

Supplementary Table S1. List of species studied in this paper and source of species.

Species	Seed/plant source
<i>Dicanthelium oligosanthes</i>	Elizabeth Kellogg, Donald Danforth Plant Science Center St. Louis Missouri, USA
<i>Panicum bisulcatum</i>	Mitsutaka Taniguchi, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan
<i>Steinchisma laxum</i>	Elizabeth Kellogg, Donald Danforth Plant Science Center St. Louis Missouri, USA
<i>Steinchisma hians</i>	Elizabeth Kellogg, Donald Danforth Plant Science Center St. Louis Missouri, USA
<i>Homolepis aturensis</i>	Costa Rica, 9°39'27.4"N 82°45'08.0"W
<i>Setaria viridis</i>	Thomas Brutnell, Donald Danforth Plant Science Center St. Louis Missouri, USA
<i>Panicum virgatum</i>	Summerhill Nursery, Toronto, Ontario, Canada

Supplementary Table S2. Additional species for which RNA-seq data was downloaded and assembled for phylogenetic analysis of the GDC subunit genes, with NCBI SRA run IDs.

SPECIES	NCBI SRA RUN ID	SEQUENCING DEPTH
<i>Dactylis glomerata</i>	SRR1632251	325.7 Million Paired-End Reads
<i>Dendrocalamus sinicus</i>	SRR2543019	27.4 Million Single-End Reads
<i>Deschampsia antarctica</i>	SRR1162371	29.7 Million Paired-End Reads
<i>Dichanthelium clandestinum</i>	SRR1158317-8	112.2 Million Single-End Reads
<i>Elymus nutans</i>	SRR1336283	77.1 Million Paired-End Reads
<i>Elymus sibiricus</i>	SRR1344836	76.7 Million Paired-End Reads
<i>Festuc aarundinacea</i>	SRR2099408	55.1 Million Paired-End Reads
<i>Holcus lanatus</i>	ERR294017	111.7 Million Paired-End Reads
<i>Leersia perrieri</i>	SRR1171869	403.9 Million Paired-End Reads
<i>Leymus arenarius</i>	SRR1812885	141.3 Million Paired-End Reads
<i>Megathyrsus maximus</i>	SRR821833	199.14 Million Paired-End Reads
<i>Phyllostachys edulis</i>	SRR2035327	144.3 Million Paired-End Reads
<i>Poa annua</i>	SRR1633980	258.7 Million Paired-End Reads
<i>Polypogon fugax</i>	SRR1721962	27.2 Million Single-End Reads
<i>Stipa grandis</i>	SRR1740003	66.0 Million Paired-End Reads
<i>Triticum urartu</i>	ERR420228-9	203.3 Million Paired-End Reads

Supplementary Table S3. Anatomical characteristics of C₃, C₂, and C₄ leaves. Values represent mean ± SE, n=3 plants per species. Values followed by the same letter are not different from one another as determined by one-way ANOVA and the Tukey's post-hoc test.

	Species	Mesophyll (%)	Bundle Sheath (%)	Mesophyll:Bundle Sheath	Distance between adjacent minor veins (μm)	mesophyll cells # between minor veins
C ₃ species	<i>Dicanthelium oligosanthos</i>	40.0±2 a, b	7.2±0.5 a	5.7±0.5 a	206±6 a	6.6±0.4 a
	<i>Panicum bisulcatum</i>	45 ±1 a	10.0±1.2 a	5.4±1.0 a	240±9 a	7.4±2.9 a
	<i>Steinchisma laxum</i>	39±2 a, b	18.0±1.5 b	2.4±0.4 b	188 ±10 a, c	6.4±0.3 a
C ₃ -proto-Kranz						
C ₂ species	<i>Homolepis aturensis</i>	42±2 a	18.7±1.1 b	2.1±0.2 b	139±7 a, c	3.9±0.4 a, c
	<i>Steinchisma hians</i>	40±1a, b	21.3±1.3 b	2.2±0.1 b	129±2 a, c	4.3±0.2 a, c
	<i>Neurachne minor</i>	47±6 b	8.5±1.3 a	6.5±1.5 a	100±19	2.5±0.3
C ₄ species	<i>Panicum virgatum</i>	35±1 b	20.9±1.0 b	1.8±0.1 b	123±3 b	2.0±0.0* b, c
	<i>Setaria viridis</i>	46±1 a	10.1±0.7a	4.7±0.4 a	84±4 b, c	2.0±0.2 b, c

* There is one colorless cell between the two mesophyll cells of *P. virgatum*.

