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Supplementary Materials for

Spatial resolution of an integrated C_4+CAM photosynthetic metabolism

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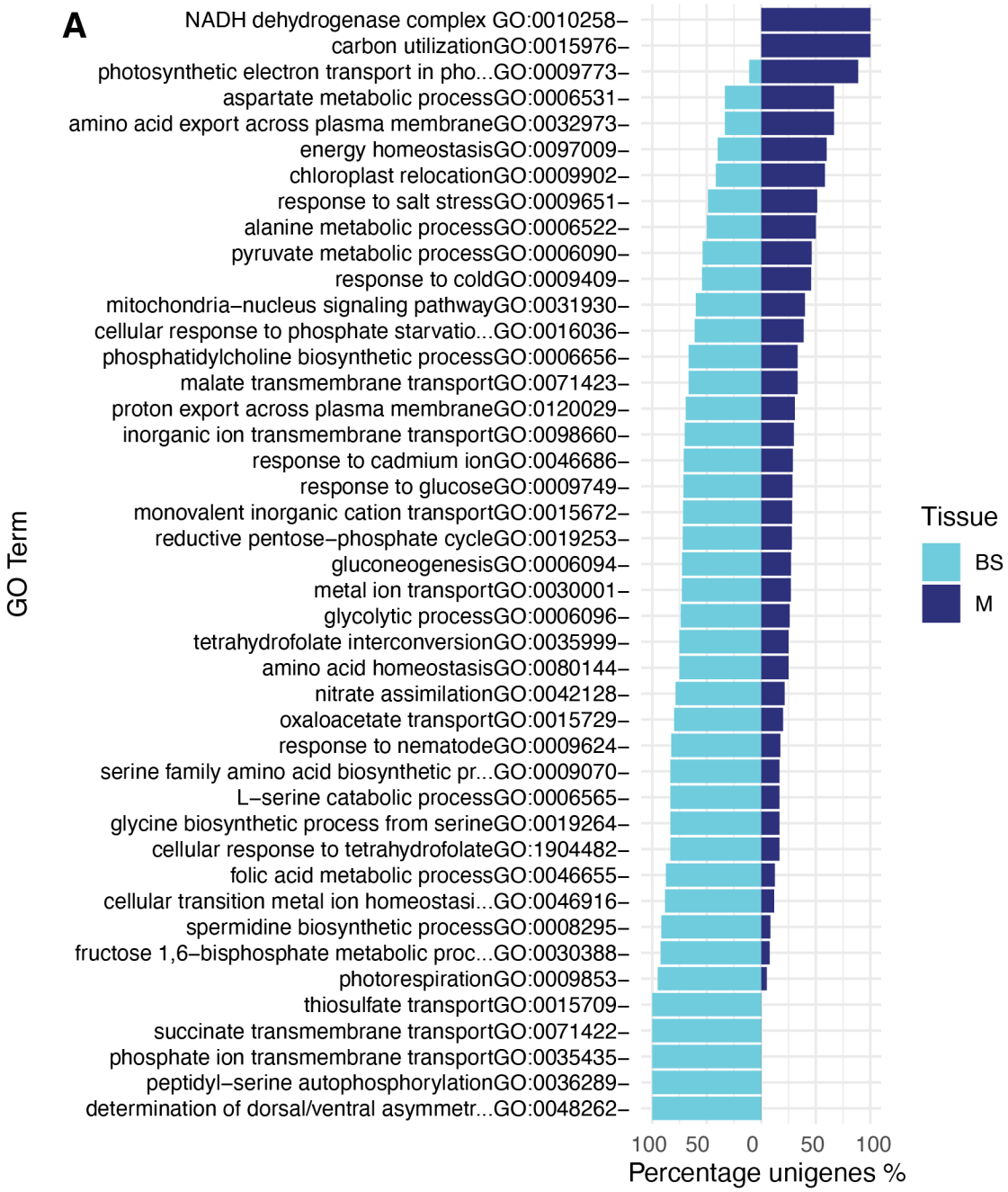
The PDF file includes:

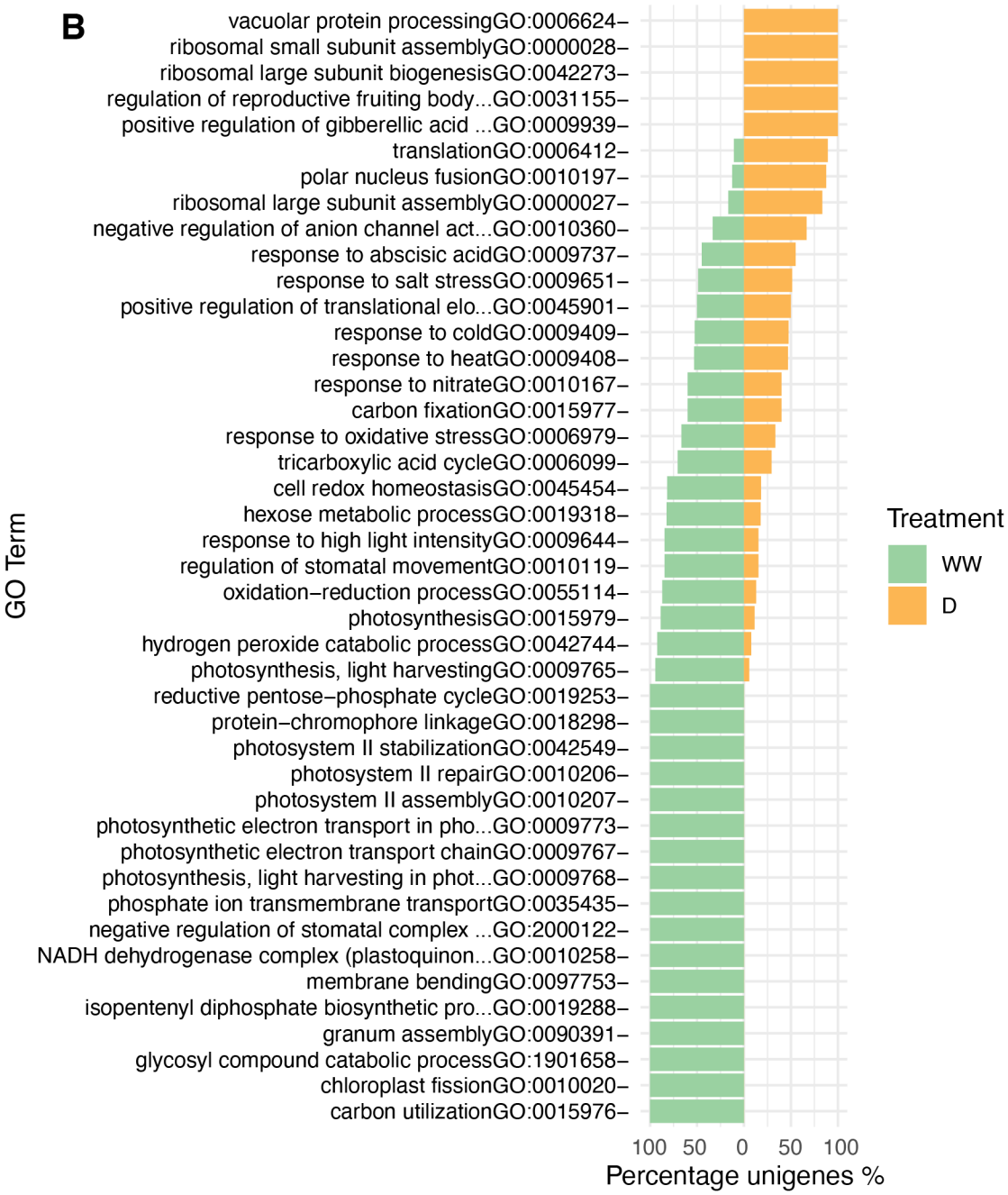
Other Supplementary Material for this manuscript includes the following:

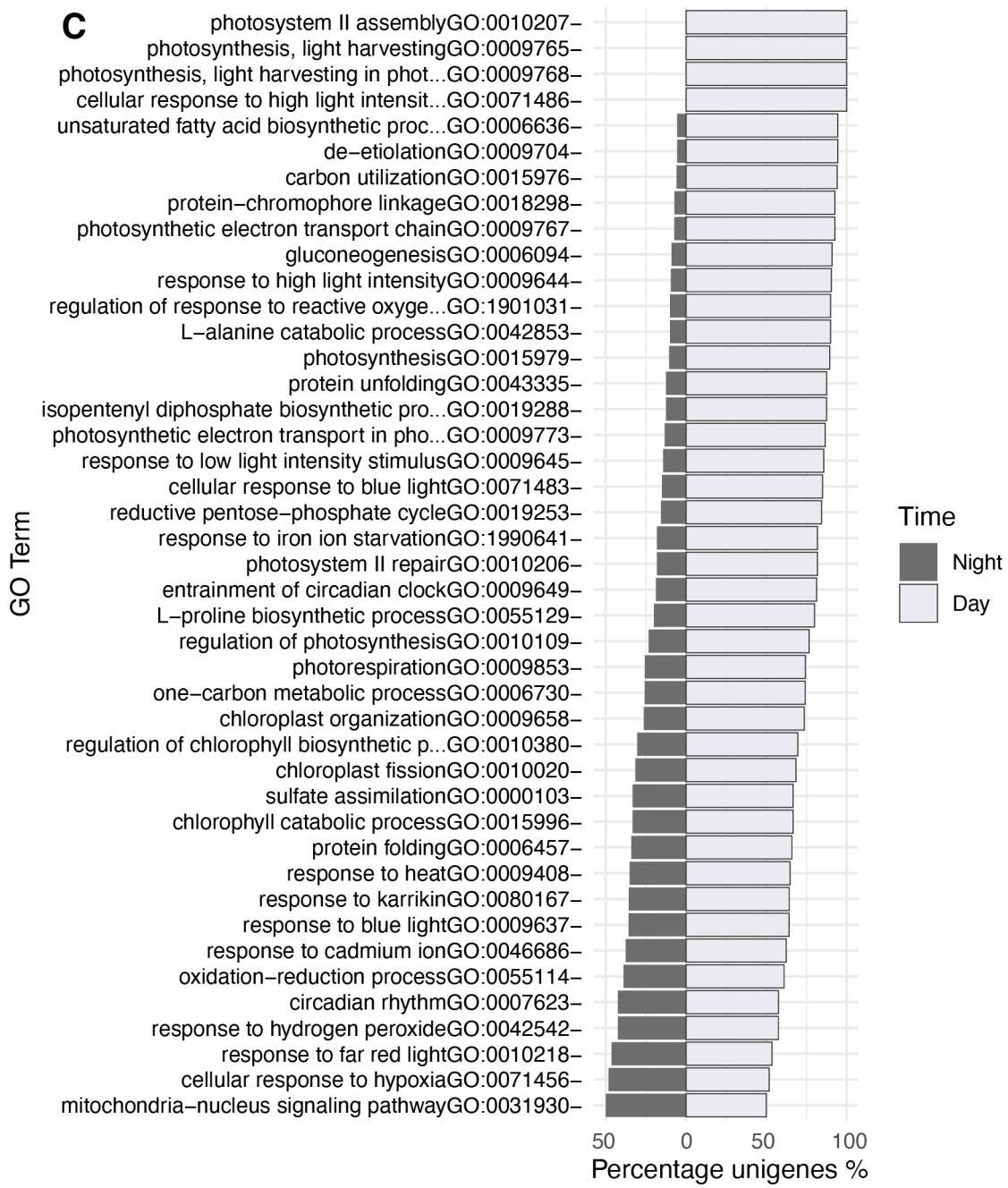
Supplementary figures

Fig. S1.

