

# HY5 Suppresses, Rather Than Promotes, Abscisic Acid-Mediated Inhibition of Postgermination Seedling Development<sup>1</sup>[OPEN]

Dear Editor,

Seed germination and postgermination seedling establishment are crucial early developmental events in angiosperms. Although the demarcations between these two successive events appear to be elusive, they have been defined as distinct developmental processes and identified to involve separate regulatory mechanisms at the molecular level. Seed germination is marked by the protrusion of embryonic root out of the seed coat. Postgermination seedling establishment denotes the developmental window after germination that involves the opening, greening, and expansion of cotyledons or foliar leaves, marking the switch to autotrophic development (Lopez-Molina et al., 2001; Weitbrecht et al., 2011).

Light is one of the most prominent environmental signals that influence early developmental events in plants. A well-coordinated regulation of light and abscisic acid (ABA) signaling pathways is crucial to optimize the timing and pace of germination and postgermination seedling establishment, especially under stress conditions (de Wit et al., 2016; Vaishak et al., 2019). The bZIP transcription factor ELONGATED HYPOCOTYL5 (HY5) is a key positive regulator of photomorphogenesis (Oyama et al., 1997; Gangappa and Botto, 2016). HY5 also acts as a major integrating factor for light and ABA pathways. A previous study identified that HY5 promotes ABA signaling by directly binding to the promoter of *ABSCISIC ACID INSENSITIVE5* (*ABI5*) and inducing its expression (Chen et al., 2008).

During seedling development, the protein level of HY5 is tightly controlled by the E3 ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC1 (*COP1*), which ubiquitinates HY5 and targets it for proteasome-mediated degradation (Ang et al., 1998; Osterlund et al., 2000). Recently, we reported that *COP1* promotes ABA-mediated inhibition of postgermination seedling

establishment (Yadukrishnan et al., 2020). We observed that *cop1* mutants show ABA hyposensitivity during postgermination seedling development (Yadukrishnan et al., 2020). Previous evidence suggests that *hy5* is less sensitive to ABA inhibition of seedling growth (Chen et al., 2008). While the germinated Columbia-0 (*Col-0*) remains in a prolonged arrested state with ABA treatment, *hy5* rapidly grows and establishes into seedlings (Chen et al., 2008). Together, these reports suggest that despite *COP1* being a negative regulator of HY5, *cop1* and *hy5* mutants do not show opposite ABA sensitivities during seedling development. This prompted us to revisit the ABA-hyposensitive phenotype of *hy5* mutants during postgermination seedling growth in light.

The *hy5* allele used by Chen et al. (2008) was a T-DNA insertion mutant (SALK\_096651) in the Columbia background. To validate the ABA-hyposensitive postgermination phenotype of *hy5*, we monitored its seedling establishment percentage under cycling light in the absence and presence of ABA. By day 6, both *Col-0* and *hy5* attained 100% seedling establishment in the absence of ABA (Fig. 1, A and B). However, in the presence of ABA, seedling establishment was considerably slower in *hy5* as compared with the wild type. While ~30% of the *Col-0* seedlings attained establishment by 6 d, *hy5* mutants had not started establishment (Fig. 1, A and B). Our observation indicated that the *hy5* mutant might be hypersensitive to ABA during postgermination seedling development, which is contradictory to the previous report.

To validate this further, we studied the postgermination ABA sensitivity of other widely used *hy5* mutant alleles: *hy5-215* and *hy5-ks50* (Fig. 1, A and B). Although some of the previous studies have shown the ABA-hyposensitive germination phenotype of *hy5-215*, its postgermination ABA sensitivity has not been quantitatively reported (Xu et al., 2014; Fernando and Schroeder, 2015; Srivastava et al., 2015; Yang et al., 2018). Thus, we monitored the seedling establishment of *hy5-215* in the presence and absence of ABA. While the wild type and *hy5-215* showed 100% seedling establishment in the absence of ABA, *hy5-215* showed significantly slower seedling establishment in 1  $\mu$ M ABA as compared with *Col-0* (Fig. 1, A and B). In the presence of ABA, ~25% of *Col-0* seedlings were established by day 6, whereas only ~5% of *hy5-215* seedlings were established (Fig. 1, A and B).