Supplementary Table S4. Characteristics of bundle sheath organelle positioning: Values represent mean \pm SE, n=3 plants per species. Values followed by the same letter are not different from one another as determined by one-way ANOVA and the Tukey's post-hoc test.

	Species	Coverage of Inner Half of Cell Area, %	Proportion in Inner Half of Cell Area, %
C ₃ species	Chloroplasts		
	<i>Dicanthelium oligosanthes</i>	1.3 \pm 0.2 a	38.6 \pm 6.0 a
	<i>Panicum bisulcatum</i>	3.4 \pm 0.3 b, c	47.5 \pm 3.2 a
C ₃ -Protokranz	<i>Steinchisma laxum</i>	2.1 \pm 0.2 a, c	52.5 \pm 2.7 a, b
C ₂ species	<i>Steinchisma hians</i>	7.2 \pm 0.5 b	60.6 \pm 2.8 b
	<i>Homolepis aturensis</i>	5.1 \pm 0.5 b	62.2 \pm 2.6 b
C ₃ species	Mitochondria		
	<i>Dicanthelium oligosanthes</i>	0.20 \pm 0.03 a	68.8 \pm 4.7 a
	<i>Panicum bisulcatum</i>	0.18 \pm 0.03 a	65.2 \pm 5.8 a
C ₃ -Protokranz	<i>Steinchisma laxum</i>	0.27 \pm 0.02 a	98.3 \pm 0.9 b
C ₂ species	<i>Steinchisma hians</i>	0.81 \pm 0.07 b	99.6 \pm 0.2 b
	<i>Homolepis aturensis</i>	0.73 \pm 0.06 b	98.8 \pm 0.6 b
C ₃ species	Peroxisomes		
	<i>Dicanthelium oligosanthes</i>	0.03 \pm 0.01	18.9 \pm 7.1 a
	<i>Panicum bisulcatum</i>	0.10 \pm 0.02 a	41.4 \pm 7.6 a
C ₃ -Protokranz	<i>Steinchisma laxum</i>	0.10 \pm 0.01 a	80.0 \pm 7.4 b
C ₂ species	<i>Steinchisma hians</i>	0.27 \pm 0.04 b	92.9 \pm 4.6 b
	<i>Homolepis aturensis</i>	0.22 \pm 0.03 b	91.4 \pm 4.0 b

Supplementary Table S5. Organelle parameters in the mesophyll and bundle sheath cells of nine species of grasses in the Panicoideae. Values represent means \pm SE. Values followed by the same letter are not different from one another as determined by one-way ANOVA and the Tukey's post-hoc test.

