

1. Supplementary Methods

Mesocosm system

The controlled mesocosm system is part of the marine experimental infrastructure and wet laboratory facilities at the Oceanographic Center of Murcia (Spain, IEO-CSIC), providing capacity for manipulative experimentation on seagrasses under semi-natural, yet tightly controlled and standardized conditions. The system was recently upgraded with components to enhance versatility, robustness, and experimental reliability, while improving energy efficiency, operational safety, and ease of use.

System development and upgrades were funded by the GRASSREC project (PID2020-118144RB-C31; Spanish Ministry of Science and Innovation) and the ThinkInAzul Programme (C17.I01 – Complementary Plan for Marine Sciences, Region of Murcia), supported by MCIU with funding from the European Union NextGenerationEU (PRTR-C17.I1) and by the Comunidad Autónoma de la Región de Murcia–Fundación Séneca.

Mesocosm configuration

The experimental platform consisted of a central support reservoir connected to 12 independent mesocosm modules housed within a thermally insulated indoor room, where ambient temperature was controlled via air conditioning. Each module functioned as an individual experimental unit while remaining hydraulically connected to the central system, ensuring environmental consistency alongside experimental replication. Each module comprised a structural frame made of high-quality tubular plastic resin supporting two fiberglass tanks (200 L each): an upper experimental tank and a lower auxiliary sump. This configuration allowed stable operation and independent control within each experimental unit.

Water supply and filtration

Seawater was continuously supplied from the Mar Menor coastal lagoon through a dedicated intake system. Incoming water was pre-filtered prior to entering the experimental facility to remove coarse suspended particles while maintaining natural salinity and nutrient composition. Following intake, seawater was routed to a central reservoir connected to the mesocosm system, allowing the filling of experimental units and periodic partial water renewal. Independent experimental units were equipped with mechanical and biological filtration systems to reduce particulate and organic matter loads and ensure stable water quality throughout the experiments.

Temperature control

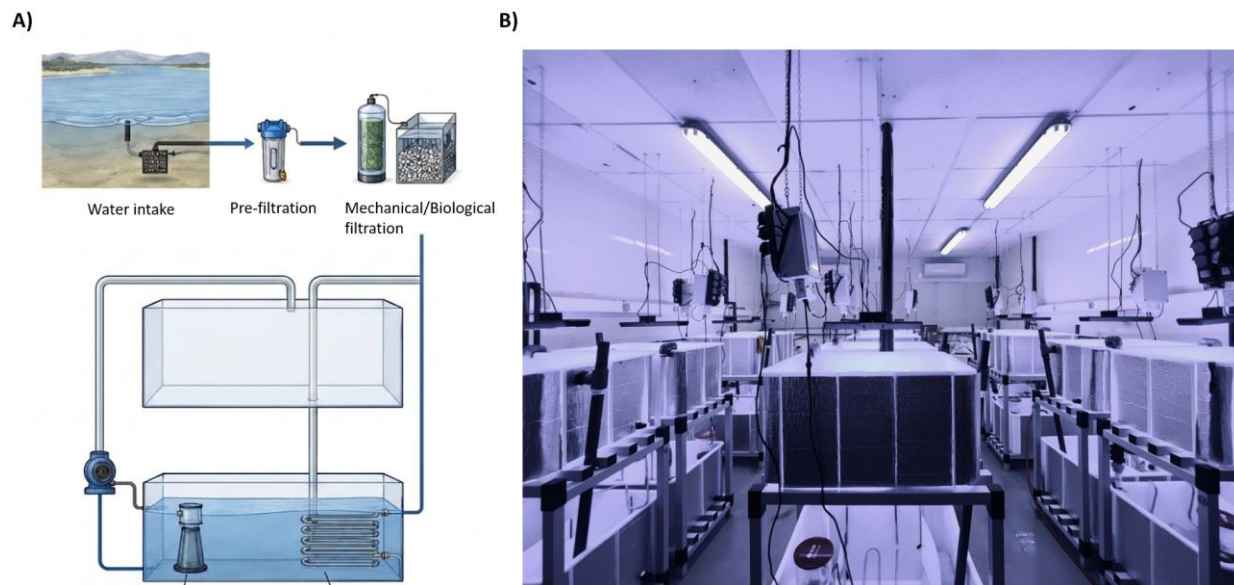
Water temperature was continuously monitored using digital probes and data loggers installed in both the reservoir and experimental aquaria. Thermal conditions were regulated through a high-precision (± 0.1 °C) automated system specifically designed for the facility, consisting of a closed water circuit connected to both a cooling unit (5 °C) and a heating unit (40 °C). Temperature deviations from programmed setpoints were detected by a thermostat coupled to in-tank sensors, triggering the injection of cooled or heated water into the system. Thermal exchange between the control circuit and the experimental water occurred through a stainless steel coil installed in the reservoir, ensuring stable, homogeneous temperature conditions across experimental units.

