

Beating the blues: engineering cryptochrome expression improves soybean yield

Phytochrome A overexpression can increase harvest index, as was shown 25 years ago in a breakthrough paper on tobacco (Robson et al., 1996). The impact of this important discovery has, however, not been fully developed. Plants at high densities often respond strongly to nearby competitors by strong elongation of their internodes and upward bending of their leaves, i.e., shade avoidance. This is crucial for plants to ascertain access to sunlight, and this navigation through vegetation occurs through light cues that are sensed with various photoreceptors. A spectacular sensitivity is displayed by sun-loving plants that can already sense their nearby competitors even before mutual shading occurs and respond through a first acceleration of shoot elongation. This anticipatory response is triggered through red (R):far-red (FR) light-sensitive phytochrome photoreceptors that detect FR light that is reflected by nearby vegetation. When the vegetation continues to grow and true shading occurs, there is also a significant depletion of red and blue light, since the latter two are absorbed for photosynthesis in the overhead leaves (reviewed in Pierik and Ballaré, 2021). Plants can respond to blue light depletion, especially when integrating it with signaling of FR enrichment in *Arabidopsis* (de Wit et al., 2016).

Much is known about the core R:FR signaling pathways: FR enrichment inactivates the phytochrome B (phyB) photoreceptor, releasing phytochrome-interacting proteins (PIFs, a subgroup of basic helix-loop-helix transcription factors) from their inactivation that typically occurs upon interaction with active phyB. As a result, under FR-enriched light conditions PIFs accumulate and promote expression of their target genes. Among these targets are a variety of genes associated with auxin homeostasis, such as YUCCAs and PINs. Auxin signaling builds up and stimulates cell elongation in target tissues, such as the hypocotyl or internodes. This is a highly simplified representation, and all aspects of the signaling pathway are in fact networks with various positive and negative regulators, as well as parallel pathways to control shade avoidance (Pierik and Ballaré, 2021).

In contrast to our relatively detailed understanding of the R:FR-triggered shade avoidance syndrome (SAS) pathway, knowledge of the blue light-mediated pathways is scant. It was shown previously in *Arabidopsis* that cryptochromes (cry; Wang and Lin, 2020) are the main photoreceptors triggering elongation responses to blue light depletion (Keller et al., 2011; Keuskamp et al., 2011) via PIF4 and PIF5 (Keller et al., 2011; de Wit et al., 2016; Pedmale et al., 2016). The downstream events, however, remain poorly understood.

In a recent study, Lyu et al. (2021) took an integrated approach, combining classic and CRISPR/Cas9-based genetic engineering, transcriptomics, physiology, and field trials, to

study these processes in soybean (*Glycine max*) rather than *Arabidopsis*. An interesting observation was that low R:FR ratios majorly induced upward bending of leaves and petiole elongation, while low blue light (LBL) caused pronounced stem elongation, implying that low R:FR and LBL are involved in distinct pathways to regulate different aspects of SAS in soybean (Figure 1A–1C). They identified seven *GmCRY* genes, representing a *CRY1* and a *CRY2* group, and created knockout and overexpression lines for these two groups in soybean. Although *cry2* knockouts were rather similar to wild type, *cry1* mutant plants (*Gmcry1s-qm*) displayed severe constitutive elongation, similar to the effect of blue light depletion on wild type. *GmCRY1b* overexpression, on the other hand, suppressed stem elongation. Consistently, the different genotypes displayed important differences in their transcriptome profiles. Expression of two *GA2-OXIDASE-7* (*GA2OX7*) genes was repressed in the *cry1* knockout and elevated in the *CRY1* overexpressor lines. GA_2 oxidases are gibberellin (GA) catabolic enzymes that reduce the levels of growth-promoting, bioactive GA, and transgenic overexpression of *GmGA2ox7a* reduced stem length and inhibited LBL-induced stem elongation in soybean. In accordance with the differential expression levels between the different *cry1* lines, GA concentrations in the *GmCRY1b* overexpressor were reduced, whereas the *Gmcry1s-qm* mutant had elevated endogenous GA_1 (Lyu et al., 2021). Together, these findings indicate that GA regulation by *cry1*-mediated blue light signaling could regulate shade avoidance in soybean, consistent with LBL-mediated shade avoidance in *Arabidopsis* (Djakovic-Petrovic et al., 2007).

The question that remained was: how is this regulated? Lyu et al. (2021) observed that two bZIP transcription factor-encoding genes, *STF1* and *STF2*, with homology to *HY5* were upregulated in the *GmCRY1b* overexpressor and downregulated in the *cry1* mutant. Overexpression of *STF1* and *STF2* led to severe dwarfing, comparable with what was seen in the *GmCRY1b* overexpression lines, consistent with elevated expression of *GmGA2ox7-a* in the *STF1* overexpression line. In an elegant follow-up, it was confirmed through chromatin immunoprecipitation that STFs physically interact with the *GmGA2ox7-a* and *GmGA2ox7-b* promoters. Moreover, *STF1* was also confirmed, using electrophoretic mobility shift and dual-luciferase assays, to promote *GmGA2ox7-a* expression, and this was further promoted by co-expression with *GmCRY1b*.

