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Supplementary Material

Pearl millet (*Pennisetum glaucum*) contrasting for the transpiration response to vapour pressure deficit also differ in their dependence on the symplastic and apoplastic water transport pathways

Murugesan Tharanya[A,B], *Kaliamoorthy Sivasakthi*[A,B], *Gloria Barzana*[A], *Jana Kholová*[A], *Thiyagarajan Thirunalasundari*[B] *and Vincent Vadez*[A,C,D]

[A]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Crop Physiology Laboratory, Patancheru 502324, Telangana, India.

[B]Bharathidasan University, Tiruchirappalli 620 024, Tamilnadu, India.

[C]Institut de Recherche pour le Developpement (IRD) – Université de Montpellier – UMR DIADE, 911 Avenue Agropolis, BP 64501, 34394 Montpellier cedex 5, France.

[D]Corresponding author. Emails: vincent.vadez@ird.fr; v.vadez@cgiar.org

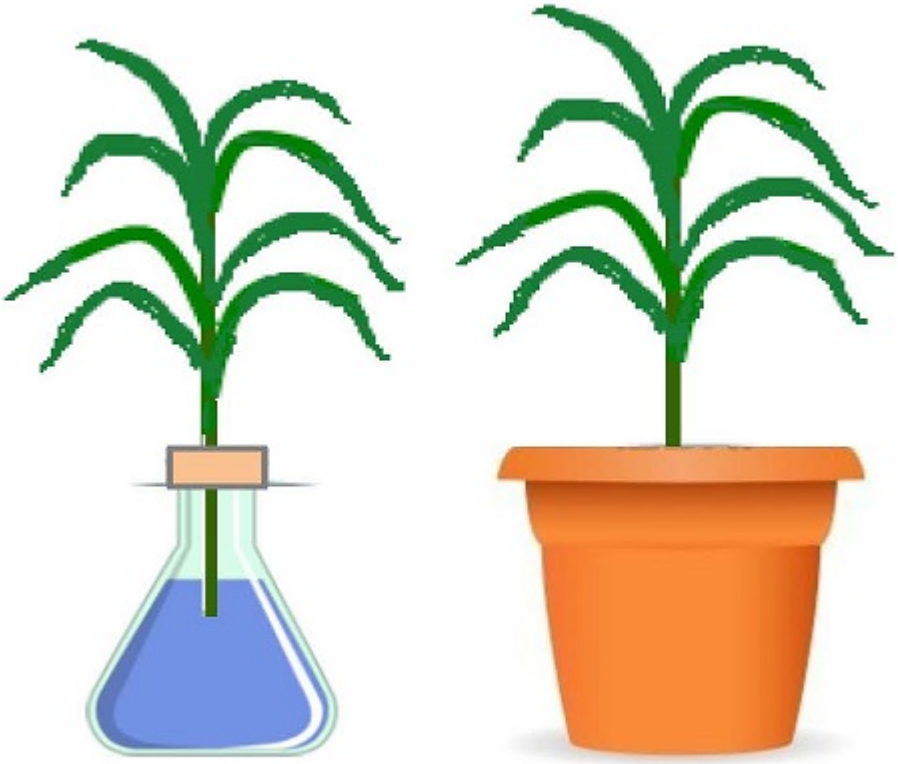

Fig. S1. Schematic diagram showing the relative height of de-rooted shoot and whole plant used in Experiment 1.

