IECPS 2020
1st International Electronic Conference on Plant Science
01–15 DECEMBER 2020 | ONLINE
plants MDPI

# 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 reinardtii* 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-l-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, respieatory 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; unfolden 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 mitochhodrial respiration

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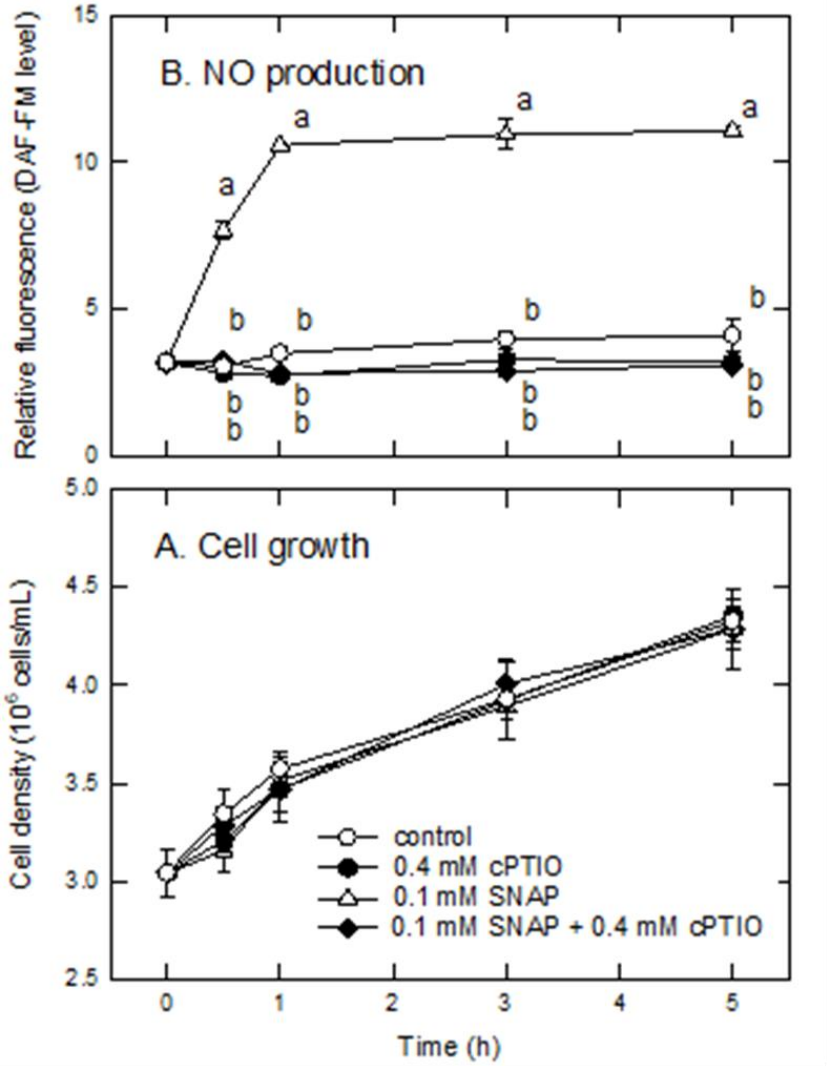
# **Results** NO production 0.1 mM SNAP – burst within 1 h

