

Water relations traits of C₄ grasses depend on phylogenetic lineage, photosynthetic pathway and habitat water availability

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Supplementary data

Table S1 Information about the 33 species used in the glasshouse experiment. Annual or perennial categories based on Kew Grassbase (Clayton *et al.*, 2006 onwards). “inter” in habitat type means “intermediate” species.

Clade	C ₄ subtype	Species	Source ¹	Seed ID	Annual/ perennial	Habitat type
Subfamily Panicoideae						
Paspaleae	NADP-me	<i>Axonopus compressus</i>	A	PI406648	P	wet
	NADP-me	<i>Panicum antidotale</i>	B	CIAT6039	P	inter
	NADP-me	<i>Paspalum dilatatum</i>	A	PI477099	P	inter
Paniceae	NAD-me	<i>Alloteropsis cimicina</i>	C	AusTRCF 59560	A/P	inter
	PCK	<i>Alloteropsis semialata</i>	D		P	arid
	PCK	<i>Brachiaria decumbens</i>	C	AusTRCF 72793	P	inter
	PCK	<i>Brachiaria nigropedata</i>	C	AusTRCF 319409	P	arid
	NADP-me	<i>Digitaria eriantha</i>	C	AusTRCF 319398	P	inter
	PCK	<i>Melinis ambigua</i>	C	AusTRCF 52263	P	inter
	PCK	<i>Melinis repens</i>	E		A/P	inter
	NADP-me	<i>Pennisetum clandestinum</i>	C	AusTRCF 60072	P	wet
	NAD-me	<i>Panicum virgatum</i>	A	PI476292	P	inter
	NADP-me	<i>Setaria incrassata</i>	C	AusTRCF 24582	P	wet
	PCK	<i>Urochloa mosambicensis</i>	C	AusTRCF 320249	P	inter
	PCK	<i>Urochloa maxima</i>	A	PI259549	P	wet
	Andropogoneae	NADP-me	<i>Andropogon gayanus</i>	C	AusTRCF24575	P
NADP-me		<i>Bothriochloa pertusa</i>	C	AusTRCF 106426	P	inter
NADP-me		<i>Heteropogon contortus</i>	A	Grif 15979	P	inter
NADP-me		<i>Imperata cylindrica</i>	E		P	inter
NADP-me		<i>Ischaemum molle</i>	C	AusTRCF84176	P	inter
NADP-me		<i>Arundinella hirta</i>	A	PI263693	P	wet
Subfamily Chloridoideae						
Cynodonteae	NAD-me	<i>Cynodon dactylon</i>	C	AusTRCF 37896	P	inter
	PCK	<i>Chloris elata</i>	C	AusTRCF 53926	P	inter
	PCK	<i>Chloris gayana</i>	C	AusTRCF 319691	P	wet
	PCK	<i>Chloris roxburghiana</i>	C	AusTRCF 59653	P	arid
	PCK	<i>Dactyloctenium aegyptum</i>	F	91323	A	arid

	PCK	<i>Dactyloctenium scindicum</i>	C	AusTRCF25452	P	arid
Eragrostideae	NAD-me	<i>Eragrostis curvula</i>	C	AusTRCF 30368	P	inter
	NAD-me	<i>Enneapogon scoparius</i>	C	AusTRCF59846	P	arid
	NAD-me	<i>Fingerhuthia africana</i>	C	AusTRCF98990	P	arid
Zoysieae	NAD-me	<i>Sporobolus nebulosus</i>	G	0082459	P	inter
	PCK	<i>Spartina pectinata</i>	D	30925	P	wet
	PCK	<i>Zoysia japonica</i>	F	91937	P	inter

¹Suppliers: A, US Department of Agriculture National Plant Germplasm System (NPGS), BARC-West Beltsville, MD, USA; B, International Center for Tropical Agriculture (CIAT), Genetic Resources Program (GRP); C, Australian Plant Genetic Resource Information Service (AusPGRIS), Queensland, Australia; D, collected in South Africa, stored in the University of Sheffield; E, Silverhill seeds, Cape Town, South Africa; F, Herbiseed, Twyford, UK; G, grown in Tapton Experimental Gardens of the University of Sheffield by Taylor *et al.* (2010).

Table S2 (a) Conventional principal component analysis (PCA) and (b) phylogenetic PCA (PPCA) for the first two principal components based on 29 plant traits of 33 C_4 grasses. PC loadings and the percentage of variance explained by the first two PCs are reported. For each PC, the first seven variables with highest loadings are in bold. See trait abbreviations in Fig. 1.