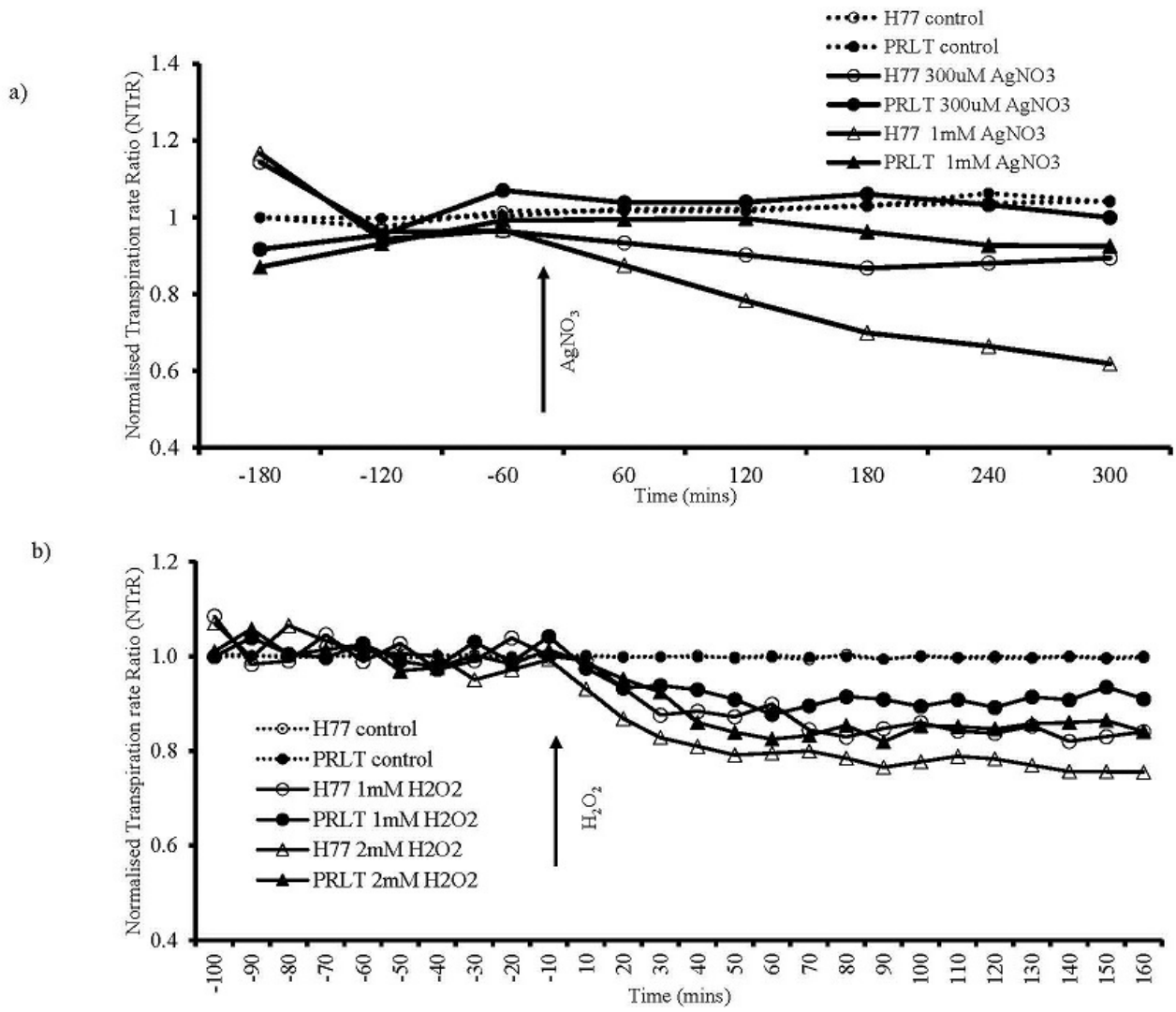

Fig. S2. Standardization of a) $AgNO_3$ (300µM and 1mM) and b) H_2O_2 (1mM and 2mM) concentrations for symplastic inhibition in the whole plants of H77/833-2 (high Tr, open markers) and PRLT-2/89/33 (low Tr, closed markers). The solid represents the response of untreated control plants and the dotted lines represents the response of treated plants exposed to constant VPD (1.8 kPa). Each point is the mean of the normalized transpiration ratio, NTrR (n=5) and the plants were tested in growth chamber at vegetative stage. The timings with negative symbols refer to time prior to inhibitor application and the timings with positive symbols refer to time post inhibitor application. In both the cases of $AgNO_3$ and H_2O_2, low concentration resulted in least inhibition of transpiration and higher concentration resulted in higher transpiration inhibition. So the moderate concentrations of 400µM $AgNO_3$ and 1.5mM H_2O_2 were chosen for further testing.

