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Functional Plant Biology

Supplementary Material

***Arabidopsis*plasma membrane intrinsic protein (AtPIP2;1) is implicated in a salinity conditional influence on seed germination**

Phan Thi Thanh Hoai[A,B,C,*], *Jiaen Qiu*[A,B], *Michael Groszmann*[D], *Annamaria De Rosa*[D], *Stephen D. Tyerman*[A,B], *and Caitlin S. Byrt*[A,B,D,*]

[A]Australian Research Council Centre of Excellence in Plant Energy Biology, Waite Research Precinct, University of Adelaide, Glen Osmond, SA, Australia.

[B]School of Agriculture, Food and Wine, and Waite Research Institute, Waite Research Precinct, University of Adelaide, Glen Osmond, SA, Australia.

[C]Faculty of Agriculture and Forestry, Tay Nguyen University, 567 Le Duan, BMT, Dak Lak, Vietnam.

[D]Australian Research Council Centre of Excellence for Translational Photosynthesis, Division of Plant Sciences, Research School of Biology, The Australian National University, Acton, ACT 2601, Australia.

[*]Correspondence to: Phan Thi Thanh Hoai Australian Research Council Centre of Excellence in Plant Energy Biology, Waite Research Precinct, University of Adelaide, Glen Osmond, SA, Australia Email: hoaiphan.agsc@gmail.com Caitlin S. Byrt Division of Plant Sciences, Research School of Biology, The Australian National University, Acton, ACT 2601, Australia Email: Caitlin.Byrt@anu.edu.au

SUPPLEMENTARY FIGURES

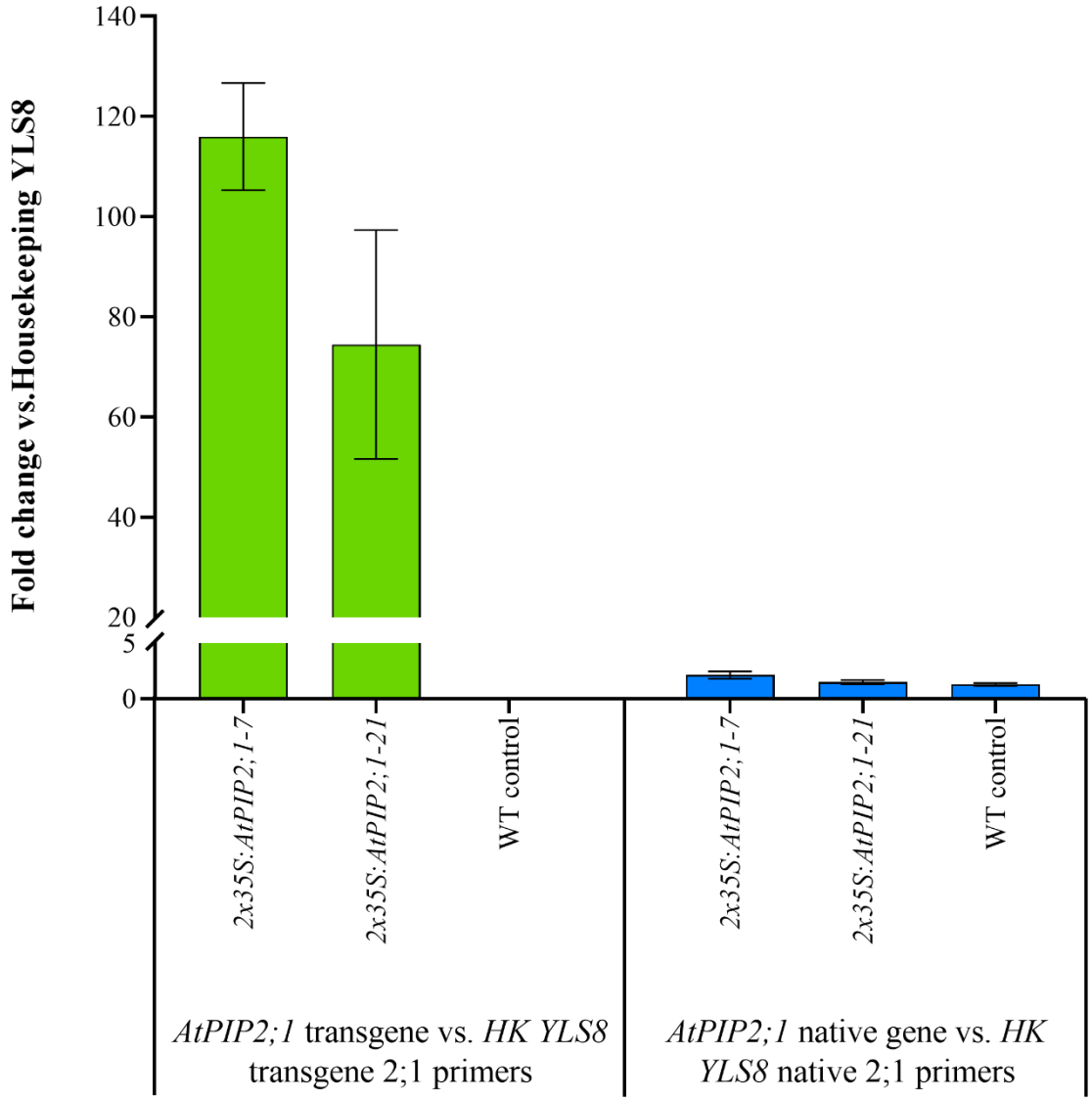


Figure S1: Relative transcript expression of for two independent lines (lines 7 and 21) that carried a single *2x35S::AtPIP2;1* insertion event vs. housekeeping gene (YLS8 - AT5G08290) as determined by segregation counts and qPCR. The over expression lines had a 71- and 45-fold greater abundance of *AtPIP2;1* transcript compared to the average abundance of the native gene from three lines. Data was generated from T_2 segregating plants selected on hygromycin media. Biological replication included aerial tissue of 8 seedlings at 18 days after germination.