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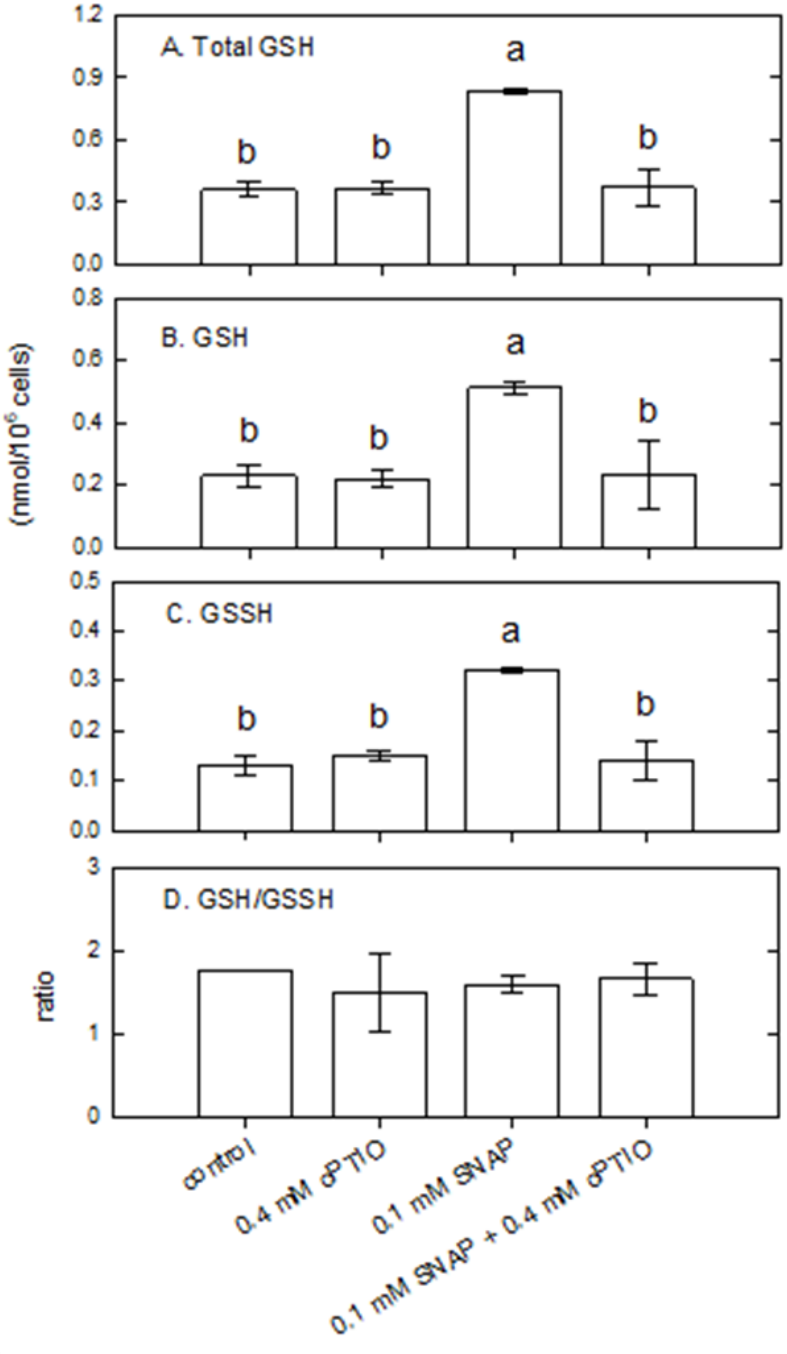
## Results

