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Supplemental Information

Local HY5 Activity Mediates Hypocotyl Growth and Shoot-to-Root Communication

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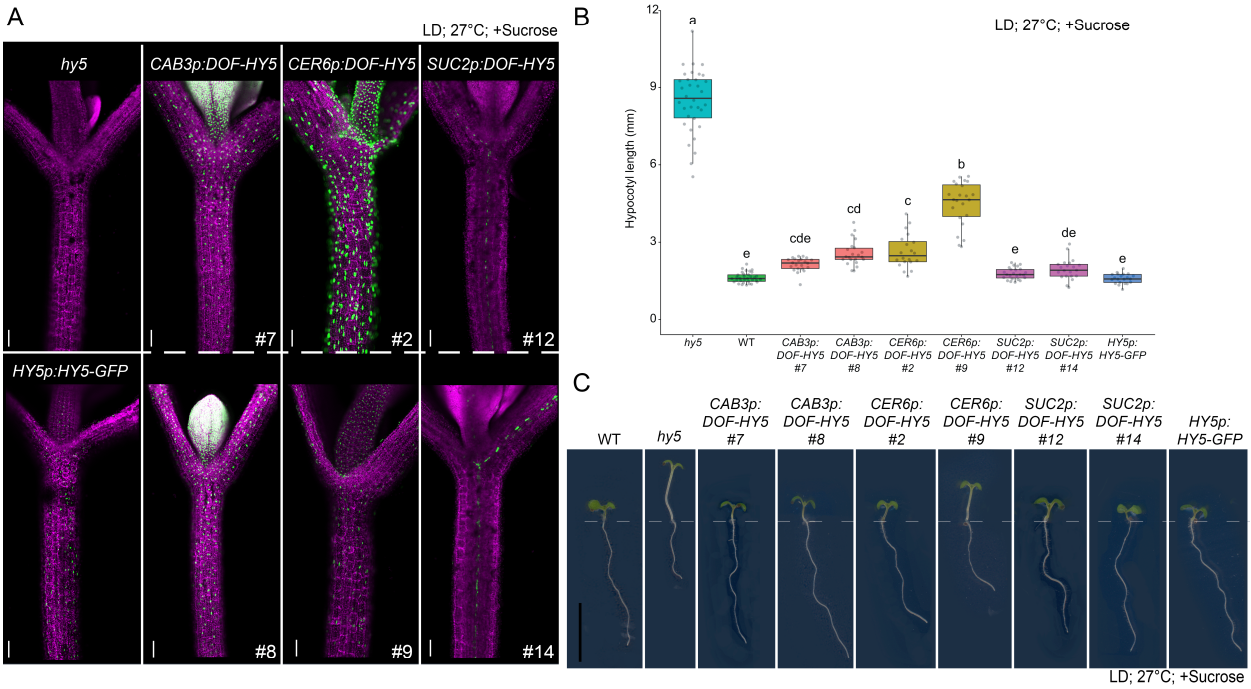
Running Title:

Local HY5 activity mediates shoot-to-root communication

Short Summary:

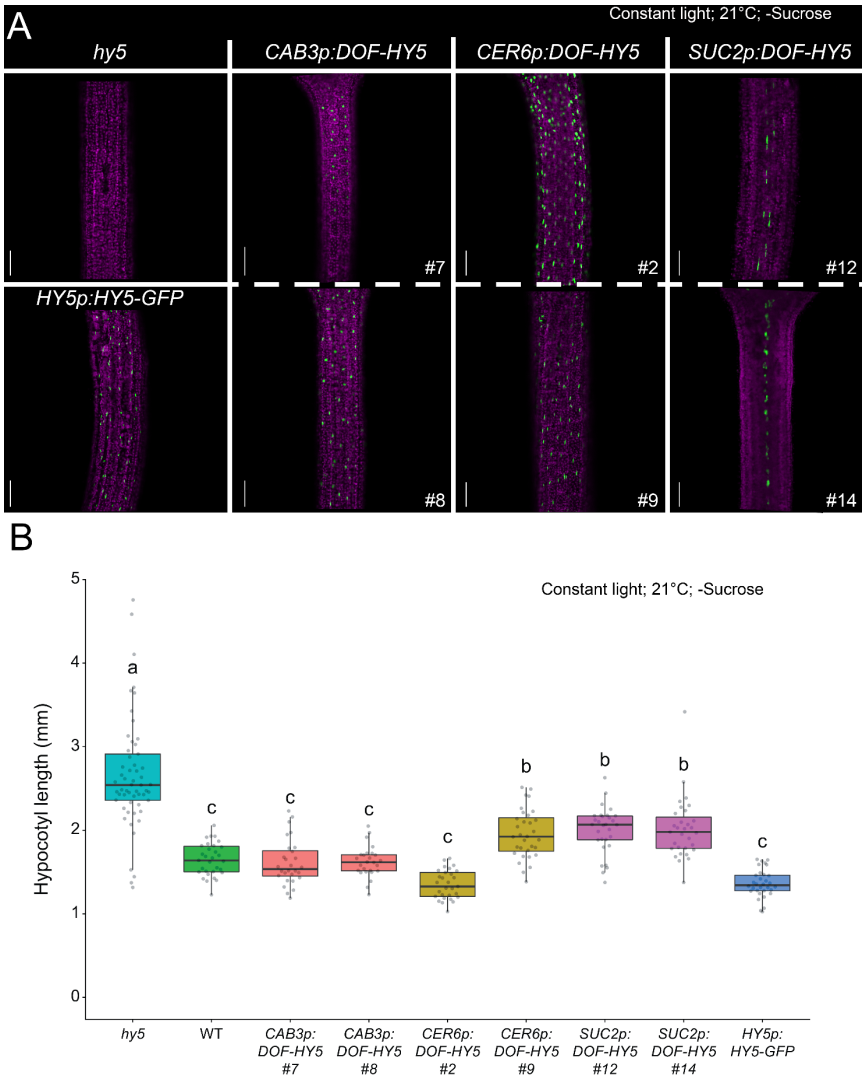
Local HY5 activity in specific cell types of the hypocotyl can rescue *hy5* primary root growth defects, in the absence of HY5 mobility to the root, and regulate hypocotyl growth. This study suggests that a mobile signal downstream of HY5 can carry out this signaling function during early seedling development.

Supplementary Figures:



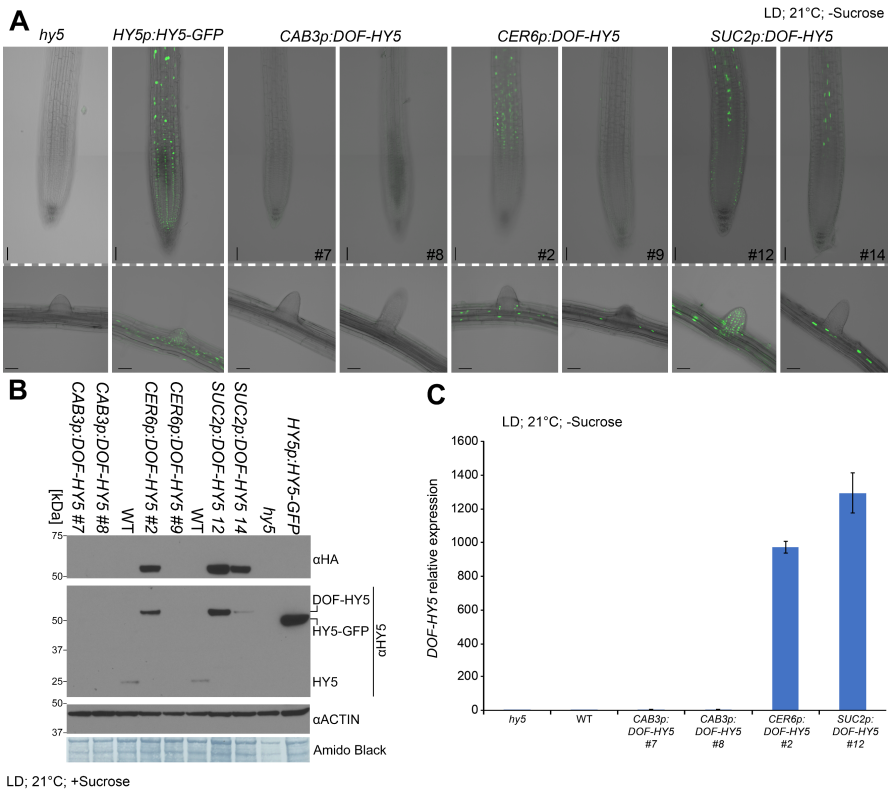
Supplementary Figure 1: Tissue specifically expressed DOF-HY5 rescues *hy5* hypocotyl and root phenotypes at 27°C.