Gene Ontology enrichment across bundle sheath and mesophyll and across watering regimens. 50 most significant Gene Ontology terms enriched across differentially expressed genes between mesophyll and bundle sheath. Barplots indicate the percentage of genes up-regulated of each GO term across (A) mesophyll and bundle sheath, (B) well-watered and drought, (C) 23h and 7h.




B
vacuolar protein processingGO:0006624
ribosomal small subunit assemblyGO:0000028
ribosomal large subunit biogenesisGO:0042273
regulation of reproductive fruiting body...GO:0031155
positive regulation of gibberellic acid ...GO:0009939
translationGO:0006412
polar nucleus fusionGO:0010197
ribosomal large subunit assemblyGO:0000027
negative regulation of anion channel act...GO:0010360
response to abscisic acidGO:0009737
response to salt stressGO:0009651
positive regulation of translational elo...GO:0045901
response to coldGO:0009409
response to heatGO:0009408
response to nitrateGO:0010167
carbon fixationGO:0015977
response to oxidative stressGO:0006979
tricarboxylic acid cycleGO:0006099
cell redox homeostasisGO:0045454
hexose metabolic processGO:0019318
response to high light intensityGO:0009644
regulation of stomatal movementGO:0010119
oxidation−reduction processGO:0055114
photosynthesisGO:0015979
hydrogen peroxide catabolic processGO:0042744
photosynthesis, light harvestingGO:0009765
reductive pentose−phosphate cycleGO:0019253
protein−chromophore linkageGO:0018298
photosystem II stabilizationGO:0042549
photosystem II repairGO:0010206
photosystem II assemblyGO:0010207
photosynthetic electron transport in pho...GO:0009773
photosynthetic electron transport chainGO:0009767
photosynthesis, light harvesting in phot...GO:0009768
phosphate ion transmembrane transportGO:0035435
negative regulation of stomatal complex ...GO:2000122
NADH dehydrogenase complex (plastoquinon...GO:0010258
membrane bendingGO:0097753
isopentenyl diphosphate biosynthetic pro...GO:0019288
granum assemblyGO:0090391
glycosyl compound catabolic processGO:1901658
chloroplast fissionGO:0010020
carbon utilizationGO:0015976
GO Term
100 50 0 50 100
Percentage unigenes %
Treatment
WW
D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photosynthesis, light harvestingGO:0009765–
photosynthesis, light harvesting in phot...GO:0009768–
cellular response to high light intensit...GO:0071486–
unsaturated fatty acid biosynthetic proc...GO:0006636–
de–etiolationGO:0009704–
carbon utilizationGO:0015976–
protein–chromophore linkageGO:0018298–
photosynthetic electron transport chainGO:0009767–
gluconeogenesisGO:0006094–
response to high light intensityGO:0009644–
regulation of response to reactive oxyge...GO:1901031–
L–alanine catabolic processGO:0042853–
photosynthesisGO:0015979–
protein unfoldingGO:0043335–
isopentenyl diphosphate biosynthetic pro...GO:0019288–
photosynthetic electron transport in pho...GO:0009773–
response to low light intensity stimulusGO:0009645–
cellular response to blue lightGO:0071483–
reductive pentose–phosphate cycleGO:0019253–
response to iron ion starvationGO:1990641–
photosystem II repairGO:0010206–
entrainment of circadian clockGO:0009649–
L–proline biosynthetic processGO:0055129–
regulation of photosynthesisGO:0010109–
photorespirationGO:0009853–
one–carbon metabolic processGO:0006730–
chloroplast organizationGO:0009658–
regulation of chlorophyll biosynthetic p...GO:0010380–
chloroplast fissionGO:0010020–
sulfate assimilationGO:0000103–
chlorophyll catabolic processGO:0015996–
protein foldingGO:0006457–
response to heatGO:0009408–
response to karrikinGO:0080167–
response to blue lightGO:0009637–
response to cadmium ionGO:0046686–
oxidation–reduction processGO:0055114–
circadian rhythmGO:0007623–
response to hydrogen peroxideGO:0042542–
response to far red lightGO:0010218–
cellular response to hypoxiaGO:0071456–
mitochondria–nucleus signaling pathwayGO:0031930–
GO Term
50
0
50
100
Percentage unigenes %
Time
Night
Day

Fig. S2.
Differential transcript abundance across cell types and experimental conditions.
(Extension fig. 3) Differential transcript abundance (measured in log2 fold change, log2FC) of selected genes in mesophyll (M) relative to bundle sheath (BS) tissue (left panel), 07h relative to 23h (middle panel) and drought relative to well-watered (right panel) across LMD samples. Gene colour backgrounds correspond with pathways in the boxes on the top. In all panels, asterisks indicate significant differential expression (*P*adj < 0.05).

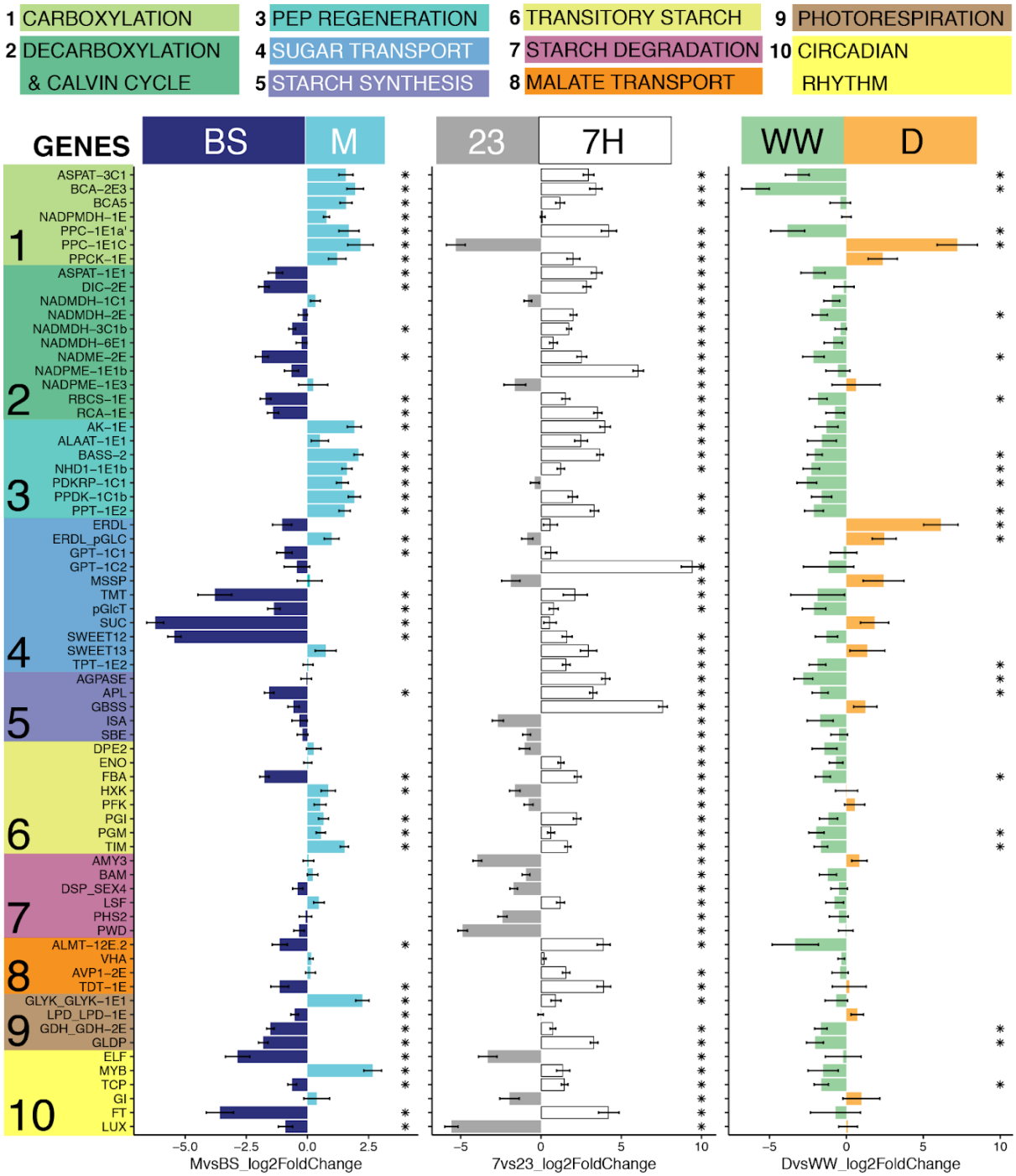


Fig. S3.