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

**NO increases GSH pool.**

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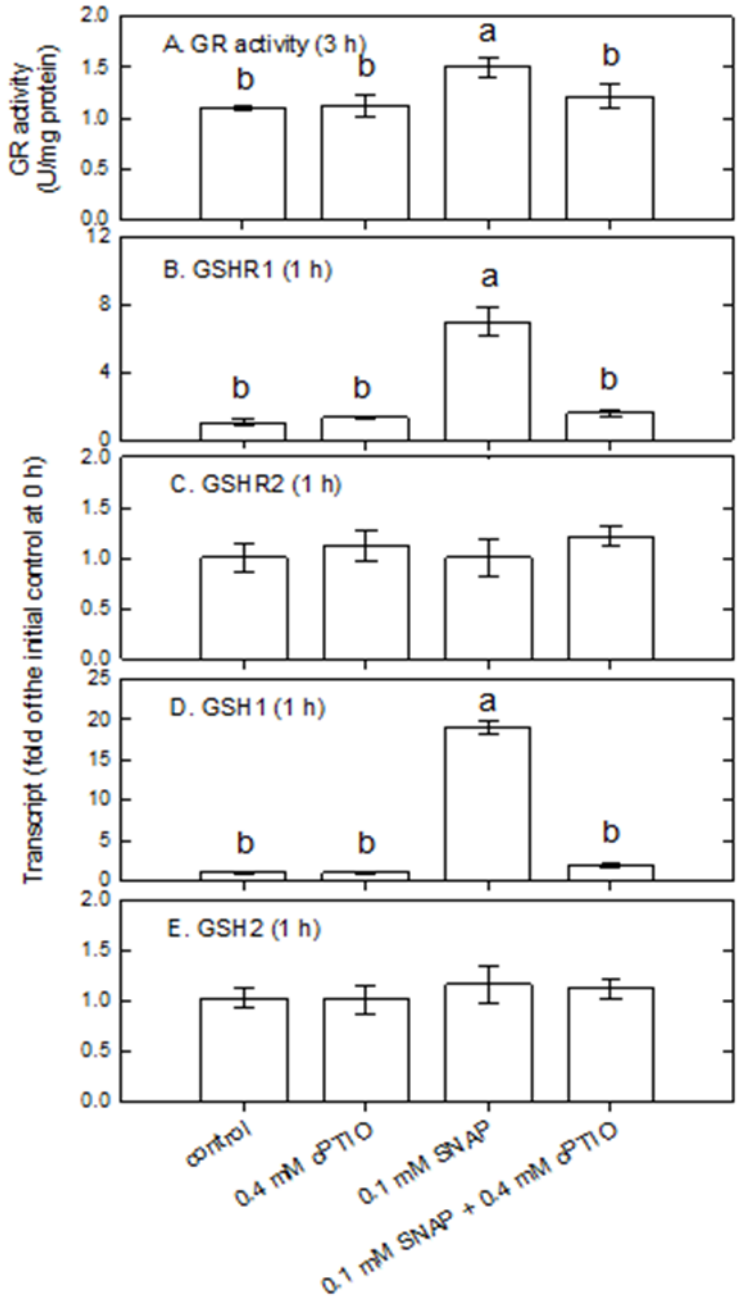
# Results

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

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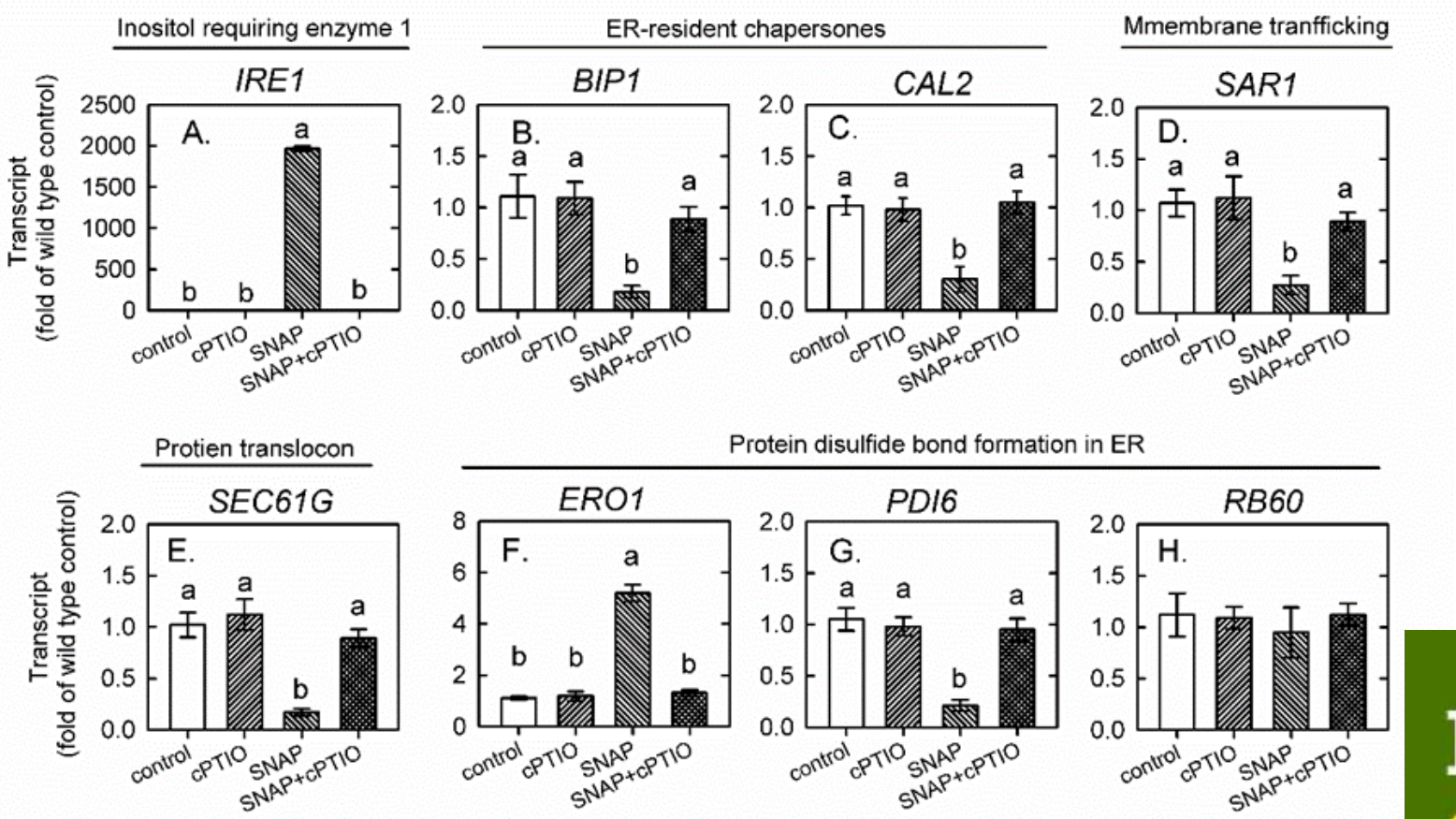
# Results