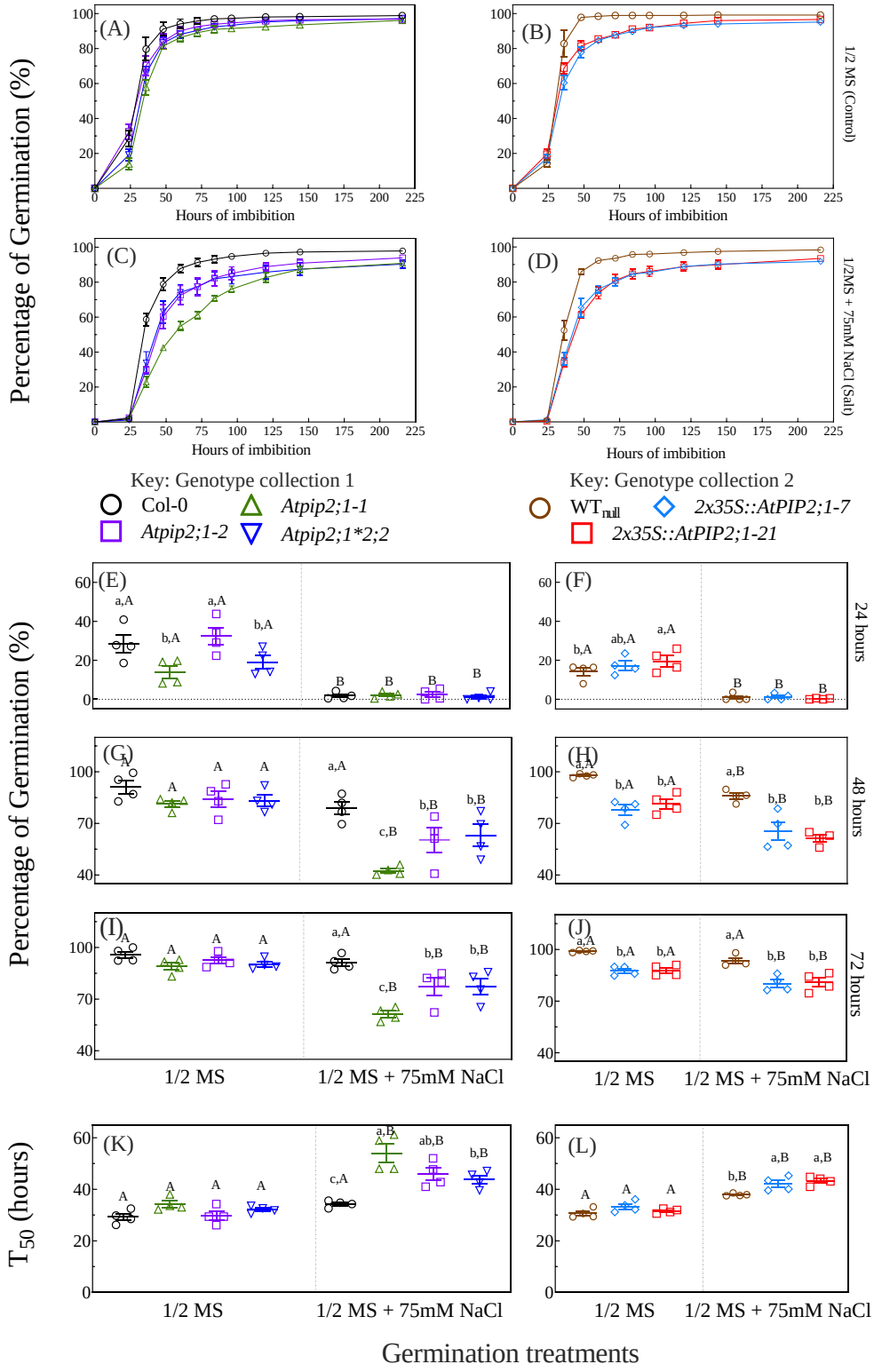


Figure S2A: Replication two of the experiment reported in Figure 1: Cumulative germination success and time to fifty percent germination (T_{50}) of Arabidopsis lines differing in PIP abundance in the presence or absence of 75 mM NaCl.

Data is reported as percentage of seed germinated under control treatment (½ MS) or saline treatment (½ MS + 75 mM NaCl). All seed with endosperm rupture were recorded over

time (0-216 h) upon exposure to 0 mM (A,B) or 75mM (C,D) added NaCl treatments. Statistical analysis of percent germination data at individual time points after stratification: 24 h (panels E-F), 48 h (panels G-H) and 72 h (panels I-J) and time to reach fifty percent germination T_{50} in h (panels K-L). Data was divided into two groups for analysis; genotype collection 1 consisting of Col-0 (○), *Atpip2;1-1* (△), *Atpip2;1-2* (□), *Atpip2;1*2;2* (▽), and genotype collection 2 consists of *2x35S::AtPIP2;1* OE 7 (◇), *2x35S::AtPIP2;1* OE 21 (□) and WT_{null} (○). T-tests indicated that there was no significant difference between WT_{null} and Col-0. Each panel includes percent seed germination in ½ MS or ½ MS + 75 mM NaCl. Data are means (±SEM) from n = 4 plates, with 150 – 250 seeds per genotype per plate. Significant difference determined by a factorial repeated measures two-way ANOVA and Tukey's HSD pair-wise tests at significant level 0.05. Different lowercase letters denote significant differences between genotypes within each germination treatment; different capital letters denote significant differences between ½ MS and ½ MS + 75 mM NaCl for each genotype. Absence of error bars indicates SE is smaller than symbol.