Effect of watering regimen in each cell type across time points (extension fig. 3).
Differential transcript abundance in droughted:D relative to well-watered plants:WW (measured in log2 fold change, log2FC). Gene colour backgrounds correspond to pathways in the boxes on the top. Triangles on the left panel represent relative abundance of D mesophyll samples vs WW mesophyll, in 7h samples (white triangles) and in 23h samples (black triangles). Right panel shows the same abundance comparisons using only bundle sheath samples. A triangle in the WW region (negative log2FC, left to the red lines) indicates higher expression during WW, while triangles within the D region (right to the red line) indicate higher expression in D. Asterisks indicate significant differential expression (*P*adj < 0.05)

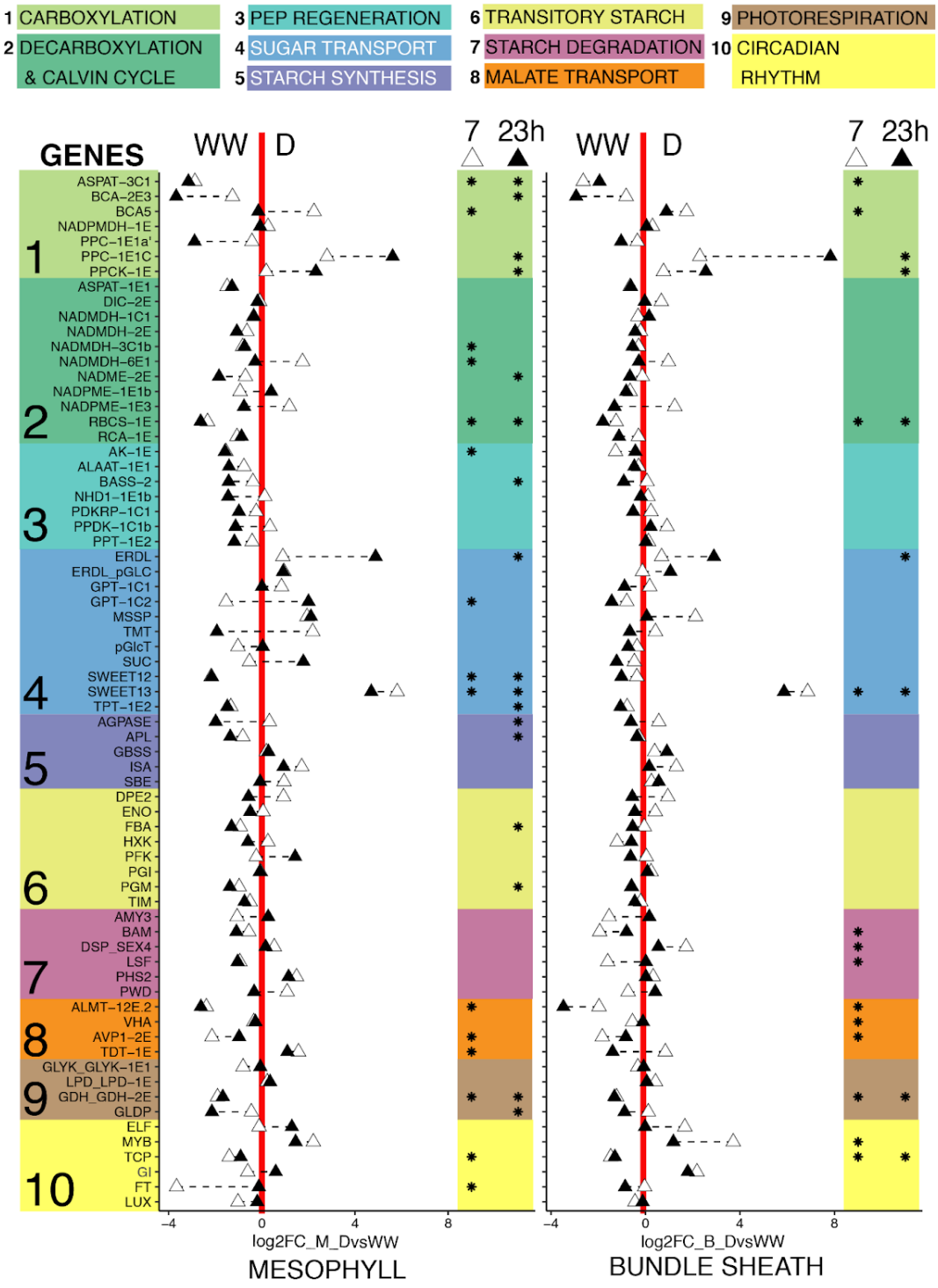
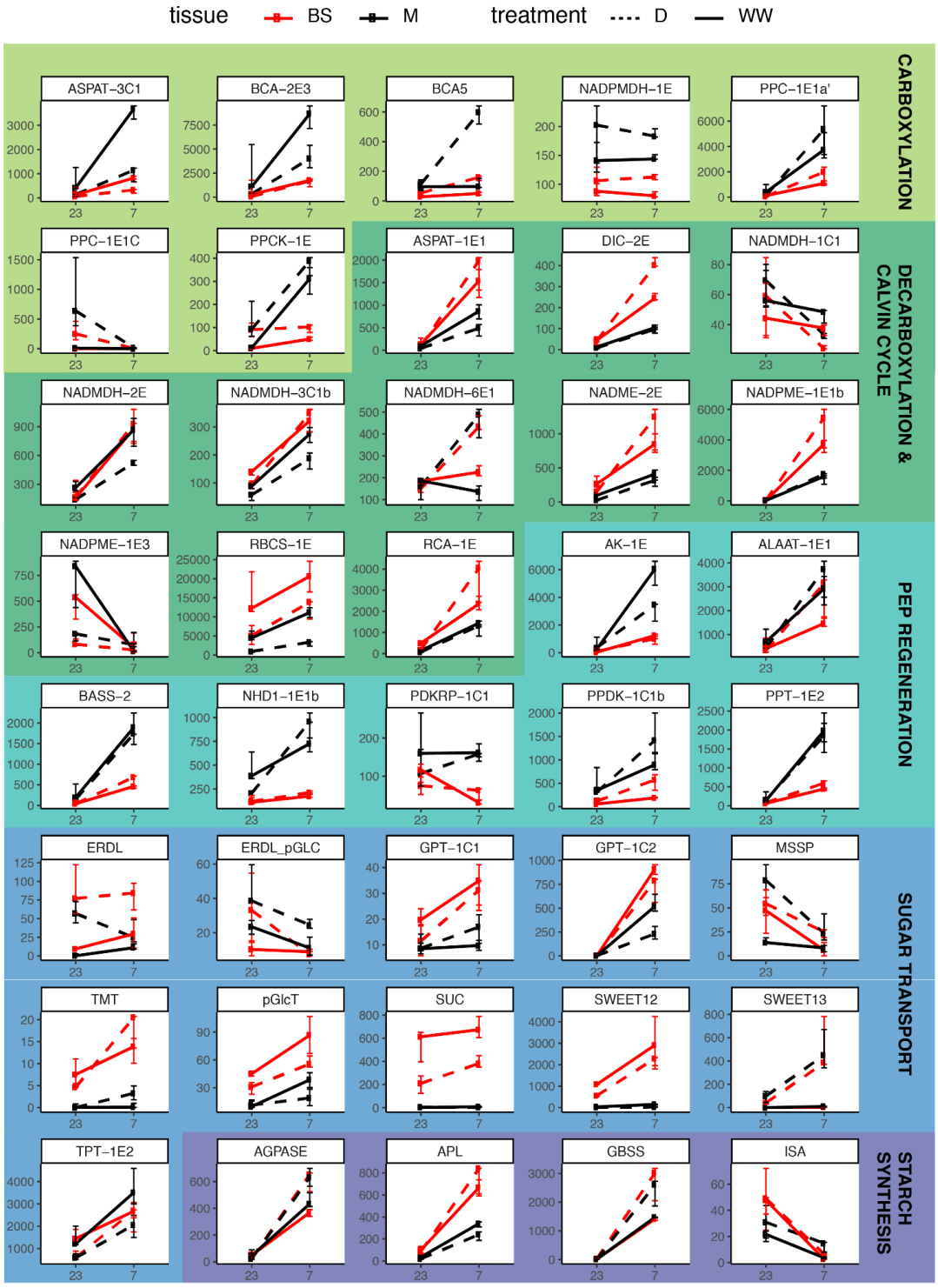
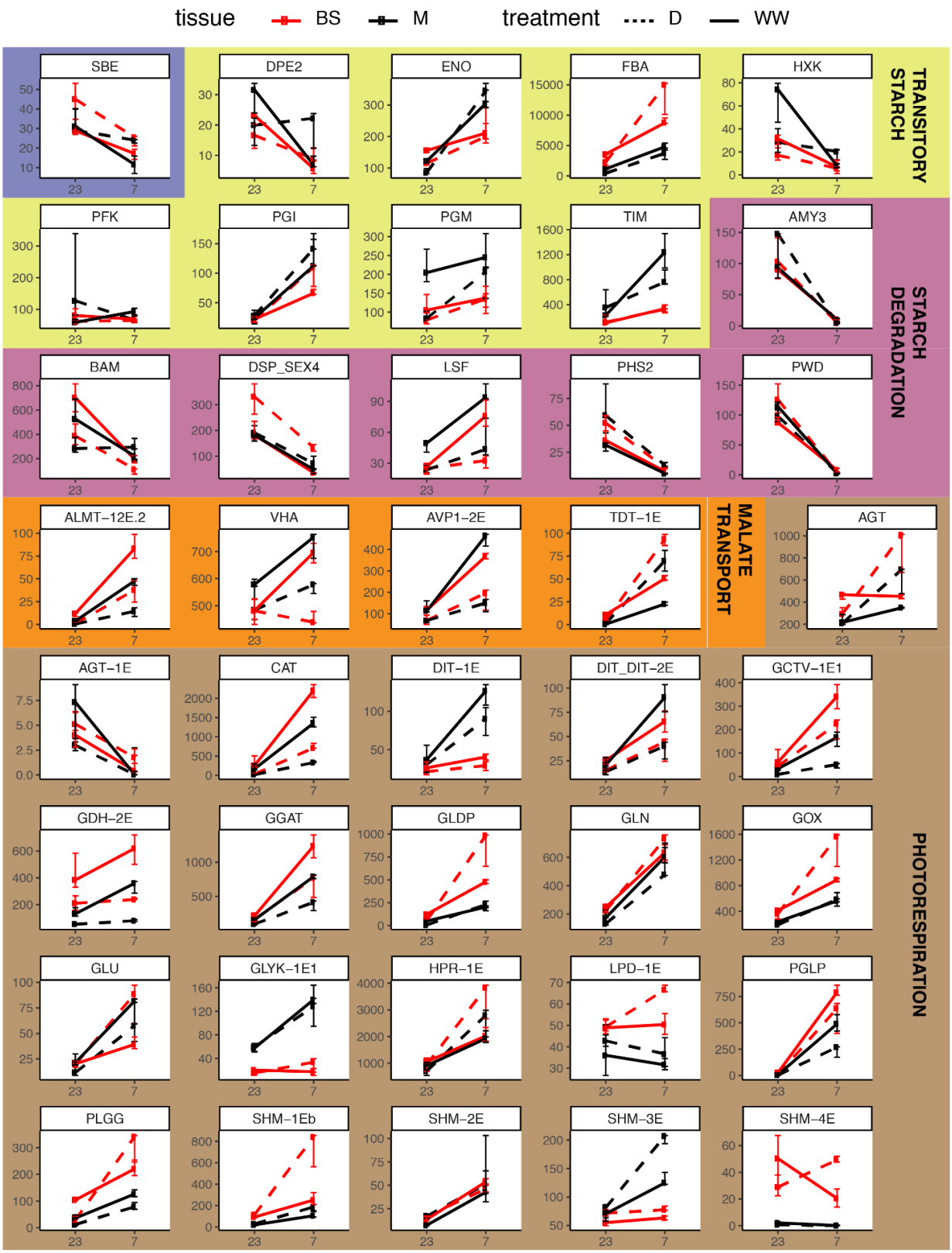


Fig. S4.

