

Full Length Research Paper

The effects of cryptochrome photoreceptors on root growth in *Arabidopsis*

Jianxin Zeng, Qiming Wang, Jianzhong Lin, Keqin Deng, Dongying Tang, Xiaoying Zhao and Xuanming Liu*

Bioenergy and Biomaterial Research Center Bioscience and Biotechnology Institute, Hunan University, Changsha 410082, China.

Accepted 8 May, 2009

Cryptochrome mutants were examined to assess their potential roles in root growth. Compared to root elongation of wild type, the cryptochrome mutant seedlings showed increased root elongation in blue light. *Cry1cry2* seedlings have the longest root elongation and *cry1* seedlings showed slightly increased root elongation than *cry2* seedlings. During this process, CRY1 play the largest role and CRY2 play lesser role, indicating that CRY1 and CRY2 act synergistic role in root growth. The auxin transport inhibitor NPA abolished the differential effect of cryptochromes on root growth. Moreover, expression of *PIN1* in CRY1 over-expression (*Oecry1*) transgenic seedlings was half of that in wild type. Taken together, these results revealed that the cryptochrome photoreceptors regulate root growth by changing auxin transport.

Key words: *Arabidopsis*, auxin, blue light, cryptochrome, root elongation.

INTRODUCTION

Light is the most important environmental factor for plant growth and development. Plants contain two cryptochrome photoreceptors (CRY1 and CRY2) that mediate blue-light dependent inhibition of hypocotyl elongation (Ahmad and Cashmore, 1993), promotion of leaf and cotyledon expansion (Jackson and Jenkins, 1995; Neff and Chory, 1998), flowering time (Bagnall et al., 1996; Guo et al., 1998), chlorophyll and anthocyanin synthesis (Ahmad et al., 1995) and other processes. The root tissue is not normally exposed to light, but many photo-responses are identified to occur in root tissue. Earlier reports have demonstrated that CRY1 promoted the primary root elongation whereas CRY2 has an opposite effect (Canamero et al., 2006). Another work indicated that redistribution of CRY1 between the nucleus and cytoplasm affects the root elongation, and also showed

that CRY1 suppressed root growth in high fluence-rate (Guosheng and Edgar, 2007). This paper examines the effects of cryptochrome photoreceptors on root growth and shows that CRY1 and CRY2 act a synergistic role in regulation of primary root elongation in *Arabidopsis*.

MATERIALS AND METHODS

Plant materials and growth conditions

The wild type (Wt, col-4) and *Arabidopsis* mutants (*cry1*, *cry2*, and *cry1cry2*) were in the Columbia background as described earlier by Mockler et al. (2003). *Oecry1* (CRY1 over-expression, Col) and *Oecry2* (CRY2 over-expression, Col) transgenic lines were as described earlier by Xuhong et al. (2007). Seeds were sown on 0.8% agar plates containing Murashige and Skoog medium with 1.5% sucrose. They were stratified at 4°C in the dark for 4 days and transferred to a temperature controlled (22 - 23°C) growth room under defined light conditions. Blue and red light used are LED-B and LED-R, respectively.

Root and cell measurements

Seedlings grew under blue light (80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 7 days. Then, the seedlings with root intact were placed on microscope slide; the root and cell length were measured under an optical microscope

*Corresponding author. E-mail: sw_xml@hnu.cn. Tel.: +86(0) 731-8821721. Fax: +86(0) 731-8822606.

Abbreviations: IAA, Indoleacetic acid; NPA, 1-naphthylphthalamic acid; PIN1, Pin-formed1; PIN2, Pin-formed2.

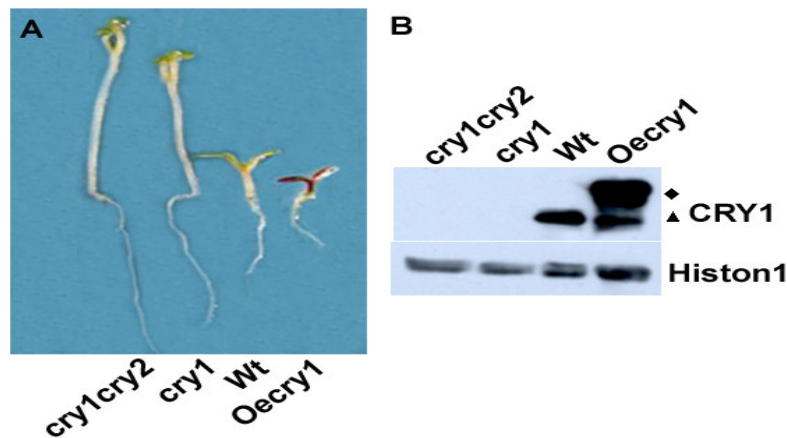


Figure 1. Root growth in cryptochrome mutants under blue light. Seedlings of Wt, *cry1*, *cry1cry2*, and *Oecry1* were grown in blue light ($80 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 7 days. Representative phenotypes (A) and Western blot analysis of CRY1 Protein levels (B). \blacklozenge indicates CRY1-GFP fusion proteins \blacktriangle indicates the endogenous CRY1 protein.

according to the methods of Canamero et al. (2006).

Western blot analysis

Proteins were extracted in $4 \times$ SDS sample buffer, fractionated in 10% SDS-PAGE mini gels. They were blotted to PVDF membrane, washed, probed with CRY1 primary antibodies and the secondary antibody conjugated with horseradish peroxidase. Blotting with histone1 was used to verify equal loading.

RNAs isolation and quantitative real-time PCR

Total RNAs were isolated using Puprep RNA easy mini kit (Ambiogen Life Tech Ltd). DNA-free RNA was obtained by RQ 1 Dnase I treatment according to the manufacturer's instructions (Promega). Real-time Q-PCR was used to quantitatively measure the expression of *PIN1* (AT1g73590) and *PIN2* (AT5g57090) genes. *ACT7* (At5g09810) was used as the internal control. SYBR green-sequence detection reagents (Invitrogen, USA), Taq polymerase, sense and anti-sense primers were assayed on an iCycler iQ Detection System (stratagene). The reaction was performed in M x 3000p (stratagene) under the following conditions: one cycle of 95°C for 10 min and then 40 cycles at 95°C for 30 s, 55°C for 30 s and 72°C for 30 s. The primers used for Q-PCR were *ACT7F*:5'-ATCCCTCAGCACCTTCCAAC-3', *ACT7R*:5'-ACAAACTCACCACCACGAAC-3'; *PIN1F*:5'-TGCGGTGATATTTGGGATGTT GA-3', *PIN1R*:5'-CTGCTTCTGATTTAATTTGTGGGTTT-3'; *PIN2F*:5'-TATATTCGGAATGCTGGTTGCTTTG-3', *PIN2R*:5'-CCATACACCTAAGCCTGACCTGGAA-3'

RESULTS AND DISCUSSION

Cryptochrome photoreceptors affect primary root growth in *Arabidopsis*

Various cryptochrome mutants were examined to ascertain their potential roles in root growth. Firstly, seedlings

of wild-type *cry1*, *crycry2* and *Oecry1* grew under blue light ($80 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 7 days. Then, clear differences in root elongation were detectable. *Cry1* and *cry1cry2* seedlings showed increased root growth whereas *Oecry1* seedlings showed significantly reduced root growth compare with wild type (Figure 1A). Western blot analysis revealed that CRY1 protein accumulation in *Oecry1* seedlings exceeding wild type seedlings, but not detectable in *cry1* and *cry1cry2* mutant seedlings (Figure 1B). Therefore, the root elongation may correlate with cryptochrome photoreceptor.

Differences in the root growth were further quantified under multiple light conditions (Figure 2). Interestingly, light promoted the root elongation with fluence-rate increasing in all seedlings. There was no differences detectable between cryptochrome mutants and wild type when growth in red light (Figure 2B), where the cryptochrome photoreceptors are inactive. By contrast, cryptochrome photoreceptors have significant effect on root growth in blue light. The *cry1cry2* double mutant seedlings shown increased root elongation than wild type, *cry1* and *cry2* single mutant seedlings (Figure 2A), the *cry1* seedlings exhibited slightly increased root elongation than *cry2* seedlings (Figure 2A), indicating that cryptochrome photoreceptors CRY1 and CRY2 suppressed root elongation in blue light. During this process, CRY1 plays the largest role and CRY2 plays lesser role, just as did inhibition of hypocotyl elongation (Lin et al., 1998), suggesting that CRY1 and CRY2 act synergistic role in root elongation. On the other hand, *Oecry1* and *Oecry2* transgenic seedlings exhibited reduced root elongation relative to wild type, consistent with cryptochrome photoreceptors play an inhibition role in root elongation.

To ascertain whether the inhibition role was caused by a reduction in cell length or cell number, the averaged

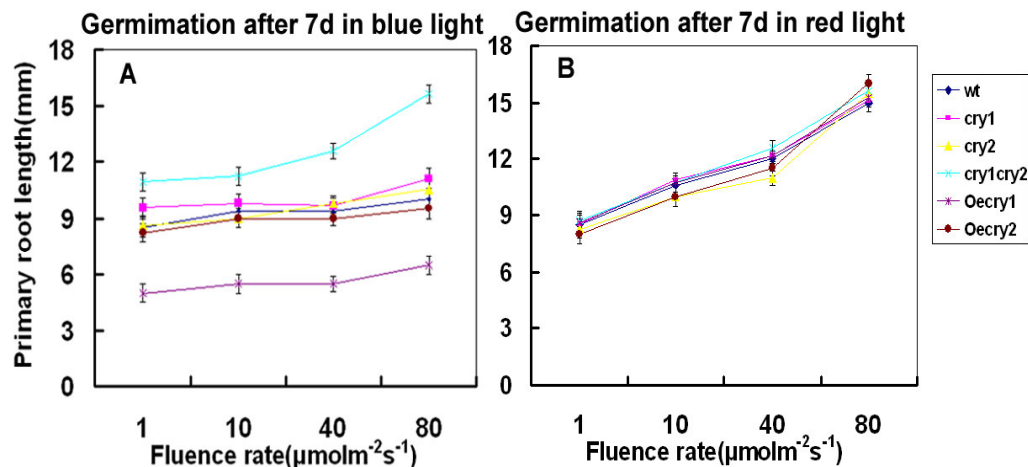


Figure 2. Effects of cryptochrome photoreceptors on root growth under defined intensity of blue light (A) and red light (B) for 7 days. Error bars represent the standard error.

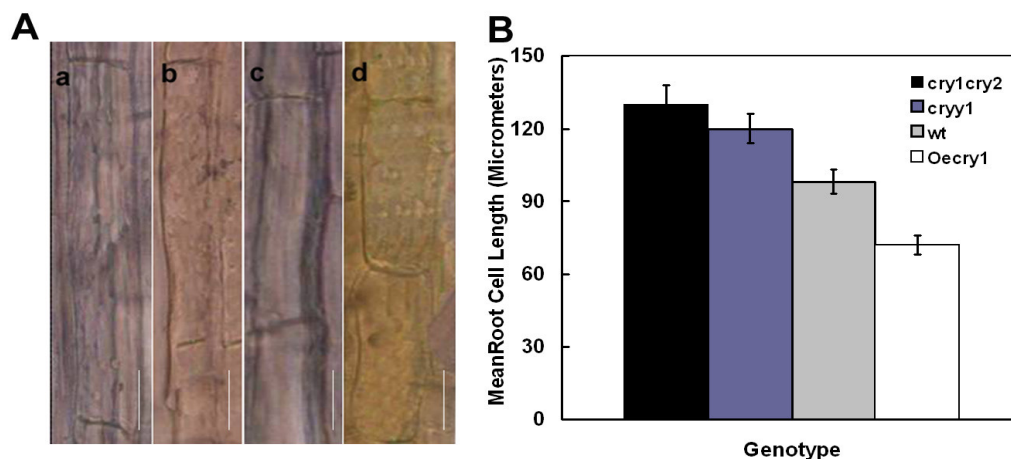


Figure 3. Effect of cryptochrome photoreceptors on root cell elongation. Mean root cell length in *cry1cry2* (a), *cry1* (b), Wt (c), and *Oecry1* (d) seedlings grown under blue light ($80 \mu\text{molm}^{-2}\text{s}^{-1}$) for 7 days. Scale bars in this images represent 25 μm . Error bars represent the standard error.

epidermal cell length of wild type, *cry1*, *cry1cry2* and *Oecry1* seedlings were measured when growth in blue light ($80 \mu\text{molm}^{-2}\text{s}^{-1}$) for 7 days (Figure 3). Compared to the wild type, average cell length was significantly reduced in *Oecry1* roots whereas that in *cry1* and *cry1cry2* seedlings was increased (Figure 3). These differences in cell elongation are consistent with the differences in primary root length in these seedlings (Figure 3). Therefore, the effect of cryptochrome photoreceptors on root growth can be explained by changing in cell length.

Cryptochrome photoreceptors regulate primary root elongation by changing auxin transport

Root growth was highly dependent on auxin. To ascertain whether auxin signaling participated in cryptochrome-

dependent inhibition of root elongation, the root elongation growth of wt, *Oecry1* and *cry1*, *cry1cry2* mutant seedlings were assayed in the presence of NPA (Figure 4). Root elongation growth in blue light was suppressed by NPA, particularly at 10 μM NPA. Furthermore, there was no difference in root growth detectable at 10 μM NPA between wild type, *Oecry1* and *cry1*, *cry1cry2* mutant *Arabidopsis* seedlings.

Transcript expression of auxin polar transport efflux carriers, *PIN1* (*pin-formed1*) and *PIN2* (*pin-formed2*) genes, was further quantified using real-time Q-PCR methods (Figure 5). Compared to the wild type, the expression of *PIN1* in *Oecry1* seedlings was halved whereas that in *cry1* and *cry1cry2* seedlings was increased. By contrast, there was no difference in expression of *PIN2* detectable between cryptochrome mutants and wild type. These results indicating that cryptochrome

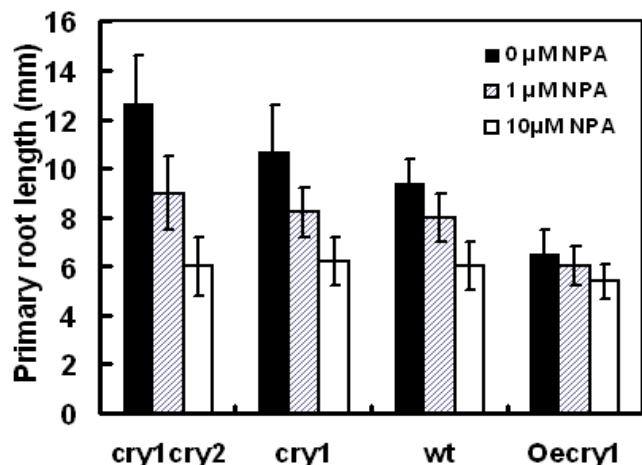


Figure 4. Inhibition of root elongation by auxin inhibitor NPA. Seedlings grew on MS salts medium supplemented with 0, 1, or 10 μ M NPA under blue light ($80 \mu\text{molm}^{-2} \text{s}^{-1}$) for 7 days prior to measure root length. Error bars represent the SD.

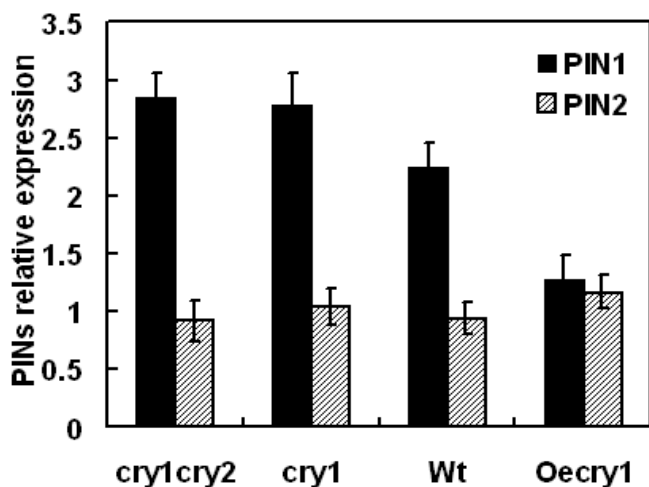


Figure 5. Q-PCR analysis of mRNA expression. The real-time Q-PCR analysis of mRNA expression changes of the *PIN1* and *PIN2* genes in *Arabidopsis* seedlings. Data are means (\pm SD) of three Q-PCR reactions of the same of RNA samples. RNAs were prepared from 7 d old seedlings growth in blue light ($80 \mu\text{molm}^{-2} \text{s}^{-1}$).

photoreceptors suppressed auxin transport in *Arabidopsis*.

In this work, data revealed that the cryptochrome photoreceptors CRY1 and CRY2 act a synergistic role in regulation of root growth, which conflicted with results given by Canamero et al. (2006). A better explanation is due to different ecotypes of *Arabidopsis* seedlings and growth condition analyzed in this works. Moreover, the CRY1 mainly mediates high-intensity blue light responses (Lin, 2000; Kleine et al., 2007). The *cry1* mutant seedlings showed increased root elongation (Figure 1A, and 2A) consisting with CRY1 play inhibition roles at higher

fluence-rates (Guosheng and Spalding, 2007). Earlier reports have shown that *cry1cry2* double mutant plants were more drought-tolerant than wild type, *cry1*, and *cry2* single mutant (Mao et al., 2005), that means the *cry1cry2* double mutant seedlings have strong root formation, consistent with our results that the *cry1cry2* seedlings have the longest primary root (Figure 2A). Earlier reports have demonstrated that NPA intensifies the inhibition of hypocotyl growth under light (Jensen et al., 1998), pointing to the involvement of light in polar auxin transport. In this work, the auxin transport inhibitor NPA abolished the differential effect of cryptochromes on root growth (Figure 4) and the expression of *PIN1* in *Oecry1* seedlings were halved compared with wild type (Figure 5). Taken together, these results suggested that the cryptochrome photoreceptors regulate root growth by changing auxin transport.

ACKNOWLEDGEMENTS

This work is supported by the National Natural Science Foundation of China (30770200), and the 211/985 higher education enhancement funds to Hunan University.

REFERENCES

- Ahmad M, Cashmore AR (1993). HY4 gene of *Athaliana* encodes a protein with characteristics of a blue-light photoreceptor. *Nat.* 366: 162-166.
- Ahmad M, Lin C, Cashmore AR (1995). Mutations throughout an *Arabidopsis* blue -light photoreceptor impair blue-light-responsive anthocyanin accumulation and inhibition of hypocotyl elongation. *Plant J.* 8: 653-658.
- Bagnall DJ, King RW, Hangarter RP (1996). Blue-light promotion of flowering is absent in *hy4* mutants of *Arabidopsis*. *Planta.* 200: 278-280.
- Canamero RC, Bakrim N, Bouly JP, Garay A, Dudkin EE, Habricot Y, Ahmad M (2006). Cryptochrome photoreceptors *cry1* and *cry2* antagonistically regulate primary root elongation in *Arabidopsis thaliana*. *Planta.* 224: 995-100.
- Enhancement of blue-light sensitivity of *Arabidopsis* seedlings by a blue light photoreceptor cryptochrome 2. *Proc. Natl. Acad. Sci. USA.* 95: 2686-2690.
- Guo H, Yang H, Mockler TC, Lin C (1998). Regulation of flowering time by *Arabidopsis* photoreceptors. *Sci.* 279: 1360-1363.
- Guosheng Wu, Spalding EP (2007). Separate functions for nuclear and cytoplasmic cryptochrome 1 during photomorphogenesis of *Arabidopsis* seedlings. *Proc. Natl. Acad. Sci. USA.* 104:18813-18818.
- Jackson JA, Jenkins GI (1995). Extension-growth responses and expression of flavonoid biosynthesis genes in the *Arabidopsis hy4* mutant. *Planta.* 197: 233-239.
- Jensen PJ, Hangarter RP, Estelle M (1998). Auxin Transport Is Required for Hypocotyl Elongation in Light-Grown but Not Dark-Grown *Arabidopsis*. *Plant Physiol.* 116: 455-462.
- Kleine T, Kindgren P, Benedict C, Hendrickson L, Strand A (2007). Genome-Wide Gene Expression Analysis Reveals a Critical Role for CRYPTOCHROME1 in the Response of *Arabidopsis* to High Irradiance. *Plant Physiol.* 144: 1391-1406.
- Lin C, Yang H, Guo H, Mockler T, Chen J, Cashmore AR (1998). Lin C. (2000). Plant blue-light receptors. *Trends Plant Sci.* 5: 337-342.
- Mao J, Zhang Y-C, Sang Y, Li Q-H, Yang H-Q (2005). A role for *Arabidopsis* cryptochromes and COP1 in the regulation of stomatal opening. *Proc. Natl. Acad. Sci. USA.* 102:12270-12275.
- Mockler T, Yang H, Yu X, Parikh D, Cheng Y-c, Dolan S, Lin C (2003).

- Regulation of photoperiodic flowering by Arabidopsis photoreceptors. Proc. Natl. Acad. Sci. USA. 100:2140-2145.
- Neff MM, Chory J (1998). Genetic interactions between phytochrome A, phytochrome B and cryptochrome 1 during Arabidopsis development. Plant Physiol. 118: 27-35.
- Xuhong Y, Shalitin D, Liu X, Maymon M, Klejnot J, Yang H, Lopez J, Zhao X, Bendehakalu KT, Lin C (2007). Derepression of the NC80 motif is critical for the photoactivation of Arabidopsis CRY2. Proc. Natl. Acad. Sci. USA. 104: 7289-7294.