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Two in one: Splice isoforms of a HY5-homolog in rice regulate plant height in light and darkness

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Plants possess intricate mechanisms to adjust their growth and development in response to the changes in the quality and quantity of environmental light. Specialized photoreceptors perceive discrete wavelengths of light and transduce the signals to the nucleus, causing widespread changes in the gene expression mediated by an array of transcription factors (Yadav et al. 2020). Seedlings growing in light form short hypocotyls and open and expanded cotyledons (photomorphogenesis), whereas in darkness they form elongated hypocotyls with an apical hook and closed cotyledons (skotomorphogenesis) (Yadav et al. 2020).

Although the homologs of several light signaling components in Arabidopsis are present in monocots, their function remains poorly understood. ELONGATED HYPOCOTYL 5 (HY5) is a central transcription factor in Arabidopsis that promotes photomorphogenesis in seedlings (Gangappa and Botto 2016). Three homologs of Arabidopsis *HY5* (*AtHY5*) have been identified in rice: *OsbZIP1*, *OsbZIP18*, and *OsbZIP48* (Burman et al. 2018). However, knowledge about the function of these genes is scarce compared with their Arabidopsis counterpart. In this issue of *Plant Physiology*, Bhatnagar et al. (2023) report that *OsbZIP1* undergoes light-regulated alternative splicing to make 2 isoforms that play conserved as well as novel functions in rice.

While amplifying the full-length coding sequence of *OsbZIP1* using cDNA, Bhatnagar et al. (2023) identified an alternatively spliced product of 345 bp in addition to the predicted 612-bp product. The full-length and the alternatively spliced isoforms were named *OsbZIP1.1* and *OsbZIP1.2*, respectively. Immunoblot analyses confirmed that both spliced isoforms make protein products. Whereas the coding sequence of *OsbZIP1.1* is constituted of 3 exons, *OsbZIP1.2* retains only partial regions of the first and second exons along with the complete third exon (Fig. 1A).

The authors then investigated the light-responsive regulation of the *OsbZIP1* isoforms. Light favored the accumulation of *OsbZIP1.1* transcripts, whereas darkness favored the accumulation of *OsbZIP1.2*. The accumulation of OsbZIP1.1 protein was decreased in darkness compared with light conditions, whereas OsbZIP1.2 accumulated in similar levels in both light and dark conditions. In Arabidopsis, HY5 protein levels are tightly controlled via 26S proteasome by the E3 ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) (Osterlund et al. 2000). Similar to AtHY5, OsbZIP1.1 harbors a basic leucine zipper (bZIP) domain in the C-terminal region and a COP1-interaction motif in the N-terminal region, whereas OsbZIP1.2 carries only the C-terminal bZIP domain and lacks the N-terminal COP1-interaction motif (Fig. 1B). Bhatnagar et al. (2023) asked whether the difference in the protein accumulation of OsbZIP1.1 and OsbZIP1.2 under darkness is because of the potential difference in their interaction with OsCOP1. Indeed, OsbZIP1.1 physically interacted with OsCOP1 and underwent degradation via 26S proteasome under dark conditions. The dark-stable OsbZIP1.2 did not interact with OsCOP1, which is in consonance with the absence of the COP1-interaction motif in its N-terminal region.

AtHY5 is known to undergo phosphorylation in its COP1-interacting domain by CASEIN KINASE 2 (CK2) (Hardtke et al. 2000). Immunoblot analyses indicated that OsbZIP1.1 potentially undergoes phosphorylation, whereas OsbZIP1.2 does not. A series of in vitro and in vivo experiments revealed that the catalytic subunit of the rice CK2 (OsCK2α3) interacts with and phosphorylates OsbZIP1.1, which enhances the stability of OsbZIP1.1 by lowering its affinity for interaction with OsCOP1.

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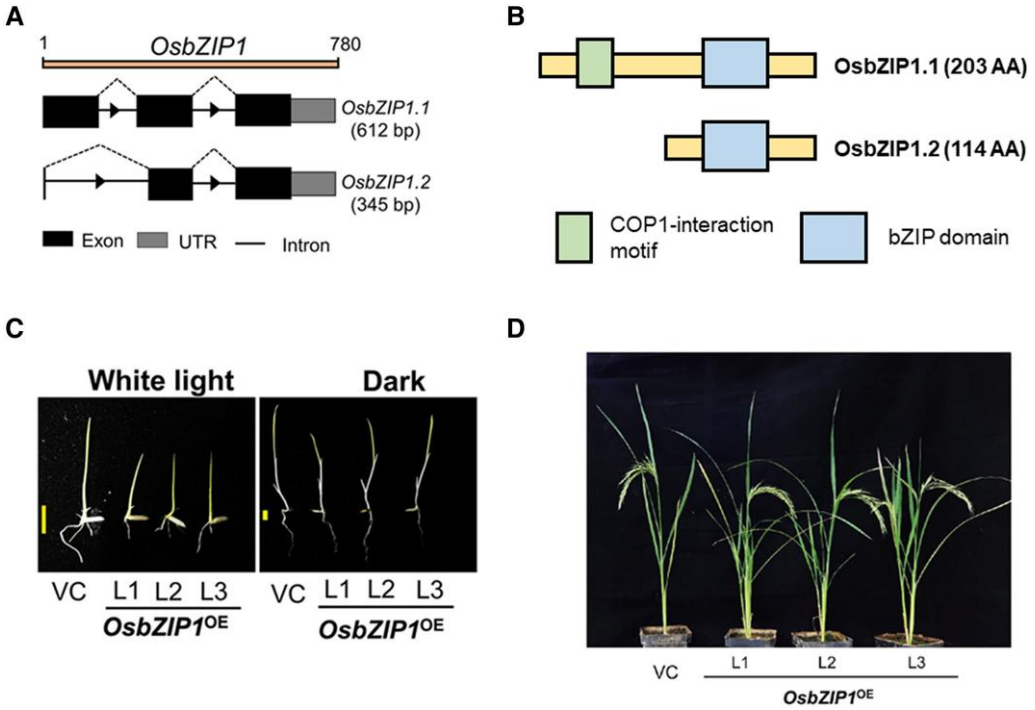

