

Expression of Heterologous *OsDHAR* Gene Improves Glutathione (GSH)-Dependent Antioxidant System and Maintenance of Cellular Redox Status in *Synechococcus elongatus* PCC 7942

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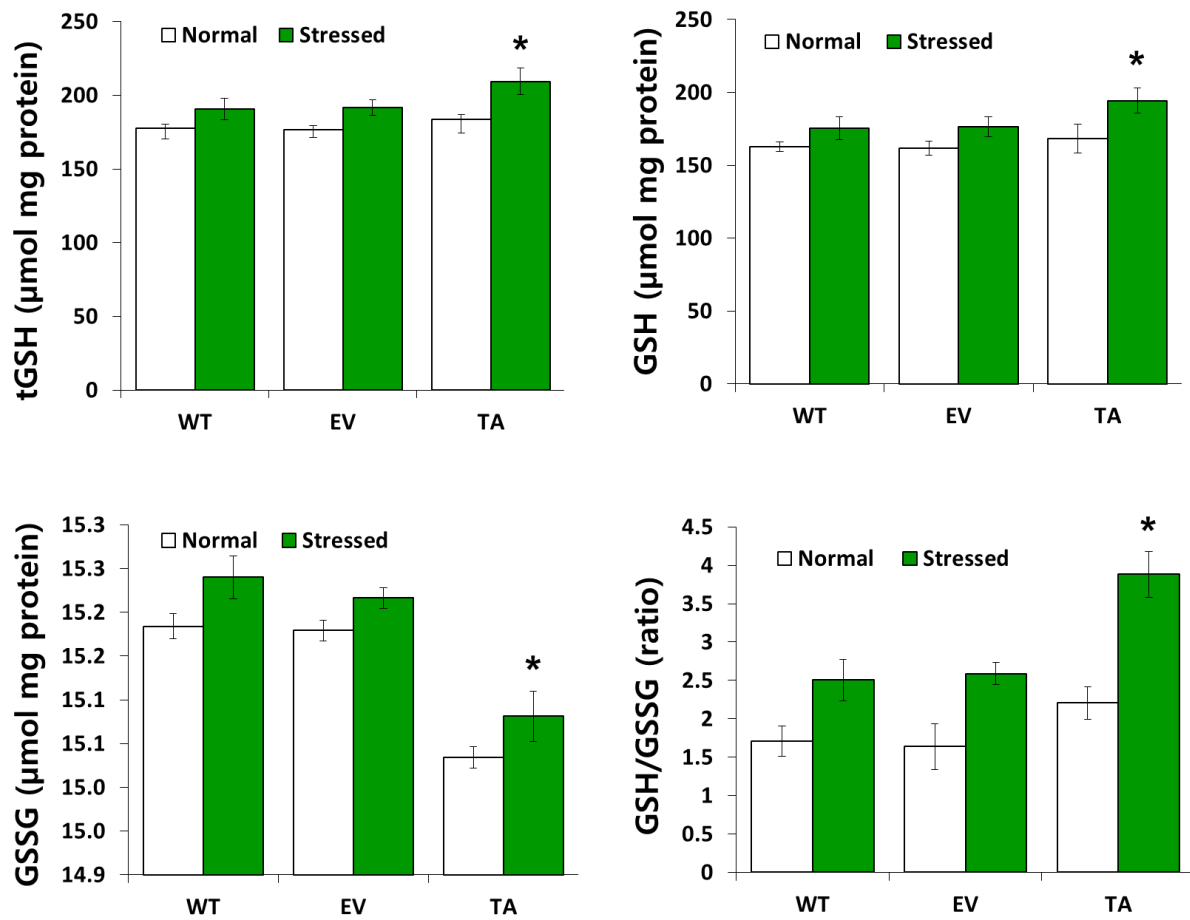
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Supplementary Figures S1 and S3