Transcription abundance of CCM-related genes. Transcription abundance of genes listed in table S3 as the median of transcripts per million (y-axis), across time points (x-axis) in LMD mRNA libraries. Black and red lines indicate mesophyll or bundle sheath, respectively. Plain lines indicate watered and dotted indicate drought. Error bars show interquartile range of expression.




tissue
BS
M
treatment
D
WW
TRANSITORY STARCH
STARCH DEGRADATION
MALATE TRANSPORT
PHOTORESPIRATION
SBE
DPE2
ENO
FBA
HXK
PFK
PGI
PGM
TIM
AMY3
BAM
DSP_SEX4
LSF
PHS2
PWD
ALMT−12E.2
VHA
AVP1−2E
TDT−1E
AGT
AGT−1E
CAT
DIT−1E
DIT_DIT−2E
GCTV−1E1
GDH−2E
GGAT
GLDP
GLN
GOX
GLU
GLYK−1E1
HPR−1E
LPD−1E
PGLP
PLGG
SHM−1Eb
SHM−2E
SHM−3E
SHM−4E

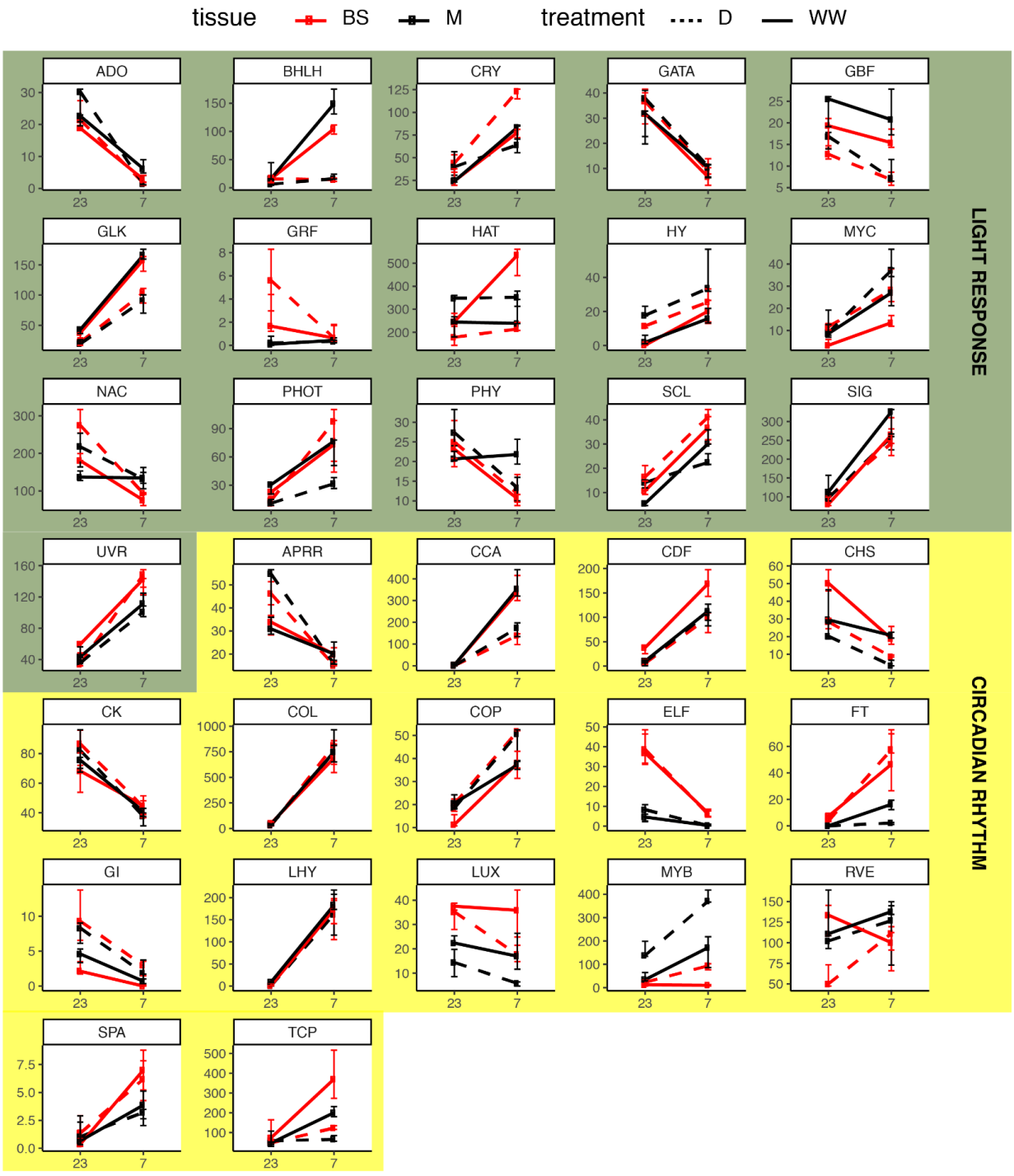

tissue
BS
M
treatment
D
WW
ADO
BHLH
CRY
GATA
GBF
GLK
GRF
HAT
HY
MYC
NAC
PHOT
PHY
SCL
SIG
LIGHT RESPONSE
UVR
APRR
CCA
CDF
CHS
CK
COL
COP
ELF
FT
GI
LHY
LUX
MYB
RVE
CIRCADIAN RHYTHM
SPA
TCP
23
7

Fig. S5.
Transcription abundance of genes with no known role in C4 or CAM. (Genes listed in table S4) Median of transcripts per million (y-axis), across time points (x-axis) in LMD mRNA libraries. Black and red lines indicate mesophyll or bundle sheath, respectively. Plain lines indicate watered and dotted indicate drought. Error bars show the interquartile range of expression.

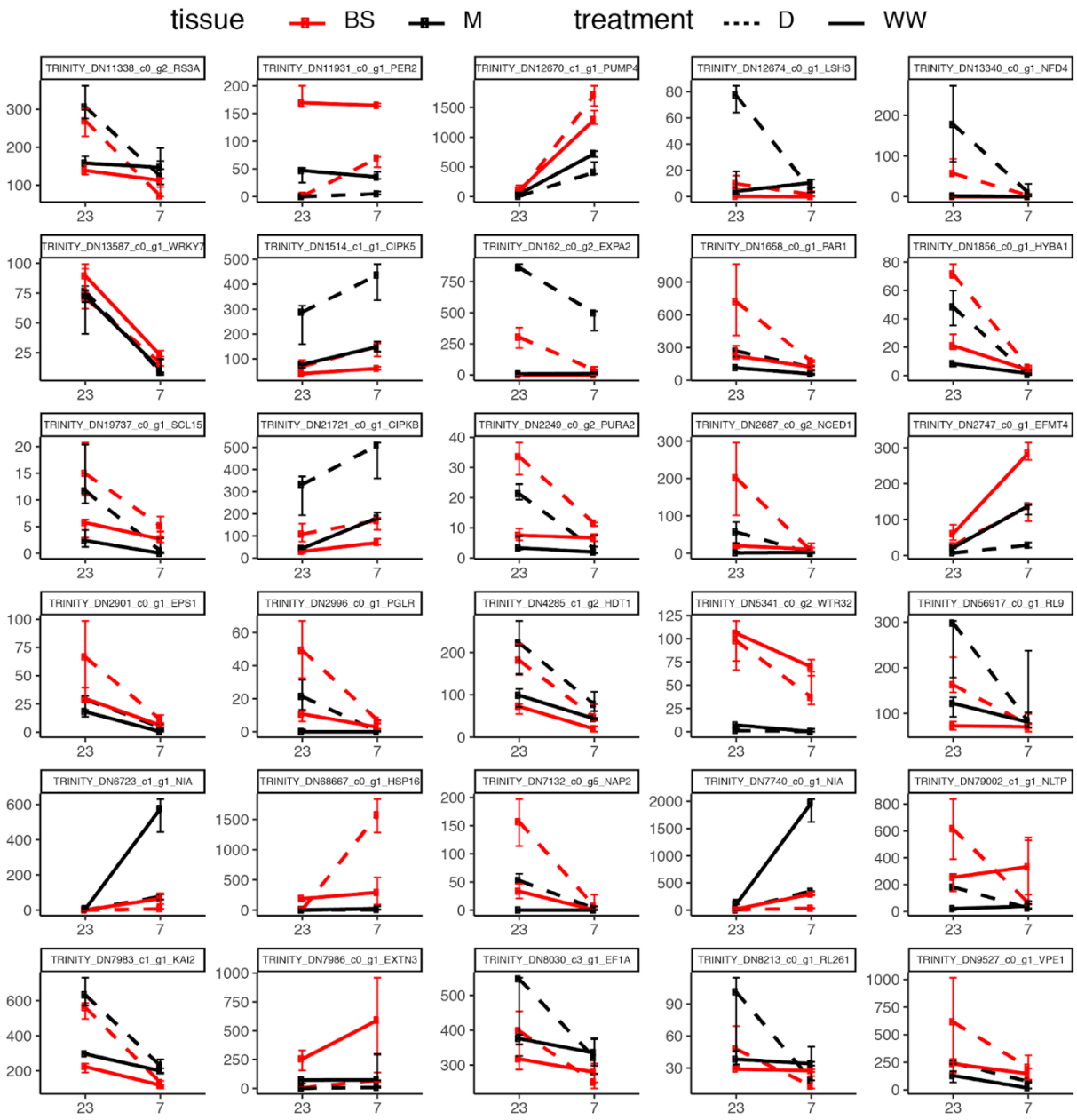


Fig. S6.

Visium spatial gene expression. The first row shows frozen leaf specimens at the moment of cryo-sectioning. The second row shows microphotographs of leaf paradermal sections under bright field (left) and K-means clustering of total gene expression (right). Successive rows show abundance of the main CCM-related genes using the 10x Genomics Visium platform. K-means clustering of sampling spots corresponds to bundle sheath (BS, dark blue), mesophyll (M, light blue), and water storage (WS, orange) tissues; abundances are shown relative to their observed unique molecule index (UMI) ranges.