Light conditions

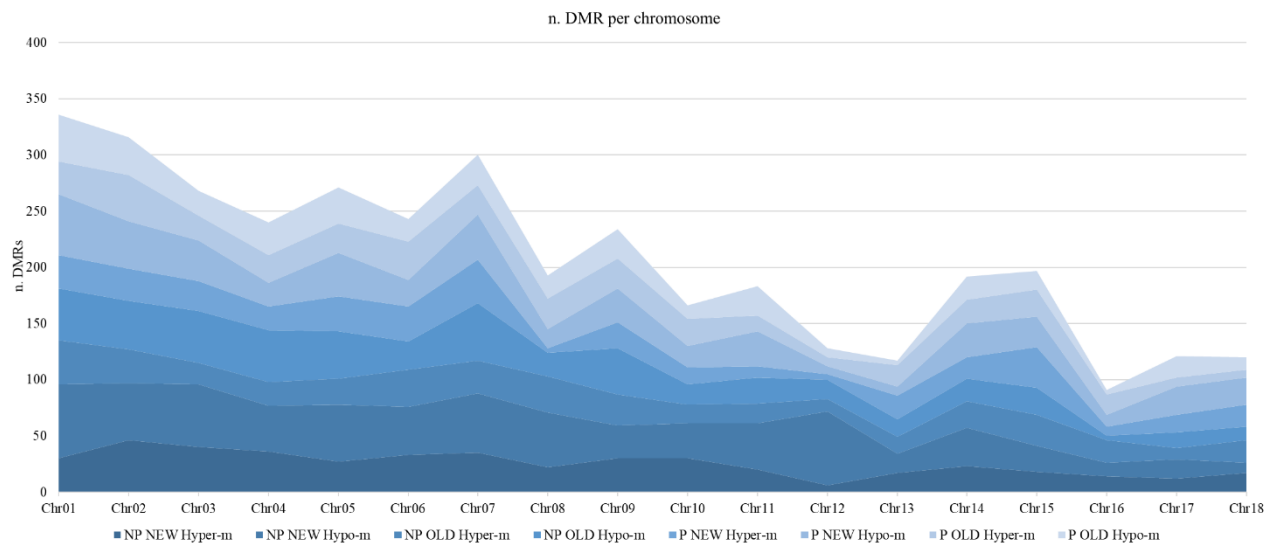
Illumination was provided by programmable 180 W LED units equipped with multi-wavelength arrays, enabling precise control of photosynthetically active radiation (PAR), spectral composition, and photoperiod, including the simulation of natural diel light cycles. Light regimes were standardized across mesocosms to ensure homogeneous irradiance exposure among treatments and were remotely controlled via a Wi-Fi-enabled system.

A schematic representation of the mesocosm facility is provided in Supplementary **Figure S1**.