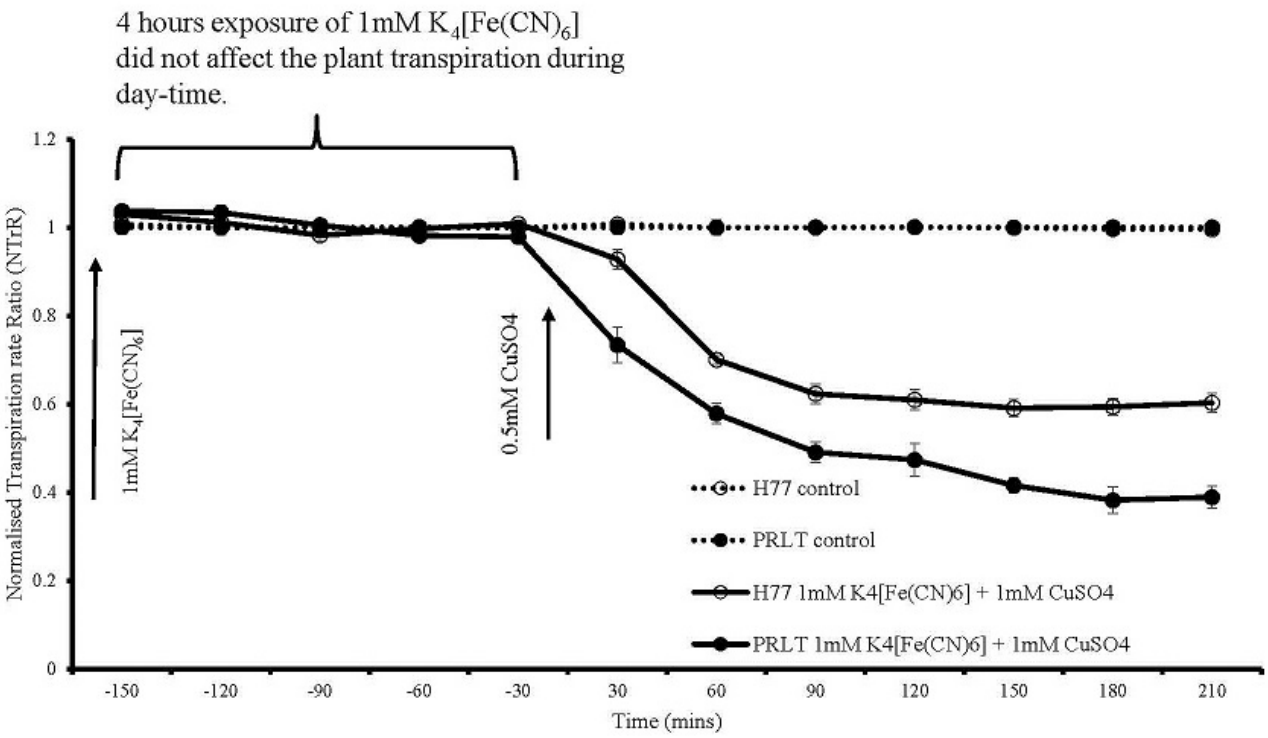

Fig. S3. Evidence showing that the application of $K_4[Fe(CN)_6]$ did not affect the plant transpiration during day time in the whole plants of H77/833-2 (high Tr, open symbols) and PRLT-2/89/33 (low Tr, closed symbols). As soon as $CuSO_4$ was added a decline in transpiration as well as genotypic difference were observed. The solid lines represent the response of untreated control plants and the dotted lines represent the response of treated plants exposed to constant VPD (1.8 kPa). Each point is the mean of the normalized transpiration ratio, NTrR (n=5) and the plants were tested in glass house at vegetative stage. The timings with negative symbols refer to time prior to inhibitor application and the timings with positive symbols refer to time post inhibitor application.