Figure 1. A) Schematic diagram of splicing patterns resulting in *OsbZIP1.1* and *OsbZIP1.2*. **B)** Schematic representation of the domain organization of OsbZIP1.1 and OsbZIP1.2 proteins. **C, D)** Five-day-old *OsbZIP1*^{OE} seedlings grown in white-light and dark conditions **(C)** and *OsbZIP1*^{OE} plants at reproductive stage **(D)** showing decreased height as compared with control plants. Scale bar represents 1 cm. L1, L2, L3, lines from independent transgenic insertions; OE, overexpressor; UTR, untranslated region; VC, vector. control; Adapted from Bhatnagar et al. (2023).

Complementing with either *OsbZIP1* genomic DNA or *OsbZIP1.2* coding sequence rescued the defective photomorphogenic development in Arabidopsis *hy5* mutant, indicating their functional homology with AtHY5. To understand the function of OsbZIP1 in rice, the authors generated stable transgenic lines in which both the isoforms are either ectopically overexpressed (*OsbZIP1*^{OE}) or knocked down using RNA interference (*OsbZIP1*^{KD}). Under white-light as well as dark conditions, *OsbZIP1*^{OE} and *OsbZIP1*^{KD} seedlings were shorter (stronger photomorphogenic response) or taller (weaker response), respectively, compared with the control seedlings (Fig. 1C). The authors also studied the effects of red, far-red, and blue monochromatic lights on the growth of *OsbZIP1*^{OE} and *OsbZIP1*^{KD} seedlings. In all 3 wavelengths, *OsbZIP1*^{OE} showed a decrease in seedling height, root length, and lengths of the first and second leaves, whereas *OsbZIP1*^{KD} showed an opposite phenotype.

Bhatnagar et al. (2023) also investigated the role of OsbZIP1 at later stages of development in rice. The adult *OsbZIP1*^{OE} plants were significantly shorter than the control plants primarily due to a decrease in the lengths of the last and penultimate internodes (Fig. 1D). Although *OsbZIP1*^{KD} plants did not show any changes in the overall height, their flowering time was significantly delayed compared with the control plants, indicating that besides its conserved role in seedling photomorphogenesis, *OsbZIP1* has also undergone neofunctionalization in rice.

Alternative splicing (AS) allows organisms to enhance protein diversity in response to dynamic changes in the environment. Bhatnagar et al. (2023) show that OsbZIP1 regulates seedling height in darkness through its alternatively spliced dark-stable isoform. Several phytochrome-interacting splicing factors have been identified in Arabidopsis and moss that modulate light-regulated AS (Kathare and Huq 2021). Retrograde signaling and changes in the kinetics of RNA polymerase II also play a role in AS under light/dark conditions (Godoy Herz et al. 2019). The detailed mechanism behind the dark-induced AS of *OsbZIP1* awaits further investigation.

The downstream mechanisms by which OsbZIP1 mediates skoto- and photo-morphogenesis in rice remains to be understood in detail. AtHY5 cannot self-activate but relies on co-factors like B-box (BBX) proteins to activate the transcription of key photomorphogenesis genes (Bursch et al. 2020). It will be interesting to explore if a similar association between HY5-homologs and BBX proteins operates in rice.

Semi-dwarfism is a key attribute of "green revolution" rice varieties (Ferrero-Serrano et al. 2019). The overexpression of *OsbZIP1* provides an effective way to decrease plant height without affecting other important agronomic traits such as flowering time, panicle length, and number of florets. OsbZIP48, another HY5-homolog in rice, has been shown to reduce plant height by promoting GA biosynthesis (Burman et al. 2018). However, the overexpression of *OsbZIP48* compromises the reproductive development and fertility in rice plants, suggesting *OsbZIP1* is a better choice.

Until his unfortunate demise in 2021, Prof. Jitendra P. Khurana and his group at the University of Delhi South

Campus in India made significant contributions toward understanding the function of several light-signaling components in Arabidopsis and other species like rice and Brassica (Tyagi and Sopory 2021). The findings from Bhatnagar et al. (2023), probably among the final contributions from Prof. Khurana, provide important insights into the mechanisms of light-regulated development in rice.

Conflict of interest statement. None declared.

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