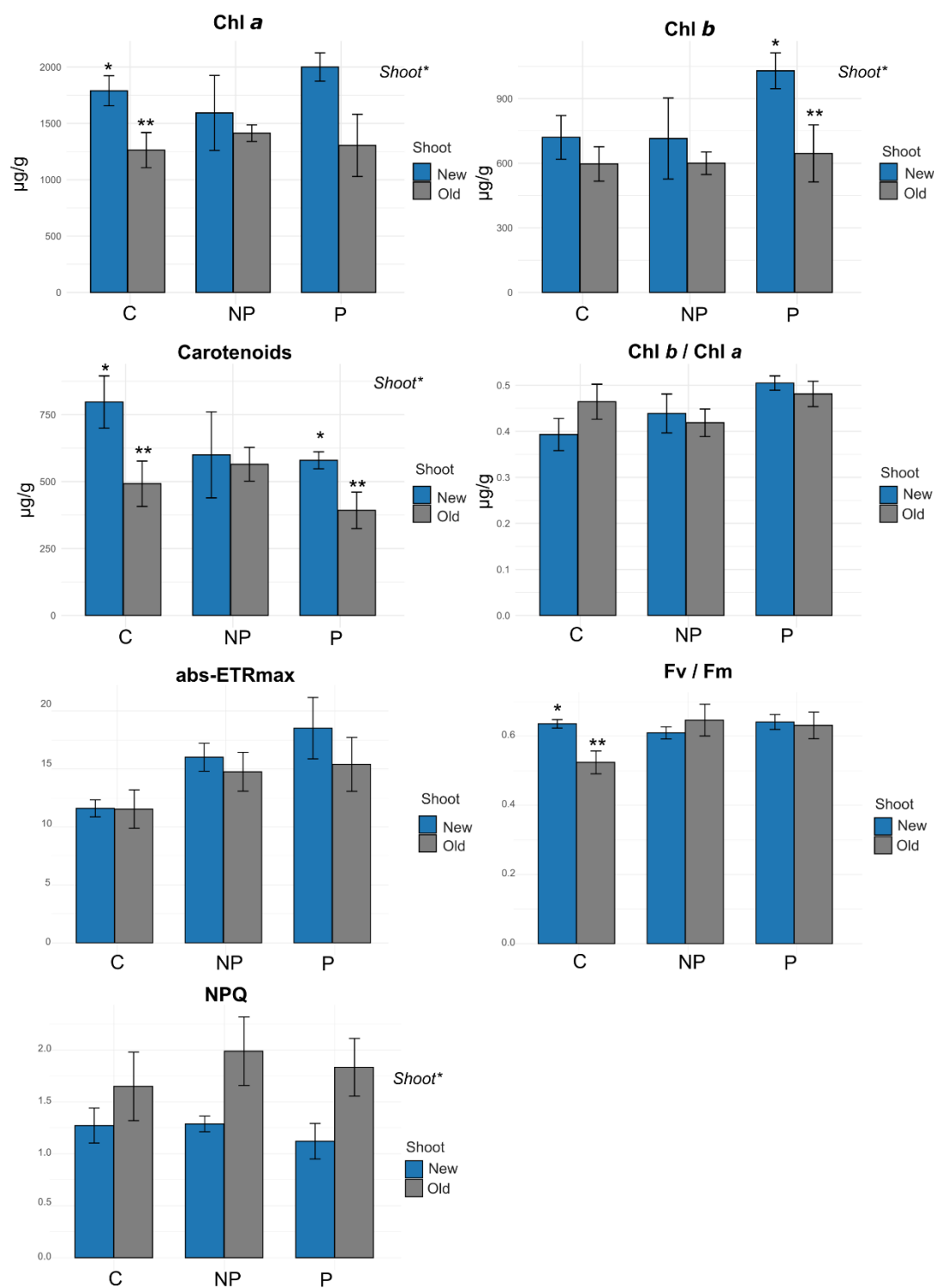
2. Supplementary Figures



Supplementary Fig. 1 Schematic workflow of the experimental mesocosm system. (A) Schematic representation of the indoor mesocosm system available at the Oceanographic Center of Murcia (IEO-CSIC), showing the seawater circulation from the Mar Menor intake through pre-filtration and mechanical/biological filtration units to a central reservoir, and its distribution to 12 independent experimental modules. Arrows indicate flow direction and water routing within the system, including temperature-controlled storage and distribution components. (B) Photograph of the mesocosm setup used for the indoor experimentation within the climate-controlled room, illustrating the arrangement of the independent experimental module.



Supplementary Fig. 2 Chromosomal distribution of differentially methylated regions (DMRs) across treatments. Line graph showing the number of hypermethylated (Hyper-m) and hypomethylated (Hypo-m) DMRs across chromosomes (Chr01–Chr18) for each condition (NP NEW, NP OLD, P NEW, P OLD) in in non-primed (NP) and primed (P) shoots.

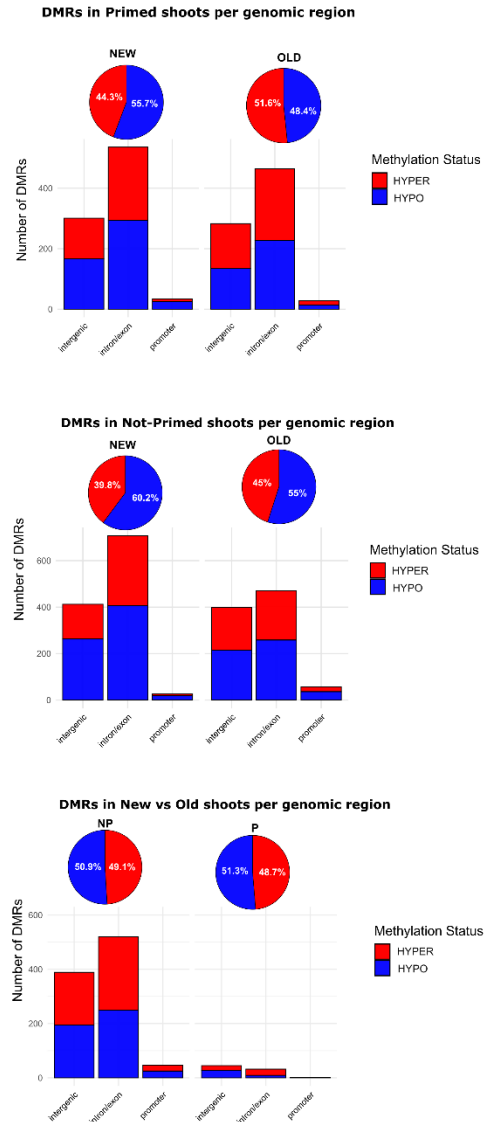


Supplementary Fig. 3 Photophysiological performance and pigment profiles across treatments and shoot types. Bar plots showing pigments content (Chl A, Chl b, carotenoids, Chl b/Chl a) Maximum electron transport rate (absETRmax, photochemical efficiency (Fv/Fm), and Non-Photochemical Quenching (NPQ) measured in new and old shoots under Control (C), Primed (P), and Not-Primed (NP) conditions. Differences between new and old shoots within each treatment are indicated; asterisks denote statistically significant differences. Statistical differences were assessed using two-way ANOVA (Treatment: Control, Primed, Not-Primed; Shoot type: New vs Old), followed by Tukey's HSD post hoc test where appropriate (Table S4).