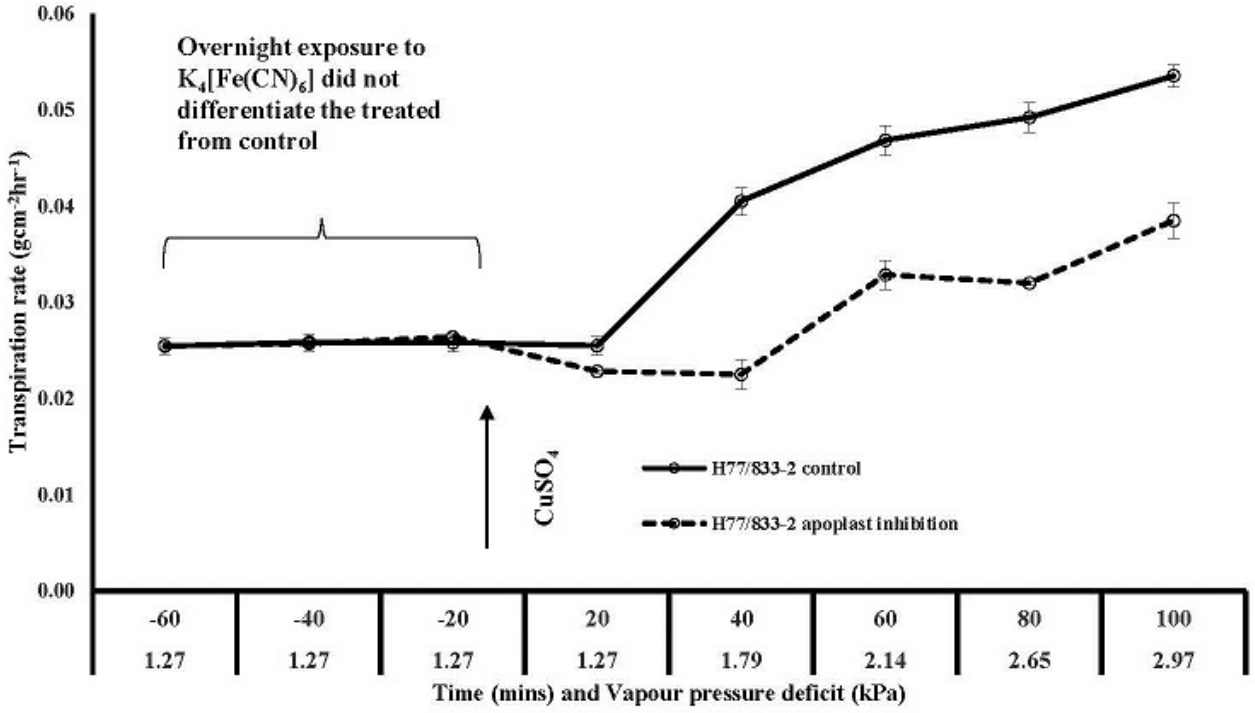

Fig. S4. Comparison of transpiration response of whole plants in H77/833-2 under untreated (dotted line) and treated conditions (solid line) with $K_4[Fe(CN_6)]$. The values immediately below the X-axis represent the time series and values further below represent the vapour pressure deficit. The timings with negative symbols refer to those prior to inhibitor application and the timings with positive symbols refer to those after the inhibitor application.The treated plants received $K_4[Fe(CN_6)]$ on the previous night while the untreated plants (control) received deionized water. The transpiration rate measured on the following morning for treated and untreated plants did not differ indicating that overnight exposure of the plants to $K_4[Fe(CN_6)]$ did not affect the transpiration.