A. Representative fluorescence images of the hypocotyl from seedlings expressing HA-YFP-HA (DOF)-HY5 protein (green) under the control of different cell-specific promoters or from *HY5p:HY5-GFP* seedlings. All constructs were expressed in the *hy5* mutant background. Magenta-chlorophyll autofluorescence. Image capture settings were the same for all constructs except *HY5p:HY5-GFP*. Scale bars: 100 µm. **B.** Hypocotyl length of six-day-old seedlings expressing the indicated constructs in the *hy5* mutant background. The boxes indicate the first and third quartiles and the whiskers indicate the minimum and maximum values, the black lines within the boxes indicate the median values. Different letters denote statistical differences ($p < 0.05$) among samples as assessed by one-way ANOVA and Tukey HSD, ($n > 20$). **C**. Representative images of the seedlings shown in A and B. scale bar: 1 cm. All images and measurements in A-C, were taken from six-day-old seedlings grown in white light (120 µE) on media with sucrose in long-day conditions (LD) (16h light, 8h dark) at 27°C.



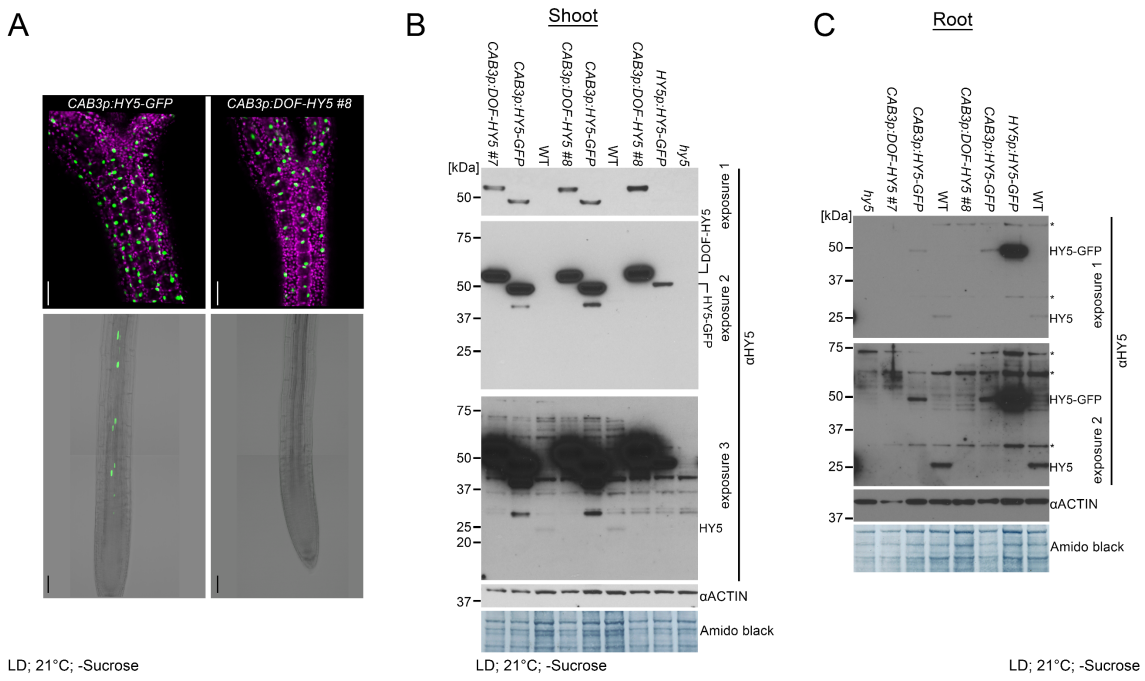
Supplementary Figure 2: Tissue specifically expressed DOF-HY5 rescues *hy5* hypocotyl phenotype in constant light.