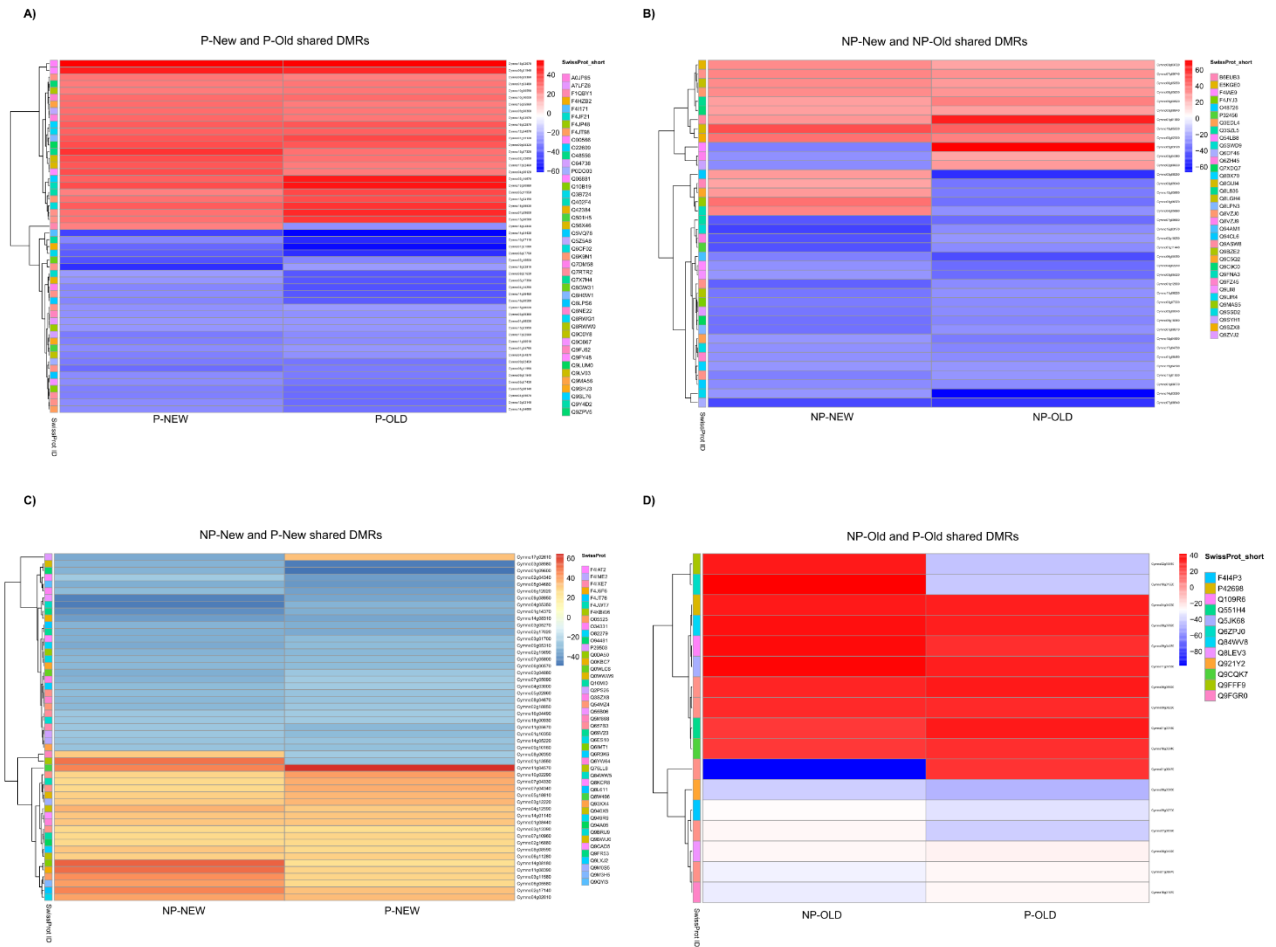
A)



B)



Supplementary Fig. 4 Differentially methylated regions (DMRs) at CG context. (A) Volcano plots showing DMRs across comparisons between new and old shoots versus controls, as well as between new and old shoots. DMRs were considered significant at $q\text{-value} < 0.05$ and $|\Delta\text{methylation}| > 0.1$. Regions with methylation differences $>40\%$ are highlighted with black circles, while extreme DMRs ($|\Delta\text{methylation}| > 80\%$) are indicated by larger circles and reported in Table S13. (B) Bar plots showing the distribution of DMRs across genomic features, separated by methylation status (HYPER, hypermethylated; HYPO, hypomethylated) for each comparison. Pie charts above each bar group indicate the proportion of hyper- and hypomethylated DMRs relative to the total number identified in each comparison.