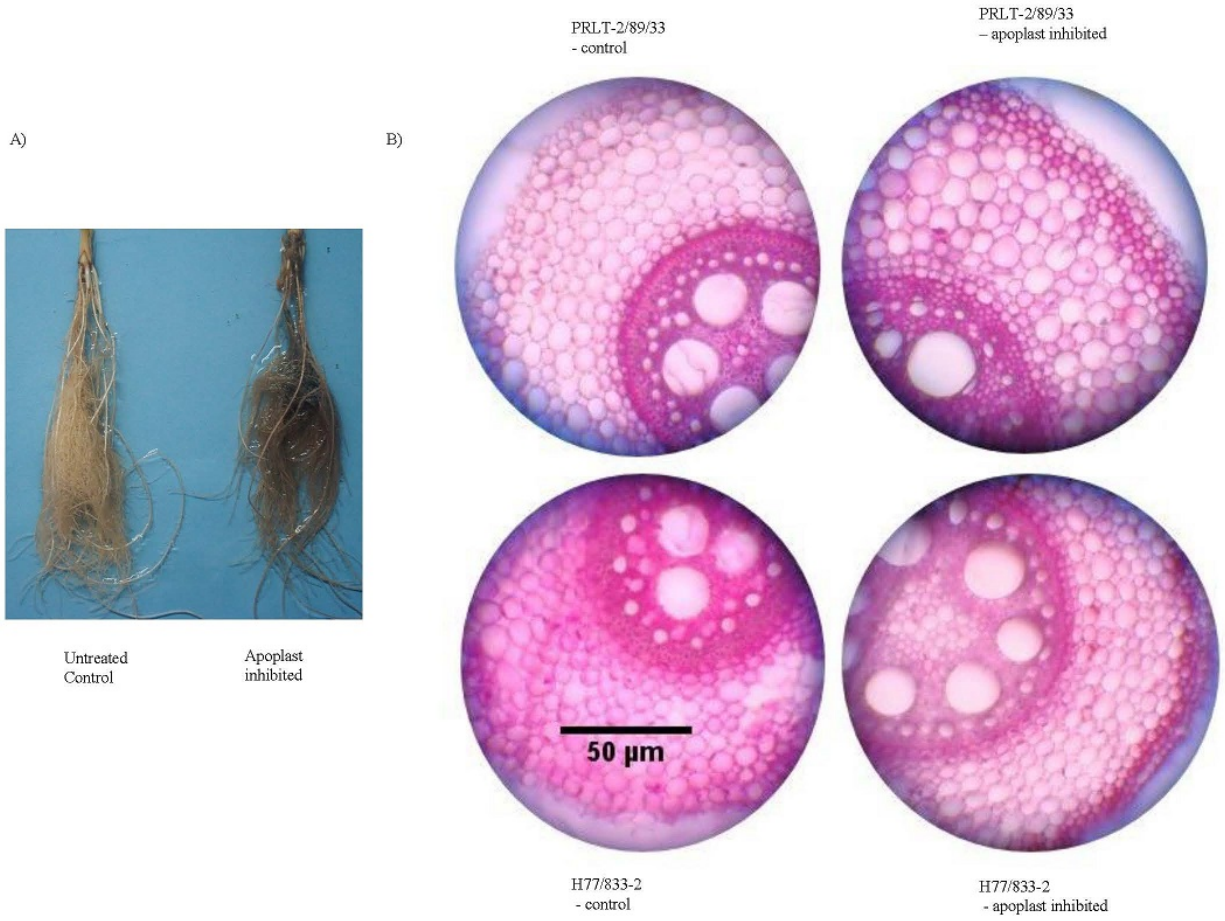

Fig. S5. A) Image of roots taken from untreated control and apoplast inhibited plants. **B**) Visualisation of the anatomical structure of H77/833-2 and PRLT-2/89/33 roots taken from untreated control and treated plants with $K_4[Fe(CN)_6$ and $CuSO_4$ tested for apoplast inhibition. The sections were cut free-hand, taken from a distance of 4-5cm from the root apex and stained with acid fuchsin dye. Anatomical structures were viewed under light microscope (Olympus) at 10 x 10 magnification.