Based on these findings, the authors propose that blue light-mediated inactivation of *CRY1* reduces *STF1* protein

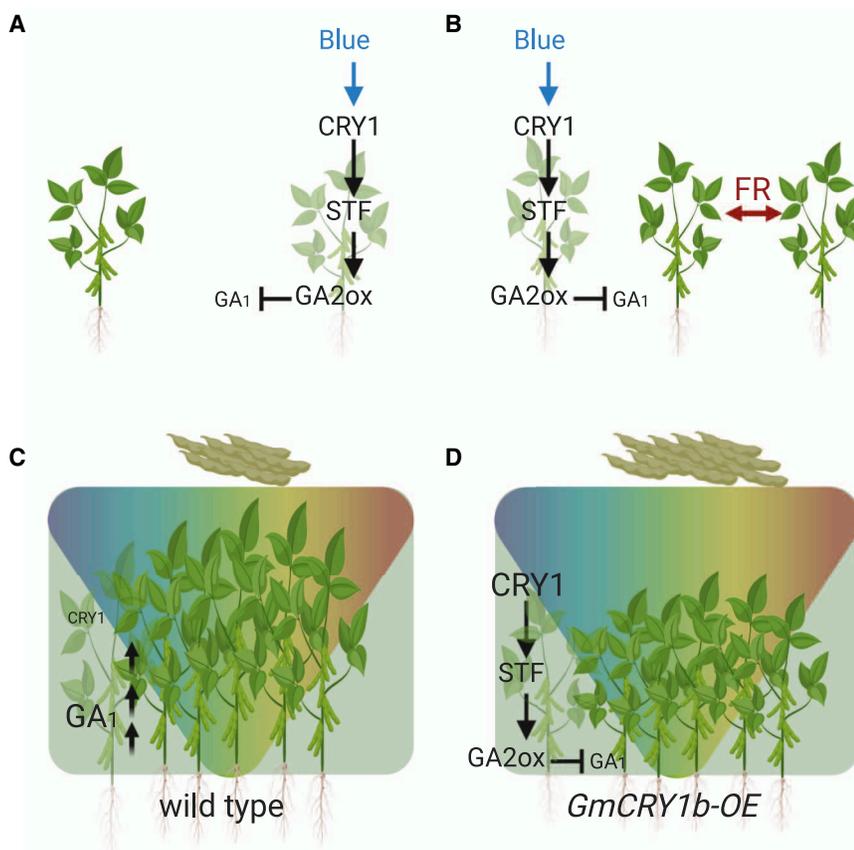


Figure 1. Soybean shade avoidance is strongly regulated by blue light depletion.

(A) Individually grown plants in full (sun)light experience high blue light that activates the cry1 photoreceptors. cry1s stimulate STF abundance, which drives the expression of *GA2OXIDASES*, thereby keeping growth-promoting GA_1 levels low.

(B) Upon neighbor proximity, far-red (FR) light is reflected and thereby enriched between plants, but this leads to only very minimal elongation in soybean.

(C) Wild-type plants grown in dense fields experience low blue (and low red) light conditions due to shading, resulting in elevated GA_1 levels that stimulate internode elongation.

(D) *CRY1* overexpression (*GmCRY1b-OE*) suppresses the low blue light-induced promotion of internode elongation, which promotes yield.

accumulation, which subsequently represses the expression of *GA2OX7* genes, resulting in elevated bioactive GA that promotes elongation in soybean (Figure 1A and 1C).

Reduced shade avoidance could result in more compact plants that may be more lodging resistant. Of particular interest is that Lyu et al. (2021) verified the performance of *CRY1* overexpression lines under high-density crop field conditions. They observed that the transgenic *GmCRY1b* overexpression lines under high planting density are highly lodging resistant and have strongly increased yield per plant as compared with their wild-type controls (Figure 1C and 1D). These findings indicate that engineering of photoreceptor signaling pathways offers a huge opportunity for food crop improvement. An important additional aspect of plant growth at high planting densities is the trade-off between (shade avoidance) growth and defense against attackers (Pierik and Ballaré, 2021). The little evidence available so far suggests that blue light signaling may not interfere strongly with resistance, unlike phytochrome-mediated R:FR signaling (Cerrudo et al., 2012). The newly developed soybean lines with engineered variation of expression of *CRY1* present a powerful tool for verifying, under agriculturally relevant conditions, to what extent cryptochromes play a role in the growth-defense trade-off. The study by Lyu et al. (2021) not only provides novel insights into soybean photobiology but also set an inspiring example showing how to boost crop yield through engineering of photobiology pathways, implying that

engineering photoreceptor expression is a promising approach towards crop improvement.

ACKNOWLEDGMENTS

No conflict of interest declared.

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<https://doi.org/10.1016/j.molp.2021.01.005>

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