(a) PCA			(b) PPCA		
Variable	PC1	PC2	Variable	PC1	PC2
LD	-0.33	0.07	LD	-0.83	0.19
LDMC	-0.31	0.10	LDMC	-0.82	0.24
LA	-0.30	-0.17	SLA	0.66	0.27
Height	-0.28	-0.15	Height	-0.65	-0.40
TLP	-0.28	0.12	WUE _i	-0.62	0.46
SLA	0.27	0.14	LA	-0.62	-0.62
ϵ	-0.26	0.10	TLP	-0.60	0.22
GL	0.06	-0.33	LV	-0.53	-0.71
SW	0.03	-0.32	LT	0.21	-0.59
Seed	-0.04	-0.29	LW	-0.46	-0.56
LT	0.06	-0.28	K_{leaf}	0.46	-0.56
LW	-0.20	-0.26	g_s	0.55	-0.54
X1	0.12	0.26	Ψ_{sat}	0.23	0.51
SD	-0.18	0.23			
Total variance %	23%	18%		24%	16%
Cumulative variance %	23%	41%		24%	40%

Table S3 Parameters derived from PV curves for different C₄ subtypes of two subfamilies in Figure 3. The highest and lowest values for each index are in bold. Abbreviations are the same as in Table 1.

	<i>n</i>	Ψ_{sat} (MPa)	Ψ_{osat} (MPa)	TLP (MPa)	RWC at TLP (%)	ε (MPa)
Chloridoideae						
NAD-me	25	-0.197	-0.937	-1.048	90.68	0.097
PCK	35	-0.195	-1.152	-1.316	91.54	0.139
Panicoidaeae						
NAD-me	10	-0.152	-0.802	-0.892	91.62	0.100
NADP-me	60	-0.159	-1.052	-1.193	91.72	0.110
PCK	35	-0.153	-0.972	-1.101	91.45	0.109

Figure legends

Fig. S1. (a) Relationship between MAP and water score, and (b) distribution of MAP for each habitat type for the 33 grass species used in the greenhouse experiment. Subfamilies are Chloridoideae (black) and Panicoideae (grey). Regression line in (a): $y = 193 \times x + 488$ ($R^2 = 0.11^{***}$).

Fig. S2. Phylogenetic relationships of the 33 species used in the greenhouse experiment. Subfamilies and lineages are labelled. C₄ photosynthetic subtypes (PT) are shown in different shapes: PCK (triangle), NAD-me (square) and NADP-me (round).

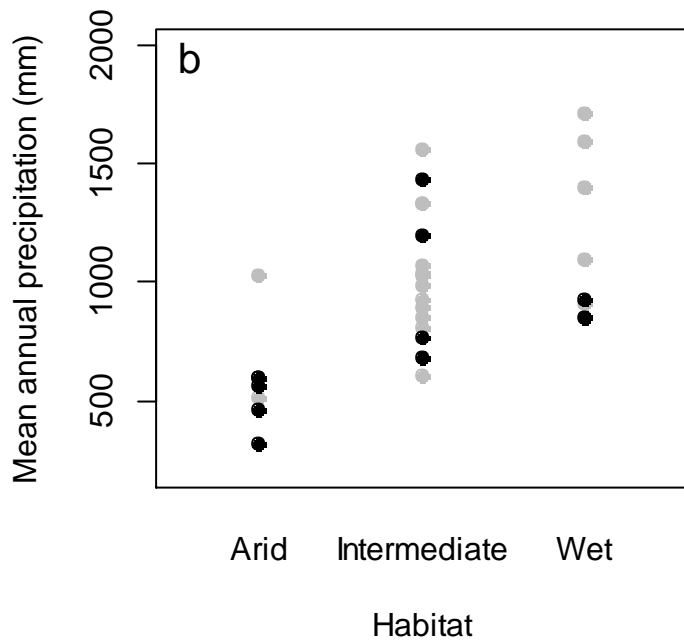
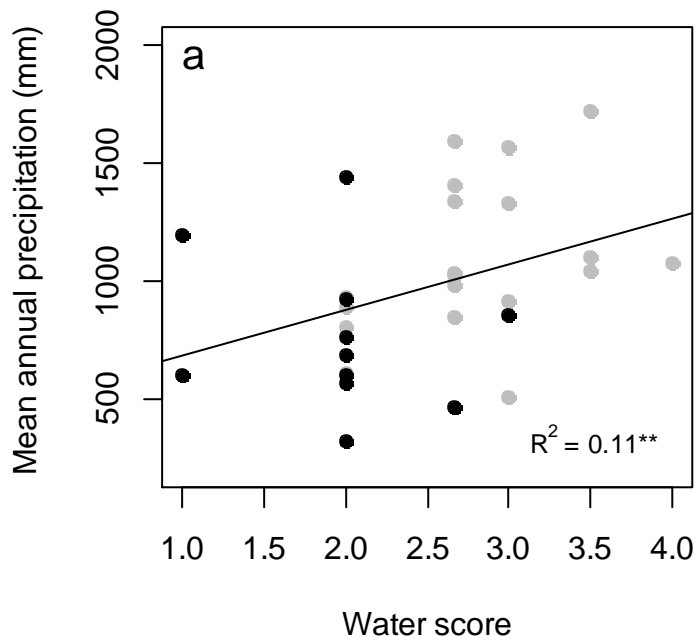


