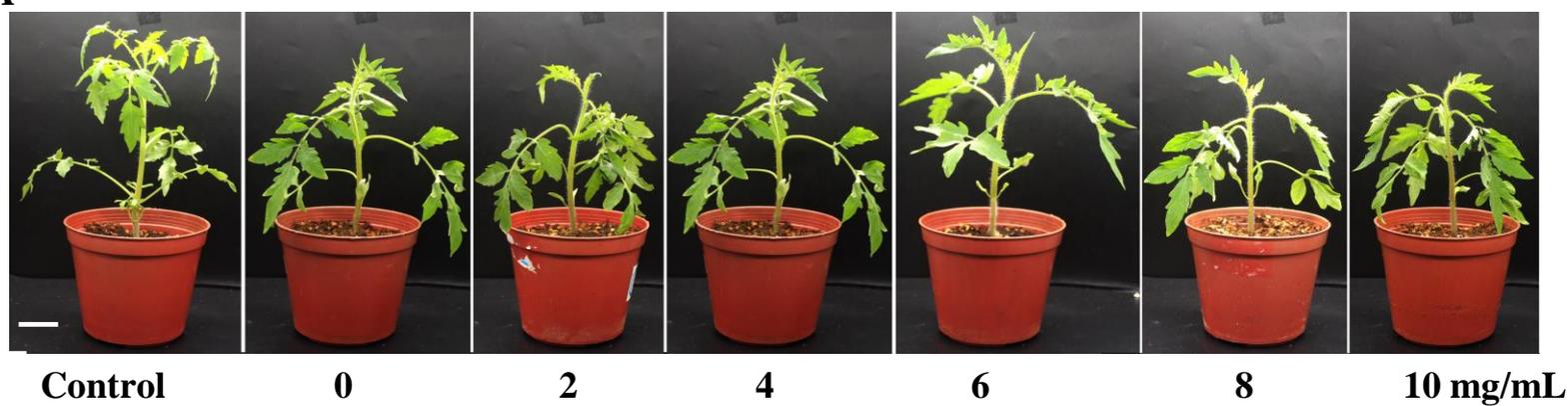


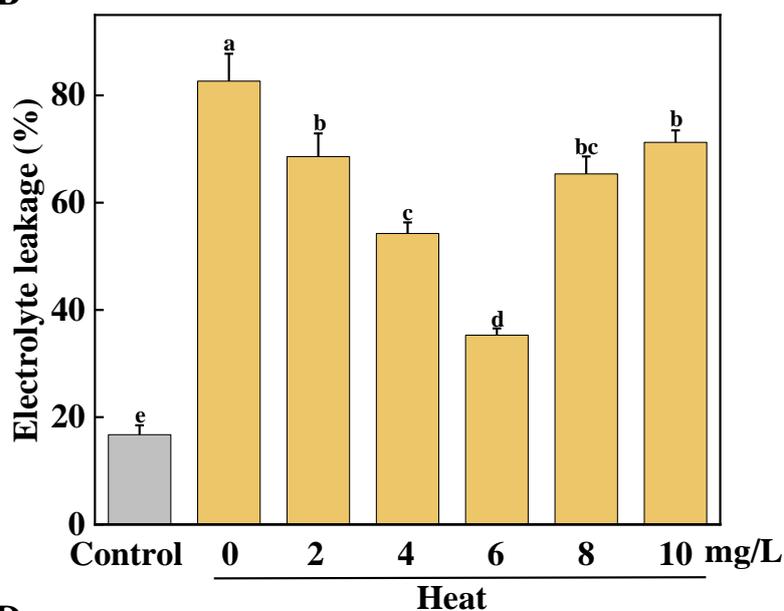
**Spermidine carbon dots enhances thermotolerance by
modulating photosynthesis and cellular redox
homeostasis in tomato**

