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 ± 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 *(hours after infiltration)*		**Transpiration rate** *(mmol H_20 m^{-2} s^{-1})*	**Photosynthetic rate** *(µmol CO_2 m^{-2} s^{-1})*
Control	0 h	5.3±1.2 ^{ns}	9.82±0.7 ^{ns}
	24h	6.0±1.3 ^{ns}	11.42±0.5 ^{ns}
	48h	6.2±1.3 ^{ns}	12.25±0.6 ^{ns}
	72h	5.5±1.5 ^{ns}	10.36±0.4 ^{ns}
MES pH_{apo} 4.0 (osmotic control)	0 h	5.2±1.4 ^{ns}	10.20±0.3 ^{ns}
	24h	5.9±1.2 ^{ns}	11.14±0.8 ^{ns}
	48h	6.0±1.3 ^{ns}	11.91±0.2 ^{ns}
	72h	6.3±1.4 ^{ns}	12.60±0.4 ^{ns}
MES pH_{apo} 6.5	0 h	5.1±1.6 ^{ns}	9.51±0.9 ^{ns}
	24h	5.9±1.0 ^{ns}	11.37±0.6 ^{ns}
	48h	6.1±1.4 ^{ns}	12.08±0.7 ^{ns}
	72h	6.0±1.5 ^{ns}	11.54±0.6 ^{ns}
MOPSO pH_{apo} 6.5	0 h	5.4±1.2 ^{ns}	10.23±0.6 ^{ns}
	24h	5.9±1.4 ^{ns}	11.65±0.5 ^{ns}
	48h	6.0±1.2 ^{ns}	11.79±0.5 ^{ns}
	72h	6.2±1.2 ^{ns}	12.35±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).

***Zea mays viviparous 14* (*Zmvp14*) 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 *Zea mays* L. [taxid:4577]; database: refseq_rna)				
		Genbank sequence ID	**Description**	**Max score/ Total score**	**Query coverage (%)/ Ident (%)**	**E value**
f 5′–3′ TTCTCGGAGGAGGAACAGAGGA r 5′–3′ CCAACTGTAACTCTGGTGTGCG	TTTCTCGGAGGAGGAACAGAGGAGCCAGCCATGGATCAGGGGAGAAGTCACCAGAGGGAGCCCAGATCAGTTCCCCGGGGTCTTCNCTGTCNCCNNCNCACNGCACACNAGAGTTACAGTTGGA	NM_001112432.3 (No other Blast hits found)	*Zea mays viviparous14* (*vp14*), 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 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 ± 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.

Treatment				Transpiration rate	Stomatal conductance	Photosynthetic rate
Name	*Specification*	*'Minute' on x-axis in Fig. 1*	*Description of time point*	*(mmol H_20 m^{-2} s^{-1})*	*(mmol H_20 m^{-2} s^{-1})*	*(µmol CO_2 m^{-2} s^{-1})*
Control	Start, before infiltration	10	No alkalisation, no ABA accumulation	5.3 ± 1.4^{a}	$252\pm42^{\alpha}$	$10.3\pm0.8^{\epsilon}$
	60 min after infiltration	100	No alkalisation, no ABA accumulation	5.5 ± 1.4^{a}	$269\pm45^{\alpha}$	$10.5\pm0.5^{\epsilon}$
	After re-acidification	200	No alkalisation, no ABA accumulation	5.4 ± 1.4^{a}	$262\pm40^{\alpha}$	$10.5\pm0.4^{\epsilon}$
	2 h after re-acidification	320	No alkalisation, no ABA accumulation	5.8 ± 1.1^{a}	$283\pm45^{\alpha}$	$11.2\pm0.4^{\epsilon}$
	4 h after re-acidification	440	No alkalisation, no ABA accumulation	6.1 ± 0.9^{a}	$294\pm48^{\alpha}$	$12.1\pm0.2^{\text{Þ}}$
MES pH_{apo} 4.0 (osmotic control)	Start, before infiltration	10	No alkalisation, no ABA accumulation	5.0 ± 1.3^{a}	$244\pm41^{\alpha}$	$10.1\pm0.5^{\epsilon}$
	60 min after infiltration	100	No alkalisation, no ABA accumulation	5.4 ± 1.5^{a}	$259\pm39^{\alpha}$	$10.4\pm0.6^{\epsilon}$
	After re-acidification	200	No alkalisation, a few ABA accumulated	5.4 ± 1.2^{a}	$264\pm43^{\alpha}$	$10.2\pm0.7^{\epsilon}$
	2 h after re-acidification	320	No alkalisation, no ABA accumulation	5.4 ± 1.3^{a}	$267\pm46^{\alpha}$	$10.5\pm0.3^{\epsilon}$
	4 h after re-acidification	440	No alkalisation, no ABA accumulation	4.7 ± 1.1^{a}	$230\pm38^{\alpha}$	$8.3\pm0.6^{\text{Þ}}$
MES pH_{apo} 6.5	Start, before infiltration	10	Before alkalisation, no ABA accumulation	5.0 ± 1.3^{a}	$242\pm41^{\alpha}$	$9.9\pm0.7^{\epsilon}$
	60 min after infiltration	100	Apoplast alkalinised, no ABA accumulation	5.4 ± 1.3^{a}	$256\pm44^{\alpha}$	$10.4\pm0.3^{\epsilon}$
	After re-acidification	200	Apoplast re-acidified, ABA accumulated	4.7 ± 1.6^{a}	$227\pm39^{\alpha}$	$8.5\pm0.3^{\text{Þ}}$
	2 h after re-acidification	320	Apoplast re-acidified, ABA accumulated	2.3 ± 0.4^{b}	$128\pm25^{\beta}$	$5.2\pm0.3^{¥}$
	4 h after re-acidification	440	Apoplast re-acidified, ABA was not measured	1.8 ± 0.3^{bc}	$97\pm26^{\beta\gamma}$	$3.1\pm0.2^{£}$
MOPSO pH_{apo} 6.5	Start, before infiltration	10	Before alkalisation, no ABA accumulation	5.5 ± 1.5^{a}	$267\pm43^{\alpha}$	$10.6\pm0.4^{\epsilon}$
	60 min after infiltration	100	Apoplast alkalinised, no ABA accumulation	5.4 ± 1.2^{a}	$258\pm37^{\alpha}$	$10.5\pm0.4^{\epsilon}$
	After re-acidification	200	Apoplast re-acidified, ABA accumulated	4.8 ± 1.5^{a}	$235\pm40^{\alpha}$	$8.4\pm0.3^{\text{Þ}}$
	2 h after re-acidification	320	Apoplast re-acidified, ABA accumulated	1.7 ± 0.1^{c}	$85\pm14^{\gamma}$	$2.9\pm0.1^{£}$
	4 h after re-acidification	440	Apoplast re-acidified, ABA was not measured	1.9 ± 0.3^{bc}	$102\pm26^{\beta\gamma}$	$3.1\pm0.1^{£}$

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 µl SYBR Safe pro 100 ml Gel (SYBR® Safe DNA Gel Stain, Invitrogen).