

pH of leaf apoplast is relevant for transcription of ABA-synthesizing key gene *vp14* and for stomatal aperture in *Zea mays* L.

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Supplementary data

Supplementary Table S1. Long-term effect of one-time infiltration on rate of photosynthesis and transpiration. 'Control', infiltration of 0.4 ml of an aqueous 25 μM OG solution; 'MES pH_{apo} 4.0 (osmotic control)', infiltration of 0.4 mL of a mixture of 7.5 mM MES at pH 4.7 and 25 μM OG; 'MES pH_{apo} 6.5', infiltration of 0.4 mL of a mixture of 5 mM MES at pH 6.5 and 25 μM OG; 'MOPSO pH_{apo} 6.5', infiltration of 0.4 mL of a mixture of 7.5 mM MOPSO at pH 6.5 and 25 μM OG. Mean \pm SD of six independent ($n = 6$) biological replications. Tukey-HSD test revealed no statistical significance ($P \leq 0.05$) between values from all groups, as indicated by ns.

Treatment		Transpiration rate	Photosynthetic rate
(hours after infiltration)		($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)
Control	0 h	5.3 \pm 1.2 ^{ns}	9.82 \pm 0.7 ^{ns}
	24h	6.0 \pm 1.3 ^{ns}	11.42 \pm 0.5 ^{ns}
	48h	6.2 \pm 1.3 ^{ns}	12.25 \pm 0.6 ^{ns}
	72h	5.5 \pm 1.5 ^{ns}	10.36 \pm 0.4 ^{ns}
MES pH_{apo} 4.0 (osmotic control)	0 h	5.2 \pm 1.4 ^{ns}	10.20 \pm 0.3 ^{ns}
	24h	5.9 \pm 1.2 ^{ns}	11.14 \pm 0.8 ^{ns}
	48h	6.0 \pm 1.3 ^{ns}	11.91 \pm 0.2 ^{ns}
	72h	6.3 \pm 1.4 ^{ns}	12.60 \pm 0.4 ^{ns}
MES pH_{apo} 6.5	0 h	5.1 \pm 1.6 ^{ns}	9.51 \pm 0.9 ^{ns}
	24h	5.9 \pm 1.0 ^{ns}	11.37 \pm 0.6 ^{ns}
	48h	6.1 \pm 1.4 ^{ns}	12.08 \pm 0.7 ^{ns}
	72h	6.0 \pm 1.5 ^{ns}	11.54 \pm 0.6 ^{ns}
MOPSO pH_{apo} 6.5	0 h	5.4 \pm 1.2 ^{ns}	10.23 \pm 0.6 ^{ns}
	24h	5.9 \pm 1.4 ^{ns}	11.65 \pm 0.5 ^{ns}
	48h	6.0 \pm 1.2 ^{ns}	11.79 \pm 0.5 ^{ns}
	72h	6.2 \pm 1.2 ^{ns}	12.35 \pm 0.6 ^{ns}

Supplementary Table S2. Specificity of the *Zmvp14* primer pair was demonstrated by sequencing the real-time quantitative RT-PCR product.

Column 1, primer pair. Column 2, DNA sequencing result. Columns 3-7, sequence was alignment against NCBI's reference mRNA sequences (refseq_rna; blastn), search was limited to *Zea mays* L. (taxid:4577). Identical sequencing results were found in our study were identical primer pair was used previous (identical sequencing result shown in the previous work by (Geilfus *et al.*, 2018).

<i>Zea mays viviparous 14</i> (<i>Zmvp14</i>) primer pair	Sequencing summary of real-time quantitative RT-PCR products (N, any base; primer sequences highlighted grey)	Significant alignments of sequences (NCBI blastn on <i>Zea mays</i> L. [taxid:4577]; database: refseq_rna)				
		Genbank sequence ID	Description	Max score/ Total score	Query coverage (%)/ Ident (%)	E value
f 5'–3' TTCTCGGAGGAGGAACAG AGGA r 5'–3' CCAACTGTA ACTCTGGTG TGCG	TTTCTCGGAGGAGGAACAGAG GAGCCAGCCATGGATCAGGGG AGAAGTCACCAGAGGGAGCCC AGATCAGTTCCCCGGGTCTT CNCTGTCNCCNNCNCACNGCA CACNAGAGTTACAGTTGGA	NM_001112432.3 (No other Blast hits found)	<i>Zea mays</i> <i>viviparous14</i> (<i>vp14</i>), mRNA	187/187	99/ 94	4e-47

Supplementary Table S3. pH_{apo} transient reduces transpiration rate, stomatal conductance and photosynthetic rate. 'Control', infiltration of 0.4 ml of an aqueous 25 μ M OG solution; 'MES pH_{apo} 4.0 (osmotic control)', infiltration of 0.4 mL of a mixture of 7.5 mM MES at pH 4.7 and 25 μ M OG; 'MES pH_{apo} 6.5', infiltration of 0.4 mL of a mixture of 5 mM MES at pH 6.5 and 25 μ M OG; 'MOPSO pH_{apo} 6.5', infiltration of 0.4 mL of a mixture of 7.5 mM MOPSO at pH 6.5 and 25 μ M OG. Mean \pm SD of six independent (n = 6) biological replications. Statistical significance between values from all groups ($P \leq 0.05$) as calculated by the Tukey-HSD test is indicated by small letters for transpiration rate and capital letters for stomatal aperture.