Supplementary data

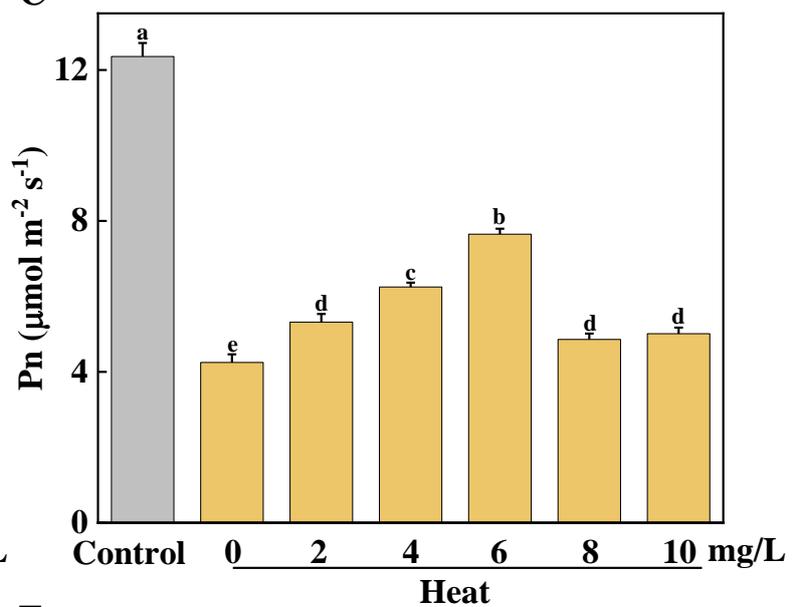
A



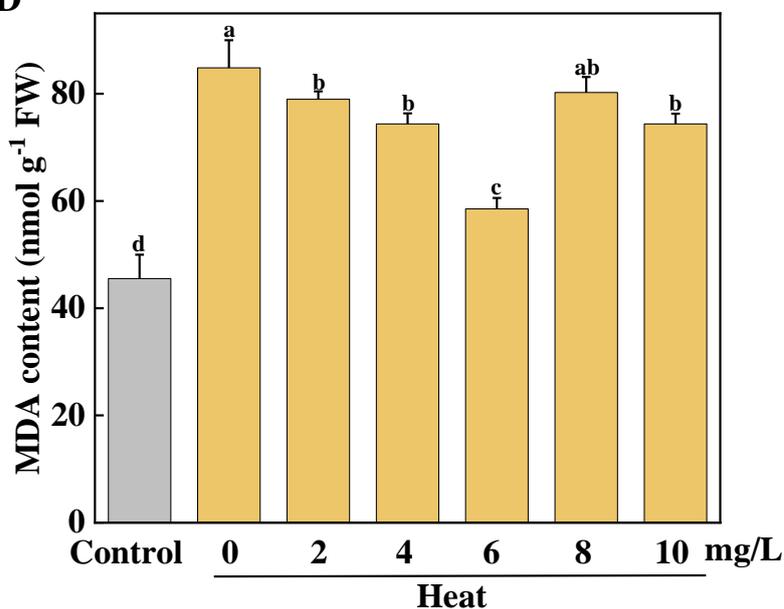
B



C



D



E

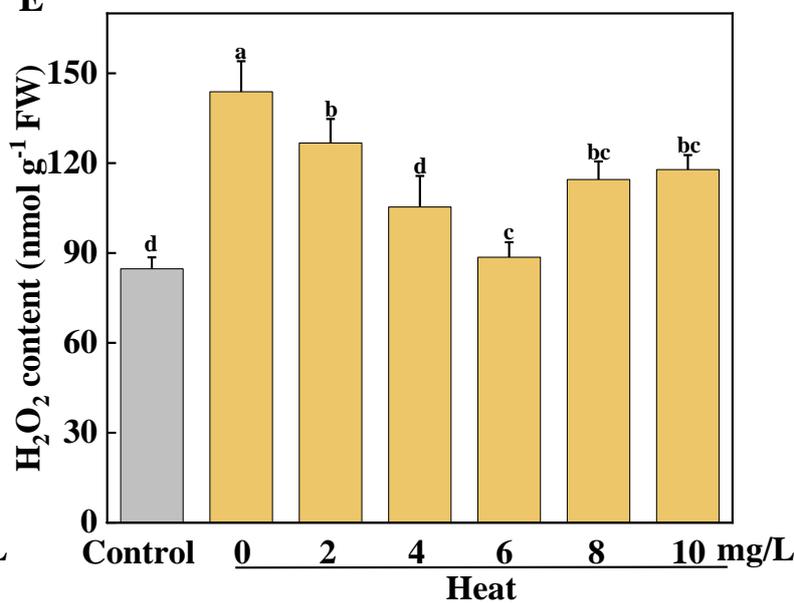


Figure S1. Phenotypic and physiological analysis of different Spd-CDs concentrations on the enhancement of thermotolerance in tomato plants. The phenotypes of 0, 2, 4, 6, 8, 10 mg/L Spd-CDs pretreated plants under normal temperature (25 °C, Control) or high temperature (45 °C, Heat) treatment, and the plant images was taken in 24 h later, bar=10 cm (A). The relative electrolyte leakage (B), the net photosynthesis rate (Pn) (C), MDA (D) and H₂O₂ (E) content of tomato leaves after 12 h of different temperature treatments. The Pn values were