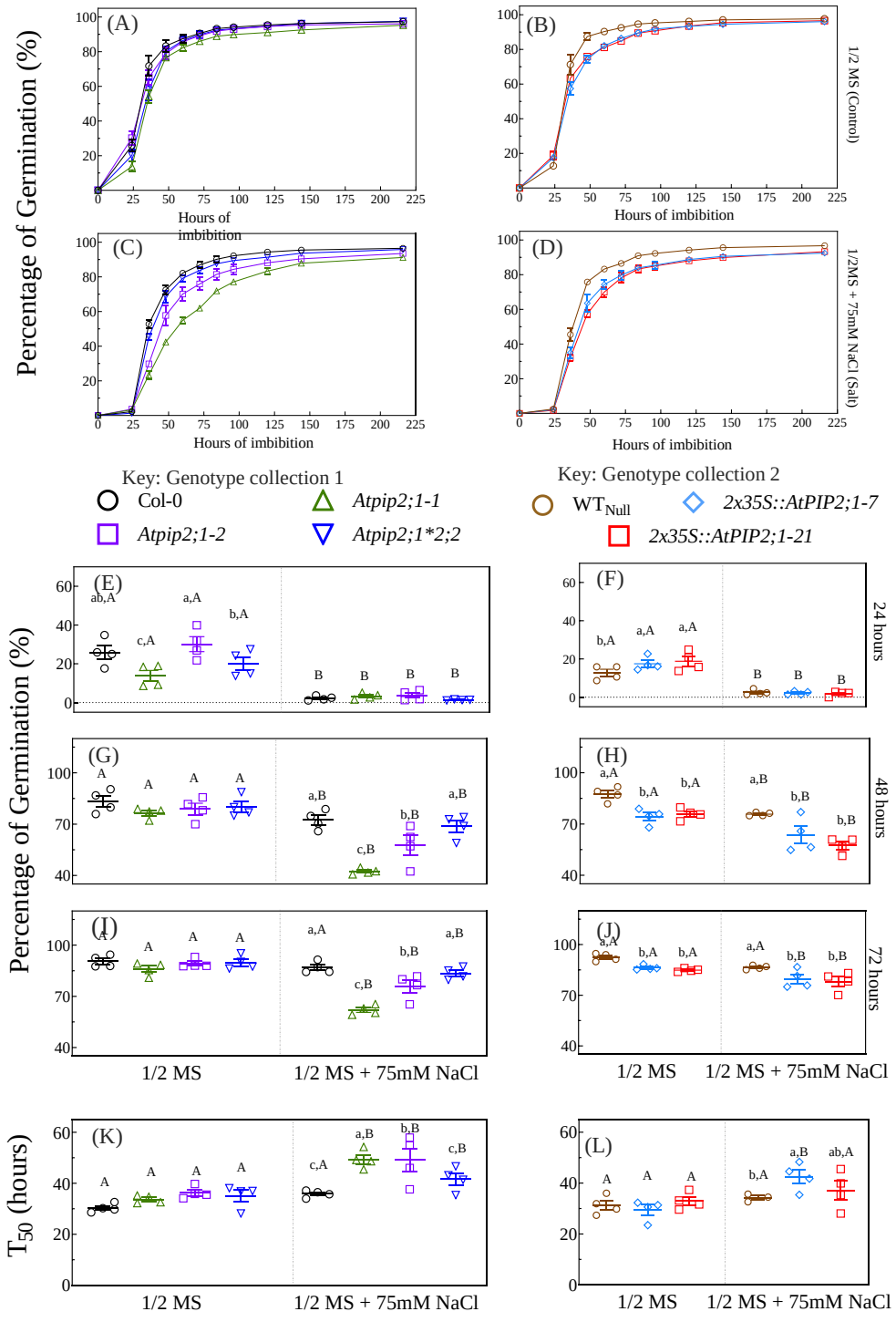


Figure S2B: Replication three of the experiment reported in Figure 1: Cumulative germination success and time to fifty percent germination (T_{50}) of Arabidopsis lines differing in PIP abundance in the presence or absence of 75 mM NaCl.

Data is reported as percentage of seed germinated under control treatment (½ MS) or saline treatment (½ MS + 75 mM NaCl). All seed with endosperm rupture were recorded over time (0-216 h) upon exposure to 0 mM (A,B) or 75mM (C,D) added NaCl treatments.

Statistical analysis of percent germination data at individual time points after stratification: 24 h (panels E-F), 48 h (panels G-H) and 72 h (panels I-J) and time to reach fifty percent germination T_{50} in h (panels K-L). Data was divided into two groups for analysis; genotype collection 1 consisting of Col-0 (○), *Atpip2;1-1* (△), *Atpip2;1-2* (□), *Atpip2;1*2;2* (▽), and genotype collection 2 consists of *2x35S::AtPIP2;1* OE 7 (◇), *2x35S::AtPIP2;1* OE 21 (□) and WT_{null} (○). T-tests indicated that there was no significant difference between WT_{null} and Col-0. Each panel includes percent seed germination in ½ MS or ½ MS + 75 mM NaCl. Data are means (±SEM) from n = 4 plates, with 150 – 250 seeds per genotype per plate. Significant difference determined by a factorial repeated measures two-way ANOVA and Tukey's HSD pair-wise tests at significant level 0.05. Different lowercase letters denote significant differences between genotypes within each germination treatment; different capital letters denote significant differences between ½ MS and ½ MS + 75 mM NaCl for each genotype. Absence of error bars indicates SE is smaller than symbol.