Fig. S1. Liu *et al.*

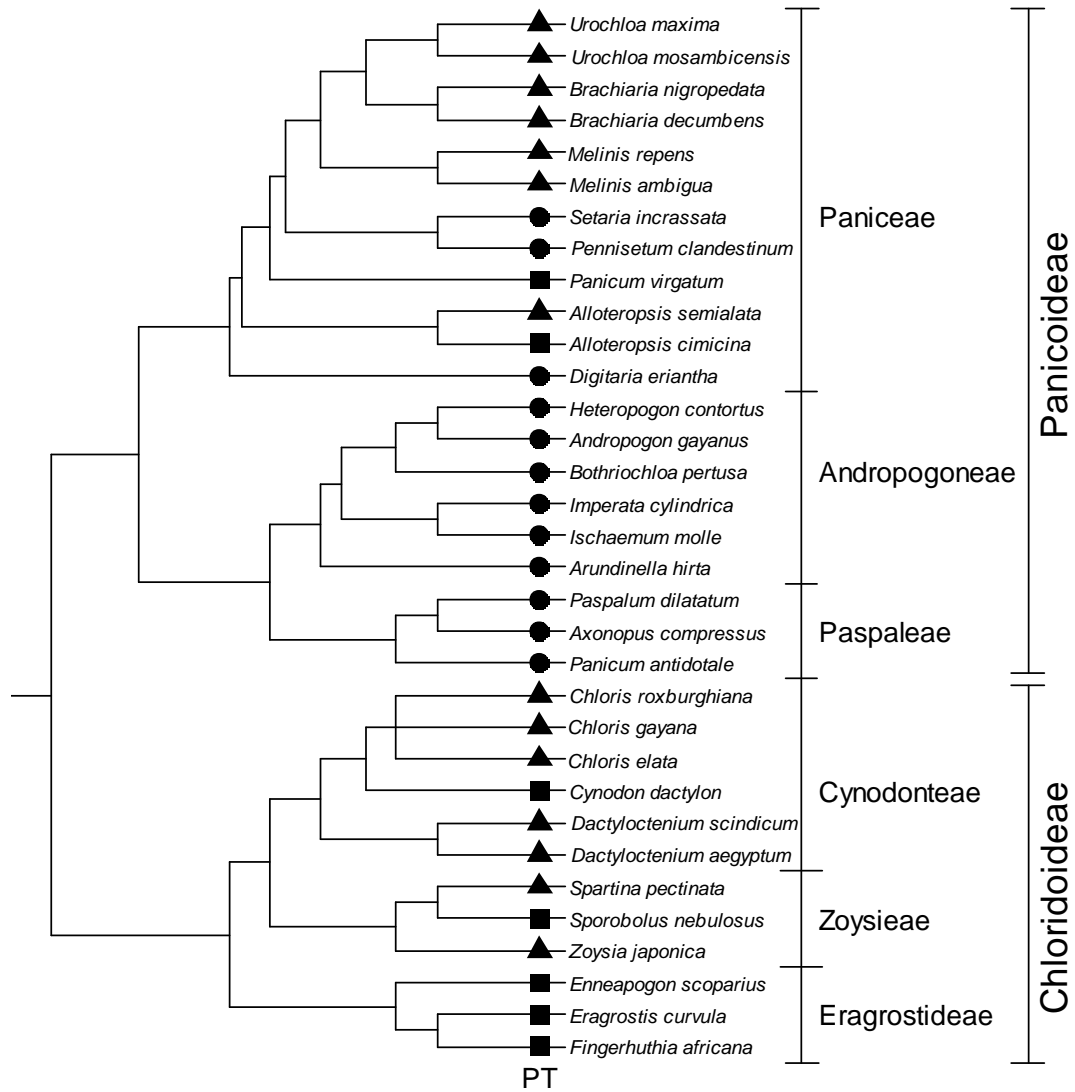


Fig. S2. Liu *et al.*