## NO decreases UPR gene expression except IRE1 and ERO1.

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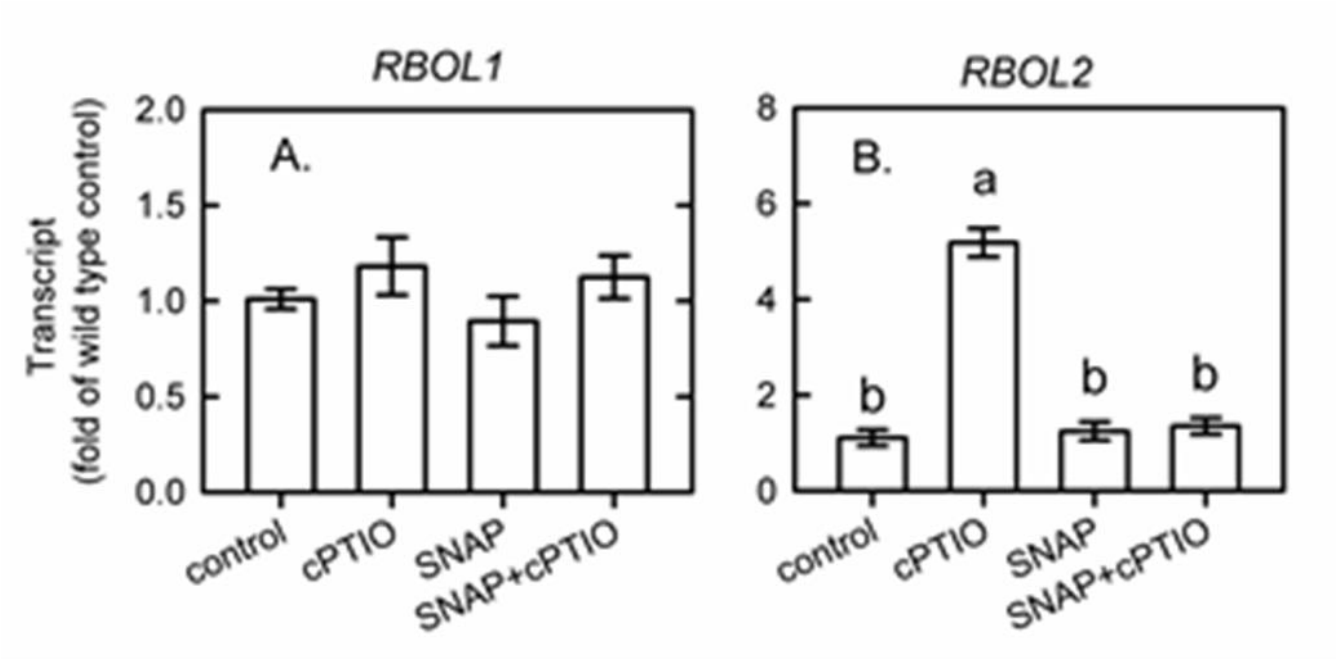
# Results

