

**The acclimation mechanisms of *Chlamydomonas reinhardtii*
against nitrosative stress: a role of NADPH oxidase (RBOL2) in
the regulation of nitric oxide-mediated ER stress and
glutathione redox state**

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Abstract:

Nitric oxide (NO) is a signal in the modulation of acclamatory responses to stress in plants. Here, the metabolic shift of *Chlamydomonas reinhardtii* to sub-lethal NO stress was approached by exposure to 0.1 mM S-nitroso-N-acetylpenicillamine (SNAP), a NO donor, in the presence or the absence of the NO scavenger, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO). NO did not cause growth impairment but induced a decrease in glutathione (GSH) levels and redox state. NO upregulated the expression of glutathione-associated genes, glutathione synthetase (GSH1), and glutathione reductase (GSHR1) genes while decreased that of the proteins associated with ER stress-induced unfolded protein response (UPR). Furthermore, the expression of NADPH oxidase isoform, respiratory burst oxygenase-like 2 (RBOL2), instead of RBOL1 increased under NO stress. NO-induced upregulation of GSH1 and GSHR1 upregulation and the downregulation of most UPR genes were not found in *rbol2* mutant. The presence of cPTIO suppressed the NO-induced changes in GSH availability, UPR, and RBOL expression. Overall, NADPH oxidase (RBOL2)-dependent- and -independent signaling pathways involve in the inhibition of UPR and the enhancement of GSH availability by NO.

Keywords: nitric oxide; glutathione; unfolded protein response; ER stress; NADPH oxidase

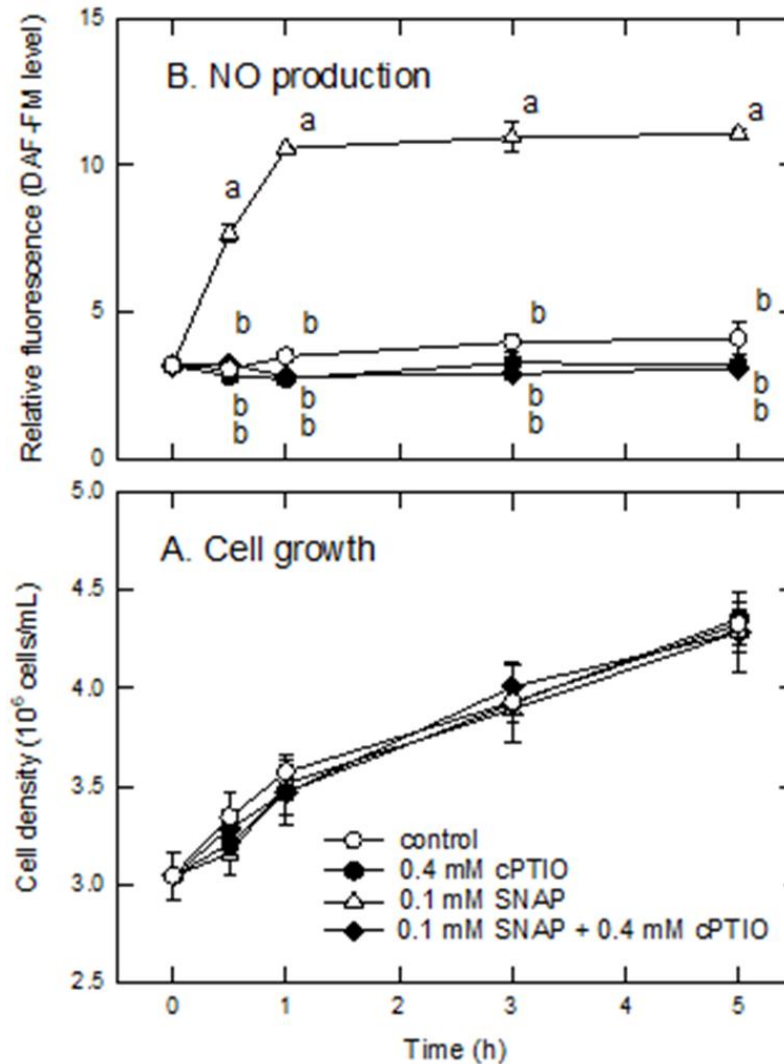
Nitric Oxide (NO)

- Nitric oxide (NO) is an important signaling molecule in the regulation of many metabolic processes in plant.

