

Supporting information for

Ozone Response of Leaf Physiological and Stomatal Characteristics in *Brassica juncea* L. at Supraoptimal Temperatures

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Table S1. Formulas and descriptions of OJIP parameters based on data obtained from OJIP transient curves (based on information from [1, 2]).

Fluorescence parameter	Description
Fluorescence parameters derived from the OJIP transient	
$F_0 = F_{50\mu s}$	Minimum fluorescence intensity (50 μs) at O-step
$F_J = F_{2ms}$	Fluorescence intensity (2 ms) at J-step
$F_I = F_{30ms}$	Fluorescence intensity (30 ms) at I-step
$F_P = F_m$	Maximum fluorescence intensity at P-step
$F_v = F_m - F_0$	Maximum variable fluorescence
$M_0 = 4(F_{300\mu s} - F_0)/(F_m - F_0)$	Approximated initial slope (in ms^{-1}) of the fluorescence transient
$V_J = (F_{2ms} - F_0)/(F_m - F_0)$	Relative variable fluorescence at 2 ms
Flux ratios or yields	
$\varphi P_0 = TR_0/ABS = 1 - (F_0/F_m) = F_v/F_m$	Maximum quantum yield of primary PS II photochemistry
$\varphi E_0 = \varphi P_0 \times (1 - V_J)$	Quantum yield of the electron transport at $t = 0$
$\varphi D_0 = 1 - \varphi P_0 = F_0/F_m$	Quantum yield (at $t = 0$) for energy dissipation
$\psi_0 = ET_0/TR_0 = 1 - V_J$	Probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond primary quinone electron acceptor in photosystem II (Q_A)
Specific energy fluxes per reaction center (RC)	
$ABS/RC = M_0 \times (1/V_J) \times (1/\varphi P_0)$	Absorption flux per RC
$TR_0/RC = M_0/V_J$	Trapped energy flux per RC at $t = 0$
$ET_0/RC = M_0 \times (1/V_J) \times (\varphi E_0)$	Electron transport flux per RC at $t = 0$
$DI_0/RC = (ABS/RC) - (TR_0/RC)$	Dissipated energy flux per RC at $t = 0$
Phenomenological energy fluxes or phenomenological activities per excited cross section (CS)	
$ABS/CS = F_0$	Absorption flux per CS at $t = 0$
$TR_0/CS = \varphi P_0 \times (ABS/CS)$	Trapped energy flux per CS at $t = 0$
$ET_0/CS = \varphi P_0 \times \psi_0 \times (ABS/CS)$	Electron transport flux per CS at $t = 0$
$DI_0/CS = (ABS/CS) - (TR_0/CS)$	Dissipated energy flux per CS at $t = 0$
$RC/CS = \varphi P_0 \times (V_J/M_0) \times (ABS/CS)$	Q_A reducing PS II reaction centers per CS
Performance index	
$PI_{ABS} = (RC/ABS) \times \varphi P_0 / (1 - \varphi P_0) \times \psi_0 / (1 - \psi_0)$	Performance index (PI) on absorption basis

Table S2. Results of analyses of variance of the main effects of temperature, O₃, and sampling date and their interactions on *A/C_i* curve response, chlorophyll content, chlorophyll *a* fluorescence, stomatal density, stomatal pore area, hydrogen peroxide and superoxide radical accumulation, and leaf growth parameters. Data were analyzed using three-way or two-way ANOVAs.

Parameters	Temp.	O ₃	Temp. × O ₃	Date	Temp. × Date	O ₃ × Date	Temp. × O ₃ × Date
Rubisco carboxylation	<0.01	<0.001	0.223	0.164	<0.01	<0.001	0.086
Electron transport	0.098	<0.001	<0.01	<0.001	<0.01	<0.001	0.34
Triose phosphate use	0.271	<0.001	<0.001	<0.001	<0.01	<0.001	0.271
Day respiration	<0.001	0.491	0.571	0.166	0.64	<0.01	0.304
Mesophyll conductance	0.138	<0.001	0.843	<0.001	0.661	0.287	0.724
Total chlorophyll	<0.01	<0.001	0.155	0.152	0.126	<0.05	0.634
Chlorophyll <i>a/b</i> ratio	0.090	0.105	0.602	0.707	<0.05	0.057	0.319
J step in Chl <i>a</i> fluorescence	<0.001	<0.001	<0.05	0.252	0.609	<0.05	0.22
I step in Chl <i>a</i> fluorescence	0.331	<0.001	0.118	0.069	0.372	<0.05	0.511
P step in Chl <i>a</i> fluorescence	0.286	<0.001	0.275	0.547	0.741	<0.01	0.952
Stomatal density	<0.01	<0.001	<0.01				
Stomatal pore area	0.107	<0.001	0.674				
DAB	<0.05	<0.001	0.746				
NBT	<0.001	<0.001	<0.001				
Leaf fresh weight	<0.001	<0.001	<0.05				
Leaf dry weight	<0.001	<0.001	<0.001				

