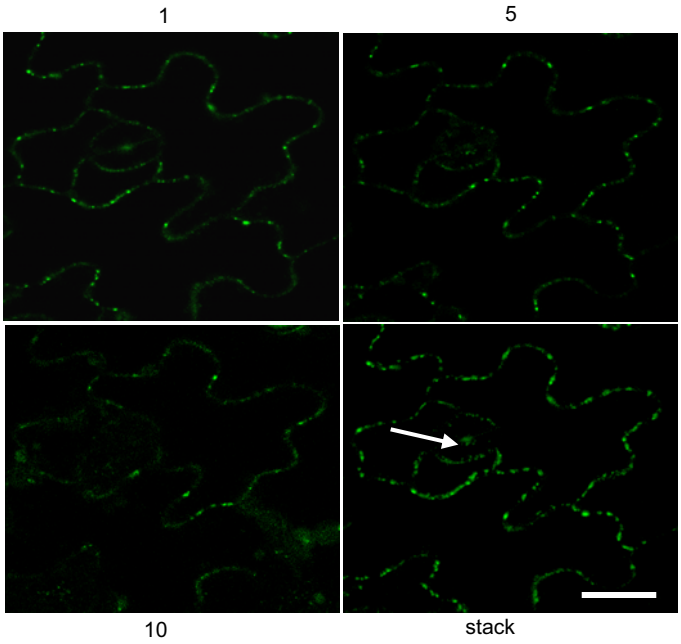
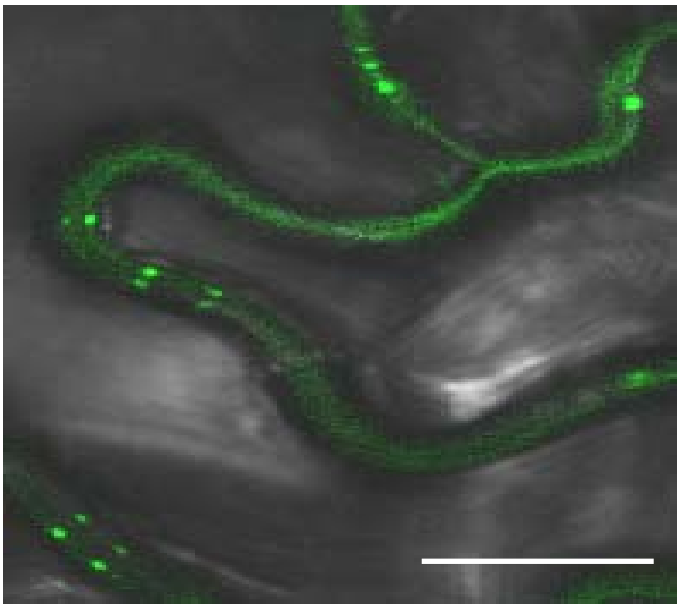
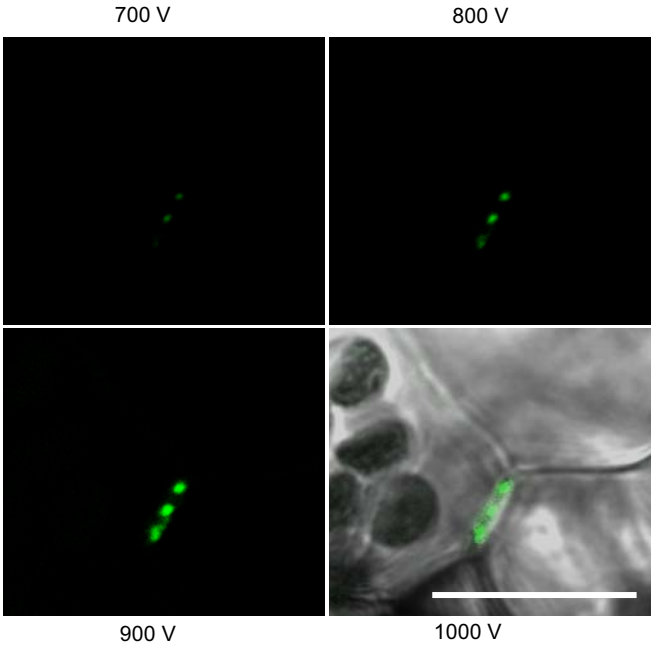
Supplemental Data. Simpson et al. (2009). An *Arabidopsis* GPI-anchor plasmodesmal neck protein with callose-binding activity and potential to regulate cell-to-cell trafficking.