A. Representative fluorescence images of the hypocotyl from seedlings expressing DOF-HY5 protein (green) under the control of different cell-specific promoters or from *HY5p:HY5-GFP* seedlings. All constructs were expressed in the *hy5* mutant background. Magenta- Chlorophyll autofluorescence. As these constructs contain different fluorophores, and they were expressed in different tissue at different levels, image capture settings were different for each image. Scale bars: 100 µm. **B.** Hypocotyl length of six-day-old seedlings expressing the indicated constructs in the *hy5* mutant background. Different letters denote statistical differences ($p < 0.05$) among samples as assessed by one-way ANOVA and Tukey HSD, ($n > 26$). All images and measurements in A and B, were taken from six-day-old seedlings grown in constant light (22 µE) on media without sucrose, at 21°C.



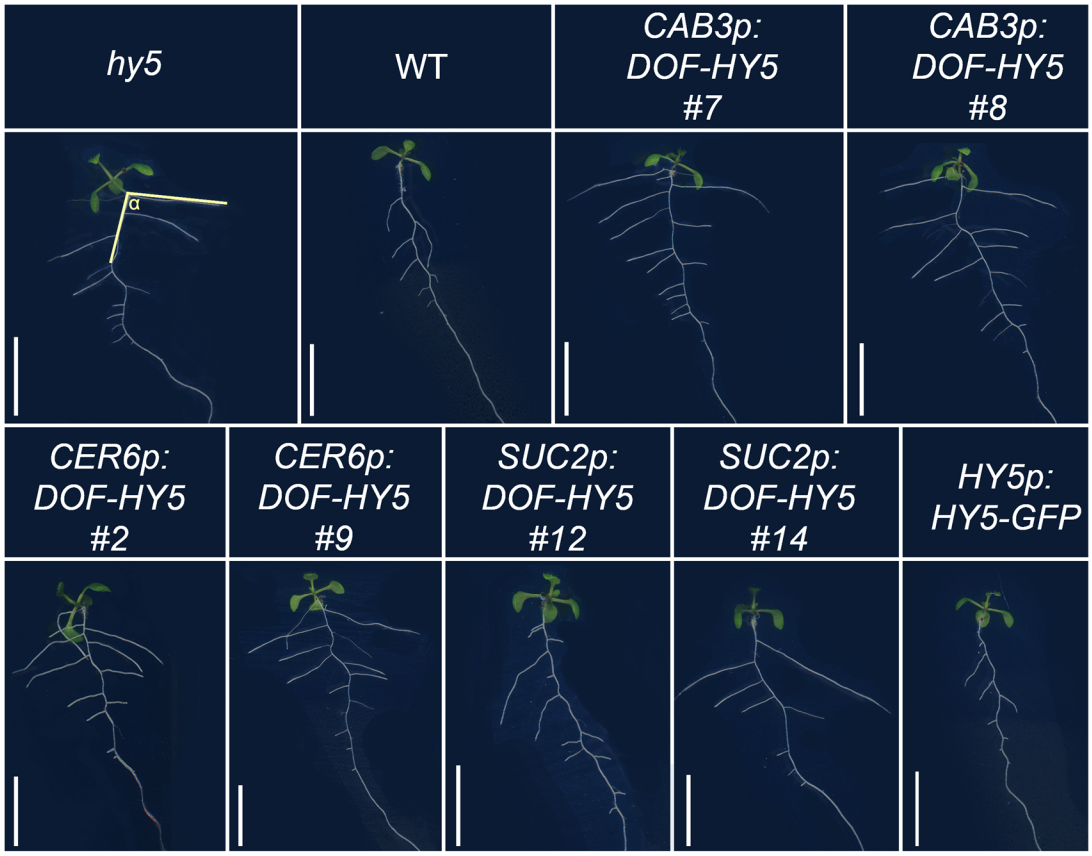
Supplementary Figure 3: Expression pattern of DOF-HY5 in the roots when expressed under tissue-specific promoters.