Number of leaves	1.000	<0.01	0.084
Leaf area	<0.001	<0.001	0.054

Table S3. Comparison of each parameter under different ambient and supraoptimal temperatures and O₃ concentrations at 7 and 14 days after exposure (DAE). Data are summarized as means $\pm$ SD (n = 5) and were analyzed using the independence t-test. Statistical significance: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$. C: optimal temperatures and ambient O₃; T: supraoptimal temperature and ambient O₃; O: supraoptimal temperatures and elevated O₃; OT: supraoptimal temperature and elevated O₃; DAE: Days after exposure.

Parameters	C		T		O		OT	
	7 DAE	14 DAE	7 DAE	14 DAE	7 DAE	14 DAE	7 DAE	14 DAE
Rubisco carboxylation ($\mu \text{ mol m}^{-2} \text{ s}^{-1}$)	164 $\pm$ 12.69	162 $\pm$ 11.30	132 $\pm$ 19.50	176 $\pm$ 17.30*	131 $\pm$ 13.32	90 $\pm$ 5.17**	104 $\pm$ 16.62	74 $\pm$ 21.89*
Electron transport ($\mu \text{ mol m}^{-2} \text{ s}^{-1}$)	207 $\pm$ 13.52	186 $\pm$ 8.36**	189 $\pm$ 17.87	221 $\pm$ 22.53*	197 $\pm$ 16.05	125 $\pm$ 10.34***	153 $\pm$ 25.19	110 $\pm$ 28.01*
Triose phosphate use ($\mu \text{ mol m}^{-2} \text{ s}^{-1}$)	13.16 $\pm$ 0.77	11.40 $\pm$ 0.30**	12.46 $\pm$ 1.14	14.40 $\pm$ 1.29*	13.80 $\pm$ 0.49	8.66 $\pm$ 0.88***	10.80 $\pm$ 1.68	7.64 $\pm$ 2.07*
Day respiration ($\mu \text{ mol m}^{-2} \text{ s}^{-1}$)	3.83 $\pm$ 1.28	4.28 $\pm$ 0.73	6.64 $\pm$ 1.09	7.75 $\pm$ 2.31	4.09 $\pm$ 1.47	2.56 $\pm$ 0.63*	8.76 $\pm$ 3.07	5.49 $\pm$ 2.39
Mesophyll conductance ($\mu \text{ mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$)	5.29 $\pm$ 1.57	3.44 $\pm$ 1.07	4.86 $\pm$ 1.50	3.06 $\pm$ 0.34	3.20 $\pm$ 0.89	1.80 $\pm$ 0.30	2.43 $\pm$ 0.63	1.54 $\pm$ 0.44*
Total chlorophyll (mg g ⁻¹ FW)	7.67 $\pm$ 0.55	8.21 $\pm$ 0.37	7.05 $\pm$ 0.21	6.76 $\pm$ 0.41	6.45 $\pm$ 0.73	5.95 $\pm$ 0.74	6.23 $\pm$ 0.96	5.29 $\pm$ 0.79
Chlorophyll a/b ratio	3.36 $\pm$ 0.10	3.41 $\pm$ 0.42	3.16 $\pm$ 0.09	3.04 $\pm$ 0.05	3.17 $\pm$ 0.15	2.98 $\pm$ 0.12	2.70 $\pm$ 0.19	2.82 $\pm$ 0.35
J step in Chl a fluorescence	2.41 $\pm$ 0.07	2.52 $\pm$ 0.04	2.30 $\pm$ 0.05	2.46 $\pm$ 0.10*	2.36 $\pm$ 0.10	2.37 $\pm$ 0.12	2.15 $\pm$ 0.03	2.03 $\pm$ 0.23
I step in Chl a fluorescence	3.76 $\pm$ 0.15	4.15 $\pm$ 0.07**	3.84 $\pm$ 0.29	4.18 $\pm$ 0.13*	3.48 $\pm$ 0.28	3.61 $\pm$ 0.37	3.39 $\pm$ 0.28	3.22 $\pm$ 0.53
P step in Chl a fluorescence	5.57 $\pm$ 0.14	5.82 $\pm$ 0.18*	5.51 $\pm$ 0.37	5.89 $\pm$ 0.04	5.20 $\pm$ 0.31	4.66 $\pm$ 0.60	4.84 $\pm$ 0.67	4.38 $\pm$ 0.78