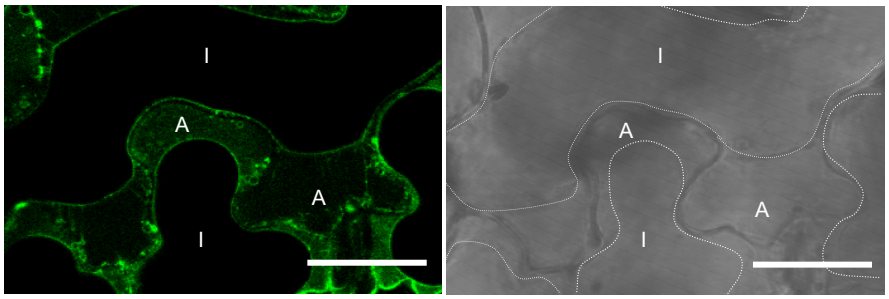
Supp- Figure 1 Confocal series through *Arabidopis* leaf epidermal pavement cells expressing *35S:YFP-PDCB1*.
The series is illustrated as individual confocal sections at positions 1, 5 and 10 through a depth of the cell comprising 6 µm. The remaining panel (stack) shows all 10 optical sections combined. The predominant labelling is as a punctate pattern on the wall with little evidence of cytoplasmic labelling. The exception is the single stoma (arrow) present in the centre of the image where the punctate patterning is less evident. Bar = 10 µm.



Supp- Figure 2 Confocal micrograph of *N. benthamiana* tissues transiently expressing *35S:YFP-PDCB1*.
PDCB1 was targeted to plasmodesmata (twin punctate spots on the cell wall) but with the high levels of transient expression in *N. benthamiana,* the protein also appeared to accumulate elsewhere on the plasma membrane. Bar = 10 μm.



Supp- Figure 3 Confocal microscopy of unplasmolysed *Arabidopsis* leaf spongy mesophyll cells expressing *35S:YFP-PDCB1* viewed at increasing power settings. Micrographs show the same confocal section viewed with the confocal power settings at 700, 800, 900 and 1000 Volts. The image at 1000 V is combined with the DIC image to show the cell wall boundaries and the restricted points of contact between adjacent cells. Even at the highest power settings the fluorescence is restricted to Pds. Bars= 10 µm.



Supp- Figure 4 *Arabidopsis* leaf epidermal pavement cells expressing *35S:YFP-PDCB1* viewed after plasmolysis without pretreatment with the phospholipase C inhibitor.
In the absence of the inhibitor the apoplastic spaces (A) have become filled with fluorescent protein leached from the wall; intracellular spaces (I) remain devoid of fluorescence. In the DIC image (right) the outline of the retracted plasma membrane is indicated with a dotted line. This leaching contrasts with the situation after pretreament with the phospholipase C inhibitor (see Figure 2D to F). Bar = 10 µm