		Mitochondria Planar Area/Planar Cell Area, %	Mitochondria number /Planar Cell Area, $\mu\text{m}^{-2} \times 10^{-3}$	Density of Gold Labeling/ Planar Mitochondria Area, μm^{-2}	Density of Gold Labeling/ Planar Cell Area, μm^{-2}	Peroxisome Planar Area/Planar Cell Area, %	Peroxisome number /Planar Cell Area, μm^{-2} $\times 10^{-3}$	Chloroplast Planar Area/Planar Cell Area, %	Chloroplast number /Planar Cell Area, μm^{-2} $\times 10^{-3}$
Mesophyll Cells									
C ₃ species	<i>Dicanthelium oligosanthes</i>	0.50 \pm 0.05 a	30 \pm 3 ac	63.1 \pm 2.5 a	0.314 \pm 0.03 a	0.323 \pm 0.038 a	13 \pm 1 ab	26.9 \pm 1.8 a	26 \pm 1 a
	<i>Panicum bisulcatum</i>	0.29 \pm 0.04 a	16 \pm 2 b	76.4 \pm 4.8 a	0.194 \pm 0.02 a	0.333 \pm 0.050 a	11 \pm 2 ab	28.7 \pm 1.7 a	27 \pm 2 a
C ₃ -Protokranz	<i>Steinchisma laxum</i>	0.39 \pm 0.03 ab	34 \pm 3 a	83.5 \pm 5.5 a	0.304 \pm 0.02 a	0.588 \pm 0.056 a	21 \pm 2 a	30.3 \pm 1.6 a	33 \pm 2 ab
C ₂ species	<i>Homolepis aturensis</i>	0.30 \pm 0.06 bc	20 \pm bc	1.3 \pm 0.3 b	0.005 \pm 0.00 b	0.376 \pm 0.059 a	7 \pm 1 b	17.7 \pm 1.2 b	27 \pm 1 a
	<i>Steinchisma hians</i>	0.39 \pm 0.04 ab	37 \pm 3 a	1.5 \pm 0.4 b	0.007 \pm 0.002b	0.414 \pm 0.052 a	20 \pm 3 a	28.2 \pm 1.6 a	48 \pm 2 b
	<i>Neurachne minor</i>	0.59 \pm 0.07	22 \pm 3	2.2 \pm 0.3	0.012 \pm 0.002	0.011 \pm 0.002	3 \pm 1	19.6 \pm 1.6	28 \pm 2
C ₄ species	<i>Setaria viridis</i>	0.13 \pm 0.02c, d	12 \pm 3 b	0.7 \pm 0.3 b	0.001 \pm 0.000b	0.005 \pm 0.003 b	1 \pm 0.00 c	14.6 \pm 1.1 b	11 \pm 1
	<i>Panicum virgatum</i>	0.09 \pm 0.02 d	12 \pm 2 b	4.61 \pm 1.1 b	0.007 \pm 0.002b	0.002 \pm 0.002 b	3 \pm 0.00 c	12.3 \pm 0.9 b	22 \pm 2 a
Bundle Sheath Cells									
C ₃ species	<i>Dicanthelium oligosanthes</i>	0.28 \pm 0.03 a	30 \pm 3 ac	6.0 \pm 0.8 a	0.02 \pm 0.00 a	0.044 \pm 0.016 a	3 \pm 1 a	3.8 \pm 0.4 a	12 \pm 1 a
	<i>Panicum bisulcatum</i>	0.24 \pm 0.03 a	25 \pm 4 a	33.7 \pm 3.2cd	0.08 \pm 0.01 ab	0.152 \pm 0.033 b, c	8 \pm 2 bcd	7.6 \pm 0.5 bc	22 \pm 1 b
C ₃ -Protokranz	<i>Steinchisma laxum</i>	0.27 \pm 0.02 a	20 \pm 2 a	109.9 \pm 6.2 b	0.27 \pm 0.02 bc	0.105 \pm 0.015 b, c	4 \pm 1 a, c	4.9 \pm 0.5 ab	11 \pm 1 a
C ₂ species	<i>Homolepis aturensis</i>	0.73 \pm 0.06 b	32 \pm 3 ac	47.2 \pm 2.1 cd	0.35 \pm 0.03 c	0.227 \pm 0.027 b	7 \pm 1 c, d	8.3 \pm 0.7 bc	16 \pm 1 ac
	<i>Steinchisma hians</i>	0.81 \pm 0.06 b	53 \pm 3 b	92.2 \pm 3.7 b	0.74 \pm 0.05 d	0.266 \pm 0.040 b	12 \pm 1 d	11.2 \pm 0.8 ce	20 \pm 1 bc
	<i>Neurachne minor</i>	6.95 \pm 0.80	183 \pm 0.018	49.6 \pm 1.7	3.39 \pm 0.34	0.002 \pm 0.001	5 \pm 2	32.5 \pm 2.4	44 \pm 3
C ₄ species	<i>Panicum virgatum</i>	0.74 \pm 0.07 b	40 \pm 4 bc	52.3 \pm 1.7 c	0.38 \pm 0.03 c, d	0.069 \pm 0.014 ac	3 \pm 1 ab	18.5 \pm 1.5 de	18 \pm 1 bc
	<i>Setaria viridis</i>	0.64 \pm 0.08 b	30 \pm 3 ac	28.0 \pm 2.4 ad	0.17 \pm 0.02 b	0.062 \pm 0.017 ac	4 \pm 1 a, bc	41.8 \pm 1.7 d	21 \pm 1 b