measured under photosynthetically active radiation (PAR) at 800 μmol m⁻² s⁻¹. Images shown in A was digitally extracted and scaled for comparison. The data in B to C are presented as mean values±SD; n=3. Different letters indicate significant differences between treatments ($P<0.05$, Duncan's multiple range test). Three independent experiments were performed with similar results.

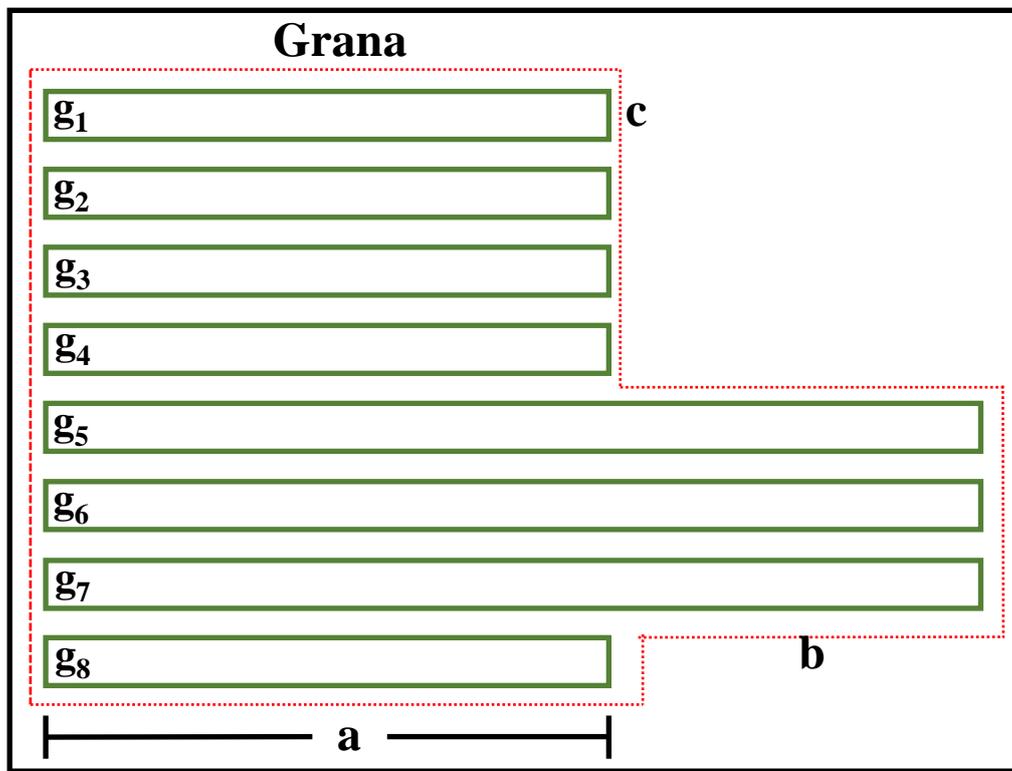


Figure S2. The schematic illustration of chloroplast membrane ultrastructure. The analysis of morphometric was performed based upon the following rules: **a**, grana width; **b**, grana surface area; **c**, single granum thylakoid height; the sum of g_1 to g_8 is granum height (GH).