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CLUSTAL W(1.60) multiple sequence alignment of X8 domains

at2g43670       WCVAKPSTDNERLQENINFACSIDCQIISEGGACYLPDSIISRASVAMNLYYQAQGRHFW
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at2g43660       WCVAKPGTPIKQLVKNLNNVCSVHCEVVSEGGACYDPINLYNSASVVMNLYYQNQGRQYS
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at3g58100       ACNFNNNAALTSLNPSCKY
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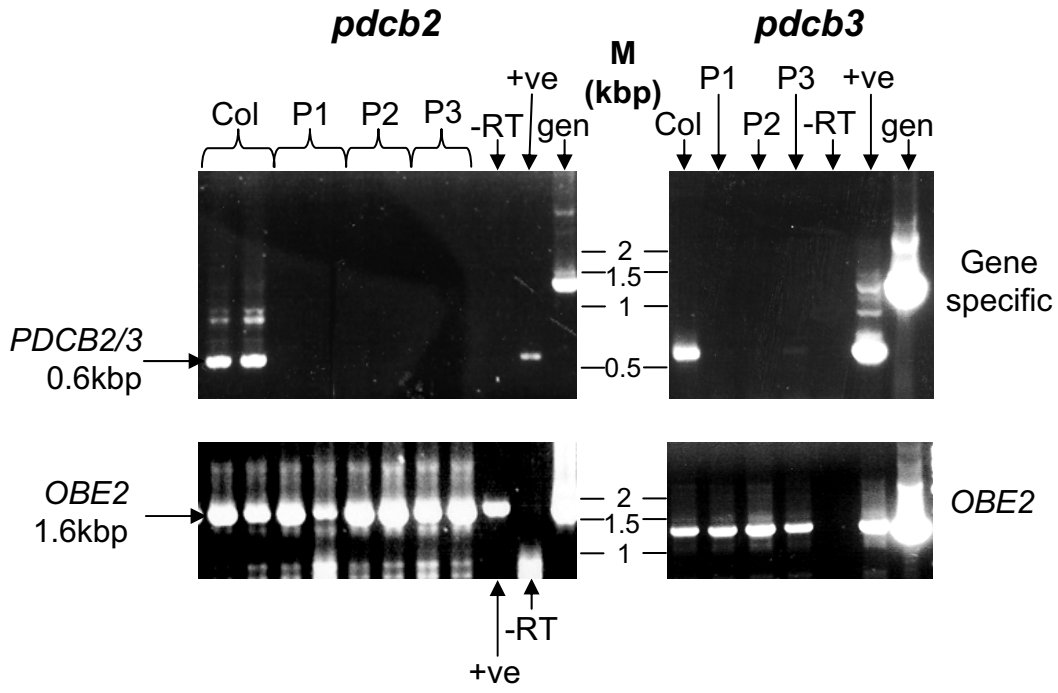
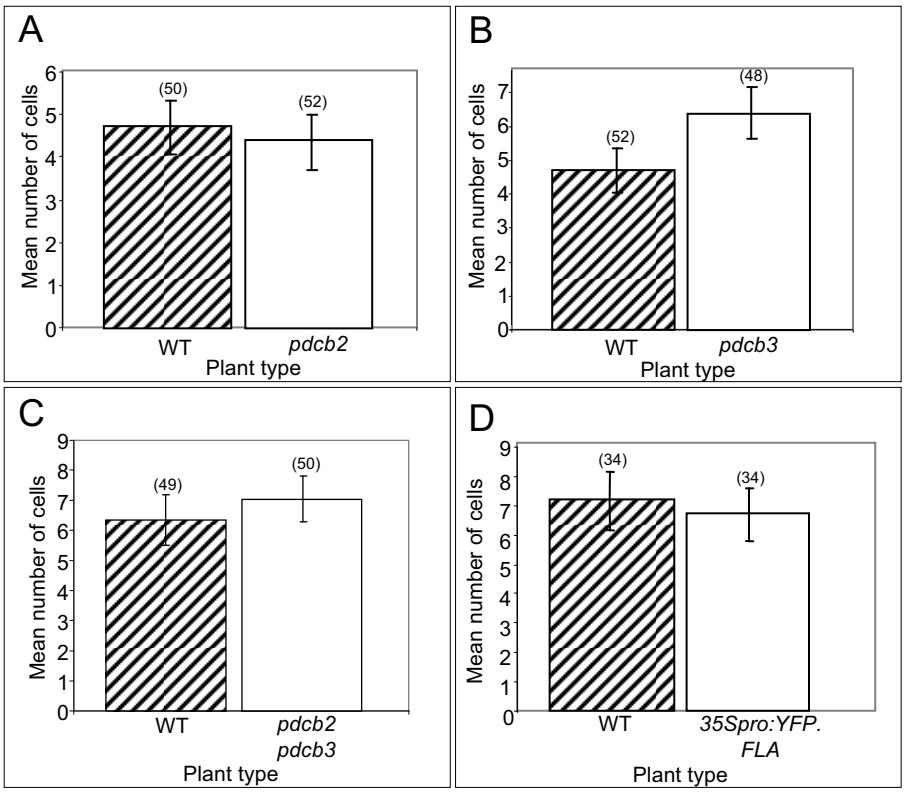
Supp- Figure 5 Alignment of the X8 domain amino acid sequences for proteins with structural similarity to PDCB1.

