

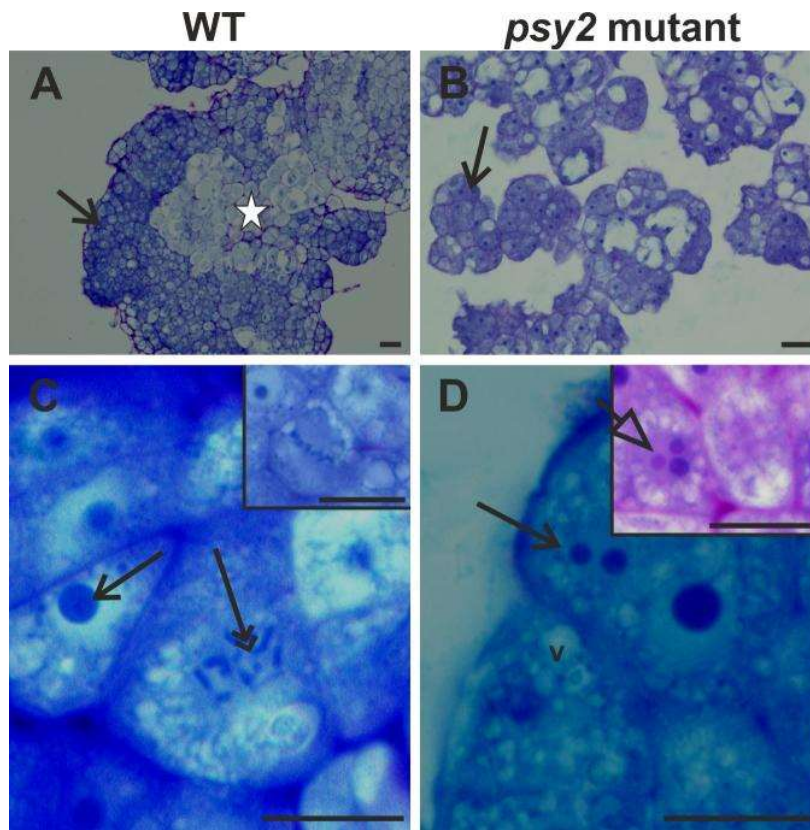
**A**

		<b>1</b>	<b>gRNA 1</b>	<b>51</b>
psy1/g1/WT		MACNFAVRVIYYPKEIHGVSVLNTRSRKSRFSCRMKLSTGVSAAVNPV		
psy1/g1/o27	[+1]	MACNFAVRVIYYPKEILWGFCVEHK*E*KE*I*LQGYEAVHWSVSCGS*SC		
		<b>239</b>	<b>gRNA 2</b>	<b>289</b>
psy1/g2/WT		LMSVPVMGIAPESKATTESVYSAALALGIANQLTNILRDVGEDARRGRIYL		
psy1/g2/o27	[-11]	LMSVPVMGIA--RHHRECLQCSFSPRNC*SAY*HSP*CWRRC*ERENISA		
psy1/g2/o27	[G/A]	LMSVPVMGIAPKSKATTESVYSAALALGIANQLTNILRDVGEDARRGRIYL		
psy1/g2/o28	[-1]	LMSVPVMGIAPDQRPQRFVTVQL*PSELLISLLTFSVMLEKMLGEGEYIC		
psy1/g2/o28	[G/A]	LMSVPVMGIAPKSKATTESVYSAALALGIANQLTNILRDVGEDARRGRIYL		
psy1/g2/o29	[-2]	LMSVPVMGIAPVKGHHRECLQCSFSPRNC*SAY*HSP*CWRRC*ERENISA		
psy1/g2/o186	[-3]	LMSVPVMGIAP-AKATTESVYSAALALGIANQLTNILRDVGEDARRGRIYL		
psy1/g2/o186	[-6]	LMSVPVMG--LPKATTESVYSAALALGIANQLTNILRDVGEDARRGRIYL		
psy1/g2/o186	[-10]	LMSVPVMG---NQRPPQRFVTVQL*PSELLISLLTFSVMLEKMLGEGEYIC		
psy1/g2/o186	[G/A]	LMSVPVMGIAPESKATTESVYSAALALGIANQLTNILRDVGEDARRGRIYL		
psy1/g2/o186	[C/A]	LMSVPVMGIATESKATTESVYSAALALGIANQLTNILRDVGEDARRGRIYL		
psy1/g2/o196	[+1]	LMSVPVMGIAPEIKGHHRECLQCSFSPRNC*SAY*HSP*CWRRC*ERENIS		
psy1/g2/o196	[-16]	LMSVPVMGIAP-----QRFVTVQL*PSELLISLLTFSVMLEKMLGEGEYIC		
psy1/g2/o196	[-20]	LMSVPVMG-----HHRECLQCSFSPRNC*SAY*HSP*CWRRC*ERENIS		
psy1/g2/o196	[G/A]	LMSVPVMGIAPKSKATTESVYSAALALGIANQLTNILRDVGEDARRGRIYL		