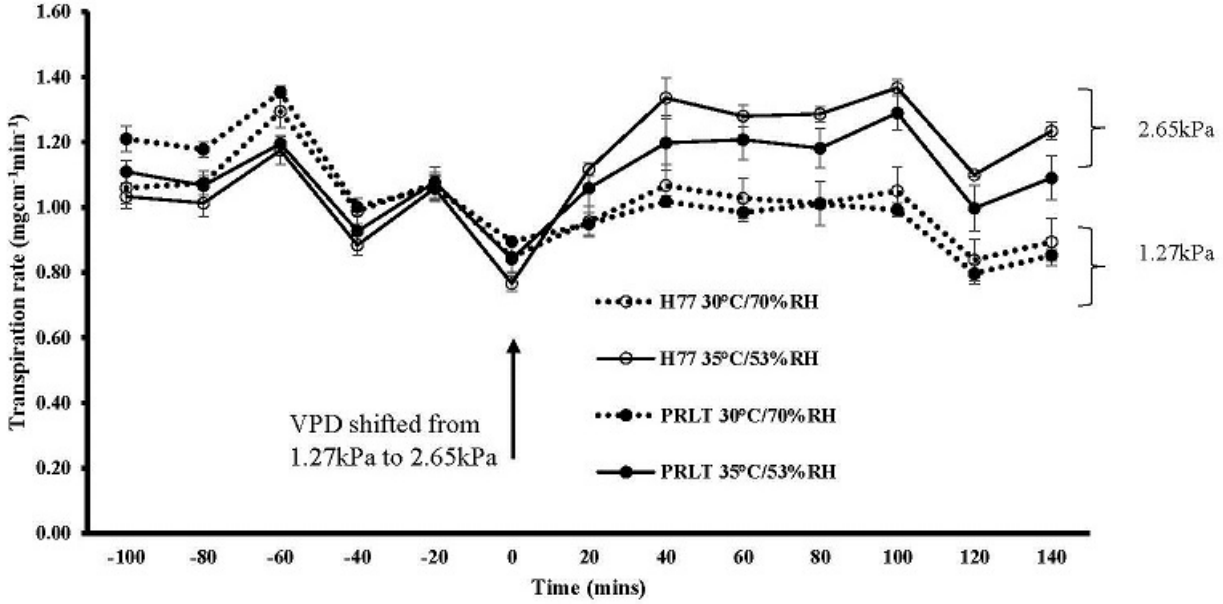

Fig. S6. Transpiration rate (Tr) in detached leaves from H77/833-2 (high Tr, open circle) and PRLT-2/89/33 (low Tr, closed circle) tested for a VPD response. The solid represents the Tr of increased VPD treatment (2.65kPa) and the dotted lines represents the Tr of untreated control plants exposed to constant VPD (1.27 kPa). Each point is the mean of Tr (n=9) and error bar at each time are the s.e. of the mean. Plants were tested in growth chamber at vegetative stage.