Chlamydomonas

- the remodelling of chloroplast proteins by degrading cytochrome b6f complex and Rubisco via FtsH and Clp proteases
- the regulation of nitrogen assimilation by repressing the expression of nitrate reductase and nitrate and ammonium transporters under N limitation
- mastoparan-induced cell death
- high light-induced oxidative stress and autophagy
- proline accumulation under copper stress
- The upregulation of alternative oxidase 1 for regulation of mitochondrial respiration

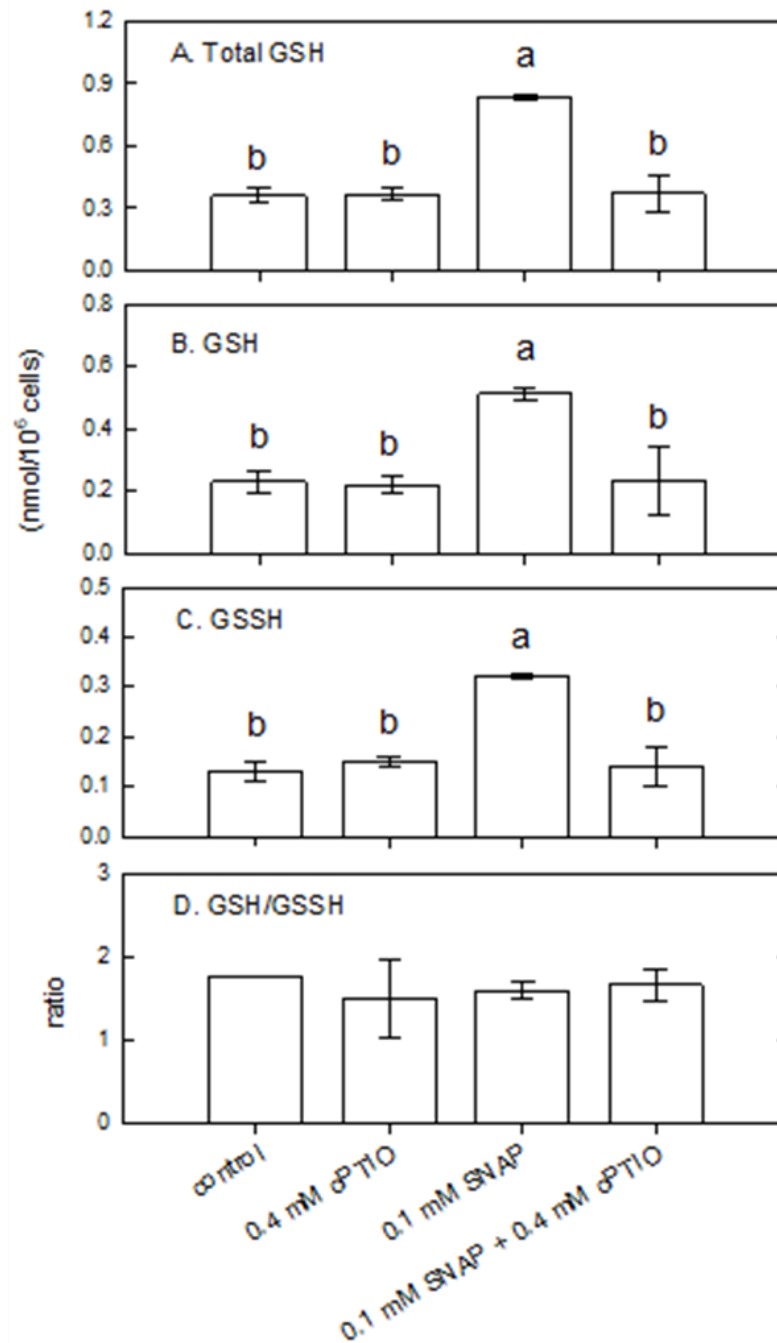
Results **NO production** 0.1 mM SNAP – burst within 1 h