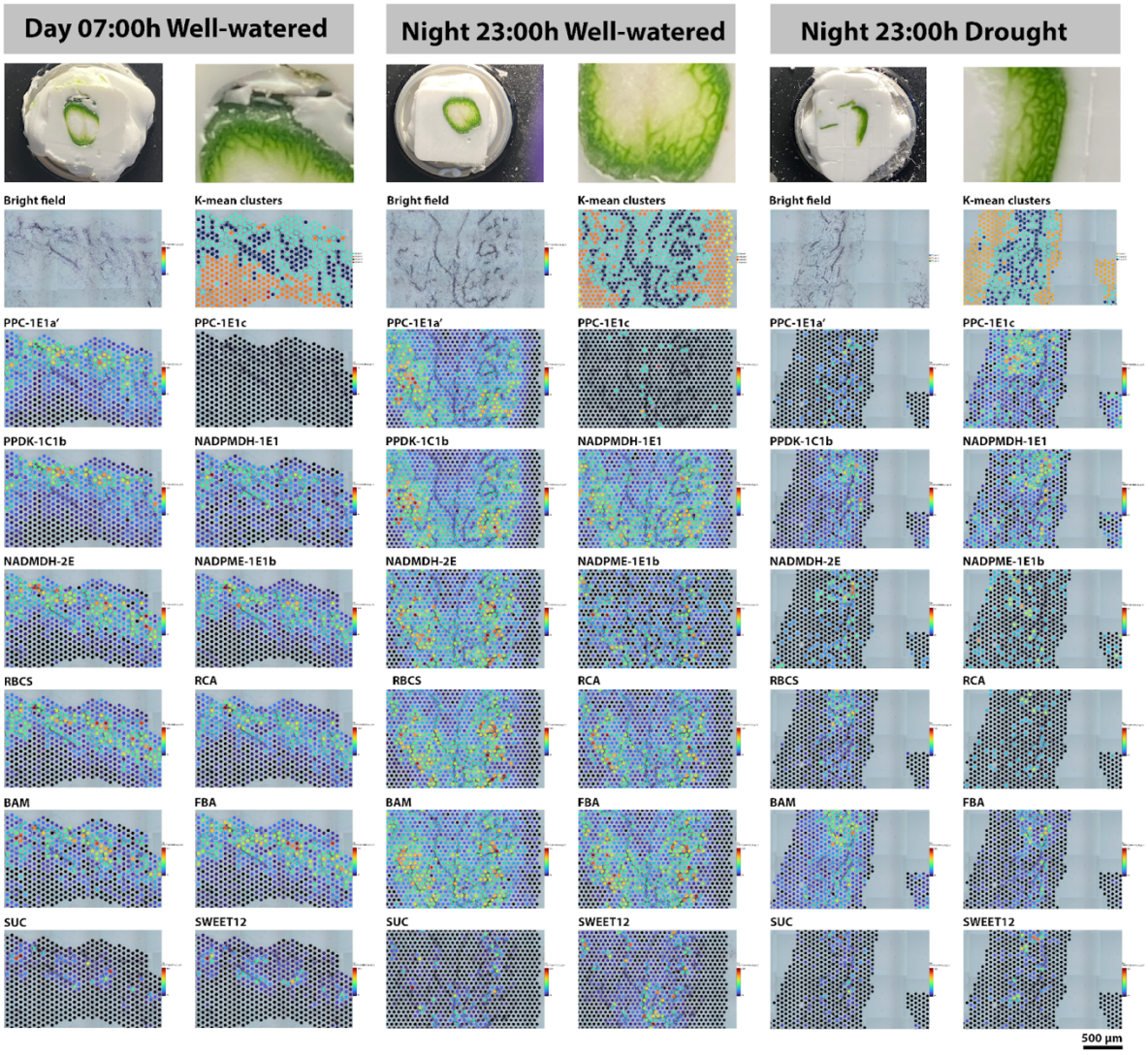


Fig. S7.

Correlations between predicted enzymatic fluxes and estimated gene expression in mesophyll and bundle sheath samples. Pearson correlation between z-score normalized (mean set to 0, SD set to 1) pFBA results and transcript abundance. The transcript abundances from different orthologs used in the same biochemical reactions were added together to be compared with the pFBA results. 23_BS_D: nighttime flux in bundle sheath under drought; 23_BS_W: nighttime flux in bundle sheath under well-water; 23_M_D: nighttime flux in mesophyll under drought; 23_M_W: nighttime flux in mesophyll under well-water; 7_BS_D: daytime flux in bundle sheath under drought; 7_BS_W: daytime flux in bundle sheath under well-water; 7_M_D: daytime flux in mesophyll under drought; 7_M_W: daytime flux in mesophyll under well-water;

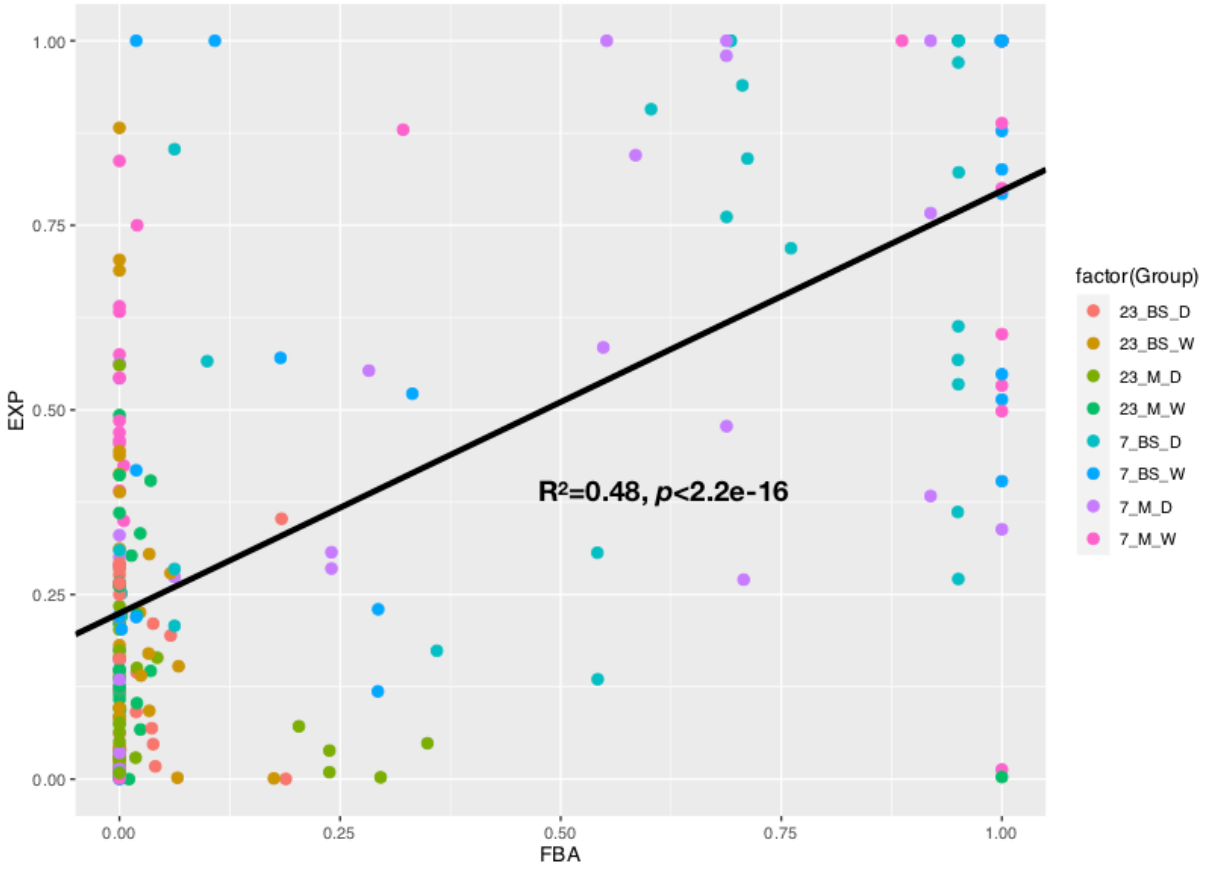
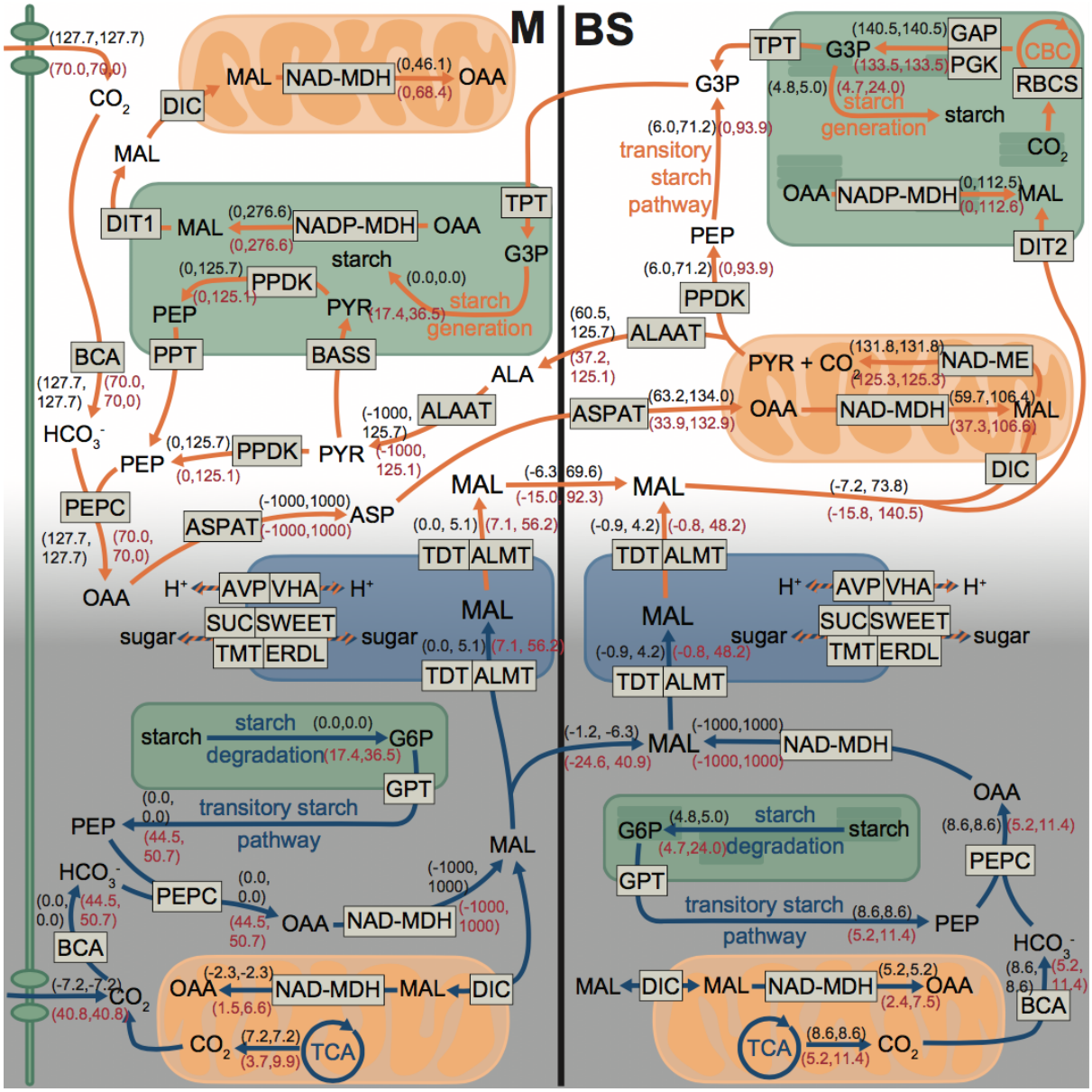


Fig. S8.