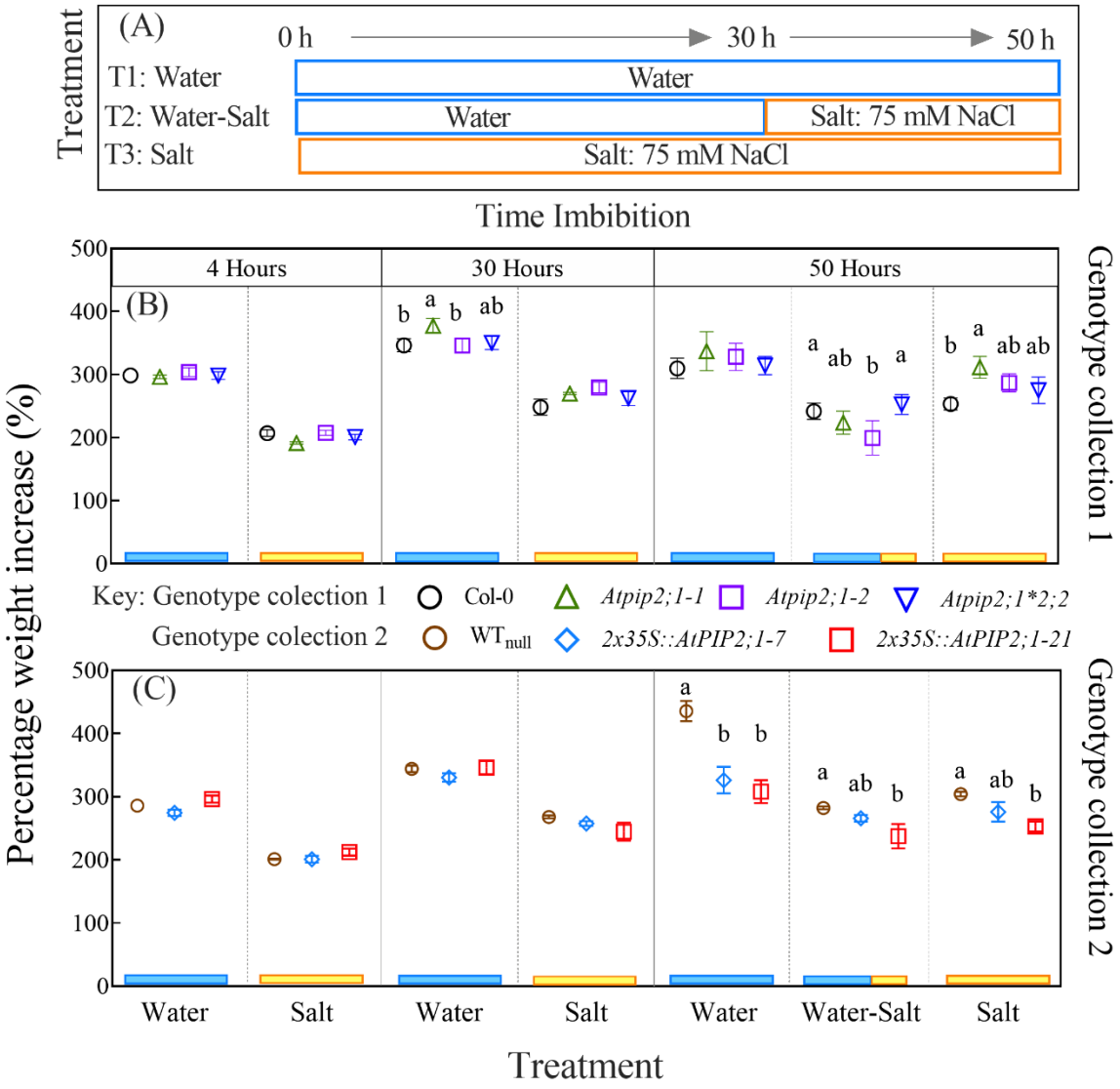


Figure S3. Seed weight increase in percentage relative to fresh dry seed.

A, Summary of the experimental set up with 3 treatments: Treatments consisted of 50 h imbibition in water only (water; T1); 30 h imbibition in water followed by 20 h in 75 mM NaCl solution (50 h total, Water-Salt; T2) and 50 h imbibition in 75 mM NaCl solution (salt; T3). B-C, Relative increase in seed weight compared to the fresh dry seed weight during 50 h of imbibition in two genotype collections. Genotype collection 1 consisting of Col-0 (○), *Atpip2;1-1* (△), *Atpip2;1-2* (□), *Atpip2;1*2;2* (▽), and genotype collection 2 consists of *2x35S::AtPIP2;1* OE 7 (◇), *2x35S::AtPIP2;1* OE 21 (□) and WT_{null} (○).