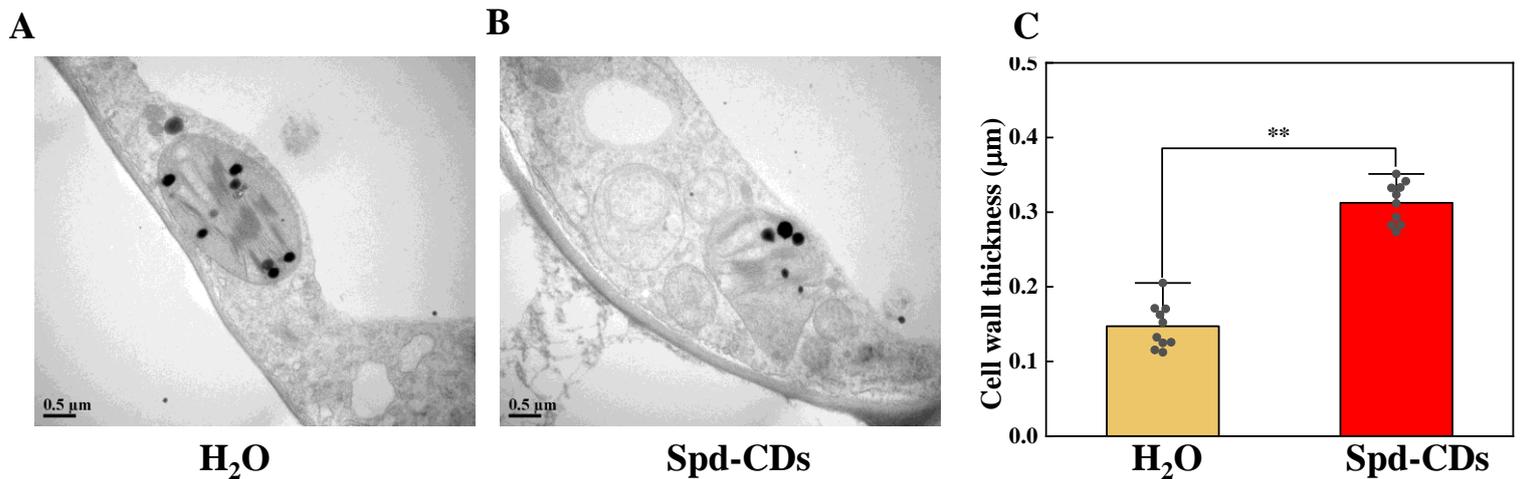


Figure S3. TEM images showing Spd-CDs increased cell wall thickness in the primary leaves of tomatoes. The effects of Spd-CDs on the ultrastructure of cell wall thickness under normal conditions (**A**, **B**). The cell wall thickness (**C**). The data are presented as mean values \pm SD; n=10. Asterisks indicate significant differences between treatments (** $P < 0.01$, Duncan's multiple range test). Three independent experiments were performed with similar results.

A

<i>PsaA</i>	1.01	1.15	0.45	0.85
<i>PsaB</i>	1.00	2.10	0.44	0.75
<i>PsaD</i>	1.00	1.35	0.31	0.95
<i>PsaE</i>	0.96	2.45	0.39	0.84
<i>PasF</i>	0.94	2.10	0.62	0.67
<i>PsaH</i>	1.01	1.45	0.32	0.77
<i>PsaJ</i>	1.12	1.32	0.85	1.33
<i>PsaK</i>	1.09	2.22	0.67	0.71
<i>PsaL</i>	1.08	1.19	0.27	0.69
<i>PsaN</i>	1.02	2.35	0.35	0.88
	<u>H₂O</u>	<u>Spd-CDs</u>	<u>H₂O</u>	<u>Spd-CDs</u>
	Control		Heat	

B

<i>PsbA</i>	1.02	3.25	0.36	3.12
<i>PsbB</i>	1.01	2.98	0.45	2.85
<i>PsbC</i>	0.99	3.50	0.55	3.40
<i>PsbE</i>	1.12	3.15	0.32	3.00
<i>PsbQ</i>	1.13	3.60	0.28	3.42
<i>PsbO</i>	0.97	4.20	0.38	3.85
<i>PsbP</i>	1.14	3.98	0.40	3.72
<i>Psb27</i>	1.10	2.36	0.51	2.26
<i>Psb28</i>	0.99	3.85	0.36	3.75
	<u>H₂O</u>	<u>Spd-CDs</u>	<u>H₂O</u>	<u>Spd-CDs</u>
	Control		Heat	

C

<i>Lhca1</i>	0.99	1.35	0.62	0.80
<i>Lhca2</i>	1.03	1.25	0.53	0.76
<i>Lhca3</i>	1.11	1.22	0.43	0.69
<i>Lhca4</i>	1.13	1.34	0.34	0.65
<i>Lhca5</i>	0.97	1.34	0.36	0.69
	<u>H₂O</u>	<u>Spd-CDs</u>	<u>H₂O</u>	<u>Spd-CDs</u>
	Control		Heat	