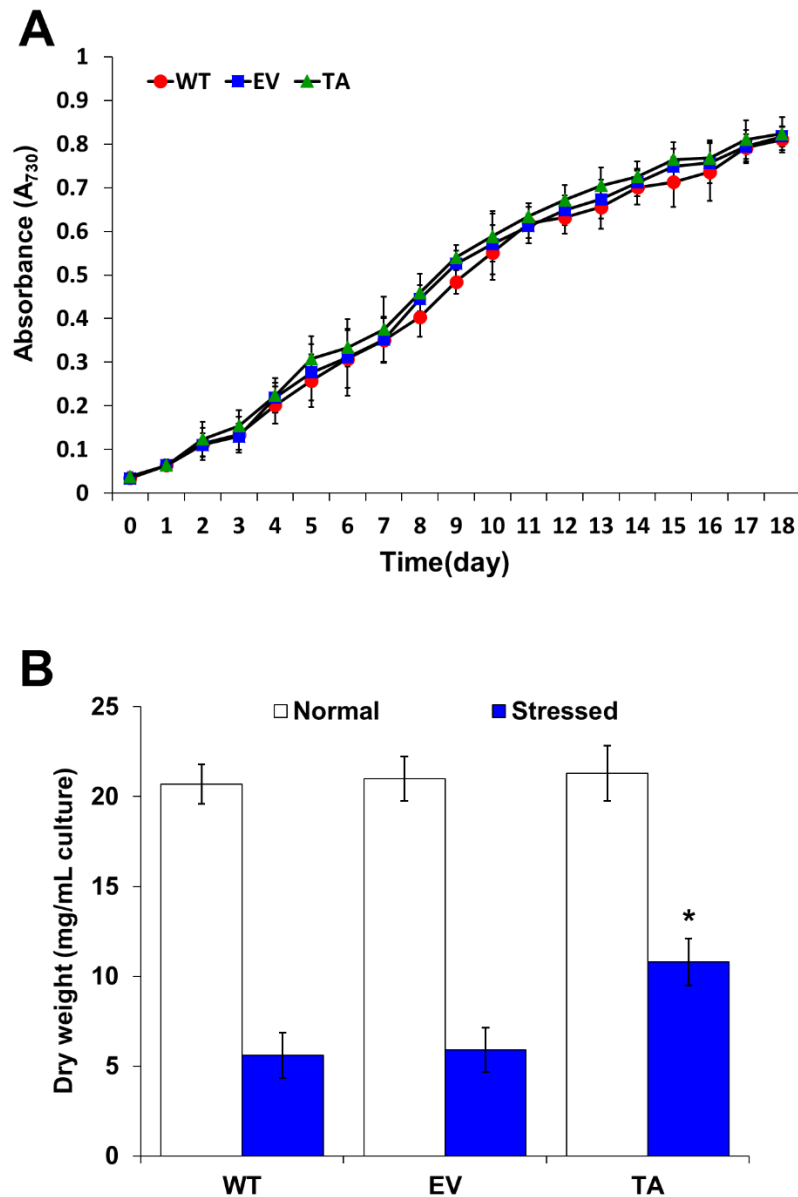
Supplementary Tables S1 to S2

Supplementary Figure S1.

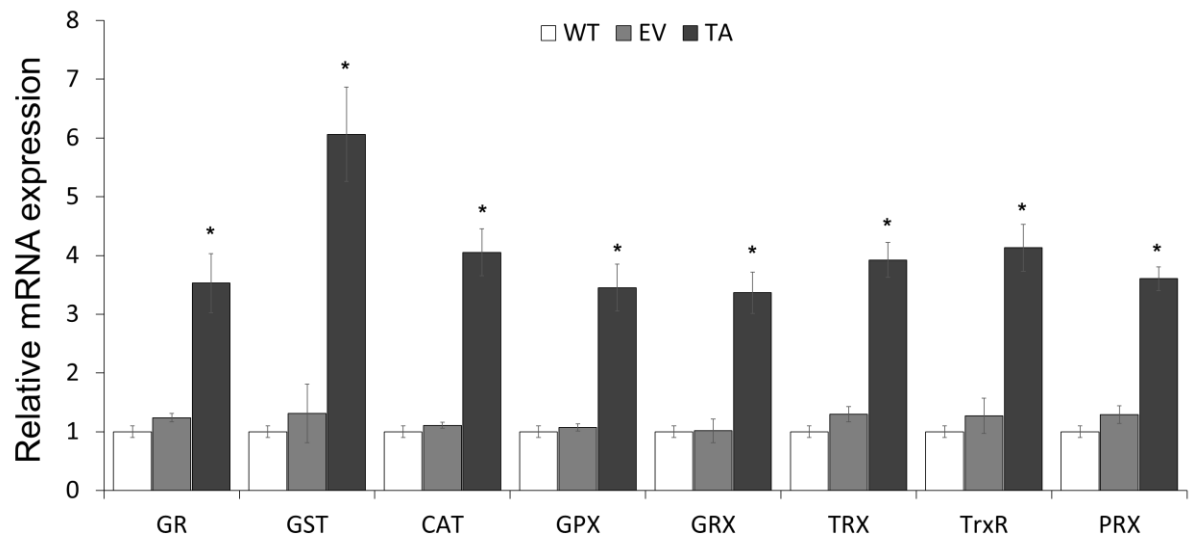


Supplementary Figure S1. Analysis of antioxidant redox status in TA strains after H_2O_2 treatment. Total glutathione (GSH+GSSG) and oxidized glutathione (GSSG) were determined by measuring absorbances at 412 nm. Reduced GSH content was calculated by subtraction of GSSG from total GSH. Total glutathione (GSH+GSSG), reduced glutathione (GSH), oxidized glutathione (GSSG), and ratio of GSH to GSSG. Error bars indicate $\pm$ SD of three independent experiments. Asterisks indicate significant differences between treatments as estimated by Student's *t*-test ($P < 0.05$).

