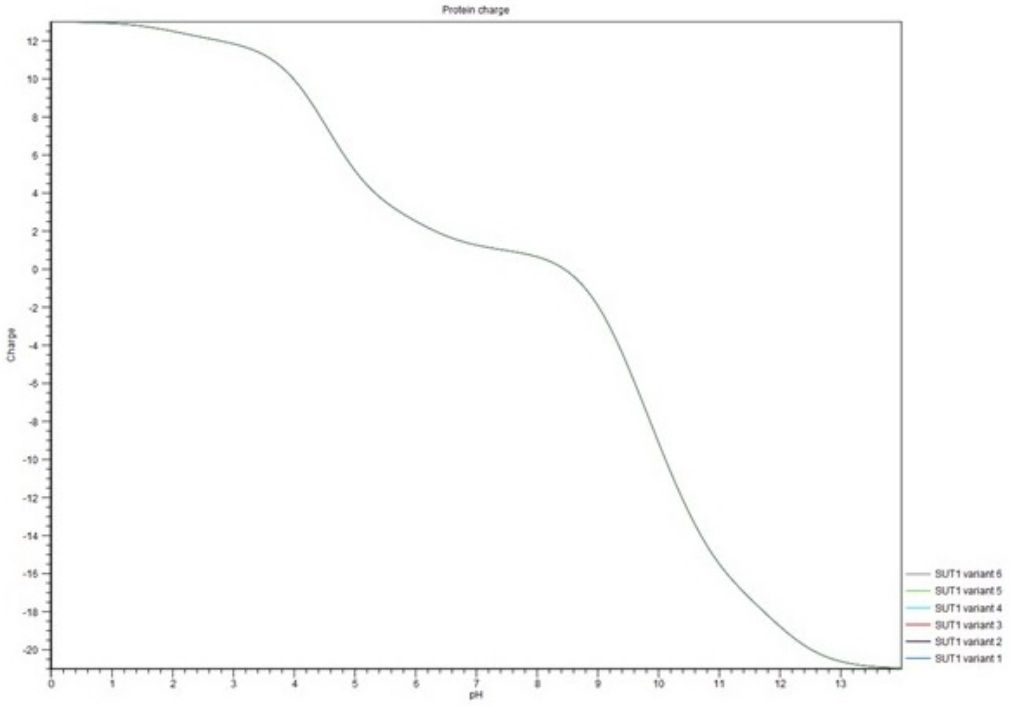


Fig. S2. Protein charge for the 416 bp region for the six SUT1 variants identified. A single line is observed as there is no difference in protein charge between the variants. Protein charge was calculated within CLC Main Workbench version 7.6.2 (Qiagen, Aarhus A/S).

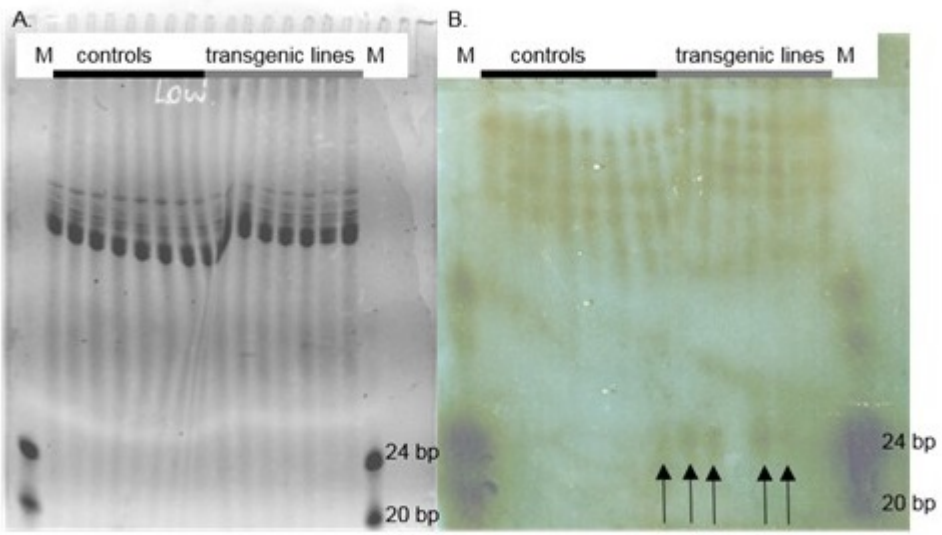


Fig. S3. Detection of smRNA. A. Electrophoretic gel prior to transfer to hybridisation membrane. M – molecular weight markers. RNA extracted from controls run in lanes under the dark line and transgenic lines under the gray line. B. X-ray film indicating the radioactive hybridisation of the ***SUT1*** gene to the smRNA produced as a result of the transgene expression. Five transgenic lines tested showed the presence of ***SUT1*** smRNA, indicated by the arrows. The control lines detected no ***SUT1*** smRNA.