D

<i>Lhcb1</i>	0.98	1.20	0.65	1.15
<i>Lhcb2</i>	1.02	1.65	0.42	1.45
<i>Lhcb3</i>	1.06	1.54	0.53	1.44
<i>Lhcb4</i>	0.97	1.72	0.48	1.53
<i>Lhcb5</i>	1.04	2.10	0.45	1.86
<i>Lhcb6</i>	1.10	1.98	0.51	1.67
	<u>H₂O</u>	<u>Spd-CDs</u>	<u>H₂O</u>	<u>Spd-CDs</u>
	Control		Heat	

E

<i>PetA</i>	1.03	1.35	0.36	0.74
<i>PetB</i>	0.97	1.44	0.39	0.85
<i>PetC</i>	1.01	1.32	0.42	0.82
<i>PetD</i>	0.99	1.25	0.46	0.79
	<u>H₂O</u>	<u>Spd-CDs</u>	<u>H₂O</u>	<u>Spd-CDs</u>
	Control		Heat	

Figure S4. Transcription of photosystem I (PSI) (A) and PSII (B), light-harvesting complex (LHCI and LHCII) (C and D) and photosynthetic electron transport (PET) (E) in Spd-CDs pretreated plants under normal temperature (25 °C) or high temperature (45 °C) treatment. 6 h after the application, leaf samples were collected for gene expression analysis. *Actin* was used as the reference gene. The data are presented as mean values±SD; n=3. Three independent experiments were performed with similar results.

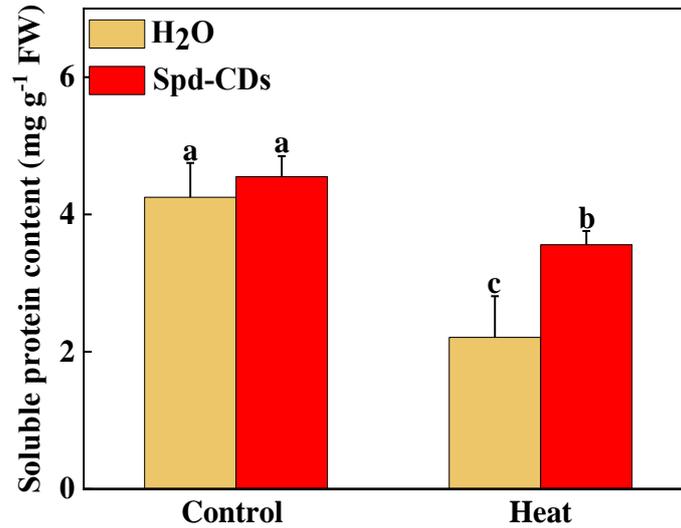


Figure S5. The protein content of leaves in Spd-CDs pretreated plants under normal temperature (25 °C) or high temperature (45 °C) treatment. The data are presented as mean values \pm SD; n=3. Different letters indicate significant differences between treatments ($P < 0.05$, Tukey's test). Three independent experiments were performed with similar results.

<i>FBA1</i>	0.98	2.12	0.35	1.21
<i>FBA2</i>	0.97	3.10	0.44	1.19
<i>FBA3</i>	1.01	2.18	0.56	1.22
<i>FBA6</i>	1.10	3.15	0.52	1.26
<i>FBA7</i>	1.20	2.21	0.49	1.28
<i>PGK1</i>	0.96	4.35	0.45	1.31
<i>PGK2</i>	0.99	3.15	0.61	1.18
<i>PGK3</i>	0.97	5.32	0.62	1.33
<i>SBPase</i>	0.98	3.26	0.54	1.34
<i>RCA1</i>	1.02	2.45	0.50	1.52
<i>RCA2</i>	1.12	4.28	0.51	1.33
<i>RCA3</i>	0.97	3.19	0.58	1.21
<i>FBP1</i>	0.98	2.20	0.61	1.23
<i>FBP2</i>	1.01	3.24	0.35	1.25
<i>FBP2</i>	1.10	2.12	0.46	1.09
	<u>H₂O</u>	<u>Spd-CDs</u>	<u>H₂O</u>	<u>Spd-CDs</u>
	Control		Heat	

Figure S6. Transcription of Calvin cycle genes in Spd-CDs pretreated plants under normal temperature (25 °C) or high temperature (45 °C) treatment. 6 h after the application, leaf samples were collected for gene expression analysis. *Actin* was used as the reference gene. The data are presented as mean values±SD; n=3. Three independent experiments were performed with similar results.