**B**

		<b>23</b>	<b>gRNA 1</b>	<b>73</b>
psy2/g1/WT		ETAREGTRVLDPSRLGSRDKNMRCGGRLEKGLRKWSSKSFNAEYSYSCLG		
psy2/g1/o270	[+1]	ETAREGTRVLDPSRLGFPG*EYEVWRQT*EG*AAEVEF*IFQC*I*LFVFG		
psy2/g1/o270	[+1]	ETAREGTRVLDPSRLGYPG*EYEVWRQT*EG*AAEVEF*IFQC*I*LFVFG		
psy2/g1/o270	[-4]	ETAREGTRVLDPSRLGWIRI*GVEADLRVSCGSGVLNLSMLNIAIRVWV		
psy2/g1/o270	[-286]	ETAREGTRVLDPSRLG-----		
psy2/g1/o280	[-42]	ETAR-----DKNMRCGGRLEKGLRKWSSKSFNAEYSYSCLG		
psy2/g1/o280	[-81]	ETAREGTRVLDPS-----FNAEYSYSCLG		
psy2/g1/o280	[-81]	ETAREGTRVLDLS-----FNAEYSYSCLG		
psy2/g1/o293	[+1, C/A]	ETAREGTRVLDPSRLGYQG*EYEVWRQT*EG*AAEVEF*IFQC*I*LFVFG		
psy2/g1/o298	[-27]	ETAREGTRVLDPSR-----CGGRLEKGLRKWSSKSFNAEYSYSCLG		
psy2/g1/o298	[C/T]	ETAREGTRVLDPSRLGFRDKNMRCGGRLEKGLRKWSSKSFNAEYSYSCLG		
		<b>119</b>	<b>gRNA 2</b>	<b>169</b>
psy2/g2/WT		QFRSDEELEVKEPILPGTSLSLSEAYDRCEVCAEYAKTFYLGTLTMTPE		
psy2/g2/o270	[-1]	QFRSDEELEVKEPILRGL*AC*VKLMIDAVKYVLSMPKHFTWEHY**PQR		
psy2/g2/o270	[-1]	QFRSDEELEVKEPILRGL*AC*VKLMIDAVKYVLSMPKHFTWEHY**PQR		
psy2/g2/o270	[-286]	-----FRGL*AC*VKLMIDAVKYVLSMPKHFTWEHY**PQR		
psy2/g2/o270	[dup]	QFRSDEELEVKEPILPG*EYEVWRQT*EG*AAEVEF*IFQC*I*LFVFGW		
psy2/g2/o280	[-1]	QFRSDEELEVKEPILRGL*AC*VKLMIDAVKYVLSMPKHFTWEHY**PQR		
psy2/g2/o280	[+1]	QFRSDEELEVKEPILAGDSELVK*SL*SMR*SMC*VCQNILLGNTTDDPR		
psy2/g2/o280	[-73]	QFRSD-----AVKYVLSMPKHFTWEHY**PQR		
psy2/g2/o293	[+1]	QFRSDEELEVKEPILAGDSELVK*SL*SMR*SMC*VCQNILLGNTTDDPR		
psy2/g2/o293	[+1]	QFRSDEELEVKEPILSGDSELVK*SL*SMR*SMC*VCQNILLGNTTDDPR		
psy2/g2/o298	[+1]	QFRSDEELEVKEPILTGSELVK*SL*SMR*SMC*VCQNILLGNTTDDPR		
psy2/g2/o298	[-2]	QFRSDEELEVKEPIL-PGDSELVK*SL*SMR*SMC*VCQNILLGNTTDDPR		
psy2/g2/o298	[-2]	QFRSDEELEVKEPIL-GDSELVK*SL*SMR*SMC*VCQNILLGNTTDDPR		
psy2/g2/o298	[-32]	QFRSDEELEVK-----VK*SL*SMR*SMC*VCQNILLGNTTDDPR		
psy2/g2/o306	[+1]	QFRSDEELEVKEPILSGDSELVK*SL*SMR*SMC*VCQNILLGNTTDDPR		
psy2/g2/o306	[-1]	QFRSDEELEVKEPILRGL*AC*VKLMIDAVKYVLSMPKHFTWEHY**PQR		