Results

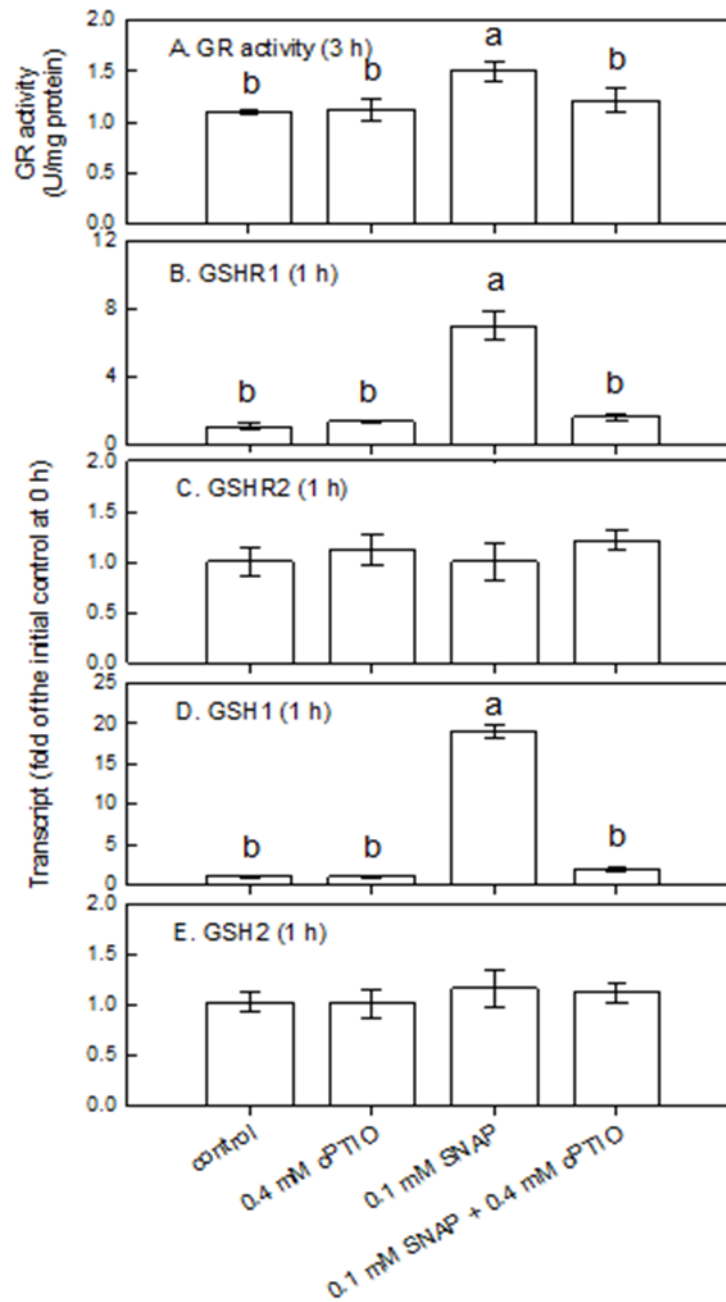
NO induces changes in GSH, GSSG, and GSH/GSSG ratio.

NO increases GSH pool.