Flux variability analysis (FVA) of mesophyll and bundle sheath with diel variation and under drought and well-watered conditions. Flux variability analysis (FVA) of mesophyll and bundle sheath with diel variation and under drought and well-watered conditions. Daytime: orange arrows and white background; nighttime: blue arrows and grey background. Reactions that occur during the day-night transition are shown with striped arrows. M: mesophyll; BS: bundle sheath. Black numbers: well-watered condition (no specific CO_2 uptake constraint, stomata open); red numbers: drought condition (daytime CO_2 uptake constraint as 45% stomata closure compared to well-watered condition). The unit for the flux values is mmol $gDW^{-1}day^{-1}$.



Supplementary tables

Table S1.
Leaf titratable acidity analysis results. Microequivalents H+ (μEq H+) per gram fresh mass was calculated as volume titrant (μL) × titrant molarity (M) / tissue mass (g).

Sample_id	Treatment	Time (24h)	μEq H+
POL1-D-7	drought	7h:00	76.22
POL1-D-7	drought	7h:00	89.52
POL2-D-7	drought	7h:00	84.13
POL2-D-7	drought	7h:00	71.11
POL1-D-19	drought	19:00:00	27.12
POL1-D-19	drought	19:00:00	22.26
POL2-D-19	drought	19:00:00	41.24
POL2-D-19	drought	19:00:00	11.05
POL1-W-7	watered	7h:00	24.32
POL1-W-7	watered	7h:00	15.63
POL2-W-7	watered	7h:00	22.32
POL2-W-7	watered	7h:00	16.73
POL1-W-19	watered	19:00:00	23.41
POL1-W-19	watered	19:00:00	25.96
POL2-W-19	watered	19:00:00	16.75
POL2-W-19	watered	19:00:00	16.97

Table S2.

LMD-RNA sequencing and read mapping statistics. BS: bundle-sheath cells, M: mesophyll cells, n_: number of reads.T: time; Ind: Plant individual; n_raw: number of raw reads; n_filtered: number of reads after filtering; n_aligned: number of aligned read to the transcriptome; %_al: percentage of reads aligned to the transcriptome.

Library_id	T	Tissue	Treatment	Ind	n_raw	n_filtered	n_aligned	%_al
22-A_PO1-D-7-M	7h	BS	Drought	PO1	37,306,803	32,891,937	25,374,659	77.10
5-2-A_PO2-D-7-BS	7h	BS	Drought	PO2	36,651,612	32,289,322	23,469,722	72.70
5-A_PO2-D-7-BS	7h	BS	Drought	PO2	32,587,781	28,351,390	20,341,689	71.70
10-B_PO2-WW-23-BS	7h	BS	Watered	PO1	39,734,057	23,311,582	17,349,512	74.40
12-B_PO1-WW-7-BS	7h	BS	Watered	PO1	34,351,505	23,369,981	17,052,138	73.00
4-12-B_PO1-WW-7-BS	7h	BS	Watered	PO1	32,181,954	20,082,323	14,527,796	72.30
19-B_PO2-WW-7-BS	7h	BS	Watered	PO2	37,865,848	19,888,745	14,497,329	72.90
24-A_PO1-D-23-M	7h	M	Drought	PO1	35,708,771	27,081,395	22,110,992	81.60
4-2-A_PO2-D-7-M	7h	M	Drought	PO2	31,310,261	27,982,988	21,524,928	76.90
6-2-A_PO2-D-7-M	7h	M	Drought	PO2	35,647,657	31,989,325	24,098,407	75.30
13-B_PO1-WW-7-M	7h	M	Watered	PO1	34,519,270	22,373,676	16,661,642	74.50
15-B_PO1-WW-7-M	7h	M	Watered	PO1	41,545,559	29,425,690	21,639,546	73.50
21-A_PO1-D-7-BS	7h	M	Watered	PO2	39,268,580	34,697,032	22,238,196	64.10
26-A_PO1-D-23-M	23h	BS	Drought	PO1	38,373,081	32,491,941	26,358,868	81.10
7-C_PO1-D-23-BS	23h	BS	Drought	PO1	35,134,577	15,276,839	10,700,178	70.00
15-A_PO2-D-23-BS	23h	BS	Drought	PO2	38,199,397	29,299,221	21,694,673	74.00
7-A_PO2-D-23-BS	23h	BS	Drought	PO2	35,729,969	30,921,888	23,884,264	77.20
3-C_PO1-WW-23-BS	23h	BS	Watered	PO1	39,224,405	17,905,370	13,410,110	74.90
1-12-B_PO1-WW-7-BS	23h	BS	Watered	PO2	33,572,474	19,664,420	14,754,741	75.00
8-B_PO2-WW-23-BS	23h	BS	Watered	PO2	38,190,910	20,875,273	15,495,580	74.20
25-A_PO1-D-23-BS	23h	M	Drought	PO1	35,543,707	31,380,508	24,151,852	77.00
2-C_PO1-WW-23-M	23h	M	Drought	PO1	35,114,605	12,695,318	10,070,151	79.30
8-C_PO1-D-23-M	23h	M	Drought	PO1	48,944,420	19,757,250	15,312,762	77.50
16-A_PO2-D-23-M	23h	M	Drought	PO2	40,444,993	34,196,837	27,231,638	79.60
8-A_PO2-D-23-M	23h	M	Drought	PO2	35,841,757	30,860,105	24,858,317	80.60
20-B_PO2-WW-7-M	23h	M	Watered	PO1	39,592,141	22,769,276	16,909,912	74.30
11-B_PO2-WW-23-M	23h	M	Watered	PO2	44,046,759	28,007,152	21,373,120	76.30
9-B_PO2-WW-23-M	23h	M	Watered	PO2	43,071,551	24,150,596	18,659,731	77.30

Table S3.

Annotation of selected genes with a role or potential role in CCM.

Phylo-annotation	Uniprot_gene	Description	unigenes	Pathway
ASPAT-3C1	AATC_DAUCA	Aspartate aminotransferase, cytoplasmic	TRINITY_DN346_c1_g3	Carboxylation
BCA-2E3		Beta carbonic anhydrase	TRINITY_DN889_c1_g2	Carboxylation
BCA5	BCA5_ARATH	Beta carbonic anhydrase 5, chloroplastic	TRINITY_DN8226_c0_g1	Carboxylation
NADPMDH-1E	MDHP_MESCR	Malate dehydrogenase [NADP], chloroplastic	TRINITY_DN11597_c0_g1	Carboxylation
PPC-1E1a'	CAPP_AMAHP	Phosphoenolpyruvate carboxylase	TRINITY_DN1747_c2_g1	Carboxylation
PPC-1E1C	CAPP2_ARATH	Phosphoenolpyruvate carboxylase 2	TRINITY_DN3235_c0_g3	Carboxylation
PPCK-1E	PPCK1_ARATH	Phosphoenolpyruvate carboxylase kinase 1	TRINITY_DN4567_c3_g1	Decarboxylation
ASPAT-1E1		Alanine aminotransferase 2, mitochondrial	TRINITY_DN18480_c0_g1	Decarboxylation
DIC-2E	PUMP5_ARATH	Mitochondrial uncoupling protein 5	TRINITY_DN1178_c2_g2	Decarboxylation
NADMDH-1C1	MDHP_ARATH	Malate dehydrogenase, chloroplastic	TRINITY_DN2897_c0_g1	Decarboxylation
NADMDH-2E		Malate dehydrogenase	TRINITY_DN12466_c1_g1	Decarboxylation
NADMDH-3C1b	MDHG_CUCSA	Malate dehydrogenase, glyoxysomal	TRINITY_DN379_c2_g1	Decarboxylation
NADMDH-6E1	MDHC_BETVU	Malate dehydrogenase, cytoplasmic	TRINITY_DN70366_c1_g1	Decarboxylation
NADME-2E	MAON_SOLTU	NAD-dependent malic enzyme 59 kDa isoform, mitochondrial	TRINITY_DN17872_c1_g1	Decarboxylation
NADPME-1E1b	MAOC_FLAPR	NADP-dependent malic enzyme, chloroplastic	TRINITY_DN1253_c0_g1	Decarboxylation
NADPME-1E3	MAOX_VITVI	NADP-dependent malic enzyme	TRINITY_DN9969_c0_g1	Decarboxylation
RBCS-1E		Ribulose bisphosphate carboxylase small chain, chloroplastic	TRINITY_DN6389_c0_g1	Calvin cycle
RCA-1E		Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic	TRINITY_DN17967_c2_g1	Calvin cycle
AK-1E	KAD2_ARATH	Adenylate kinase 2, chloroplastic	TRINITY_DN1795_c0_g4	PEP generation
ALAAT-1E1	ALAT2_ARATH	Alanine aminotransferase 2, mitochondrial	TRINITY_DN8707_c0_g1	PEP generation
BASS-2	BASS2_ARATH	Sodium/pyruvate cotransporter BASS2, chloroplastic	TRINITY_DN6617_c4_g1	PEP generation
NHD1-1E1b	NHD1_ARATH	Sodium/proton antiporter 1	TRINITY_DN1372_c2_g1	PEP generation
PDKRP-1C1	PDRP1_ORYSI	Probable pyruvate, phosphate dikinase regulatory protein, chloroplastic	TRINITY_DN7731_c0_g1	PEP generation
PPDK-1C1b	PPDK_MESCR	Pyruvate, phosphate dikinase, chloroplastic	TRINITY_DN4872_c3_g1	PEP generation
PPT-1E2	PPT2_ORYSJ	Phosphoenolpyruvate/phosphate translocator 2, chloroplastic	TRINITY_DN13020_c1_g1	PEP generation
ERDL	EDL16_ARATH	Sugar transporter ERD6-like 16	TRINITY_DN1122_c0_g1	Starch/sugar transport
ERDL_pGLC	ERDL7_ARATH	Sugar transporter ERD6-like 7	TRINITY_DN2349_c0_g1	Starch/sugar transport
GPT-1C1	GPT2_ARATH	Glucose-6-phosphate/phosphate translocator 2, chloroplastic	TRINITY_DN6031_c0_g1	Starch/sugar transport
GPT-1C2	GPT2_ARATH	Glucose-6-phosphate/phosphate translocator 2, chloroplastic	TRINITY_DN6322_c1_g1	Starch/sugar transport
MSSP	MSSP2_ARATH	Monosaccharide-sensing protein 2	TRINITY_DN17666_c0_g1	Starch/sugar transport
TMT	MSSP2_ARATH	Monosaccharide-sensing protein 2	TRINITY_DN6869_c0_g2	Starch/sugar transport
pGlcT	PLST4_ARATH	Plastidic glucose transporter 4	TRINITY_DN7812_c0_g2	Starch/sugar transport
SUC	SUT_SPIOL	Sucrose transport protein	TRINITY_DN6710_c1_g1	Starch/sugar transport
SWEET12	SWT12_ARATH	Bidirectional sugar transporter SWEET12	TRINITY_DN19841_c0_g1	Starch/sugar transport
SWEET13	SWT13_ARATH	Bidirectional sugar transporter SWEET13	TRINITY_DN8938_c4_g2	Starch/sugar transport
TPT-1E2	TPT_SPIOL	Triose phosphate/phosphate translocator, chloroplastic	TRINITY_DN1249_c1_g1	Starch/sugar transport
AGPASE	GLGS2_VICFA	Glucose-1-phosphate adenylyltransferase small subunit 2, chloroplastic	TRINITY_DN7789_c1_g1	Starch synthesis
APL	GLGL1_ARATH	Glucose-1-phosphate adenylyltransferase large subunit 1, chloroplastic	TRINITY_DN4352_c0_g1	Starch synthesis
GBSS	SSG1_MANES	Granule-bound starch synthase 1, chloroplastic/amyloplastic	TRINITY_DN11204_c1_g1	Starch synthesis
ISA	ISOA1_ARATH	Isoamylase 1, chloroplastic	TRINITY_DN4229_c0_g1	Starch synthesis
SBE	GLGB1_PEA	1,4-alpha-glucan-branching enzyme 1, chloroplastic/amyloplastic	TRINITY_DN2618_c0_g1	Starch synthesis
DPE2	DPE2_ARATH	4-alpha-glucanotransferase DPE2	TRINITY_DN6357_c0_g1	Transitory starch pathway
ENO	ENO_MESCR	Enolase	TRINITY_DN6068_c1_g2	Transitory starch pathway
FBA	ALFP_ORYSJ	Fructose-bisphosphate aldolase, chloroplastic	TRINITY_DN1271_c0_g1	Transitory starch pathway
HXK	HXK2_ORYSJ	Hexokinase-2	TRINITY_DN7391_c0_g1	Transitory starch pathway
PFK	PFKA6_ARATH	ATP-dependent 6-phosphofructokinase 6	TRINITY_DN8265_c0_g1	Transitory starch pathway
PGI		Glucose-6-phosphate isomerase	TRINITY_DN1518_c1_g1	Transitory starch pathway
PGM	PGMC_MESCR	Phosphoglucomutase, cytoplasmic	TRINITY_DN23625_c0_g1	Transitory starch pathway
TIM	TPIC_SPIOL	Triosephosphate isomerase, chloroplastic	TRINITY_DN4168_c0_g2	Transitory starch pathway
AMY3	AMY3_ARATH	Alpha-amylase 3, chloroplastic	TRINITY_DN2216_c0_g1	Starch degradation
BAM	BAM3_ARATH	Beta-amylase 3, chloroplastic	TRINITY_DN1866_c0_g1	Starch degradation
DSP_SEX4	DSP4_CASSA	Phosphoglucan phosphatase DSP4, amyloplastic	TRINITY_DN12877_c0_g1	Starch degradation