Supplementary Fig. 5 DNA methylation patterns of shared DMRs across primed and non-primed conditions and shoots. Heatmaps showing hierarchical clustering of differentially methylated regions (DMRs) shared between primed (P) and non-primed (NP) conditions across new and old shoots. **(A)** DMRs shared between P new and P old shoots. **(B)** DMRs shared between NP new and NP old shoots. **(C)** DMRs shared between NP and P new shoots. **(D)** DMRs shared between NP and P old shoots. Color gradients represent relative methylation levels (blue: hypomethylation; red: hypermethylation). Annotation legend (SwissProt ID) for each gene was also reported.

3. Supplementary Tables

Supplementary Tab. 1 Read counts for all sequenced samples. Number of reads (in millions) produced for each sample. In the Sample_name, "C" refers to the control treatment, "P" to the priming treatment, and "NP" to the triggering treatment. Additionally, "A" corresponds to the apical shoot, "N" to the new shoot, and "O" to the old shoot.

Sample name	Reads (M)
ID3238_1-C31B-A1	42.929
ID3238_2-C32A-B1	52.785
ID3238_3-C33A-C1	48.028
ID3238_4-C63A-D1	47.83
ID3238_5-C91B-E1	48.177
ID3238_6-C31BN-A2	44.009
ID3238_7-C32N-B2	49.549
ID3238_8-C33N1-C2	47.979
ID3238_9-C63N1-D2	42.784
ID3238_10-C91N1-E2	60.175
ID3238_11-C31O-A3	47.203
ID3238_12-C32O-B3	54.338
ID3238_13-C33O1-C3	44.906
ID3238_14-C63O-D3	48.77
ID3238_15-C91O-E3	53.014
ID3238_16-P12B-A4	45.884
ID3238_17-P14B-B4	48.626
ID3238_18-P21B-C4	44.031
ID3238_19-P24B-D4	44.425
ID3238_20-P83B-E4	47.085
ID3238_21-P12N1-A5	45.726
ID3238_22-P14N-B5	44.287
ID3238_23-P21N1-C5	50.176
ID3238_24-P24N1-D5	45.769
ID3238_25-P83N1-E5	52.971
ID3238_26-P12O-A6	46.973
ID3238_27-P14O-B6	54.573
ID3238_28-P21O1-C6	47.853
ID3238_29-P24O-D6	43.795
ID3238_30-P83O-E6	55.17
ID3238_31-NP112A-A7	47.621
ID3238_32-NP41A-B7	52.984
ID3238_33-NP42A-C7	51.653
ID3238_34-NP71A-D7	44.896
ID3238_35-NP74A-E7	57.063
ID3238_36-NP112N-A8	49.713
ID3238_37-NP41N1-B8	55.761
ID3238_38-NP43BN-C8	44.863
ID3238_39-NP71BN-D8	44.344

ID3238_40-NP74BN-E8	51.375
ID3238_41-NP42O1-A9	77.984
ID3238_42-NP41O-B9	56.956
ID3238_43-NP4301-C9	51.381
ID3238_44-NP71O1-D9	57.691
ID3238_45-NP7O1-E9	52.244

Supplementary Tab. 2 Effects of treatment and shoot type on DMR abundance in different sequence contexts. ANOVA 2-ways performed on DMRs data obtained by comparing treatments versus controls in different treatments (primed, not-primed) and shoots (new, old) across different sequence contexts (CG, CHG, CHH).

CG	Df	Sum Sq	Mean Sq	F value	P value	Tukey test	
shoot	2	10938	5469	4.55	0.01	old-new	0.01
treatment	1	29366	29366	24.41	0.00	primed-not_primed	0.00
shoot:treatment	2	1246	623	0.52	0.60		
Residuals	5453	6560840	1203				

CHG	Df	Sum Sq	Mean Sq	F value	P value		
shoot	2	7	4	0.003	0.10		
treatment	1	4797	4797	4.258	0.04	primed-not_primed	0.04
shoot:treatment	2	4521	2260	2.007	0.13		
Residuals	2309	2601032	1126				

CHH	Df	Sum Sq	Mean Sq	F value	P value		
shoot	2	75	38	0.043	0.96		
treatment	1	1342	1342	1.52	0.22		
shoot:treatment	2	10187	5093	5.769	0.00	new:primed-new:not_primed	0.02
Residuals	77	67984	883				

Supplementary Tab. 3 Chromosome-wise distribution and methylation range of DMRs in the CG sequence context. Summary of differentially methylated regions (DMRs) identified in primed and non-primed old and new shoots in CG sequence context. Comparisons include Primed_old vs Control (P OLD) and Primed_new vs Control (P NEW), Not Primed_old vs control (NP OLD), Not Primed_new vs control (NP NEW), Not Primed_new vs old (NP), and Primed_new vs old (P) across chromosomes (Chr01–Chr11). For each chromosome, the table reports the maximum (Δ Max meth.) and minimum (Δ Min meth.) methylation differences between treatments and controls, expressed as percentage change. The number of DMRs located in intergenic regions, intron/exon regions, and promoter regions, together with the total number of DMRs (n. DMRs) detected for each comparison were also reported. These values describe the range of methylation variation and the genomic distribution of DMRs across chromosomes.