A. Representative fluorescence and brightfield images of roots tip and lateral roots primordia, from seedlings expressing DOF-HY5 protein (green) under the control of different tissue-specific promoters in the *hy5* mutant background or from *HY5p:HY5-GFP*. Images were taken from six-day-old seedlings grown in white light (117 µE) LD, at 21°C on media without sucrose. As these constructs contain different fluorophores, and they were expressed at different levels, image capture settings were different for each image (capture settings for *CAB3p:DOF-HY5* lines were equal to the highest sensetive level). Scale bars: 50 µm. **B**. Western blot of root samples from six-day-old seedlings grown in 21°C LD on media with sucrose and expressing HY5 under the control of different tissue-specific promoters in the *hy5* mutant background. DOF-HY5 protein was visualized by anti-HA antibody (upper panel). DOF-HY5, HY5-GFP and native HY5 were visualized by anti-HY5 (middle panel). Anti-ACTIN and Amido Black staining used as loading controls (lower panels). **C**. Expression of *DOF-HY5* in the roots of six-day-old seedlings grown in 21°C LD on media without sucrose. Relative expression was assayed using qRT-PCR relative to the reference gene *IPP2* and normalized to expression in *hy5*. The average of three biological replicates per condition +/- SE is shown.



Supplementary Figure 4: Expression of *CAB3p:DOF-HY5* and *CAB3p:HY5-GFP* in shoot and root.

A. Representative fluorescence images of the hypocotyl (upper panel) and root (lower panel) from seedlings expressing DOF-HY5 or HY5-GFP protein (green) under the control of CAB3 promoter. Both constructs were expressed in the *hy5* mutant background. Magenta- Chlorophyll autofluorescence, Scale bars: 50 µm. **B**,**C**. Western blot of shoot (B) and root (C) samples from seedlings expressing *DOF-HY5* or *HY5-GFP* under the control of the *CAB3* or *HY5* promoters in the *hy5* mutant background. DOF-HY5, HY5-GFP, and native HY5 were visualized by anti-HY5. Anti-ACTIN and Amido black were used as a loading control. * - Cross-reacting band. All images and measurements were taken from six-day-old seedlings grown in 21°C LD on media without sucrose.

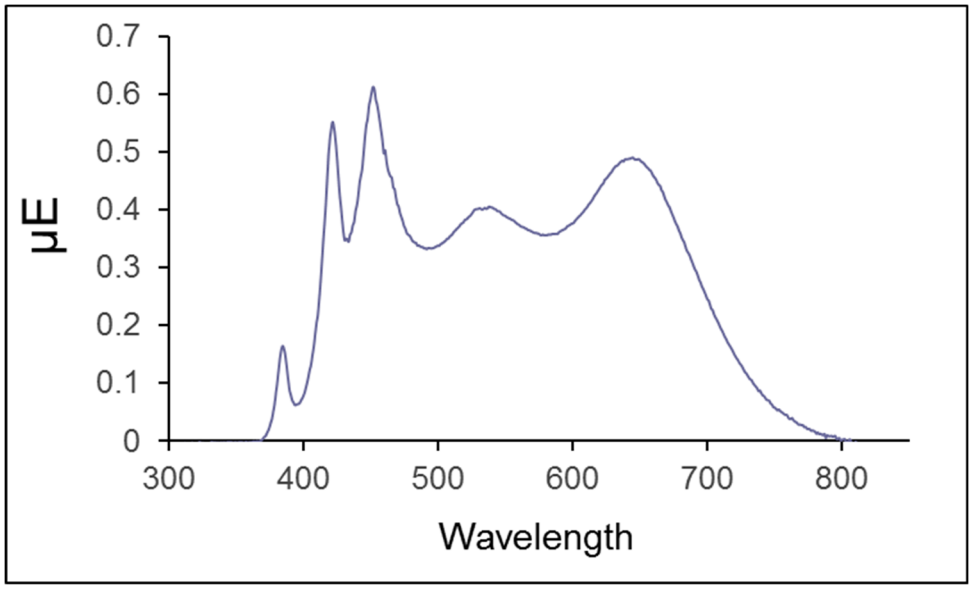


Supplementary Figure 5: Lateral root phenotypes of the different plants expressing DOF-HY5 under tissue-specific promoters.

Representative images of seedlings expressing *HY5* under the control of different tissue-specific promoters or from *HY5p:HY5-GFP*. Seedlings were grown in white light supplement with far-red (127 µE, R:FR= 0.6) on media with sucrose at 21°C LD for 10 days. All constructs were expressed in the *hy5* mutant background. Scale bar: 1 cm. Example of the LR angle shown in Fig. 3D is shown in yellow.