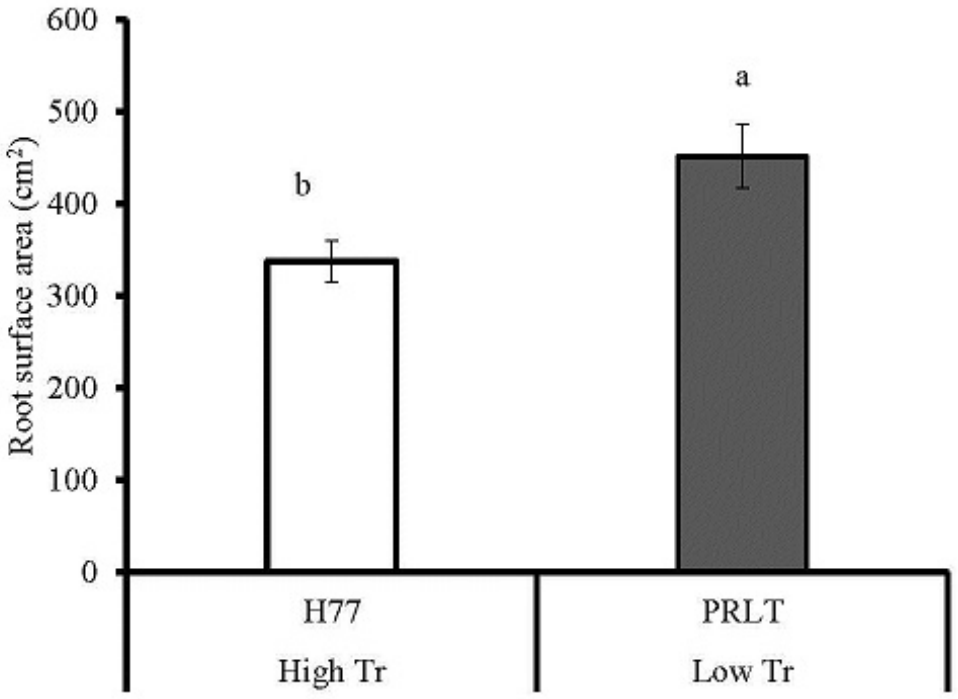

Fig. S7. Root surface area (cm^2) of hydroponically grown H77/833-2 (high Tr, open bar) and PRLT-2/89/33 (low Tr, closed bar) at vegetative stage. Error bars at each column are the s.e. of the mean (n=10). Bars with different letters are significantly different ($p<0.05$; Tukey Kramer test).

Table S1. Details on the temperature (T), relative humidity (RH) and vapour pressure deficit (VPD) at the time of plant growth and experimentations

Note: The experimental period consists only day-time. The environments used for plant growth and experimentation includes glasshouse (GH) and growth chambers (GC).

				Growth period						Experimental period		
		Environment		Day			Night			Day		
Experiment no.	**Size of pot/flask**	**Growth**	**Experiment**	**T (ºC)**	**RH (%)**	**VPD (kPa)**	**T (ºC)**	**RH (%)**	**VPD (kPa)**	**T (ºC)**	**RH (%)**	**VPD (kPa)**
1	7 inch pot	GH	GC	29.5	55	1.85	22	65	0.90	27 - 39	40 - 80	0.67 - 4.2
2	7 inch pot	GH	GC	31	40	2.69	22	55	1.10	31 - 38	48 -60	1.8 - 4.2
3	350 ml flask	GH	GC	30	48	2.2	23	70	0.84	37	44	3.5
4a	350 ml flask	GH	GC	27	60	1.43	18	80	0.41	30 -35	53 - 73	1.27 - 2.97
4b		GH	GC	30	45	2.26	22.5	63	1.00	31	60	1.8
4c		GH	GC	27	60	1.43	18	80	0.41	31	60	1.8
5	350 ml flask	GH	GC	28	60	1.51	18	75	0.55	27 - 39	40 - 70	1 - 4.2
6a	350 ml flask	GH	GC	28	60	1.51	18	75	0.55	28 - 34	55 - 70	1.27 - 2.65
6b		GH	GC	28	60	1.51	18	75	0.55	37	44	3.5
6c		GH	GC	28	60	1.51	18	75	0.55	37	44	3.5
7	350 ml flask	GH	GH	27	65	1.25	16.5	85	0.3	27	65	1.25