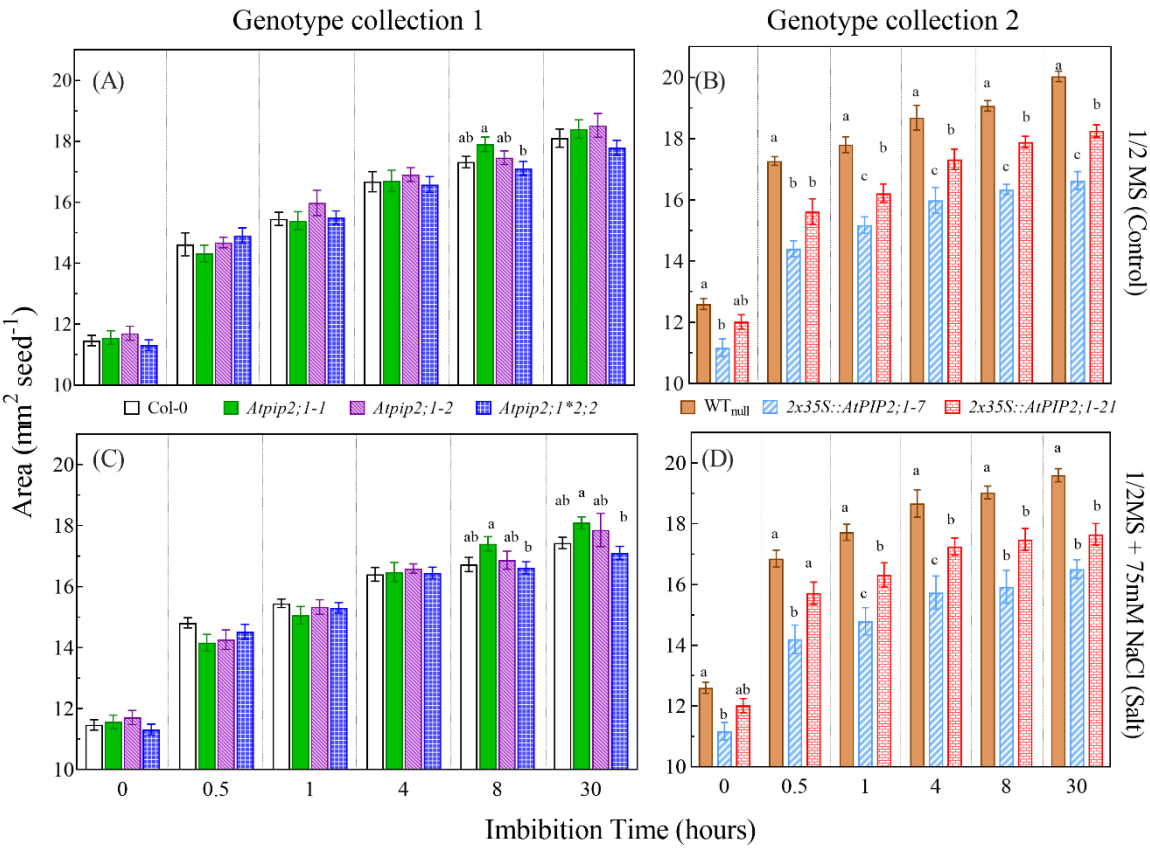


Figure S4: Seed MCS area increase upon imbibition in control (½ MS - panel A and B) and saline treatment (½ MS + 75 mM NaCl - panel C and D).

Data are means (±SE) from n=4-8 plates, with 200 seeds per genotype per plate. At some time points the error bars are so tight as to be obscured by the symbols. Significant difference determined by two-way ANOVA and Fisher's LSD pair-wise tests.