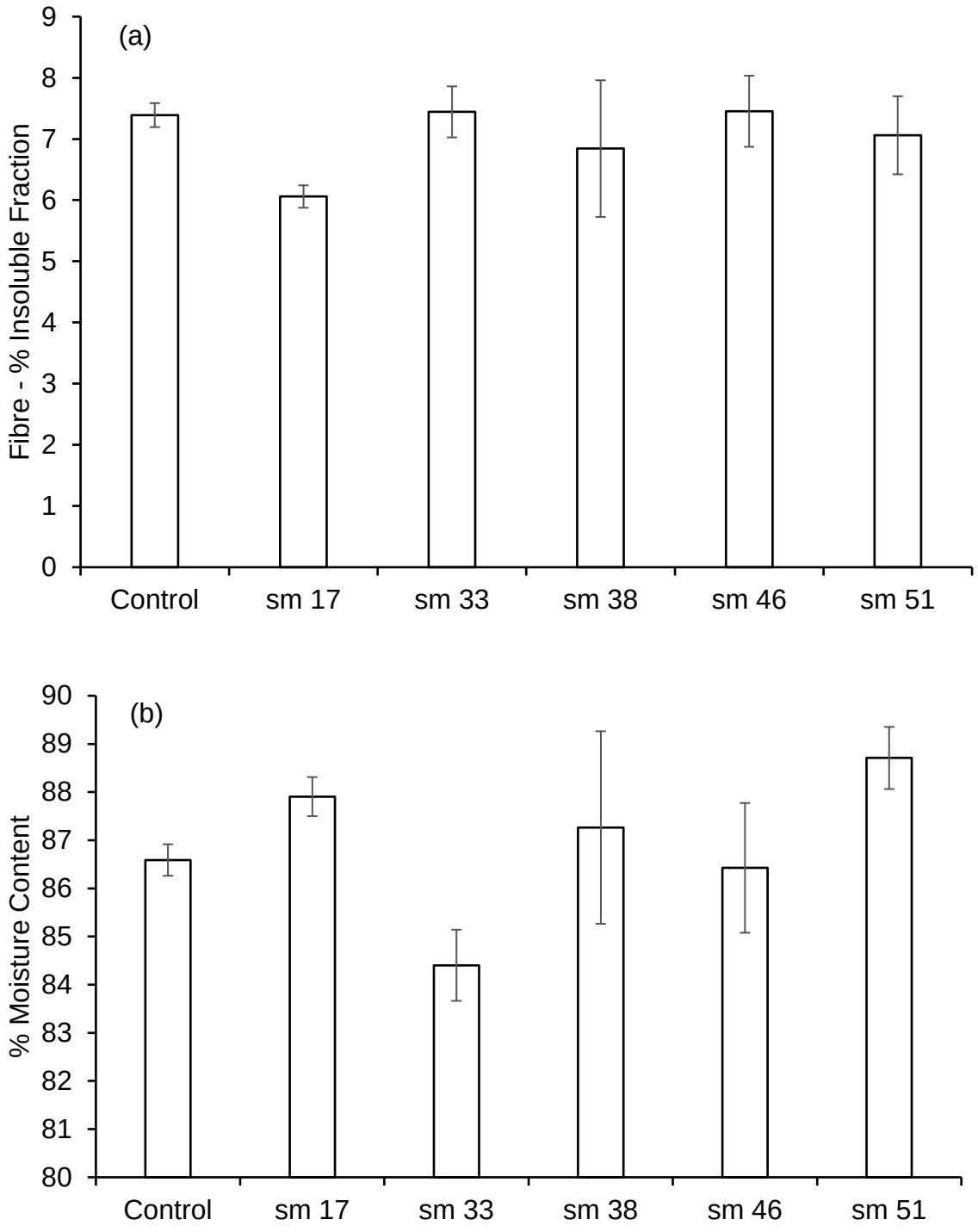


Fig. S4. Percentage of fibre (a) and moisture content (b) in internode seven from transgenic and control plants. The results are shown as averages (n=43 for control lines, n=4 for each transgenic line) with bars indicating standard errors.

