Regulation of phototropin expression by abiotic and biotic factors

ABSTRACT

Phototropins are UV and blue light photoreceptors, which are responsible for the growth and development of plants in changing light conditions as well as the optimization of photosynthetic efficiency. In the *Arabidopsis thaliana* genome, two genes encode phototropins: *PHOT1* and *PHOT2.* Light regulation of phototropin expression is maintained at every stage of plant development. The *PHOT1* transcript is downregulated, whereas the *PHOT2* transcript is upregulated by light. Phototropins expression can be regulated on the transcriptional level based on *in silico* analysis of phototropin promoter sequences, which are rich in light-regulated motifs and transcription factors binding sites. The results of the Yeast One Hybrid assay revealed putative transcription factors that bind to phototropin promoter regions.

The first aim of the study was to analyse the activity of phototropin promoter regions in transgenic lines of *A. thaliana* with the GUS reporter gene under the control of *PHOT1* and *PHOT2* putative promoter sequences in different light conditions and at several developmental stages of the plant. Additionally, *PHOT1* and *PHOT2* mRNA levels were evaluated in wild-type plants growing in the same conditions. We show that the light-regulated activity of phototropin promoters matched the expression levels of phototropin genes. In addition, the activity of *PHOT1* and *PHOT2* promoters was distinct within organs and this fact may be associated with physiological processes controlled by phototropins at the plant developmental stages.

The second aim of the study was to confirm the role of transcription factors in the regulation of phototropin expression. *PHOT1* and *PHOT2* transcript levels were investigated in *A. thaliana* T-DNA mutant lines of transcription factor coding genes in two distinct light conditions: in the darkness and after illumination with white light. In mutants of transcription factor genes phototropin light regulation was maintained, in particular, dark-adapted plants had elevated levels of *PHOT1* mRNA whereas those illuminated with white light had decreased levels of *PHOT2* mRNA. We did not observe the differences in the expression levels of phototropins in T-DNA mutant lines of *ptf-1, tcp2, anac102, arf19, eil2,* and *athb-12* when compared to wild-type plants. Moreover, no differences in phototropin expression levels were detected between ARF19 mutant lines: *arf19-2* and *arf7arf19,* and wild-type plants growing in the presence of exogenous auxins. Leaf blade downward curling (LEI index) was also evaluated in T-DNA mutant lines of TF genes: *ptf-1, tcp2, anac102, arf19, eil2, athb-1* along

with double mutant lines of *phot1phot2* and *arf7arf19*. The leaves of *phot1phot2* and *arf7arf19* were curled downward to a greater extent, while the *tcp2* leaf blade was less curled than in wild-type plants. We also investigated whether transcription factors are capable of transactivating *pPHOT2*::GUS using transient transformation of *Nicotiana benthamiana* leaves. Following the transformation, tobacco plants were either incubated in the photoperiod or in the darkness. We did not observe the activation of the *PHOT2* promoter by transcription factors *in planta.* However, we noticed that the *PHOT2* promoter is highly activated by light in *N. benthamiana* leaves. With the competitive electrophoretic mobility shift assay (cEMSA) we show that DNA binding domains of TCP2 and ANAC102 interacted with the *PHOT2* promoter *in vitro.* Among transcription factors identified in the Yeast One-Hybrid assay TCP2 and ANAC102 possibly regulate phototropin expression.

The third part of the study examines the role of biotic factors – endophytic fungi on the regulation of phototropin expression and plant growth and development in light supplemented with UV-A radiation. Our data shows that *PHOT2* expression was increased in plants inoculated with endophytic fungi. Two of the examined microorganisms: *Paraphoma chrysantehmicola* and *Mucor* inhibited the growth of *A. thaliana* shoots. Plants growing in the presence of UV-A had shorter root systems before inoculation (8^{th} day of plant growth), but after 10 additional days, the effect was no longer significant. The impact of endophytic fungi on phototropin expression is small in magnitude, however, we observed an increase in the expression level of *PHOT2.* UV-A radiation did not influence the colonisation of *A. thaliana* by endophytic fungi.

In summary, this work demonstrated that the transcription step is important in the regulation of phototropin expression. Moreover, among examined transcription factors, TCP2 and ANAC102 bound to fragments of *PHOT2* promoter *in vitro*. Biotic factors such as endophytic fungi had a minor role in the regulation of phototropin expression.