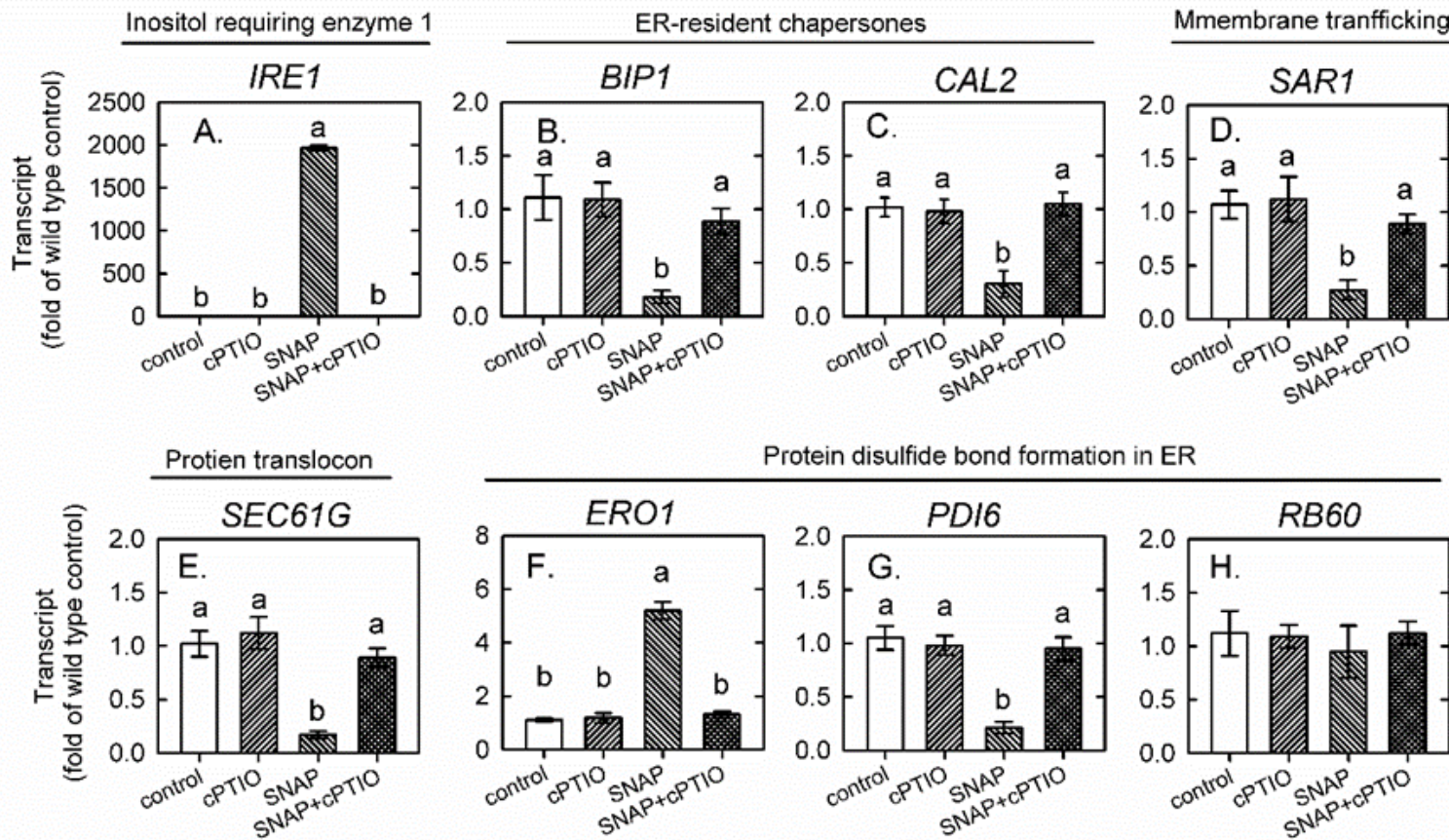
Results

- NO increases GR activity and GSHR expression.
- NO also activates GSH1 expression.



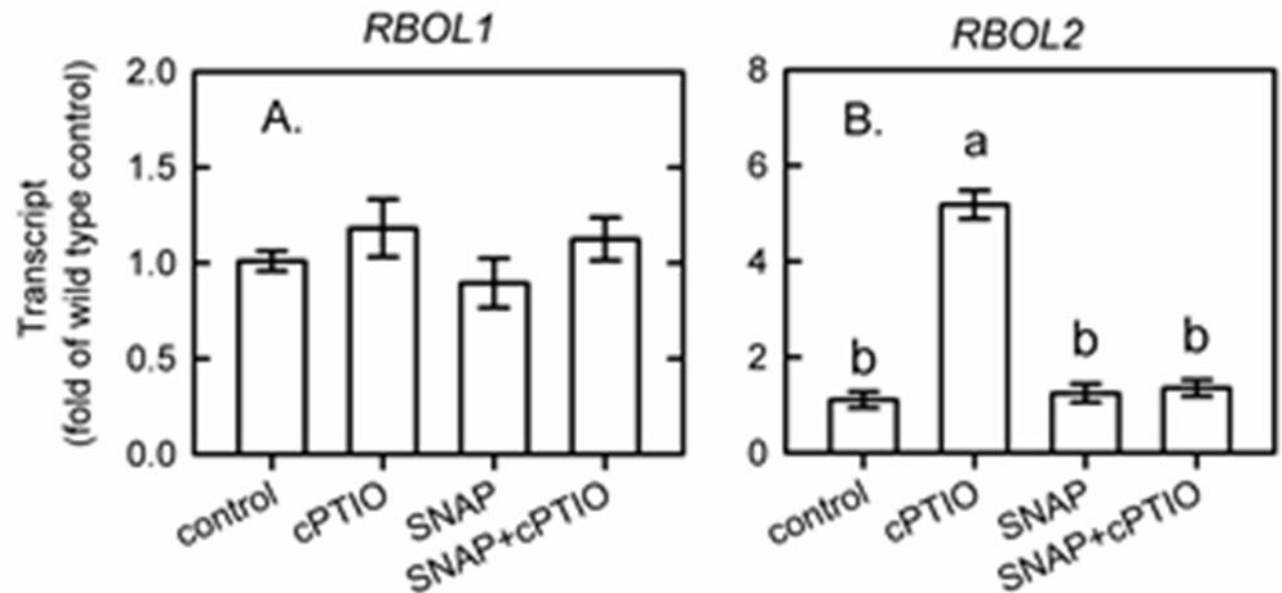
Results

NO decreases UPR gene expression except IRE1 and ERO1.