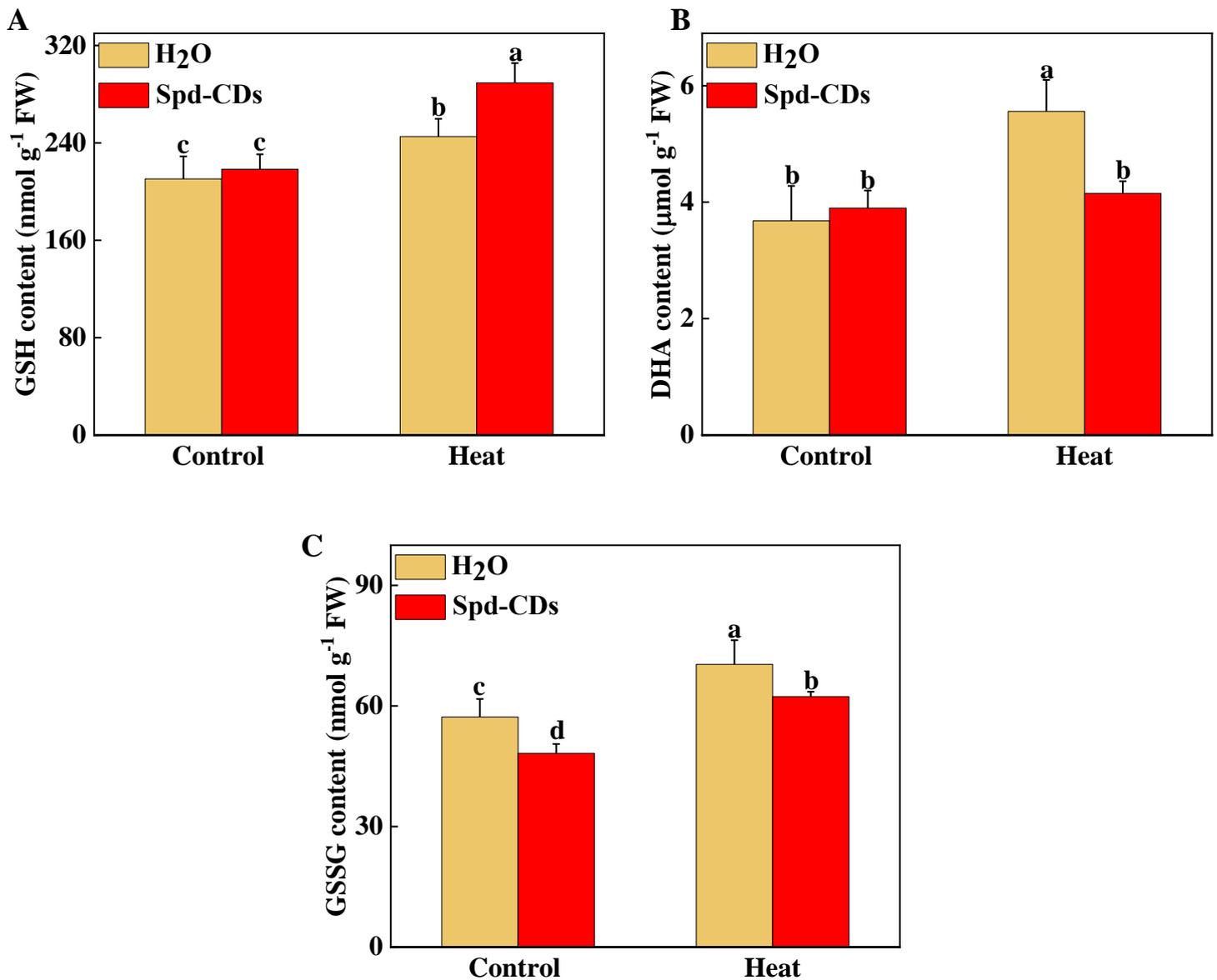


Figure S7. Antioxidants in Spd-CDs pretreated plants under normal temperature (25 °C) or high temperature (45 °C) treatment. The effects of Spd-CDs on antioxidants (GSH, glutathione; DHA, dehydroascorbate; GSSG, glutathione disulfide) in response to high temperature (A, B, C). The data are presented as mean values \pm SD; n=3. Different letters indicate significant differences between treatments ($P < 0.05$, Duncan's multiple range test). Three independent experiments were performed with similar results.