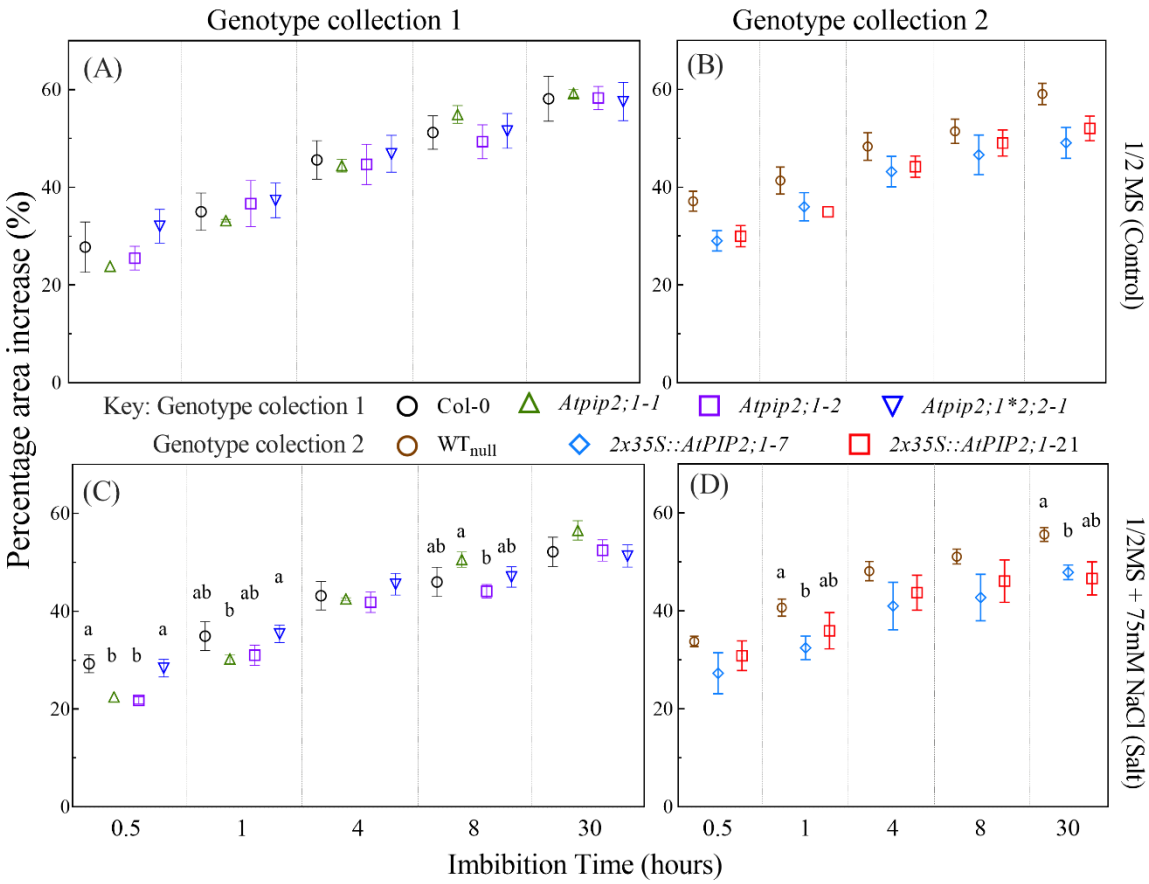


Figure S5. Changes in percentage of imbibing seed MCS area over time compared to seed at which had been imbibing for two minutes.

Data is divided into two groups: Genotype collection 1 consisting of Col-0 (○), *Atpip2;1-1* (△), Atpip*2;1-2* (□), *Atpip2;1*2;2* (▽), and Genotype collection 2 consists of *2x35S::AtPIP2;1* OE 7 (◇), *2x35S::AtPIP2;1* OE 21 (□) and WT_{null} (○).

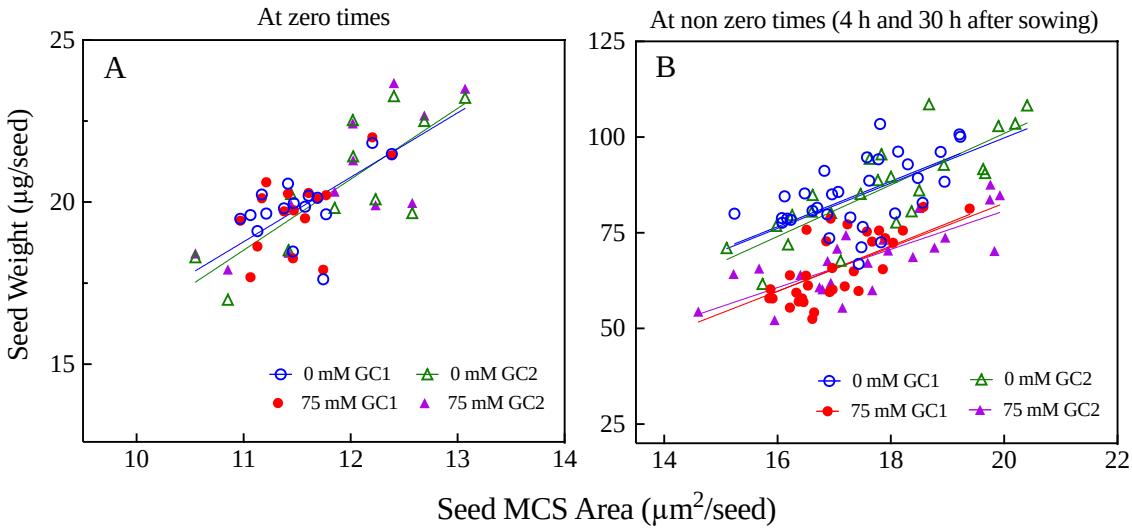


Figure S6. Positive relationships between seed MCS area and its weight change from dry seed to 30 h imbibition of two genotypes collections. Genotype collection 1 (GC1) consists of WT Col-0, *Atpip2;1-1*, *Atpip2;1-2*, *Atpip2;1*2;2*, and genotype collection 2 (GC1) includes *2x35S::AtPIP2;1* OE 7, *2x35S::AtPIP2;1* OE 21 and WT_{null}. Data at zero times (0 h) (Figure S4A) and at non zero times (4 and 30 h) (Figure S4B) after the start of imbibition are shown. As seed swells during imbibition, it is expected that both the weight and seed area would increase, so the positive relationship was expected. The seed area measures that were captured represent maximum cross-sectional (MCS) rather than total seed area because images of seed swelling were captured from an aerial view which does not capture vertical swelling, hence it was expected that the R^2 values would be less than 1.