Chr	intergenic	intron/ exon	promoter	n. DMRs	intergenic	intron/ exon	promoter	n. DMRs
Chr01	27	41	3	71	34	46	4	84
Chr02	33	39	3	75	24	43	4	71
Chr03	8	36	0	44	17	46	0	63
Chr04	19	34	1	54	18	22	2	42
Chr05	16	41	1	58	10	60	0	70
Chr06	27	24	3	54	21	32	2	55
Chr07	22	29	2	53	33	46	0	79
Chr08	23	21	4	48	7	12	2	21
Chr09	10	42	1	53	14	37	2	53
Chr10	25	9	2	36	21	13	0	34
Chr11	9	29	2	40	7	34	0	41
Chr12	8	7	1	16	6	5	1	12
Chr13	4	19	0	23	11	18	0	29
Chr14	11	31	0	42	8	41	0	49
Chr15	16	22	3	41	29	26	8	63
Chr16	11	11	0	22	6	9	4	19
Chr17	10	15	2	27	22	17	2	41
Chr18	4	14	0	18	13	29	2	44

Chr	intergenic	intron/exon	promoter	n. DMRs	intergenic	intron/exon	promoter	n. DMRs
Chr01	32	49	4	85	34	60	2	96
Chr02	29	40	4	73	33	59	5	97

Chr03	24	35	6	65	19	76	1	96
Chr04	31	29	7	67	29	46	2	77
Chr05	17	46	2	65	13	64	1	78
Chr06	27	25	6	58	36	38	2	76
Chr07	36	37	7	80	34	54	0	88
Chr08	27	23	3	53	31	37	3	71
Chr09	35	30	4	69	17	41	1	59
Chr10	12	21	2	35	33	26	2	61
Chr11	15	25	1	41	14	46	1	61
Chr12	17	11	0	28	59	13	0	72
Chr13	21	8	2	31	15	19	0	34
Chr14	14	28	2	44	8	48	1	57
Chr15	27	21	4	52	12	27	2	41
Chr16	11	11	2	24	11	13	2	26
Chr17	11	12	1	24	10	19	0	29
Chr18	12	20	0	32	4	21	1	26

Chr	intergenic	intron/ exon	promoter	n. DMRs	intergenic	intron/ exon	promoter	n. DMRs
Chr01	30	42	1	73	2	2	0	4
Chr02	35	43	1	79	3	4	0	7
Chr03	31	62	4	97	4	0	0	4
Chr04	19	32	8	59	4	2	1	7
Chr05	16	59	4	79	6	8	0	14
Chr06	22	26	4	52	6	0	0	6
Chr07	33	18	6	57	2	3	0	5
Chr08	21	21	4	46	5	2	0	7
Chr09	35	42	3	80	6	3	0	9
Chr10	12	25	3	40	3	2	0	5
Chr11	15	24	1	40	0	2	0	2
Chr12	25	12	0	37	-	-	-	-
Chr13	26	17	1	44	-	-	-	-
Chr14	15	27	1	43	0	1	0	1
Chr15	22	26	2	50	1	2		3
Chr16	13	12	1	26	2	0	0	2

Chr17	8	8	1	17	-	-	-	-
Chr18	11	24	2	37	1	1	0	2

Supplementary Tab. 4 Two-way ANOVA results for photo-physiological and pigment-related traits.

Results of the two-way ANOVA testing the effects of Treatment (Control, Primed, Not-Primed) and Shoot type (New vs Old) on physiological and pigment-related variables. For each variable, degrees of freedom (Df), sum of squares (Sum Sq), mean squares (Mean Sq), F-values (F), and p-values are reported. Significant effects ($p < 0.05$) are highlighted in bold, indicating the factors that significantly influence each variable. The Treatment $\times$ Shoot interaction tests whether the effect of shoot type differs among treatments. When the Treatment factor was significant, Tukey's post hoc test was performed to identify pairwise differences among treatments.