<i>RbohA</i>	1.02	1.12	3.60	1.35
<i>RbohB</i>	0.98	1.21	4.50	1.45
<i>RbohC</i>	1.12	1.10	5.10	1.62
<i>RbohD</i>	0.99	1.00	3.50	1.35
<i>DHAR</i>	1.12	2.23	4.30	5.68
<i>MDHAR</i>	1.15	2.25	4.10	6.25
<i>APX</i>	1.19	3.98	3.78	6.37
<i>CAT</i>	1.21	2.13	3.35	6.58
<i>SOD</i>	1.01	2.14	3.20	6.89
<i>GPX</i>	0.96	3.15	2.58	7.01
<i>GR</i>	0.97	3.17	2.78	7.05
	<u>H₂O</u>	<u>Spd-CDs</u>	<u>H₂O</u>	<u>Spd-CDs</u>
	Control		Heat	

Figure S8. Transcription of ROS production and scavenging enzymatic activities genes in Spd-CDs pretreated plants under normal temperature (25 °C) or high temperature (45 °C) treatment. 6 h after the application, leaf samples were collected for gene expression analysis. *Actin* was used as the reference gene. The data are presented as mean values \pm SD; n=3. Three independent experiments were performed with similar results.

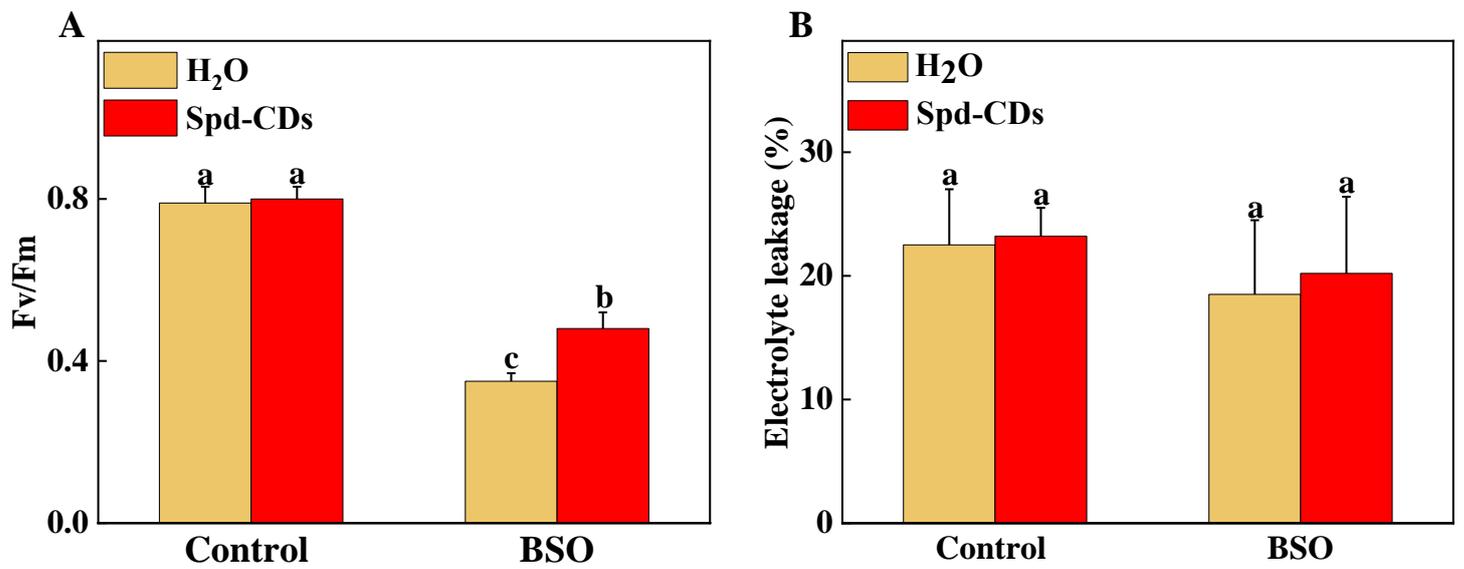


Figure S9. Effects of BSO on the maximum quantum yield of PSII (F_v/F_m) (**A**) and the relative electrolyte leakage (**B**) under normal conditions in Spd-CDs treated plants. The data are presented as mean values ± SD; n=3. Three independent experiments were performed with similar results. Different letters indicate significant differences between treatments ($P < 0.05$, Duncan's multiple range test). Three independent experiments were performed with similar results.