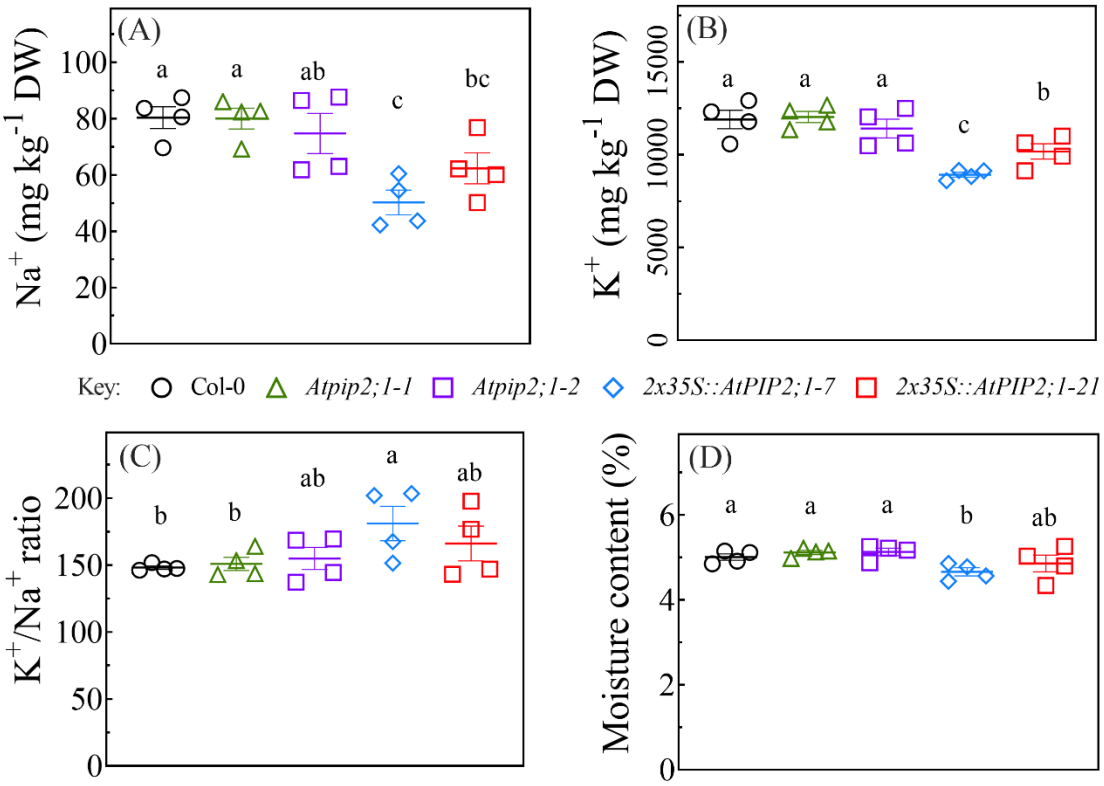
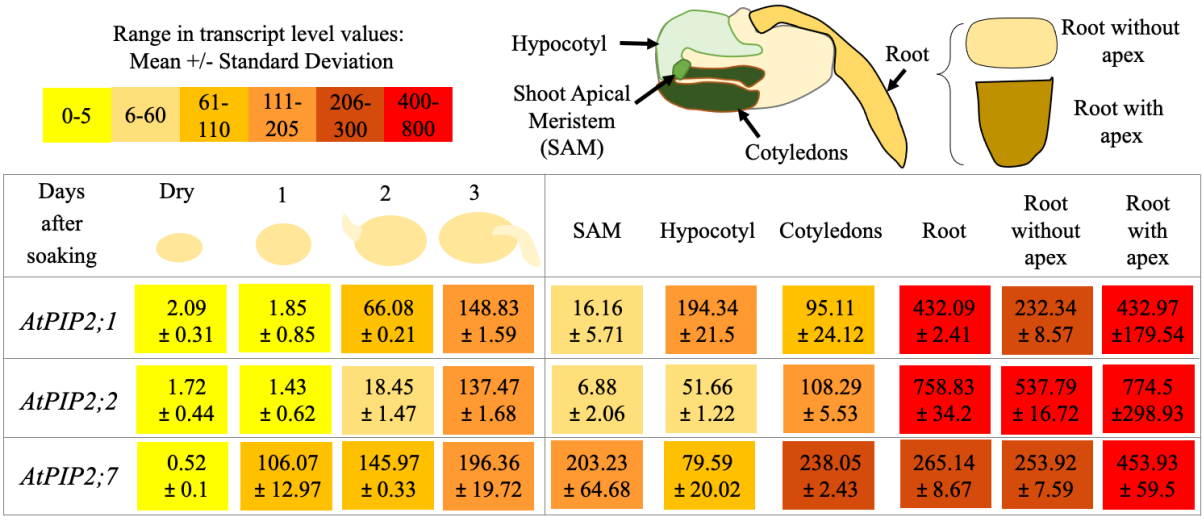


Figure S7. The K^+ and Na^+ content and moisture content of fresh dry seed grown in water conditions.

A-C, The K^+ and Na^+ ion content and the ration of ions in seed of five genotypes where parent plants were grown in control conditions and seeds were collected when mature and dry. D, Moisture content of seed (%) = (fresh dry seed weight – oven dried seed weight) x 100/ fresh dry seed weight. Seeds were weighed, dried in an oven temperature at 65°C in 72 h and then dried seeds were weighed. Data for Col-0 (○), *Atpip2;1-1* (△), Atpip*2;1-2* (□), *2x35S::AtPIP2;1* OE 7 (◇) and *2x35S::AtPIP2;1* OE 21 (□). Data points are means and s.e.m. (n = 4 plates per condition, 600 - 900 seeds per genotype in each plate). The experiment was repeated 3 times. Significant difference determined by two-way ANOVA and Fisher's LSD pair-wise tests. This seed is a different batch of seed relative to seed in Figure 3.