Variable	Factor	Df	Sum Sq	Mean Sq	F	p-value	Tukey test
Fv/Fm	Treatment	2	0.02952	0.01	1.90	0.17	
	Shoot	1	0.00558	0.00	0.72	0.40	
	Treatment $\times$ Shoot	2	0.02780	0.01	1.79	0.18	
NPQ	Treatment	2	0.182	0.09	0.33	0.72	
	Shoot	1	2.623	26.233	9.50	0.00	
	Treatment $\times$ Shoot	2	0.155	0.08	0.28	0.76	
abs-ETRmax	Treatment	2	118.1	59.03	2.73	0.09	
	Shoot	1	21.1	21.09	0.97	0.33	
	Treatment $\times$ Shoot	2	11.0	5.48	0.25	0.78	
pmax_cm2	Treatment	2	193.0	96.51	1.97	0.15	
	Shoot	1	159.6	159.55	3.25	0.08	
	Treatment $\times$ Shoot	2	30.5	15.25	0.311	0.73	
rd_cm2	Treatment	2	32.21	16.11	29.99	3.11e-08	P.NP≠C p < 0.001
	Shoot	1	1.72	1.72	3.21	0.08	
	Treatment $\times$ Shoot	2	0.49	0.24	0.45	0.64	
pmax_rd_cm2	Treatment	2	89.70	44.85	21.21	1.05e-06	P.NP≠C p < 0.001
	Shoot	1	5.81	5.81	2.75	0.11	
	Treatment $\times$ Shoot	2	0.19	0.10	0.05	0.95	
Chla	Treatment	2	75336	37668	0.15	0.86	
	Shoot	1	1895341	1895341	7.77	0.01	
	Treatment $\times$ Shoot	2	324629	162315	0.67	0.52	
Chlb	Treatment	2	175573	87786	1.32	0.28	
	Shoot	1	389442	389442	5.84	0.023	
	Treatment $\times$ Shoot	2	126640	63320	0.95	0.40	
chlb/chla	Treatment	2	0.02781	0.01391	2.54	0.10	
	Shoot	1	0.00045	0.00045	0.08	0.78	
	Treatment $\times$ Shoot	2	0.01428	0.00714	1.30	0.29	
Carotenoids	Treatment	2	140845	70422	2.07	0.15	
	Shoot	1	253986	253986	7.47	0.01	

Treatment × Shoot 2 79537 39769 1.17 0.33

Supplementary Tab. 5 Unpaired t-test comparisons between new and old shoots within each treatment. Results of unpaired t-tests comparing “New” versus “Old” shoots for each variable and treatment. For each comparison, the t statistic (t), degrees of freedom (df), p-value, mean values for New and Old shoots, 95% confidence interval (CI), and direction of change (trend) are reported. Significant differences ($p < 0.05$) are indicated in bold. Positive differences indicate an increase in New shoots relative to Old shoots, while negative differences indicate a decrease. Significant effects ($p < 0.05$) are highlighted in bold.

Variable	Treatment	t	df	p-value	Media New	Media Old	95% CI / commento	trend
Carotenoids	Control	23.58	6.88	0.05	797.20	492.11	-1.95 – 612.13	New > Old
	Primed	24.52	10.69	0.03	1029.32	645.51	38.13 – 729.47	New > Old
	Not-Primed	0.20	2.64	0.85	599.85	564.71	-559.66 – 629.93	New > Old
Fv/Fm	Control	31.74	6.27	0.02	0.63	0.52	0.03 – 0.20	New > Old
	Primed	0.22	11.57	0.83	0.64	0.63	-0.09 – 0.12	New > Old
	Not-Primed	-0.74	6.32	0.48	0.61	0.65	-0.16 – 0.082	New < Old
NPQ	Control	10.18	4.46	0.36	12.71	16.48	-1.36 – 0.61	New < Old
	Primed	21.84	6.66	0.07	11.20	18.32	-1.49 – 0.07	New < Old
	Not-Primed	20.64	4.41	0.10	12.87	19.87	-1.61 – 0.21	New < Old
abs-ETRmax	Control	0.034	4.07	0.98	116.07	115.46	-4.93 – 5.05	New > Old
	Primed	0.89	8.98	0.40	185.29	154.02	-4.84 – 11.09	New > Old
	Not-Primed	0.61	6.80	0.56	160.22	147.63	-3.64 – 6.15	New > Old
Pmax	Control	16.16	9.20	0.14	164.58	111.73	-2.00 – 12.57	New > Old
	Primed	17.52	12.11	0.10	128.04	77.81	-1.21 – 11.26	New > Old
	Not-Primed	0.24	7.90	0.81	160.53	147.21	-11.24 – 13.90	New > Old
DR	Control	0.82	7.52	0.44	0.74	0.64	-0.20 – 0.41	New > Old
	Primed	12.84	13.99	0.22	29.69	23.29	-0.43 – 1.71	New > Old
	Not-Primed	11.85	9.93	0.26	28.63	24.39	-0.37 – 1.22	New > Old
Pmax/DR	Control	0.71	8.44	0.50	5.18	4.52	-1.46 – 2.79	New > Old
	Primed	15.08	10.96	0.16	1.87	0.94	-0.43 – 2.29	New > Old
	Not-Primed	0.70	6.84	0.50	2.217	1.58	-1.51 – 2.79	New > Old
Chl a	Control	2574	7.92	0.03	1789.68	1262.61	53.98 – 1000.16	New > Old
	Primed	2295	9.48	0.05	2000.05	1304.47	15.23 – 1375.93	New > Old