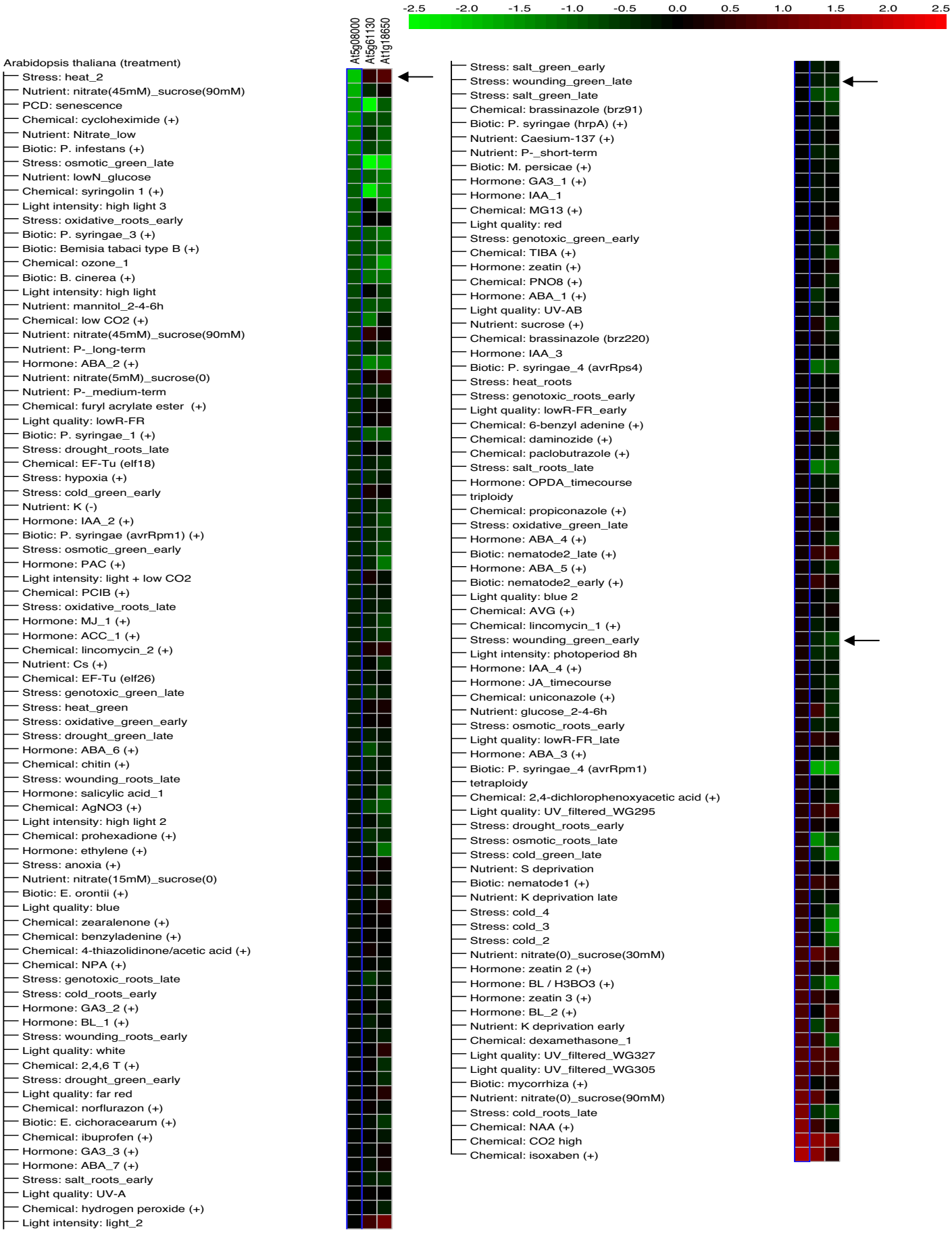


Supp- Figure 6 RNA analysis for *pdcb2* and *pdcb3* mutant lines. RNAs from three independent plants (P1-3) each for *pdcb2* and *pdcb3*, and RNA and genomic DNA (gen) from wild type Col-0 (Col) were analysed by RT-PCR using gene specific primers for the accumulation of transcripts from the mutated genes (upper panels). Negative (-RT; reaction lacking RT) and positive (+ve; specific plasmid DNA) control were run in parallel. In addition the samples were analysed for the expression of the OBERON2 gene (Saiga et al., 2008) using specific primers (lower panels). In this qualitative assay, gels were stained with ethidium bromide. The 0.6 kbp diagnostic fragment for *PDCB2* or *PDCB3* RNAs was not detected from any of mutant plants tested (*pdcb2*: three biological and two technical replicates, *pdcb3*: three biological and one technical replicate) . M shows the migration of DNA size markers in kbp.