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

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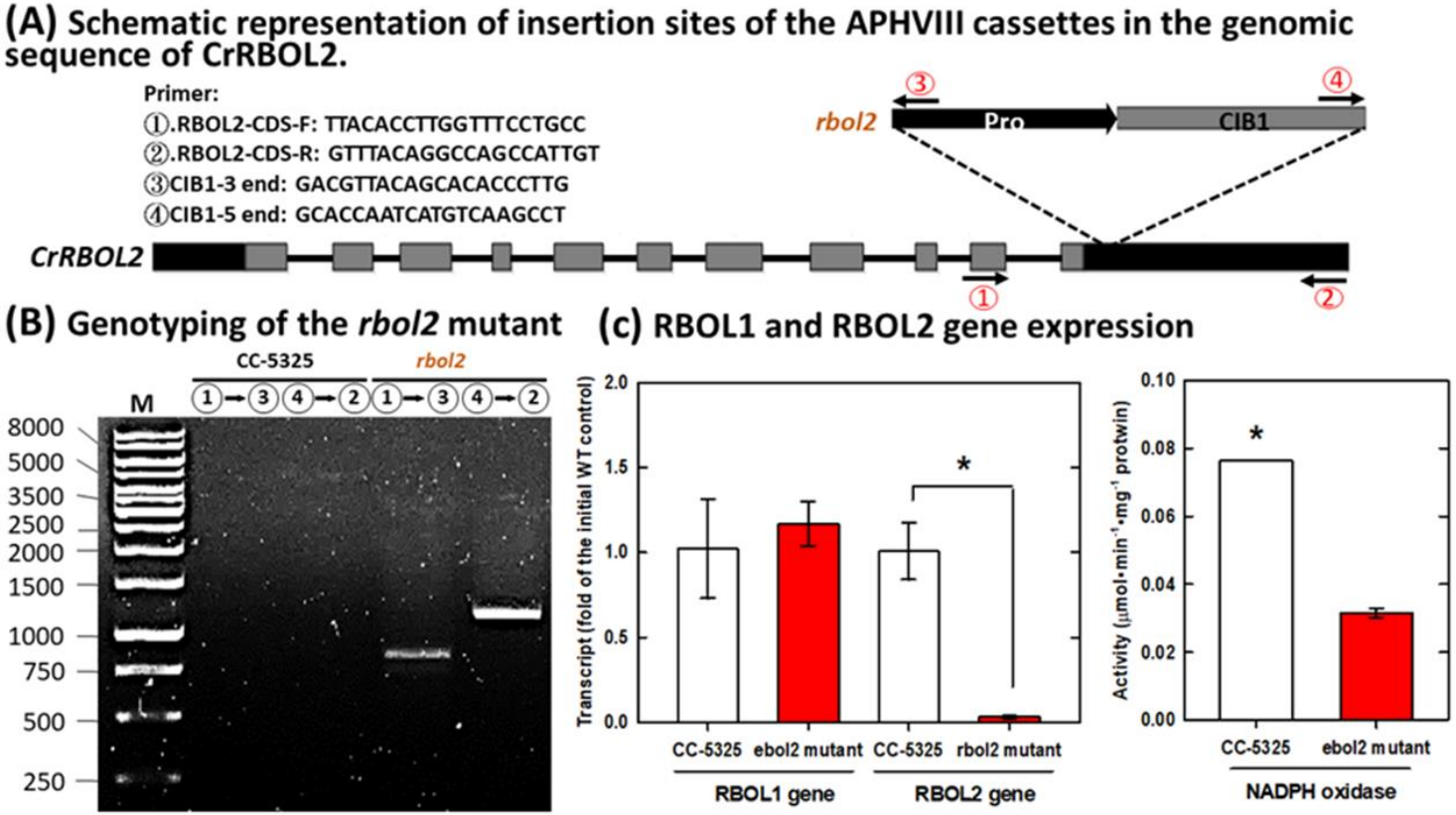
# Results