Results

**NO induces
NADPH oxidase
(RBOL2)
expression.**



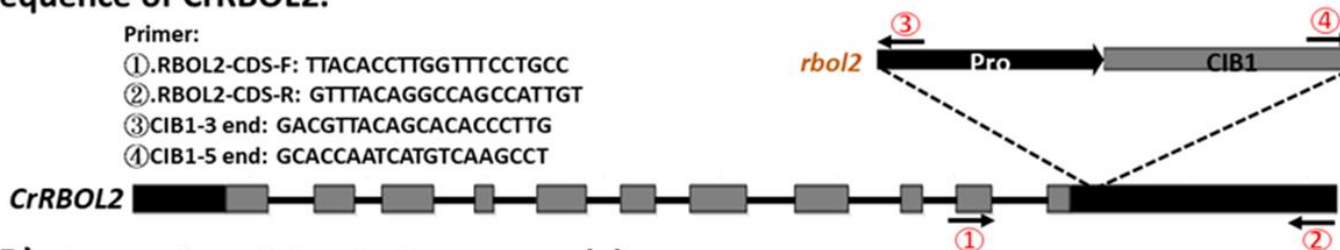
Results

rbol2 mutant-low RBOL2 expression and NADH oxidase activity

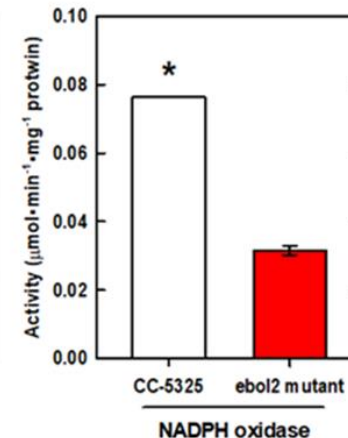
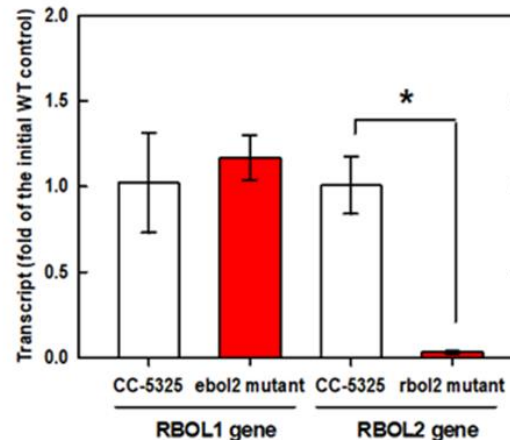
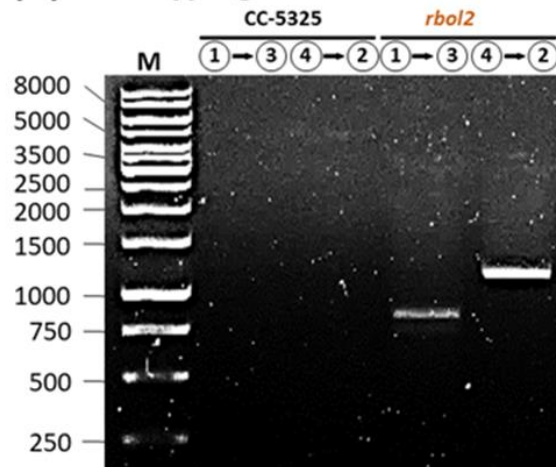
(A) Schematic representation of insertion sites of the APHVIII cassettes in the genomic sequence of CrRBOL2.