Reference: Saiga, S., Furumizu, C., Yokoyama, R., Kurata, T., Sato, S., Kato, T., Tabata, S., Suzuki, M. and Komeda, Y. (2008). The Arabidopsis OBERON1 and OBERON2 genes encode plant homeodomain finger proteins and are required for apical meristem maintenance. *Development* **135**: 1751-1759.

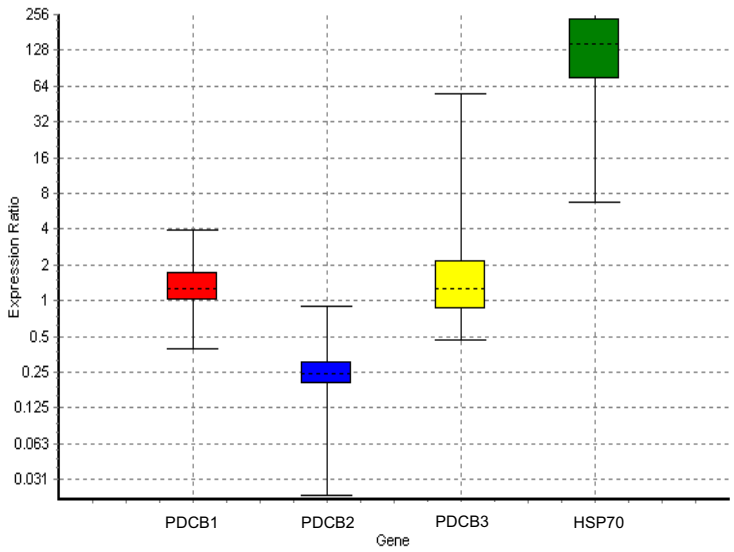


Supp. Figure 7 GFP trafficking is unchanged in *pdcb2 and -3* knockout lines and in a transgenic line expressing the unrelated GPI-anchor protein FLA13.
A GFP diffusion assessed at 48 h post-bombardment in WT and *pdcb2* lines.
B GFP diffusion assessed at 48 h postbombardment in WT and *pdcb3* lines.
C GFP diffusion assessed at 48 h postbombardment in WT and the *pdcb2 pdcb3* lines.
D GFP diffusion assessed in WT and *35Spro:YFP.FLA13* lines.
Numbers in parentheses indicate the numbers of bombardment sites assayed. Bars = +SE. There was no significant difference in GFP movement in any of the treatments.