## rbol2 mutant-low RBOL2 expression and NADH oxidase activity

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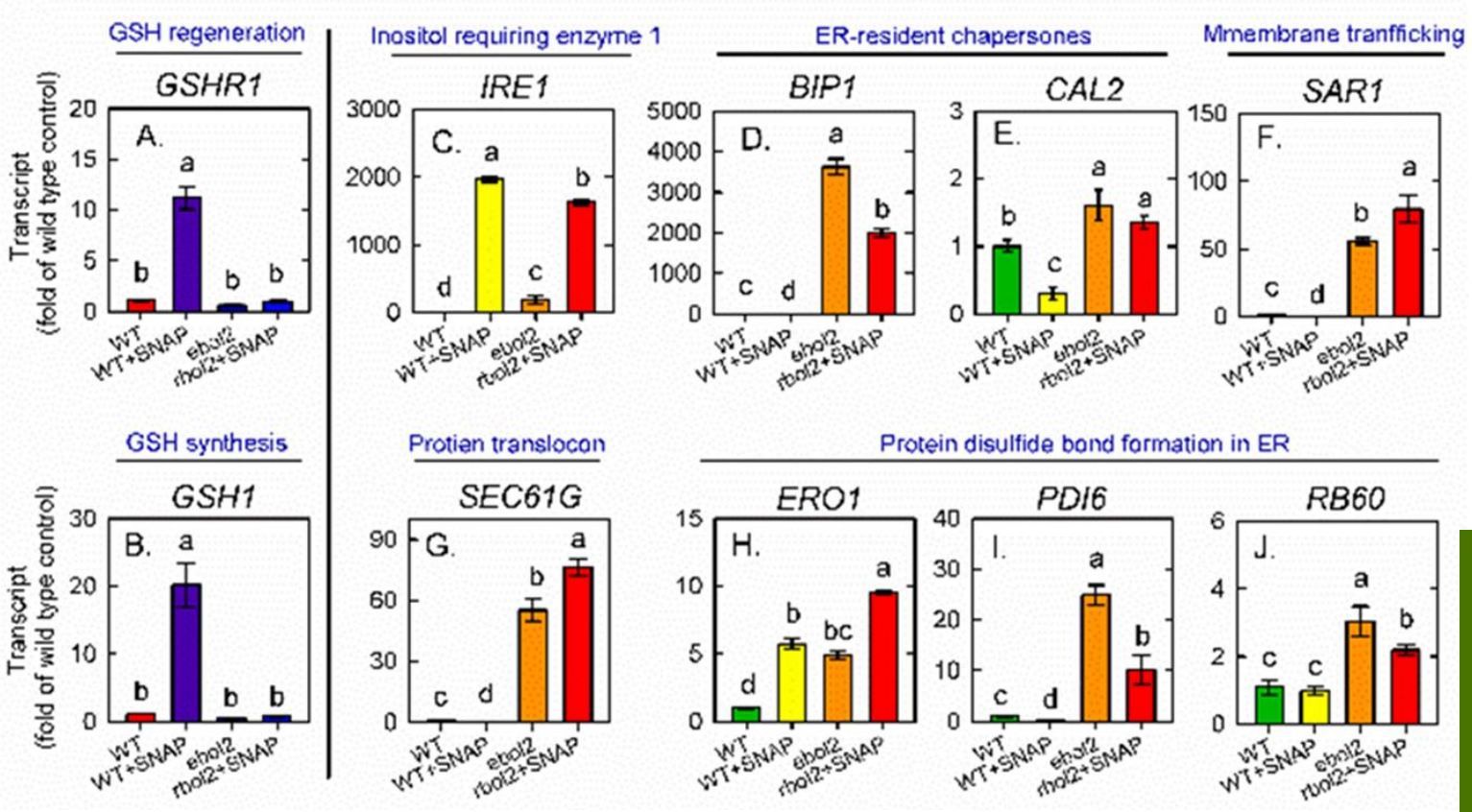
# Results

- **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.**

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## 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*.

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# Acknowledgments

This work was supported by the Ministry of Science Technology, Executive Yuan, Taiwan (Grant Number: MOST 103-2311-B-110-001MY3, MOST 107-2311-B-110-003-MY3) to Tse-Min Lee.

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