**Figure S1** Amino acid sequences of PSY in the wild type (WT; not transformed control) and mutant lines. The sequence changes in mutants in comparison to WT are marked in red. (A) Sequences of PSY1 in psy1-g1g2 lines. (B) Sequences of PSY2 in psy2-g1g2 lines.



**Figure S2** Morphology and structure of (A and C) the wild type (WT; not transformed control) and (B and D) *psy2* mutant callus. (A) Dividing cells (arrow) localized at the periphery of callus clumps surrounding the parenchymatic cells (white star). (B) *psy2* mutant callus composed mainly of dividing cells (arrow) characterised by dense cytoplasm. (C) The dividing WT cells were characterised by large nucleus with prominent nucleolus (arrow), and numerous cells in different phases of mitosis (double arrow; inset – metaphase plate). (D) *psy2* mutant callus cells with different number of nuclei (two nuclei – arrow; three nuclei – inset, empty arrow) and numerous small vacuoles (v). Scale bars = 20  $\mu\text{m}$ .

**Table S1** Primers used for PCR and qPCR

Amplified fragment	Primer name	Primer sequence 5' - 3'
<i>aph</i>	aph-F	AAGGAATCGGTCAATACACTACATGG
	aph-R	AAGACCAATGCGGAGCATATACG
35S: <i>Cas9</i>	CaMV-35S-F	CCACGTCTTCAAAGCAAGTGG
	AteCas9-R	TTGGGTGTCTCTCGTGCTTC
<i>psy1</i> (gRNA1 region)	psy1-F1	ACAACCCAACTCAACTCTCTCT
	psy1-R1	TACTCAGCACAAACTTCGCCA
<i>psy1</i> (gRNA2 region)	psy1-F2	CCTCCCATATCACGCCCAAG
	psy1-R2	ACTCTTCTCCAACATCACGG
<i>psy2</i> (gRNA1+gRNA2)	psy2-F	GTTGGTGTGAATGGTTGAGGC
	psy2-R	AGTAATTTAGAGGCAGTCAGCA
<i>psy1</i> (qPCR)	psy1-qpcr-F	GGGAATAACACCAGAATAC
	psy1-qpcr-R	ATCTACTTGCTGAACTTAAC
<i>psy2</i> (qPCR)	psy2-qpcr-F	TATGTTGCTGGTACTGTTGG
	psy2-qpcr-R	CGTTGTTGCCTGTGAATTAG

**Table S2**

Cellular epitopes localization and degree of their occurrence within the callus of different lines

Epitope	WT	<i>psy2</i> mutant
<b>pectins</b>		
JIM5	walls, intercellular spaces +++	walls, cytoplasmic compartments +
JIM7	walls, cytoplasmic compartments and secreted outside the walls of peripheral cells +++	walls, cytoplasmic compartments and secreted outside the walls of peripheral cells +++
<b>AGPs</b>		
JIM13	walls, cytoplasmic compartments +++	walls, cytoplasmic compartments in selected cells +
LM2	walls, cytoplasmic compartments +	present in new cell walls, cytoplasmic compartments in some cells +/-
<b>extensin</b>		
JIM20	walls, intercellular spaces -	extracellular location +

The percentage of cells that contain the epitope was taken into account: – no labelling, indicates the lack of epitope or the presence below 1% of cells, +/- weak labelling indicates the presence of the epitope between 2-10% of callus cells, + moderate labelling indicates the presence of epitope between 20-40% of callus cells, and +++ 100% callus cells expressing the epitope; the values are derived from three samples of each type of callus.