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Transpirational demand affects aquaporin expression in poplar roots
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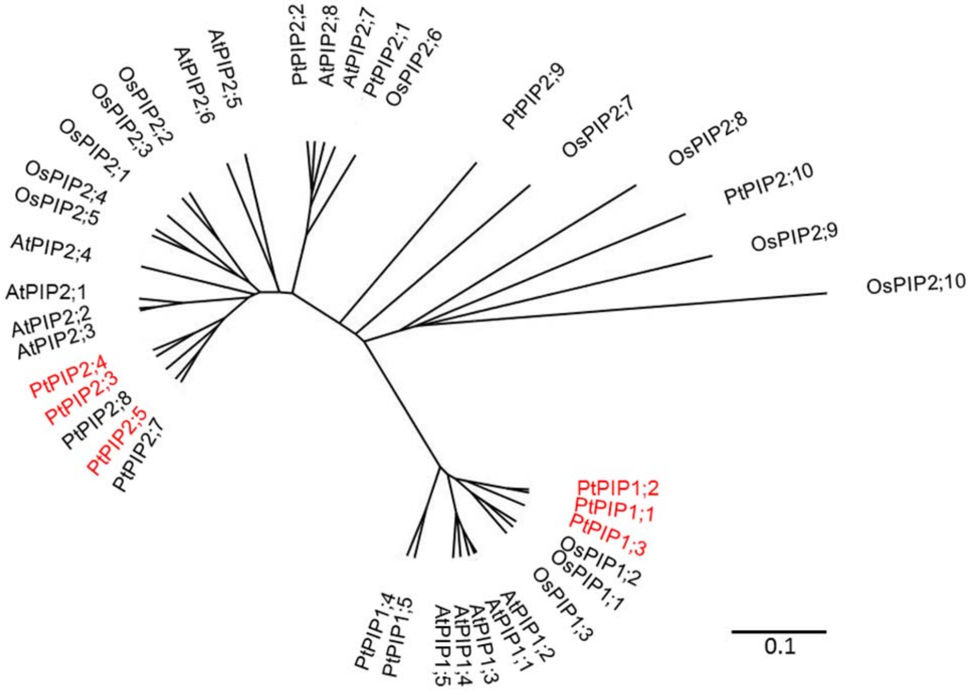
Supplementary material for on-line publication

Supplemental Fig. S1. Phylogenetic relationships of plasma membrane intrinsic proteins (PIPs) in *Arabidopsis thaliana*, *Oryza sativa* and *Populus trichocarpa*. The phylogenetic tree was constructed using Genomics Workbench version 5.5 (CLC Bio, Cambridge, MA, USA) and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method. The tree was visualized by FigTree (tree.bio.ed.ac.uk/software/figtree/). The scale bar represents the number of amino acid substitutions per site.



Supplemental Fig. S2. Effect of step changes light and humidity on transpiration rate (*E*). (A) Transpiration rate of control plants ('Light control'). (B) Transpiration rate of shaded plants ('Shade'), of plants removed from shade after 4 h ('Light increase, 4h'), and of plants removed from shade after 28 h ('Light increase, 28h'). (C) Transpiration rate of plants grown at high relative humidity (RH) after a step change in RH. Transpiration was measured 5 minutes ('RH decrease, 5 min'), 4 h ('RH decrease, 4h'), and 28 h ('RH decrease, 28h') after the decrease in humidity. Data shows means ± SE; n = 5 plants. Significant differences are indicated by unique letters ($P \leq 0.05$).