Supplementary Figure S2.



Supplementary Figure S2. Dry weights of *OsDHAR*-overexpressing transgenic TA strains under normal and stress conditions. **(A)** Growth profiles of WT, EV, and TA strains. growth levels of the different *S. elongatus* PCC 7942 strains were compared to those of the TA strain under normal conditions. Samples were grown for 18 days at 30°C with shaking under normal conditions. **(B)** Dry weights measured after growth under normal condition for 14 days and stress conditions for 7 days after H₂O₂ treatment at 7 days. WT, WT strains; EV, empty vector strains; TA, transgenic strains. Error bars indicate $\pm$ SD of three or more independent experiments. Asterisks indicate significant differences between treatments as estimated by Student's *t*-test ($P < 0.05$).



Supplementary Figure S3. Expression analysis of qRT-PCR identified GSH-related genes. GSH-related genes expression level was considered to be 1 of WT strains; described as relative values. GSH-related genes expression difference was calculated relative to the expression level in WT strains. WT, WT strains; EV, empty vector strains; TA, transgenic strains. Error bars indicate $\pm$ SD of three or more independent experiments. Asterisks indicate significant differences between treatments as estimated by Student's *t*-test ($P < 0.05$).

Supplementary Table S1. Cyanobacterial strains and plasmids used in this study.

Strain or Plasmid	Characteristic(s)	Antibiotic resistance	Source or reference
Strains			
AMC06	Wild type <i>Synechococcus elongatus</i> strain PCC 7942	none	Lab collection
DH5 α	<i>Escherichia coli</i> cloning host	none	Lab collection
Plasmids			
pAM4957 (pCV0069)	<i>S. elongatus</i> carrying <i>pCV0069</i> as a single recombinant; cloNAT ^r	NAT	Lab collection
<i>pAM4957::OsDHAR</i>	S7942NS2-NAT1-Ptrc/SwaI-OsDHAR-RBS_ <i>OsDHAR</i> , 5672 bp, cloNAT ^r , for IPTG-inducible <i>OsDHAR</i> expression	NAT	This work

* Abbreviations for antibiotics: Nourseothricin (NAT, cloNAT).

Supplementary Table S2. Primers used in this study.

Name	Sequence
OsDHAR-F-SwaI	5'-TGCGGATCCTAAGGAGGAAAATTTATGGGCGTGGAGGTGTGCGTCAAGGCCGCCGTCG-3'
OsDHAR-R-SwaI	5'-GCCGGGGAGCTCCTTCATTATTTTACGCATTCACCTTTGGTGCCCATCCAGCAATCAGG-3'
OsDHAR-qRT-PCR-F	5'-ATGGGCGTGGAGGTGTGCGTCAAGGCCGCC-3'
OsDHAR-qRT-PCR-R	5'-TGACAAGAGACGGGGTTGGGTAC-3'
rpoA-F	5'-TCGGTTCGGGCTTACAAC TG-3'
rpoA-R	5'-AGCAAGTCAGCAACGGAGTTC-3'
NS2-F	5'-GTCAACGTAAATGCATGCCGC-3'
NS2-R	5'-GGTCACTACTTGGTAGCAACTC-3'
Pttrc-F	5'-AATTGTGAGCGGATAACAATT-3'
OsDHAR-R	5'-ATTTATTTTACGCATTCACCTTTGG-3'
GR-F	5'-ATGAGTTTTGACTACGACCTC-3'
GR-R	5'-GCCCCCGACCAAATCGGTTTC-3'
GST-F	5'-ATGTATAAGGTTTTTCGGAGA-3'
GST-R	5'-ATGAGTTTCGTTAGCAAGTA-3'
CAT-F	5'-ATGACAGTCGTTATCTACTAC-3'
CAT-R	5'-GATCGCTAACCAACGAAACCA-3'
GPX-F	5'-ATGCCTTTCAAAGCAAAAGA-3'
GPX-R	5'-TGTTTGACAGAAAGGACAAA-3'
GRX-F	5'-ATGATGACTCCTGAACTCC-3'
GRX-R	5'-TTGAACAACGTTGTTGGAA-3'
TRX-F	5'-ATGAACGTTGGCGATCGCGTGC-3'
TRX-R	5'-AGTTAGAATCGCTGCAATTCG-3'
TrxR-F	5'-ATGCTTCCAGTCGTACAGC-3'
TrxR-R	5'-GAGGCAGGGAGTTAAGAGTC-3'
PRX-F	5'-ATGACCGAAGGAGCCCTGCGCG-3'
PRX-R	5'-CAGAACGACGTATTTGCCCCGG-3'