LSF	LSF2_ARATH	Phosphoglucan phosphatase LSF2, chloroplastic	TRINITY_DN2348_c0_g1	Starch degradation
PHS2	PHSL2_SOLTU	Alpha-1,4 glucan phosphorylase L-2 isozyme, chloroplastic/amyloplastic	TRINITY_DN12375_c0_g1	Starch degradation
PWD	GWD1_CITRE	Alpha-glucan water dikinase, chloroplastic	TRINITY_DN3424_c0_g1	Starch degradation
ALMT-12E.2	ALMTC_ARATH	Aluminum-activated malate transporter 12	TRINITY_DN10391_c0_g2	Metabolite transport
VHA		V-type proton ATPase subunit	TRINITY_DN16658_c0_g1	Metabolite transport
AVP1-2E		Pyrophosphate-energized vacuolar membrane proton pump	TRINITY_DN1954_c0_g1	Metabolite transport
TDT-1E	TDT_ARATH	Tonoplast dicarboxylate transporter	TRINITY_DN161_c0_g1	Metabolite transport
AGT	SGAT_ARATH	Serine--glyoxylate aminotransferase	TRINITY_DN6907_c0_g1	Photorespiration
AGT-1E	AGT23_ARATH	Alanine--glyoxylate aminotransferase 2 homolog 3, mitochondrial	TRINITY_DN3579_c0_g1	Photorespiration
CAT	CATA2_GOSHI	Catalase isozyme 2	TRINITY_DN193_c1_g1	Photorespiration
DIT-1E	DIT1_SPIOL	Dicarboxylate transporter 1, chloroplastic	TRINITY_DN2503_c0_g1	Photorespiration
DIT-2E	DIT2_SPIOL	Dicarboxylate transporter 2, chloroplastic	TRINITY_DN4387_c0_g2	Photorespiration
GCTV-1E1	GCST_MESCR	Aminomethyltransferase, mitochondrial	TRINITY_DN2504_c1_g1	Photorespiration
GDH-2E	GCSH_MESCR	Glycine cleavage system H protein, mitochondrial	TRINITY_DN6744_c1_g1	Photorespiration
GGAT		Glutamate--glyoxylate aminotransferase	TRINITY_DN2102_c0_g1	Photorespiration
GLDP	GCSPA_FLAPR	Glycine dehydrogenase (decarboxylating) A, mitochondrial	TRINITY_DN6006_c0_g1	Photorespiration
GLN	GLNA1_LOTJA	Glutamine synthetase cytosolic isozyme	TRINITY_DN4141_c1_g1	Photorespiration
GOX	GOX_SPIOL	Peroxisomal (S)-2-hydroxy-acid oxidase	TRINITY_DN3751_c0_g1	Photorespiration
GLU	GLTB_SPIOL	Ferredoxin-dependent glutamate synthase, chloroplastic	TRINITY_DN425_c0_g1	Photorespiration
GLYK-1E1	GLYK_ARATH	D-glycerate 3-kinase, chloroplastic	TRINITY_DN2228_c0_g1	Photorespiration
HPR-1E	HPR1_ARATH	Glycerate dehydrogenase HPR, peroxisomal	TRINITY_DN7689_c0_g3	Photorespiration
LPD-1E	DLDH1_ARATH	Dihydrolipoyl dehydrogenase 1, mitochondrial	TRINITY_DN7076_c1_g1	Photorespiration
PGLP	PGP1A_ARATH	Phosphoglycolate phosphatase 1A, chloroplastic	TRINITY_DN17754_c0_g1	Photorespiration
PLGG		Plastidal glycolate/glycerate translocator	TRINITY_DN10229_c0_g2	Photorespiration
SHM-1Eb	GLYM_SOLTU	Serine hydroxymethyltransferase, mitochondrial	TRINITY_DN302_c1_g2	Photorespiration
SHM-2E	GLYC6_ARATH	Serine hydroxymethyltransferase 6	TRINITY_DN1750_c0_g1	Photorespiration
SHM-3E		Serine hydroxymethyltransferase	TRINITY_DN3209_c0_g1	Photorespiration
SHM-4E	GLYP3_ARATH	Serine hydroxymethyltransferase 3, chloroplastic	TRINITY_DN1307_c0_g1	Photorespiration
ADO	ADO1_ARATH	Adagio protein 1	TRINITY_DN3633_c0_g1	Light response
BHLH	BH062_ARATH	Transcription factor bHLH62	TRINITY_DN360_c1_g1	Light response
CRY	CRY1_ARATH	Cryptochrome-1	TRINITY_DN2794_c2_g1	Light response
GATA		Putative GATA transcription factor	TRINITY_DN9003_c0_g1	Light response
GBF	GBF1_ARATH	G-box-binding factor 1	TRINITY_DN2605_c2_g1	Light response
GLK	GLK1_ARATH	Transcription activator GLK1	TRINITY_DN969_c0_g1	Light response
HAT	HAT1_ARATH	Homeobox-leucine zipper protein HAT1	TRINITY_DN4311_c1_g1	Light response
HY		Transcription factor HY5-like	TRINITY_DN34596_c0_g1	Light response
MYC	MYC2_SOLLC	Transcription factor MYC2	TRINITY_DN4962_c0_g1	Light response
NAC		NAC domain-containing protein	TRINITY_DN3383_c0_g1	Light response
PHOT		Phototropin-2	TRINITY_DN282_c3_g1	Light response
PHY	PHYA1_TOBAC	Phytochrome A1	TRINITY_DN2460_c0_g1	Light response
SIG	SIGA_ARATH	RNA polymerase sigma factor sigA	TRINITY_DN4315_c1_g1	Light response
UVR	UVR8_ARATH	Ultraviolet-B receptor UVR8	TRINITY_DN1267_c1_g1	Light response
CCA	LHY_ARATH	Protein LHY	TRINITY_DN22210_c0_g1	Circadian rhythm
CDF	CDF2_ARATH	Cyclic dof factor 2	TRINITY_DN3894_c1_g1	Circadian rhythm
CK	CSK21_ARATH	Casein kinase II subunit alpha-1	TRINITY_DN1064_c0_g2	Circadian rhythm
COL	COL4_ARATH	Zinc finger protein CONSTANS-LIKE 4	TRINITY_DN3547_c1_g1	Circadian rhythm
COP	COP1_ARATH	E3 ubiquitin-protein ligase COP1	TRINITY_DN2263_c1_g1	Circadian rhythm
ELF		Protein EARLY FLOWERING 3	TRINITY_DN11682_c0_g1	Circadian rhythm
FT		Glutaminyl-peptide cyclotransferase	TRINITY_DN17372_c0_g1	Circadian rhythm
GI	GIGAN_ARATH	Protein GIGANTEA	TRINITY_DN8078_c0_g1	Circadian rhythm
LHY	LHY_ARATH	Protein LHY	TRINITY_DN7854_c1_g1	Circadian rhythm
LUX	MYBC1_ARATH	Transcription factor MYBC1	TRINITY_DN1624_c0_g1	Circadian rhythm
MYB	MYB4_ARATH	Transcription repressor MYB4	TRINITY_DN2554_c0_g1	Circadian rhythm
RVE	RVE7L_ARATH	Protein REVEILLE 7-like	TRINITY_DN10821_c0_g1	Circadian rhythm
SPA	SPA1_ARATH	Protein SUPPRESSOR OF PHYA-105 1	TRINITY_DN1322_c1_g1	Circadian rhythm
TCP	PIP25_ARATH	Probable aquaporin PIP2-5	TRINITY_DN9818_c0_g1	Circadian rhythm

Table S4.
Annotation of selected genes differentially expressed across experimental variables without a known role in CCM.