Days after soaking	Dry	1	2	3	SAM	Hypocotyl	Cotyledons	Root	Root without apex	Root with apex
AtPIP2;1	2.09 ± 0.31	1.85 ± 0.85	66.08 ± 0.21	148.83 ± 1.59	16.16 ± 5.71	194.34 ± 21.5	95.11 ± 24.12	432.09 ± 2.41	232.34 ± 8.57	432.97 ±179.54
AtPIP2;2	1.72 ± 0.44	1.43 ± 0.62	18.45 ± 1.47	137.47 ± 1.68	6.88 ± 2.06	51.66 ± 1.22	108.29 ± 5.53	758.83 ± 34.2	537.79 ± 16.72	774.5 ±298.93
AtPIP2;7	0.52 ± 0.1	106.07 ± 12.97	145.97 ± 0.33	196.36 ± 19.72	203.23 ± 64.68	79.59 ± 20.02	238.05 ± 2.43	265.14 ± 8.67	253.92 ± 7.59	453.93 ± 59.5

Figure S8. Summary of previously reported endogenous transcript abundance of *AtPIP2;1*, *AtPIP2;2* and *AtPIP2;7* (Gene ID: *AT3G53420*, *AT2G37170* and *AT5G05290* respectively) in germinating Arabidopsis Columbia-0 seed. There are five PIP1 and eight PIP2 encoding genes in Arabidopsis and of the eight PIP2s the transcripts of *AtPIP2;1* and *AtPIP2;7* were most abundant at 48 hrs into germination (see Figure 9 of Groszmann et al. (2021). Transcript data values are from Klepikova et al (2016) available via http://bar.utoronto.ca/eplant/.

Table S9a: Statistical analysis outcome for the data for timing of when different genotypes achieved 50% seed germination in saline and control treatment, the data is included in Figure 1K-L.

Uncorrected Fisher's LSD	**Predicted (LS) mean diff.**	**95.00% CI of diff.**	**Below threshold?**	**Summary**	**Adjusted P Value**
Water - Salt					
Col-0	-5.206	-12.71 to 2.303	No	ns	0.2757
pip2;1-1	-18.30	-25.81 to -10.80	Yes	****	<0.0001
pip2;1-2	-15.75	-23.26 to -8.240	Yes	****	<0.0001
*pip2;1*2;2-1*	-7.330	-12.08 to -2.581	Yes	**	0.0011
Null (35S:PIP2;1)	-5.164	-8.841 to -1.487	Yes	**	0.0050
35S:PIP2;1 (7)	-7.945	-11.62 to -4.268	Yes	****	<0.0001
35S:PIP2;1 (21)	-11.03	-14.70 to -7.348	Yes	****	<0.0001

Table S9b: Statistical analysis outcome for when the Col-0 genotype achieved 50% seed germination in saline and control treatment in Figure 1K-L.

Table Analysed	**T50 Col-0: Paired t test**
Column B	Treated
vs.	vs.
Column A	Control
Paired t test	
P value	0.0121
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=5.452, df=3
Number of pairs	4

Table S9c: Outcome of two-way ANOVA where Factor 1 is genotype and Factor 2 is treatment; ½ MS or Salt

Šídák's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
1/2 MS - 1/2 MS + 75mM NaCl					
Col-0	-5.206	-12.10 to 1.692	No	ns	0.2487
pip2;1-1	-18.30	-25.20 to -11.41	Yes	****	<0.0001
pip2;1-2	-15.75	-22.65 to -8.851	Yes	****	<0.0001
*pip2;1*2;2*	-7.330	-11.69 to -2.967	Yes	***	0.0001
Null (35S::PIP2;1)	-5.164	-12.06 to 1.734	No	ns	0.2571
35S::PIP2;1 (7)	-7.945	-14.84 to -1.047	Yes	*	0.0155
35S::PIP2;1 (21)	-11.03	-17.92 to -4.127	Yes	***	0.0003

Table S10: Statistical analysis of germination percentage data for the overexpression lines and the wild type null control; the data is presented in Figure 1.

Time	Šídák's multiple comparisons test	Summary	Stat*	Adjusted P Value
0d		No	ns	
24h		No	ns	
36h		No	ns	
48h				
	WTnull vs. 35S::PIP2;1(7)	Yes	*	0.0356
	WTnull vs. 35S::PIP2;1(21)	Yes	*	0.0230
	35S::PIP2;1(7) vs. 35S::PIP2;1(21)	No	ns	0.9960
60h				
	WTnull vs. 35S::PIP2;1(7)	Yes	*	0.0226
	WTnull vs. 35S::PIP2;1(21)	Yes	**	0.0083
	35S::PIP2;1(7) vs. 35S::PIP2;1(21)	No	ns	0.9598
72h				
	WTnull vs. 35S::PIP2;1(7)	Yes	*	0.0168
	WTnull vs. 35S::PIP2;1(21)	Yes	*	0.0117
	35S::PIP2;1(7) vs. 35S::PIP2;1(21)	No	ns	0.9998
84h				
	WTnull vs. 35S::PIP2;1(7)	Yes	*	0.0107
	WTnull vs. 35S::PIP2;1(21)	Yes	*	0.0338
	35S::PIP2;1(7) vs. 35S::PIP2;1(21)	No	ns	0.9466
96h				
	WTnull vs. 35S::PIP2;1(7)	Yes	**	0.0040
	WTnull vs. 35S::PIP2;1(21)	No	ns	0.0568
	35S::PIP2;1(7) vs. 35S::PIP2;1(21)	No	ns	>0.9999
120h				
	WTnull vs. 35S::PIP2;1(7)	Yes	**	0.0015
	WTnull vs. 35S::PIP2;1(21)	No	ns	0.0640
	35S::PIP2;1(7) vs. 35S::PIP2;1(21)	No	ns	0.7242
144h				
	WTnull vs. 35S::PIP2;1(7)	Yes	**	0.0033
	WTnull vs. 35S::PIP2;1(21)	No	ns	0.1312
	35S::PIP2;1(7) vs. 35S::PIP2;1(21)	No	ns	0.3337
216h				
	WTnull vs. 35S::PIP2;1(7)	Yes	**	0.0014
	WTnull vs. 35S::PIP2;1(21)	No	ns	0.1609
	35S::PIP2;1(7) vs. 35S::PIP2;1(21)	No	ns	0.3972