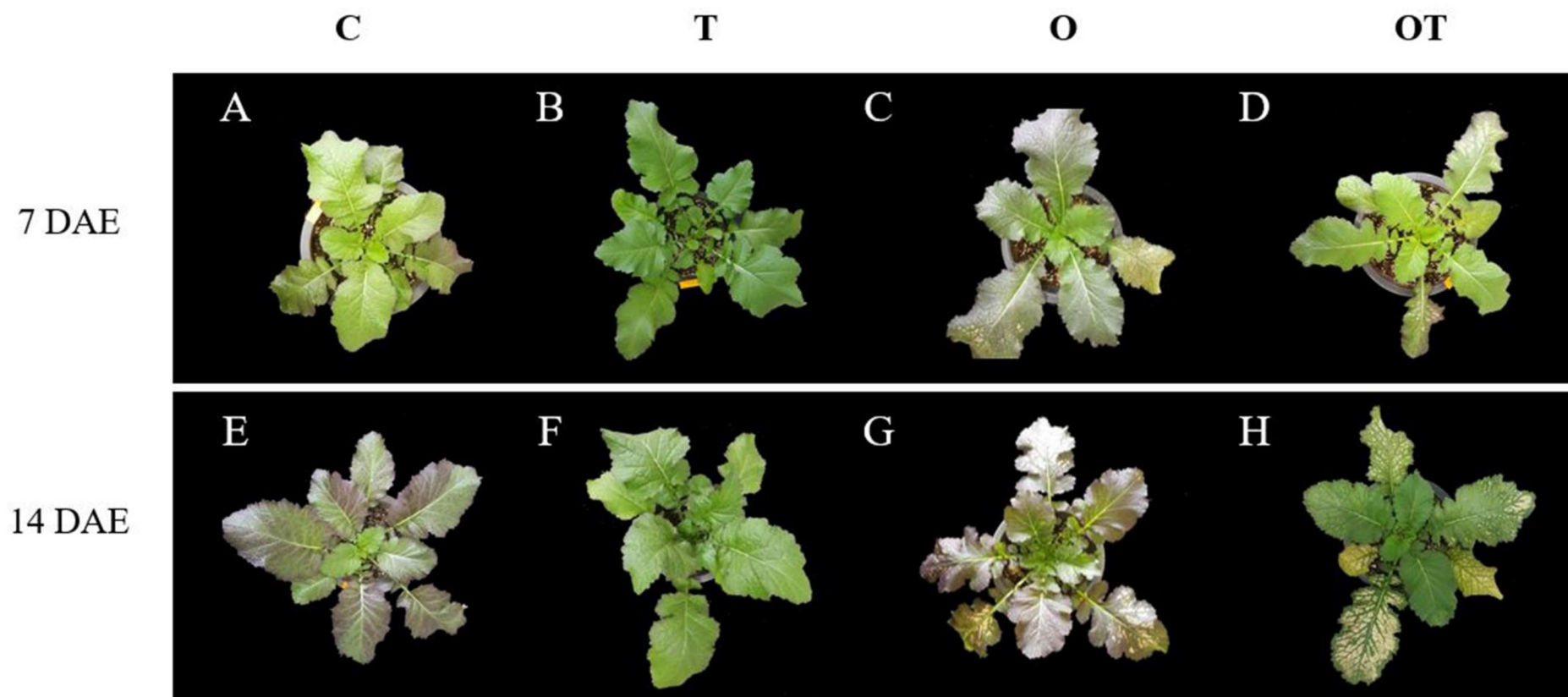


Figure S1. Visible ozone symptoms of *Brassica juncea* L. under different optimal and supraoptimal temperatures and O₃ concentration at 7 and 14 DAE. C: optimal temperatures and ambient O₃; T: supraoptimal temperature and ambient O₃; O: supraoptimal temperatures and elevated O₃; OT: supraoptimal temperature and elevated O₃; DAE: Days after the beginning of exposure.

Supporting information references

1. Sharkey, T.D.; Bernacchi, C.J.; Farquhar, G.D.; Singaas, E.L. Fitting photosynthetic carbon dioxide response curves for C₃ leaves. *Plant. Cell Environ.* **2007**, *30*, 1035–1040, doi:10.1111/j.1365-3040.2007.01710.x.
2. Strasser, R.J.; Tsimilli-Michael, M.; Srivastava, A. Analysis of the Chlorophyll *a* Fluorescence Transient. In; Springer, Dordrecht: Dordrecht, Netherlands, 2004; pp. 321–362.