Phytochrome A and phytochrome B regulate the biosynthesis of chlorophyll during cytokinin-dependent de-etiolation of *A. thaliana*

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Introduction

Seedling de-etiolation is controlled by a complicated network of light and hormonal signaling. This process involves chloroplasts development and initiation of chlorophyll biosynthesis resulting in seedling greening. In this work we investigated the regulation of the expression of key genes of chlorophyll biosynthesis: glutamyl-tRNA reductase (*HEM1A*), Mg-chelatase (*GUN4* and *GUN5*) in wild-type (WT) seedling of *Arabidopsis thaliana* and knockout mutant for red light receptors phytochromes A and B during cytokinin-dependent de-etiolation under white and red light.

Materials and methods

Wild-type seedlings of *Arabidopsis thaliana* (Landsberg *erecta*) and mutant for red light receptors *phyAphyB* were grown for 4 days under dark condition on half-strength Murashige-Skoog medium without cytokinin (CK) (0 μM *tZ*) or with the addition of *trans*-zeatin (1 μM *tZ*). Then they were fixed in liquid nitrogen under low green light (dark). The rest of the seedlings were illuminated with white light (15 mmol/m^{-2} s^{-1}) or red light (4 mmol/m^{-2} s^{-1}) and fixed after 1, 3, 6 and 9 hours. Quantification of transcripts (relative to ubiquitin 10) was performed by real-time PCR after reverse transcription.

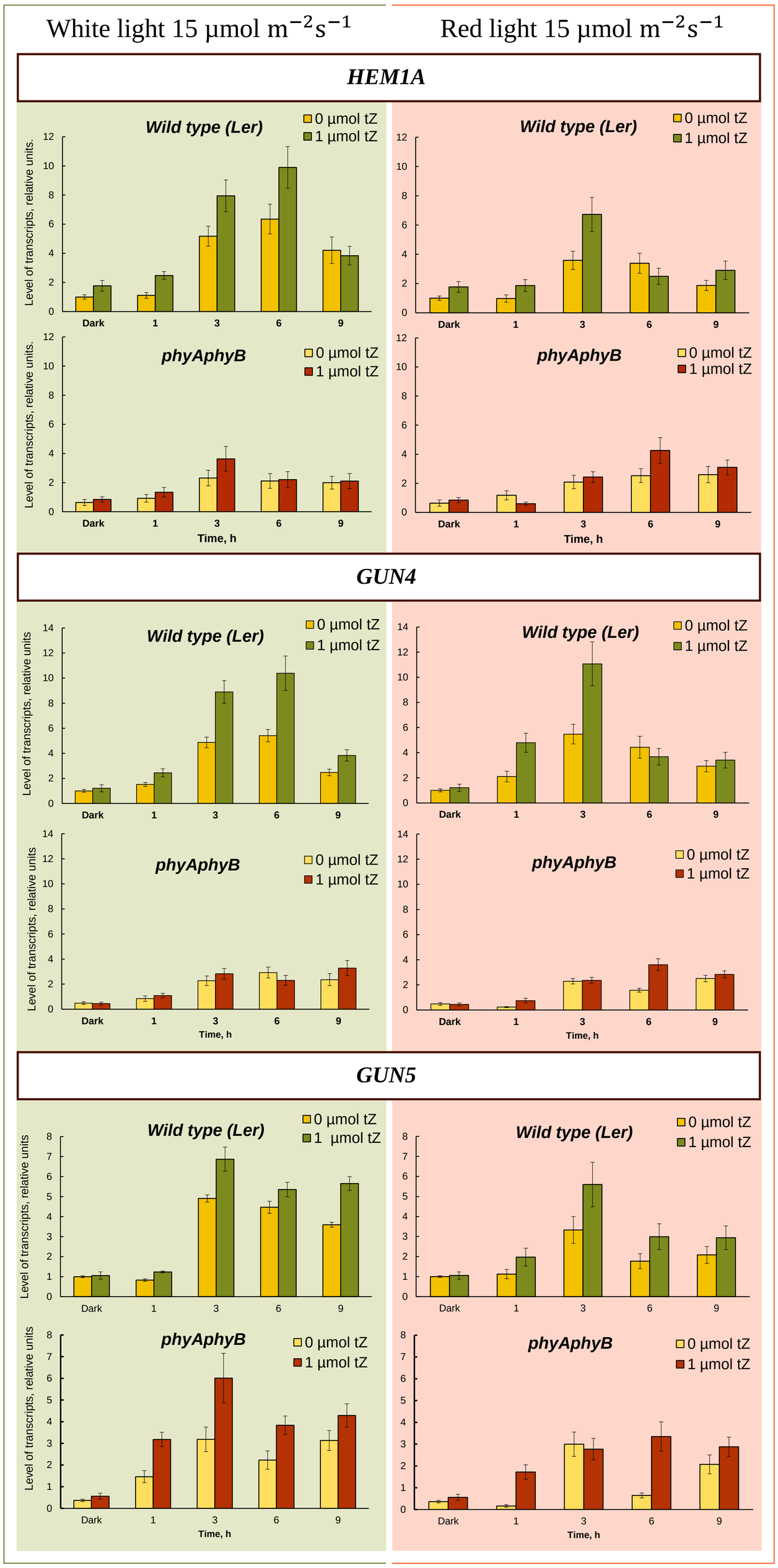
Red light and phytochromes A and B regulate the expression of *HEM1A*, *GUN4* and *GUN5* genes during cytokinin-dependent de-etiolation.

In experiments on de-etiolation both white and red light illumination stimulated the accumulation of transcripts of *HEM1A*, *GUN4* and *GUN5* genes.

Under white light cytokinin treatment resulted in increased mRNA levels of tested genes in WT seedlings compared to control without CK. When the phytochrome A and B receptors were knocked out, no activating effect of CK on *HEM1A* and *GUN4* genes was observed under white light, but positive regulation of *GUN5* gene by this phytohormone was maintained.

Illumination of the seedlings with red light resulted in lower mRNA levels of *HEM1A*, *GUN4* and *GUN5* genes in *phyAphyB* mutant than in WT. Exogenous CK stimulated an increased transcripts accumulation of tested genes in WT seedlings during the first hours of de-etiolation under red light exposure. In the absence of phytochromes A and B, CK did not regulate the mRNA content of *HEM1A*, *GUN4*, and *GUN5* genes in *phyAphyB* seedlings under red light, except points "1 h" and "6 h", when a weak response to the hormone was observed.

Results



Conclusion

During the de-etiolation of *Arabidopsis thaliana* seedlings phytochromes A and B involved in the positive effect of cytokinin, mediated by the regulation of mRNA levels of genes encoding enzymes of chlorophyll biosynthesis: *HEM1A* and *GUN4* under white light and *HEM1A*, *GUN4*, *GUN5* under red light.

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