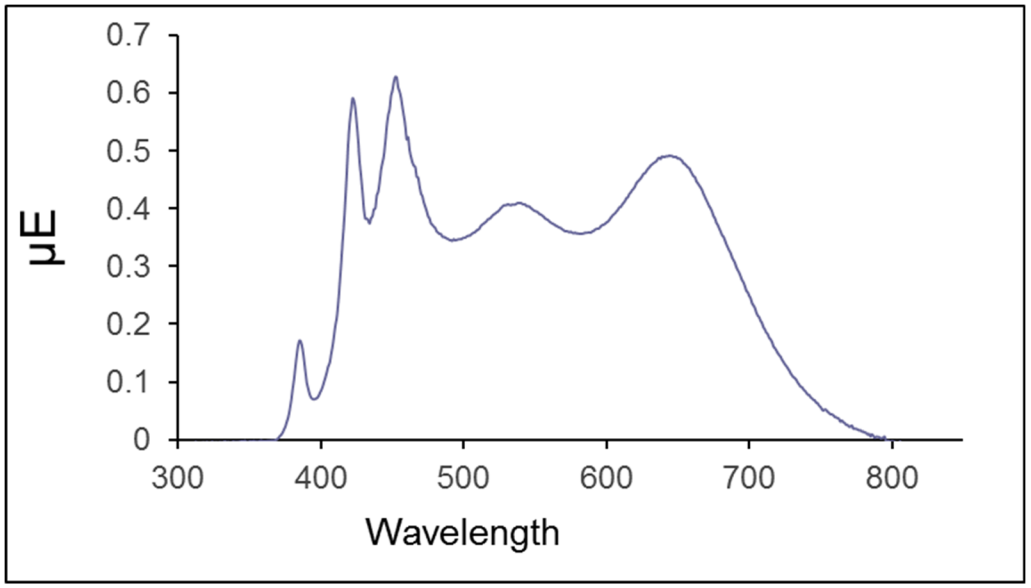
Supplementary Table 1: Light conditions used in this work.

21°C Long day:



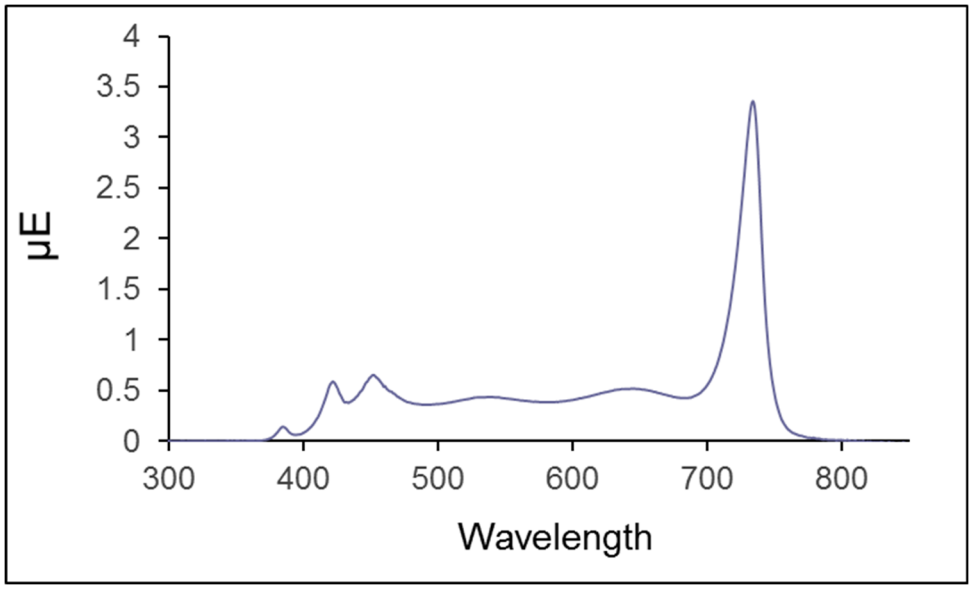
B	R	FR	R:FR	PAR
38.79389	41.30892	8.305457	4.973708	117.6788

27°C Long day:



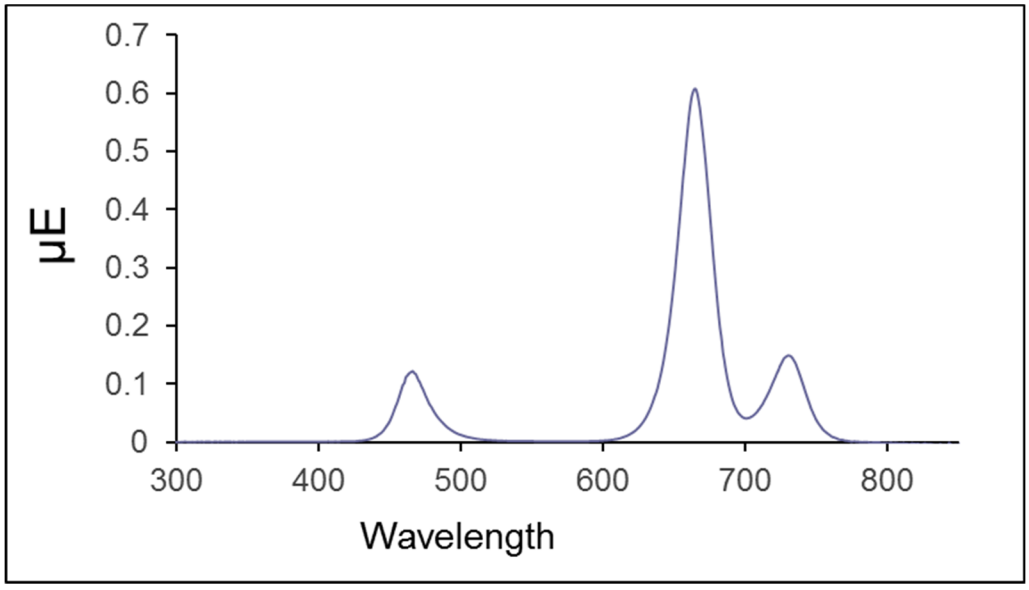
B	R	FR	R:FR	PAR
40.67178	41.67484	8.113381	5.136557	120.4865

21°C Long day white light + far-red (shade):



B	R	FR	R:FR	PAR
41.1521	46.43036	86.60099	0.536141	127.908

21°C Constant light:



B	R	FR	R:FR	PAR
3.40901	18.83746	4.770781	3.948506	22.44523

Supplementary Table 2: Summary of all growth conditions.

Figure #	Experiment	Light Condition	Temperature	Sucrose
Figures				
1A-D	Microscopy; Hypocotyl length; Western blot shoots	Long day	21°C	No
2A,B,D	Microscopy; Western blot roots; Root growth	Long day	27°C	Yes
2C	Root growth	Long day	21°C	Yes
2F	Root growth	Shoot Long day– root in dark	21°C	Yes
2G	Root growth	Shoot Long day – root in dark	21°C	No
3A-D	Lateral root	Long day + FR (R/FR=0.6)	21°C	Yes
Supplementary Figures				
S1A-C	Microscopy; Hypocotyl length; Plant images	Long day	27°C	Yes
S2A,B	Microscopy; Hypocotyl length;	Constant light	21°C	No
S3A,C	Microscopy; qRT-PCR	Long day	21°C	No
S3B	Western blot roots	Long day	21°C	Yes
S4	Microscopy; Western blot shoots; Western blot roots.	Long day	21°C	No
S5	Lateral root images	Long day + FR (R/FR=0.6)	21°C	Yes

Supplementary Table 3: Primers used in this work.

Primer name	Sequence 5' >>> 3'	
B2r-HY5_F	GGGGACAGCTTTCTTGTACAAAGTGGCTATGCAGGAACAAG CGACTAG	Cloning *HY5*
B3-HY5stop_R	GGGGACAACTTTGTATAATAAAGTTGCTCAAAGGCTTGCATC AGC	
B4-CAB3p_F	GGGGACTGCTTTTTTGTACAAACTTGCGGTGACTAACTTGTG AGTGAGAGTG	Cloning *CAB3* promoter
B1r-CAB3p_R	GGGGACAACTTTGTATAGAAAAGTTGCCAAATCAAGAGAAAA TGTGATTCTCG	
qDOF-HY5 _F	CCGGATCTAGATACCCATACGA	qRT-PCR
qDOF-HY5 _R	GTCGCTTGTTCCTGCATAGC	