Table S1. Sequences of primers used for amplification of the *ShSUT1* sequence and for qPCR

Primer name	Sequence 5' – 3'	Underlined restriction enzyme site	Italics restriction enzyme site
ShSUT1 stop F	gcg<u>ccccggg</u>*tacc*tcgagcccgggccgatcgc c	Xma1	Kpn1
ShSUT1 end R	gcgc<u>ggcgcgcc</u>*actagt*tcgaataatccttttgtttctcc	Asc1	Spe1
PST6s908	tcccgttcatcctctacgac		
PST6a530	cgacgccacgccgaacgccgggat		
ScSTDF	taccatacagaggaaacgag		
UIF	tgcacaagcttggatcctc		
OCSR	accgaaaccggcggtaag		
ShSUT1 199f	ctcacttcattcatgtggctatgc		
ShSUT1 299r	cgtcttccccatcttgctgt		
ShSUT1-fp01	ataagaagcaggcgaaacga		
ShSUT1-rp03	gagacctgaccagaccttgg		
ADF.f1[a]	ctactactgtggatttgtacgccattatag		
ADF.r1[a]	ggacctttttacacagcaacaaac		
SUT1 F1	ggctgctactcaactcgatt		
SUT1 R1	ctgggggatgacgatggagatg		

[a](Iskandar *et al.* 2004)

Table S2. Concentrations of sugars in mature leaves from control and transgenic sugarcane lines

Results are shown as averages with standard errors in brackets. (n = 44 for controls, n = 4 for each transgenic plant line) (GFS, mg g^{-1} FM glucose, fructose and sucrose combined). Within the column, concentrations marked by the same superscript letter were not significantly different from each other ($P < 0.05$)

Plant	GFS (mg g^{-1} FM)
Control	14.8 (0.8)^{c}
sm 17	9.2 (0.7)^{ab}
sm 33	12.0 (0.7)^{abc}
sm 38	8.3 (0.5)^{a}
sm 46	10.9 (1.8)^{abc}
sm 51	15.8 (2.4)^{bc}

Table S3. Fisher's protected least significant difference letter groups for concentrations of sugars in a series of internodes down the sugarcane stalk

These letters indicate the statistical significance of results shown in Fig. 6. Results with the same letter are not significantly different from each other column ($P < 0.05$). Those internodes not presented did not show any significant difference for that sugar at that internode or across all of the internodes

Plant	Fructose					Glucose				Sucrose
	All internodes	I4	I7	I10	I13	I4	I7	I10	I13	I7
Control	ab	a	b	b	a	a	bc	b	a	ab
sm17	c	b	d	b	c	b	d	bc	bc	ab
sm33	a	ab	a	a	a	ab	a	a	a	c
sm38	abc	ab	ab	b	ab	ab	ab	c	ab	b
sm46	bc	b	bc	b	bc	b	bcd	c	bc	b
sm51	abc	a	c	b	c	a	cd	c	c	a

References

Chen L-Q, Hou B-H, Lalonde S, Takanaga H, Hartung ML, Qu X-Q, Guo W-J, Kim J-G, Underwood W, Chaudhuri B, Chermak D, Antony G, White FF, Somerville SC, Mudgett MB, Frommer WB (2010) Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature* **468**, 527–532. doi:10.1038/nature09606

Grof CPL, Byrt CS, Patrick JW (2013) Phloem transport of resources. In 'Sugarcane: physiology, biochemistry and functional biology'. (Eds PH Moore, FC Botha) pp. 267–305. (John Wiley & Sons Ltd: Chichester, UK)

Iskandar HM, Simpson RS, Casu RE, Bonnett GD, Maclean DJ, Manners JM (2004) Comparison of reference genes for quantitative real-time polymerase chain reaction analysis of gene expression in sugarcane. *Plant Molecular Biology Reporter* **22**, 325–337. doi:10.1007/BF02772676