	Not-Primed	0.53	2.20	0.65	1592.72	1413.08	-1167.21 – 1526.48	New > Old
	Control	0.95	6.41	0.37	720.13	596.73	-188.29 – 435.10	New > Old
Chl <i>b</i>	Primed	2452	10.69	0.03	1029.32	645.51	38.13 – 729.47	New > Old
	Not-Primed	0.59	2.32	0.61	714.81	600.15	-623.13 – 852.45	New > Old
	Control	- 13.81	7.78	0.20	0.39	0.46	-0.19 – 0.05	New < Old
Chl <i>b</i> /Chl <i>a</i>	Primed	0.75	10.36	0.47	0.50	0.48	-0.05 – 0.09	New > Old
	Not-Primed	0.39	4.08	0.71	0.44	0.42	-0.12 – 0.16	New > Old
	Control	2358	6.88	0.05	797.20	492.11	-1.95 – 612.13	New > Old
Carotenoids	Primed	2493	9.59	0.03	579.58	392.52	18.91 – 355.21	New > Old
	Not-Primed	0.20	2.64	0.85	599.85	564.71	-559.66 – 629.93	New > Old

Supplementary Tab. 6 One-way ANOVA results for treatment effects in old shoots. One-way ANOVA results for the effects of Treatment (Control, Primed, Not-Primed) in Old shoots on physiological and pigment-related variables. For each variable, degrees of freedom (Df), sum of squares (Sum Sq), mean squares (Mean Sq), F-values (F), and p-values are reported. Significant effects ($p < 0.05$) are highlighted in bold, indicating the factors that significantly influence each variable. When a significant treatment effect was detected, Tukey's post hoc tests were performed to identify pairwise differences among treatments.

Variable	Df	Sum Sq	Mean Sq	F	p-value	TukeyHSD	
Fv/Fm	2	0.1	0	2.47	0.11		
NPQ	2	0.3	0.1	0.28	0.76		
abs-ETRmax	2	40	20	0.82	0.46		
Pmax	2	166	83	1.22	0.32		
DR	2	13	6	11.69	0.00	<i>P.NP≠C</i>	0.002
Pmax/DR	2	1035	52	12.32	0	<i>P.NP≠C</i>	0.004
Chl a	2	73277	36638	0.12	0.89		
Chl b	2	10672	5336	0.07	0.93		
Chl b/Chl a	2	0	0	1.05	0.37		
Carotenoids	2	104450	52225	1.49	0.25		

Supplementary Tab. 7 GO enrichment analysis of DMR-associated genes. Gene Ontology (GO) enrichment analysis of genes associated with differentially methylated regions (DMRs) identified in primed and non-primed conditions, comparing new and old to controls. The table reports the significantly enriched GO terms (GO ID), their p-values, and the corresponding functional annotations for each condition. Only GO terms showing significant or near-significant enrichment are presented.

	NEW			OLD		
	GO ID	p value	Function	GO ID	p value	Function
PRIMED	GO:0005622	0.03	intracellular anatomical structure	GO:0030684	0.03	preribosome
	GO:0051179	0.03	localization	GO:0140657	0.05	ATP-dependent activity
	GO:0140657	0.04	ATP-dependent activity	-		
	GO:0007417	0.09	central nervous system development	-		
NOT-PRIMED	-			GO:0140640	0.02	catalytic activity
	-			GO:0032991	0.03	protein-containing complex
	-			GO:0140657	0.03	ATP-dependent activity
	-			GO:0016661	0.04	oxidoreductase activity

Supplementary Tab. 8 One-way ANOVA of treatment effects on shoot production and biomass.

Results of the one-way ANOVA testing the effect of Treatment (Control, Primed, Not-Primed) on Shoot Production Rate (SPR) and on the total biomass of shoots, roots, and rhizomes. For each variable, degrees of freedom (Df), sum of squares (Sum Sq), mean squares (Mean Sq), F-values, and p-values (Pr(>F)) are reported. Significant effects ($p < 0.05$) indicate variables significantly influenced by the treatment. When the treatment effect was significant, Tukey's HSD post hoc test was performed to identify pairwise differences among treatments.

Variable	Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)	TukeyHSD
SPR	<i>Treatment</i>	2	6811	3405	3.70	0.03	<i>P≠C</i>
	Residuals	36	33084	919			
Shoots biomass	Treatment	2	0.4	0.2	0.50	0.62	
	Residuals	15	5.4	0.4			
Roots biomass	Treatment	2	0	0	0.17	0.84	
	Residuals	15	0.3	0			
Rhizome biomass	Treatment	2	0.4	0.2	1.05	0.37	
	Residuals	15	2.5	0.2			