Uniprot_gene	unigenes	Description
ATHB7	TRINITY_DN2613_c0_g1	Homeobox-leucine zipper protein ATHB-7
BH062	TRINITY_DN360_c1_g1	Light response
BHLH	TRINITY_DN7311_c0_g1	Light response
CIPK5	TRINITY_DN1514_c1_g1	CBL-interacting serine/threonine-protein kinase 5
CIPKB	TRINITY_DN21721_c0_g1	CBL-interacting serine/threonine-protein kinase 11
EF1A	TRINITY_DN8030_c3_g1	Elongation factor 1-alpha
EFMT4	TRINITY_DN2747_c0_g1	EEF1A lysine methyltransferase 4
EPS1	TRINITY_DN2901_c0_g1	Protein ENHANCED PSEUDOMONAS SUSCEPTIBILITY 1
EXPA2	TRINITY_DN162_c0_g2	Expansin-A2
EXTN3	TRINITY_DN7986_c0_g1	Extensin-3
HDT1	TRINITY_DN4285_c1_g2	Histone deacetylase HDT1
HSP16	TRINITY_DN68667_c0_g1	18.5 kDa class I heat shock protein
HYBA1	TRINITY_DN1856_c0_g1	Non-reducing end beta-L-arabinofuranosidase
KAI2	TRINITY_DN7983_c1_g1	Probable esterase KAI2
LSH3	TRINITY_DN12674_c0_g1	Protein LIGHT-DEPENDENT SHORT HYPOCOTYLS 3
LSH6	TRINITY_DN12674_c0_g1	Protein LIGHT-DEPENDENT SHORT HYPOCOTYLS 6
MYB4	TRINITY_DN2554_c0_g1	Transcription repressor MYB4
NAP2	TRINITY_DN7132_c0_g5	NAC domain-containing protein 2
NCED1	TRINITY_DN2687_c0_g2	9-cis-epoxycarotenoid dioxygenase NCED1, chloroplastic
NFD4	TRINITY_DN13340_c0_g1	Protein NUCLEAR FUSION DEFECTIVE 4
NIA	TRINITY_DN6723_c1_g1	Nitrate reductase [NADH]
NLTP	TRINITY_DN79002_c1_g1	Probable non-specific lipid-transfer protein AKCS9
PAR1	TRINITY_DN1658_c0_g1	Phenylacetaldehyde reductase
PER2	TRINITY_DN11931_c0_g1	Cationic peroxidase 2
PGLR	TRINITY_DN2996_c0_g1	Probable polygalacturonase
PUMP4	TRINITY_DN12670_c1_g1	Mitochondrial uncoupling protein 4
PURA2	TRINITY_DN2249_c0_g2	Adenylosuccinate synthetase 2, chloroplastic
RL261	TRINITY_DN8213_c0_g1	60S ribosomal protein L26-1
RL9	TRINITY_DN56917_c0_g1	60S ribosomal protein L9
RS3A	TRINITY_DN11338_c0_g2	40S ribosomal protein S3a
SCL15	TRINITY_DN19737_c0_g1	Scarecrow-like protein 15
VPE1	TRINITY_DN9527_c0_g1	Vacuolar-processing enzyme
WRKY7	TRINITY_DN13587_c0_g1	Probable WRKY transcription factor 7
WTR32	TRINITY_DN5341_c0_g2	WAT1-related protein At4g08300

Table S5.
Sequencing and reads mapping statistics across Visium mRNA libraries.

Sample ID	n_spots	m_Genes	n_reads	m_reads_per_spot	n_reads_map_confidently	Fraction Reads in Spots Under Tissue	Total Genes Detected	Median UMI Counts per Spot	Fraction of Spots Under Tissue
S1_A1_PO1_7h_WW	1426	53	150099218	105258.92	0.04	0.92	138	368.5	0.29
S2_B1_PO1_23h_WW	1972	57	199760709	101298.53	0.03	0.98	139	352.5	0.40
S3_D1_PO1_23h_D	1751	26	121129683	69177.43	0.01	0.80	136	44	0.35
S4_A1_PO1_7h_WW	1925	40	192674632	100090.72	0.03	0.90	137	167	0.39
S5_B1_PO1_23h_WW	3757	34	322216248	85764.24	0.02	0.96	139	95	0.75
S6_D1_PO1_23h_D	1575	25	117371115	74521.34	0.01	0.75	135	41	0.32

Table S6
Sensitivity analysis FBA results of major reactions for different drought conditions. Well-watered: well-watered conditions. Drought20%: 20% stomata closure compared to well-watered condition; Drought45%: 45% stomata closure compared to well-watered condition, which is the regular drought condition; Drought70%: 70% stomata closure compared to well-watered condition. Unit for the fluxes is mmol $gDW^{-1}day^{-1}$. Major reactions are Phloem output, PEP carboxylation, Rubisco carboxylation and CO_2 absorption directly from the atmosphere (CO_2). All the scenarios are modelled in mesophyll (M) or bundle sheath (BS) at daytime (day) and night time (night)

SCENARIO	Well-watered	Drought20%	Drought45%	Drought70%
Phloem output	3.42	3.29	3.14	2.99
PEP carboxylation_M_day	127.67	100.00	70.00	40.00
PEP carboxylation_BS_day	0	0	0	0
PEP carboxylation_M_night	0	20.27	47.23	74.12
PEP carboxylation_BS_night	8.55	8.86	8.68	8.58
Rubsico carboxylation_M_day	0	0	0	0
Rubsico carboxylation_BS_day	140.53	136.94	133.51	130.07
Rubsico carboxylation_M_night	0	0	0	0
Rubsico carboxylation_BS_night	0	0	0	0
CO2_M_day	127.67	100.00	70.00	40.00
CO2_BS_day	0	0	0	0
CO2_M_night	-7.18	16.07	40.79	65.51
CO2_BS_night	0	0	0	0

Table S7.
FBA results for major reactions of additional modelling scenarios in mesophyll (M) or bundle sheath (BS) at daytime (day) and night time (night). Major reactions are Phloem output, PEP carboxylation (PEP), Rubisco carboxylation (Rubisco) and CO_2 absorption directly from the atmosphere (CO_2). All the scenarios are modelling under the drought condition (45% stomata closure). Scenario 1): blocking malate transfer between mesophyll and bundle sheath (bMalT). Scenario 2):blocking malate storage in mesophyll (bMS), bundle sheath (bBSS), or both (bMBSS). Scenario 3): CAM with C_3 or C_4 anatomy. C3+CAM: both C_3 and CAM activity (both daytime and night time CO_2 uptake) allowed with a C_3 anatomy (CO_2 directly diffuses into mesophyll, bundle sheath is considered an inner mesophyll with a longer distance to stomata); CAM_C3: only CAM (nighttime CO_2 uptake) occurswith C_3 anatomy ; CAM_C4: CAM process (nighttime CO_2 uptake) with C_4 anatomy (CO_2 can not directly diffuse into bundle sheath); C4+CAM_C4: C4 and CAM (both daytime and night time CO_2 uptake) can occur with C_4 anatomy (CO_2 can not directly diffuse into bundle sheath), which can be used as the reference for all the above scenarios.

SCENARIO	bMalT	bMS	bBSS	bMBSS	C3+CAM_ C3	CAM_C3	CAM_C4	C4+CAM_ C4
Phloem output	3.14	3.14	3.14	1.64	2.56	2.48	2.79	3.14
PEP_M_day	70.00	70.00	70.00	70.00	0	0	0	70.00
PEP_BS_day	0	0	0	0	0	0	0	0
PEP_M_night	47.49	49.53	50.72	0	29.91	77.05	108.65	47.23
PEP_BS_night	8.42	6.38	5.19	2.79	4.28	29.99	9.76	8.68
Rubsico_M_day	0	0	0	0	88.94	85.33	0	0
Rubsico_BS_day	133.51	133.51	133.51	74.4	36.40	33.49	125.49	133.51
Rubsico_M_night	0	0	0	0	0	0	0	0
Rubsico _BS_night	0	0	0	0	0	0	0	0
CO2_M_day	70.00	70.00	70.00	70.00	40.00	0	0	70.00
CO2_BS_day	0	0	0	0	30.00	0	0	0
CO2_M_night	40.79	40.79	40.79	-12.22	24.83	67.89	98.48	40.79
CO2_BS_night	0	0	0	0	-4.42	19.77	0	0

Table S8.
Sensitivity analysis of FBA results of major reactions for Rubisco carboxylation to oxygenation ratio (V_c/V_o) for C_4 in bundle sheath. Major reactions are Phloem output, PEP carboxylation , Rubisco carboxylation and CO_2 absorption directly from the atmosphere (CO_2). All the scenarios are modelling under the drought condition in mesophyll (M) or bundle sheath (BS) at daytime (day) and night time (night). Unit for the fluxes is mmol $gDW^{-1}day^{-1}$.

V_c/V_o	**10**	**20**	**40**	**80**	**200**
Phloem output	3.14	3.32	3.43	3.48	3.51
PEP carboxylation_M_day	70.00	70.00	70.00	70.00	70.00
PEP carboxylation_BS_day	0	0	0	0	0
PEP carboxylation_M_night	47.23	54.48	58.23	60.15	61.31
PEP carboxylation_BS_night	8.68	8.64	8.66	8.67	8.67
Rubsico carboxylation_M_day	0	0	0	0	0
Rubsico carboxylation_BS_day	133.51	137.53	136.63	140.71	141.36
Rubsico carboxylation_M_night	0	0	0	0	0
Rubsico carboxylation_BS_night	0	0	0	0	0
CO2_M_day	70.00	70.00	70.00	70.00	70.00
CO2_BS_day	0	0	0	0	0
CO2_M_night	40.79	47.44	50.91	52.69	53.77
CO2_BS_night	0	0	0	0	0

Online Supplementary Materials

Aavailable on Dryad (https://datadryad.org) DOI: 10.5061/dryad.931zcrjm6

ONLINE TABLES:

Captions for Online Table S1:
Estimated counts of reads mapped to each unigene per LMD library.

Captions for Online Table S2:
Transcriptome-wide gene annotation and differential expression statistics.

Captions for Online Table S3:
CCM-related (or hypothetically related) genes. Functional and phylogenetic annotation into gene lineage and/or gene families. Differential expression statistics.

Captions for Online Table S4:
Representative contigs (highest expressed) by gene lineage and/or gene family. Differential expression statistics.

Captions for Online Table S5:
FBA results summary.

ONLINE VISIUM METADATA:
Space Ranger Output files. Metadata containing quantification and analysis summaries of transcripts abundance across Visium samples. Files can be visualized using the software Loupe Browser (10X Genomics; https://www.10xgenomics.com/products/loupe-browser). The list of gene features to display in Loupe are found in table S3

Captions for Online Data S1
Visium spatial gene expression: Loupe file with metadata for 7h well-watered sample replicate 1

Captions for Online Data S2
Visium spatial gene expression: Loupe file with metadata for 7h well-watered sample replicate 2

Captions for Online Data S3
Visium spatial gene expression: Loupe file with metadata for 23h well-watered sample replicate 1

Captions for Online Data S4
Visium spatial gene expression: Loupe file with metadata for 23h well-watered sample replicate 2

Captions for Online Data S5
Visium spatial gene expression: Loupe file with metadata for 23h droughted sample replicate 1

Captions for Online Data S6
Visium spatial gene expression: Loupe file with metadata for 23h droughted sample replicate 2