Primer:

- ①.RBOL2-CDS-F: TTACACCTTGGTTTCCTGCC
- ②.RBOL2-CDS-R: GTTACAGGCCAGCCATTGT
- ③CIB1-3 end: GACGTTACAGCACACCTTG
- ④CIB1-5 end: GCACCAATCATGTCAAGCCT

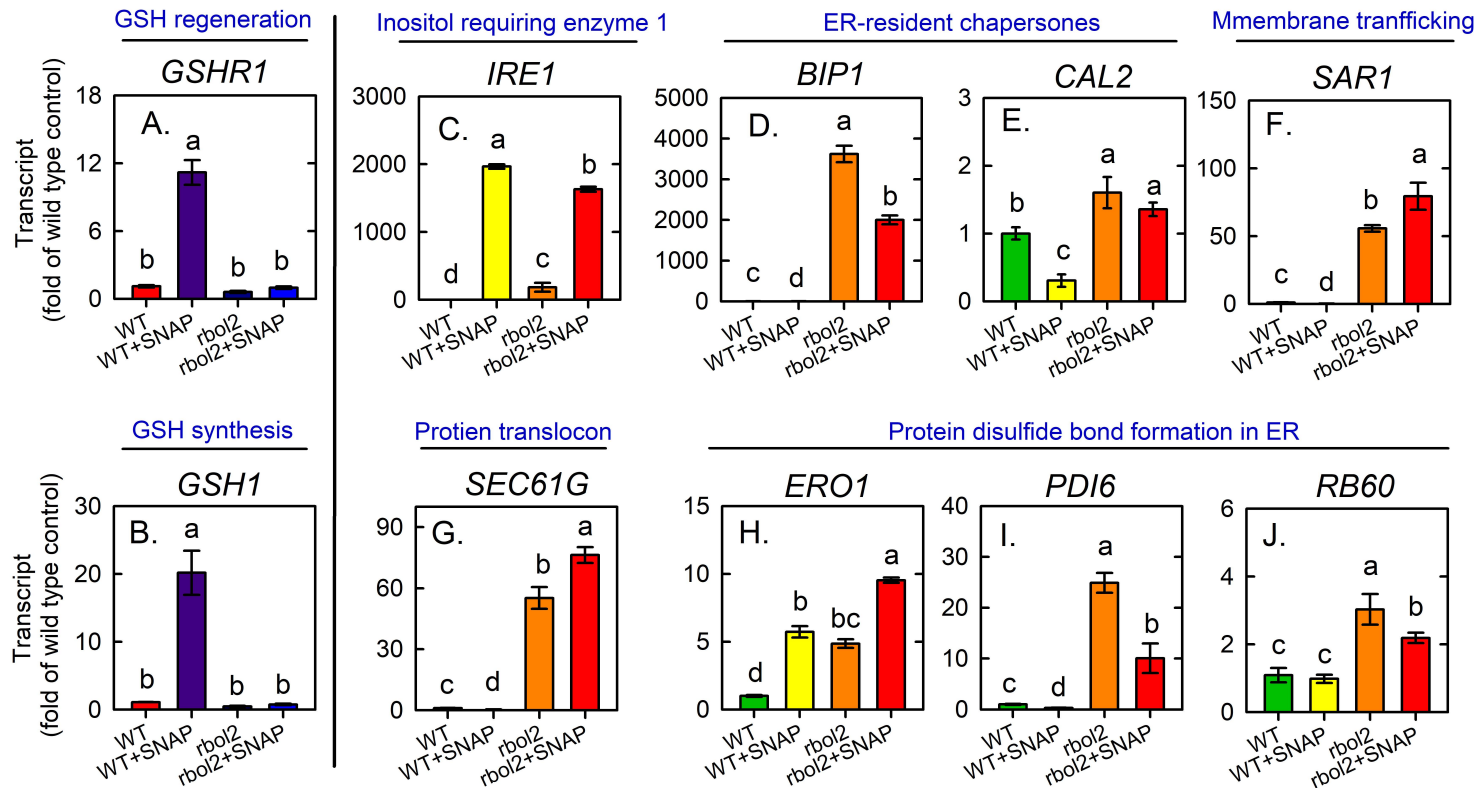


(B) Genotyping of the *rbol2* mutant (c) RBOL1 and RBOL2 gene expression



Results

- SNAP does not increase the transcript abundance of GSHR1 and GSH1.
- The transcript abundance of IRE1, BiP1, CAL2, SAR1, SEC61G, ERO1, PDI6, and RB60 in *rbol2* mutant.



Conclusion

NADPH oxidase (RBOL2)-dependent and -independent signaling pathways are involved in the inhibition of UPR and the enhancement of GSH availability by NO in *Chlamydomonas*.

Acknowledgments

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