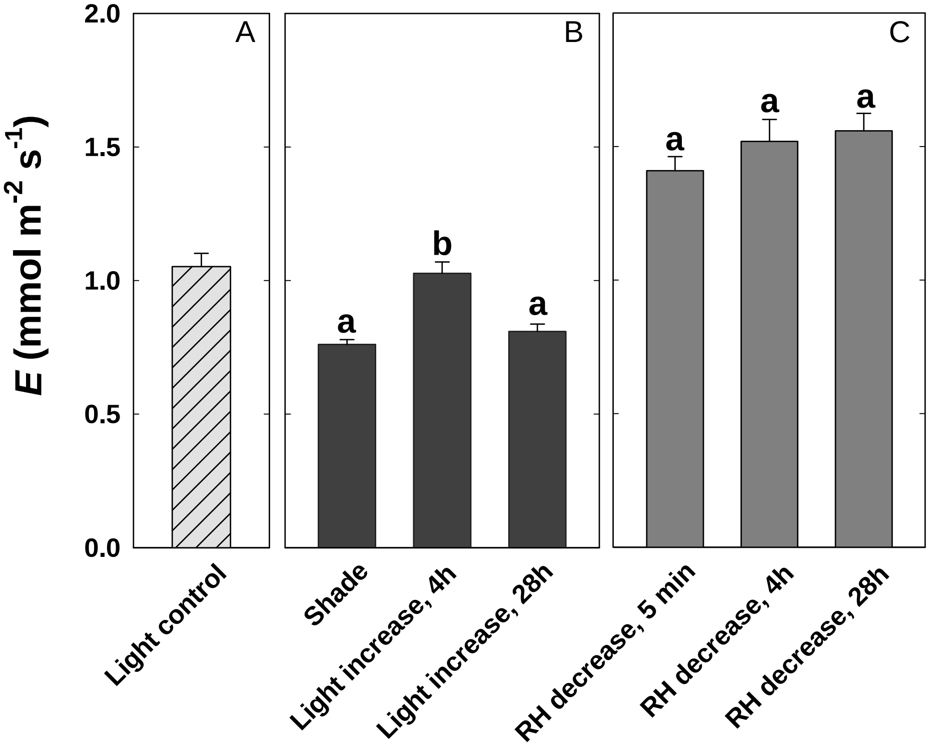


Table S1. Summary of *PIP* expression patterns. The poplar gene names follow the nomenclature of Gupta and Sankararamakrishnan (2009). Findings from a previous experiment (Almeida-Rodriguez *et al*. 2011) are summarized as: * for differentially expressed genes; *nd* for non-differentially expressed genes; blank spaces indicate genes that were not investigated. The *Populus* eFP browser (Wilkins *et al*. 2009) was used to check the tissue specificity of *PIP*s as well as putative regulation by light in seedlings. Relative transcript abundance of a particular gene is indicated as a fold change ratio between the tissue specific probe signal normalized to the control signal (value=1) as indicated in the eFP Browser. Grey background highlights the genes used in this present study.

P. trichocarpa gene name			Almeida-Rodriguez *et al.* (2011)		Poplar eFP Browser - Wilkins *et al.* (2009)				
			-------Root tips -------		-------- Mature tissue --------			-------- Seedlings -------	
Gene name	Phytozome v2.0	Affymetrix probe ID	Acclimation	Dynamic	Leaf	Root	Xylem	Dark grown	+3h light
PtPIP1;1	POPTR_0010s19930	PtpAffx.7686.1.S1_a_at	*		0.24	1.16	0.25	1.15	1.04
PtPIP1;2	POPTR_0008s06580	Ptp.4455.1.S1_s_at	*	*	0.09	2.06	0.14	1.4	1.87
PtPIP1;3	POPTR_0003s12870	PtpAffx.12342.2.S1_s_at			0.13	0.87	1.89	0.74	0.65
PtPIP1;4	POPTR_0006s09920	PtpAffx.54577.1.S1_at	*	*	0.13	1.26	2.09	0.52	0.84
PtPIP1;5	POPTR_0016s12070	PtpAffx.2848.1.S1_a_at	*		0.13	0.8	1.87	0.12	0.24
PtPIP2;1	POPTR_0009s13890	PtpAffx.5465.1.A1_x_at		*	0.18	1.18	1.58	1.13	0.93
PtPIP2;2	POPTR_0004s18240	PtpAffx.5465.2.A1_x_at	*nd*	*nd*	0.05	1.13	4.1	1.05	0.58
PtPIP2;3	POPTR_0010s22950	Ptp.1588.1.S1_s_at	*	*	0.05	0.9	4.42	0.83	0.52
PtPIP2;4	POPTR_0008s03950	PtpAffx.249.108.A1_x_at	*	*	0.2	0.96	0.11	1.04	1.29
PtPIP2;5	POPTR_0006s12980	PtpAffx.7681.3.A1_x_at		*	0.28	12.52	1.68	7.25	6.88
PtPIP2;7	POPTR_0016s09090	Ptp.139.1.S1_at	*nd*	*nd*	0.03	12.46	3.31	0.85	2.96
PtPIP2;8	POPTR_0009s01940	PtpAffx.5992.1.S1_at			0.02	10.31	1.46	1.19	1.25
PtPIP2;9	POPTR_0005s11110	PtpAffx.221954.1.S1_at			0.28	0.04	0.03	1.21	1.1
PtPIP2;10	POPTR_0005s11100	PtpAffx.221953.1.S1_s_at		*	0.08	0.26	0.09	4.85	1.38

Table S2. Primer sequences used for the gene expression study. Primers were designed based on *Populus trichocarpa* reference gene sequences. Primer sequences of the selected candidate genes are represented as well as the specific amplicon length.

P. trichocarpa gene Name		Amplicons		
Gene name	Phytozome v2.0	Forward Primer (5´→3´)	Reverse Primer (5´→3´)	Length (bp)
PtPIP1;1	POPTR_0010s19930	TGCAGAGTTCATGGCCACCTTC	TCGTGTCCTTAAACACGCCCATC	74
PtPIP1;2	POPTR_0008s06580	TGGCCTTGGTGCTGAGATTGTC	GCACTACGCTTGGCATCAGTTG	78
PtPIP1;3	POPTR_0003s12870	AACTGGCATTAACCCGGCAAGG	AATGGGCCAACCCAGAAGATCCAG	96
PtPIP2;3	POPTR_0010s22950	AGTCTGGGAGCCGCTGTTATCTAC	GGGTCCAACCCAGAAGATCCAATG	72
PtPIP2;4	POPTR_0008s03950	GTCATTCAGGAGCAACCCGAATGTC	CCATCATGCACGCACAAGCACTC	81
PtPIP2;5	POPTR_0006s12980	TGTGTTGGCACCACTTCCCATC	GTCATCCCATGCCTTGTCTTCGT	139