Name	Specification	Treatment		Transpiration rate ($mmol H_2O m^{-2} s^{-1}$)	Stomatal conductance ($mmol H_2O m^{-2} s^{-1}$)	Photosynthetic rate ($\mu mol CO_2 m^{-2} s^{-1}$)
		'Minute' on x-axis in Fig. 1	Description of time point			
Control	Start, before infiltration	10	No alkalinisation, no ABA accumulation	5.3 \pm 1.4 ^a	252 \pm 42 ^a	10.3 \pm 0.8 ^e
	60 min after infiltration	100	No alkalinisation, no ABA accumulation	5.5 \pm 1.4 ^a	269 \pm 45 ^a	10.5 \pm 0.5 ^e
	After re-acidification	200	No alkalinisation, no ABA accumulation	5.4 \pm 1.4 ^a	262 \pm 40 ^a	10.5 \pm 0.4 ^e
	2 h after re-acidification	320	No alkalinisation, no ABA accumulation	5.8 \pm 1.1 ^a	283 \pm 45 ^a	11.2 \pm 0.4 ^e
	4 h after re-acidification	440	No alkalinisation, no ABA accumulation	6.1 \pm 0.9 ^a	294 \pm 48 ^a	12.1 \pm 0.2 ^p
MES pH_{apo} 4.0 (osmotic control)	Start, before infiltration	10	No alkalinisation, no ABA accumulation	5.0 \pm 1.3 ^a	244 \pm 41 ^a	10.1 \pm 0.5 ^e
	60 min after infiltration	100	No alkalinisation, no ABA accumulation	5.4 \pm 1.5 ^a	259 \pm 39 ^a	10.4 \pm 0.6 ^e
	After re-acidification	200	No alkalinisation, a few ABA accumulated	5.4 \pm 1.2 ^a	264 \pm 43 ^a	10.2 \pm 0.7 ^e
	2 h after re-acidification	320	No alkalinisation, no ABA accumulation	5.4 \pm 1.3 ^a	267 \pm 46 ^a	10.5 \pm 0.3 ^e
	4 h after re-acidification	440	No alkalinisation, no ABA accumulation	4.7 \pm 1.1 ^a	230 \pm 38 ^a	8.3 \pm 0.6 ^B
MES pH_{apo} 6.5	Start, before infiltration	10	Before alkalinisation, no ABA accumulation	5.0 \pm 1.3 ^a	242 \pm 41 ^a	9.9 \pm 0.7 ^e
	60 min after infiltration	100	Apoplast alkalinised, no ABA accumulation	5.4 \pm 1.3 ^a	256 \pm 44 ^a	10.4 \pm 0.3 ^e
	After re-acidification	200	Apoplast re-acidified, ABA accumulated	4.7 \pm 1.6 ^a	227 \pm 39 ^a	8.5 \pm 0.3 ^B
	2 h after re-acidification	320	Apoplast re-acidified, ABA accumulated	2.3 \pm 0.4 ^b	128 \pm 25 ^{β}	5.2 \pm 0.3 ^{γ}
	4 h after re-acidification	440	Apoplast re-acidified, ABA was not measured	1.8 \pm 0.3 ^{bc}	97 \pm 26 ^{$\beta\gamma$}	3.1 \pm 0.2 ^e
MOPSO pH_{apo} 6.5	Start, before infiltration	10	Before alkalinisation, no ABA accumulation	5.5 \pm 1.5 ^a	267 \pm 43 ^a	10.6 \pm 0.4 ^e
	60 min after infiltration	100	Apoplast alkalinised, no ABA accumulation	5.4 \pm 1.2 ^a	258 \pm 37 ^a	10.5 \pm 0.4 ^e
	After re-acidification	200	Apoplast re-acidified, ABA accumulated	4.8 \pm 1.5 ^a	235 \pm 40 ^a	8.4 \pm 0.3 ^B
	2 h after re-acidification	320	Apoplast re-acidified, ABA accumulated	1.7 \pm 0.1 ^c	85 \pm 14 ^{γ}	2.9 \pm 0.1 ^e
	4 h after re-acidification	440	Apoplast re-acidified, ABA was not measured	1.9 \pm 0.3 ^{bc}	102 \pm 26 ^{$\beta\gamma$}	3.1 \pm 0.1 ^e

Supplementary Fig. S1. Specificity of the *vp14* primer pair. Real-time qRT-PCR products were separated on agarose gels. Only one single DNA band appeared. *In-silico* analysis using the Primer-BLAST software predicted a band size of ~ 117 bp. Gels contain 2% agarose, Tris-borate-EDTA, 5 μ l SYBR Safe pro 100 ml Gel (SYBR® Safe DNA Gel Stain, Invitrogen).