Since both *hy5* (SALK\_096651) and *hy5-215* are in the genetic background of ecotype Columbia, we further verified the phenotype in the *hy5-ks50* allele in the *Ws* background. When grown in the presence of 1  $\mu$ M ABA, the seedling establishment rate of the wild-type *Ws* ecotype was higher than in the *Col-0* ecotype (Fig. 1, A and B).

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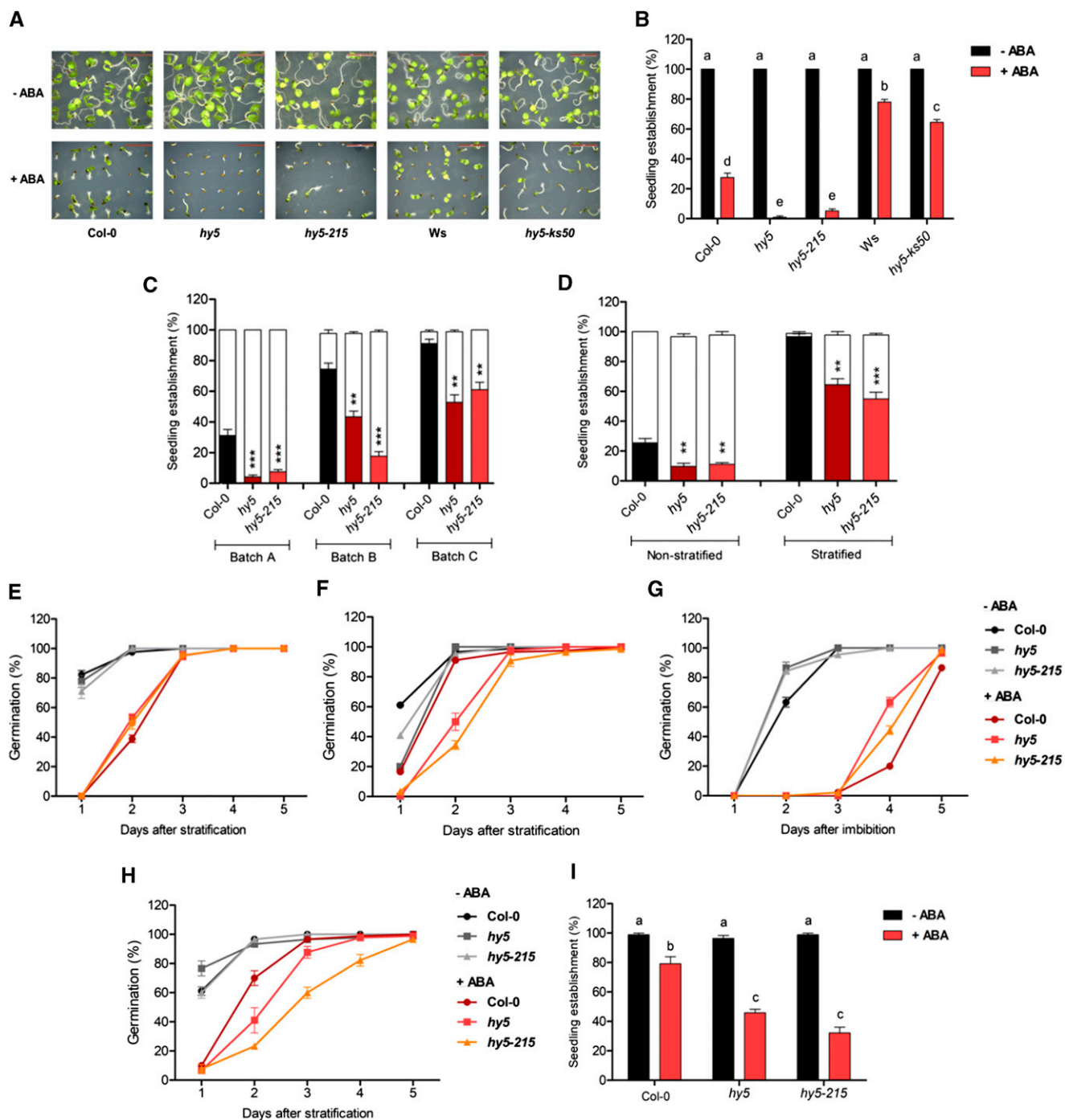
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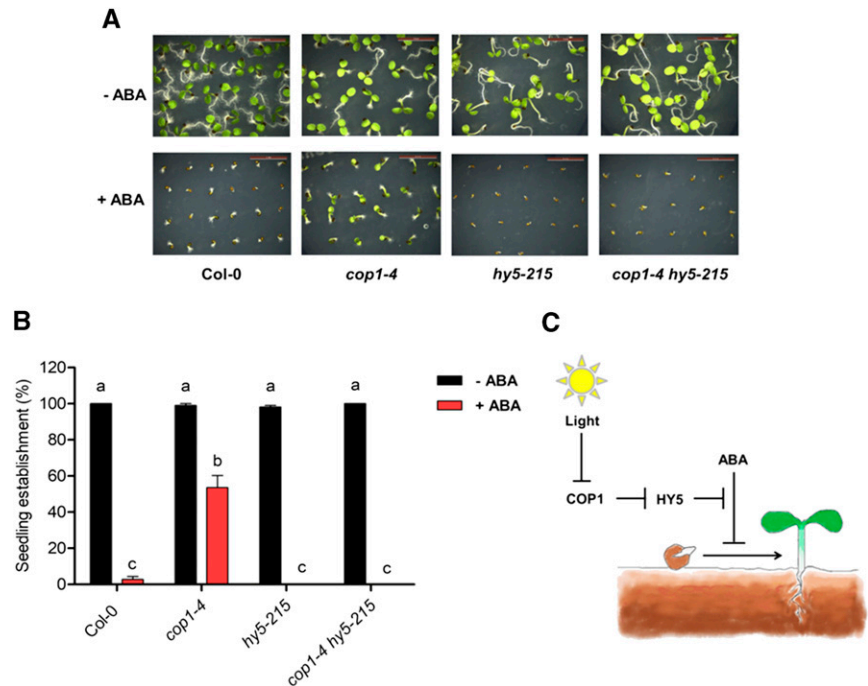
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**Figure 1.** HY5 negatively regulates ABA-mediated inhibition of postgermination seedling establishment. A and B, Representative images (A) and seedling establishment rates (B) of Col-0, *hy5* (SALK\_096651), *hy5-215*, Wassilewskija (Ws), and *hy5-ks50* grown on  $0.5 \times$  Murashige and Skoog (MS) plates complemented with and without  $1 \mu\text{M}$  ABA for 6 d. Scale bars = 5 mm. C, Seedling establishment rates of three different batches of Col-0, *hy5*, and *hy5-215* seeds grown on  $0.5 \times$  MS plates without (white bars) and with (colored bars)  $1 \mu\text{M}$  ABA for 6 d. Batches A, B, and C represent freshly harvested, 6-month-old, and 1-year-old seed batches, respectively. D, Seedling establishment rates of nonstratified and stratified Col-0, *hy5*, and *hy5-215* seeds (batch C) on  $0.5 \times$  MS plates without (white bars) and with (colored bars)  $1 \mu\text{M}$  ABA for 6 d. Values represent means  $\pm$  SE of three experiments with 50 or more seeds used in each experiment. Asterisks represent statistically significant differences between the individual mutants and the wild type ( $***P < 0.001$  and  $**P < 0.01$ ) as determined by one-way ANOVA followed by Dunnett's posthoc test. E and F, Germination rates of batch A (E) and batch C (F) Col-0, *hy5*, and *hy5-215* seeds on  $0.5 \times$  MS plates without and with  $1 \mu\text{M}$  ABA counted up to 5 d after stratification treatment. G, