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Article Addendum

Plant cryptochromes employ complicated mechanisms for subcellular localization and are involved in pathways apart from photomorphogenesis

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Cryptochromes (CRYs) are photoreceptors mediating developmental responses to blue light throughout the life of plants. Function and signal transduction of CRYs in photomorphogenesis have been well characterized in Arabidopsis. Studies on rice CRYs demonstrate that monocots CRYs may function similarly to their Arabidopsis counterparts. However, there is inconsistency in subcellular localization of CRYs in different species and little has been known about the effects of environmental cues on CRYs except for light. We recently reported that TaCRY1a of monocot wheat displays a light-responsive nucleocytoplasmic shuttling pattern similar to Arabidopsis CRY1 but differs from AtCRY1 and OsCRY1 by containing nuclear localization domains in both its N and C termini and the sequence for nuclear export in its N-terminal domain. *TaCRY1a* and *TaCRY2* are transcriptionally regulated by osmotic stress/ABA and overexpression of *TaCRY1a-GFP* and *TaCRY2-GFP* led to higher sensitivity to high salinity, osmotic stress and ABA treatment. Mining wheat EST database provided additional clues for CRY's involvement in pathways apart from photomorphogenesis.

Blue light receptor cryptochorome proteins (CRYs) in model plant species have been extensively investigated for their roles in photomorphogenesis and photoperiodic timing. Arabidopsis CRY1 (AtCRY1) is the primary receptor mediating blue light regulation of seedling de-etiolation and circadian clock;[1-3] AtCRY2 works redundantly with AtCRY1 under relatively low light and plays a key role in the control of photoperiodic flowering.[4,5] Rice CRYs function similarly to their Arabidopsis counterparts and their corresponding N-terminal domains are functionally interchangeable.[6-8] This indicates conserved CRY signaling in monocots and dicots. However, the underlying mechanisms may not be identical. For example, AtCRY2 is a constitutively nuclear localized protein and AtCRY1 shuttles between nucleus and cytoplasm in a light-dependant manner;[9-11] while rice CRY1 (OsCRY1) distributes both in nucleus and cytoplasm irrespective of the light condition.[6] This raises an interest to explore the subcellular localization of CRYs in more plant species and its relation to the functions.

In a recent paper,[12] we reported the cloning and characterization of two *CRY* genes, *TaCRY1a* and *TaCRY2*, from hexaploid wheat, an important monocot cereal crop. We presented evidence that TaCRY2, like AtCRY2,[9,10,13] is a nuclear protein degrading when exposed to light. Similar to AtCRY1, TaCRY1a is a light-dependent nucleocytoplasmic shuttling protein. But they differ in that TaCRY1a possesses nuclear localization domains in both its N and C termini and carries sequence for nuclear export in its N-terminal domain. Through examining sub-cellular distributions of various TaCRY1a segment-GFP fusions, we determined that the N-terminal segment (AA136-260) is important for both nuclear targeting and blue light responsive nuclear export. Interestingly, even though the AA136-260 segment alone is capable for nuclear import and export, a complete N-terminal domain is necessary for nuclear export when the highly hydrophilic C-terminus of TaCRY1a exists.[12] These results, together with other reports, demonstrate that plant CRYs utilize complicated mechanisms to regulate their subcellular localization. A few studies have indicated that CRY homodimerization[14] and partner binding[8,15] might be required for correct subcellular localization of Arabidopsis and rice CRY1 proteins.

Growth and development of plants is the expression of interactions between genes and the environment. It has been known that light quality[16,17] and circadian clock[17,18] affect the expression of *CRY* genes. To investigate whether environmental stresses affect the behavior of CRYs, we examined the expression of *TaCRY1a* and *TaCRY2* under various treatments. *TaCRY1a* and *TaCRY2*, especially the latter, are clearly transcriptionally regulated by osmotic stress and ABA in roots and germinating embryos. By analyzing the responses of transgenic Arabidopsis to osmotic stress, we found that overexpression of *TaCRY1a* or *TaCRY2* resulted in decreased seed germination,

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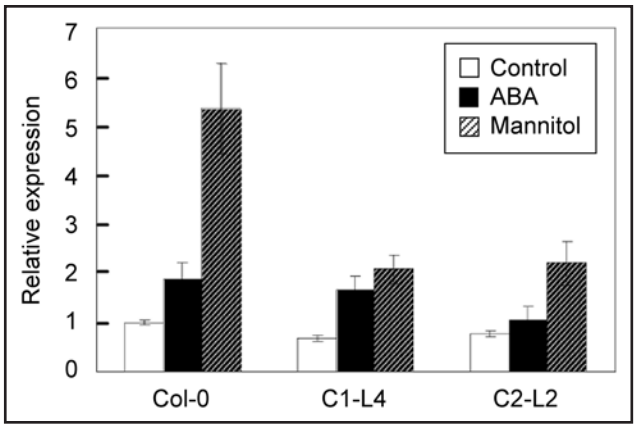

Figure 1. Expression of *ABA2* in Col-0 and transgenic Arabidopsis lines overexpressing *TaCRY* genes. Total RNA was extracted from 15-day-old T_2 transgenic seedlings grown on MS plates (control), on MS plates for 12 days and then on MS plates supplemented with 300 mM mannitol or 10 μM ABA for three days. Error bars indicate SD. Col-0: wild type; C1-L4: *TaCRY1a-GFP* overexpressing line; C2-L2: *TaCRY2-GFP* overexpressing line.

impaired cotyledon opening, and less tolerance to ABA and high salinity at the vegetative growth stage, suggesting that the high level of *CRY* expression compromises plant resistance to osmotic stress. In the attempt to uncover the mechanisms by which CRY signals affect osmotic stress/ABA responses, we examined in the transgenic lines the expression of some marker genes for stress responses, and found that the upregulation of both *RD29A* and *ADH1* under osmotic stress/ABA was impaired.[12] Since their upregulation has been positively associated with stress tolerance,[19,20] their decreased induction is in accordance with the increased susceptibility of these transgenic lines to osmotic stresses. Besides these two genes, a few ABA biosynthesis/metabolism-related genes in the transgenic lines were also examined for their expression under the osmotic stress/ABA treatment. *ABA2*, an ABA biosynthesis fine-tuning gene, was less induced under the ABA treatment in the line overexpressing *TaCRY1a-GFP* and under the mannitol treatment in lines overexpressing either *TaCRY1a-GFP* or *TaCRY2-GFP* (Fig. 1).

Involvement of CRY proteins in stress responses may exist in a broader extent. By mining wheat EST database with 1,050,000 ESTs (Genbank 159th release) from tissues under various growth conditions, we found that the *TaCRY2* transcript in roots has ~ten-fold increase under desiccation compared with that under normal growing conditions. In a 24267-clone cDNA library prepared with crown and leaf tissues after a long exposure to low temperature, the *TaCRY1a* ESTs increased 7-fold. *TaCRY2* EST frequency also increased in leaves after *septoria tritici* infection. Thus, the crosstalk of CRY signalling with other pathways should be more extensively explored.

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