Supp- Figure 8 Stress-induced changes in the expression of *PDCB* genes (arbitrary units). Public dataset (https://www.genevestigator.ethz.ch) for the expression for At5g61130 (*PDCB1*), At5g08000 (*PDCB2*) and At1g18650 (*PDCB3*), displayed as a heat map. Treatments are ranked with respect to the magnitude of the expression changes from down-regulation (green) to up-

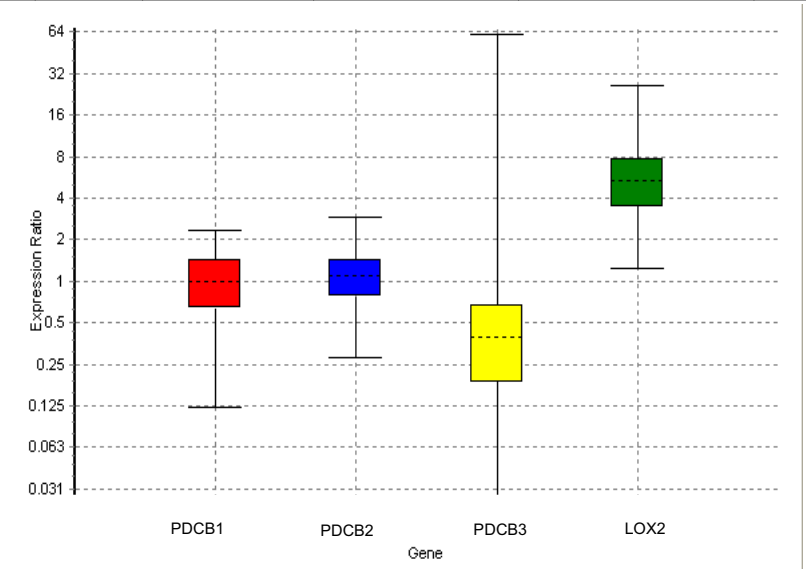
A



B

Gene	Type	Expression	Std. Error	95% C.I.	P value	Result
PDCB1	TRG	1.282	0.850 - 2.129	0.443 - 2.898	0.157	
PDCB2	TRG	0.213	0.172 - 0.360	0.025 - 0.629	0.000	DOWN
PDCB3	TRG	1.709	0.782 - 3.277	0.519 - 23.615	0.138	
eIF1α	REF	1.000				
HSP70	TRG	129.310	49.262 - 305.593	20.573 - 685.441	0.000	UP

C



D

Gene	Type	Expression	Std. Error	95% C.I.	P value	Result
PDCB1	TRG	0.906	0.483 - 1.765	0.249 - 2.299	0.696	
eIF1α	REF	1.000				
PDCB2	TRG	1.034	0.560 - 1.779	0.335 - 2.600	0.859	
PDCB3	TRG	0.512	0.140 - 0.858	0.056 - 57.004	0.304	
LOX2	TRG	5.391	2.954 - 9.416	1.759 - 23.641	0.000	UP

Supp- Figure 9 qRT-PCR data for *PDCB* expression following stress treatments
A Box plot of qRT-PCR data for heat treated seedlings.
B Numerical data as presented in A. TRG=treatment; Ref=Reference
C Box plot of qRT-PCR data for wounded seedlings.
D Numerical data as presented in C.
Data from three biological and three technical replicates were analysed using REST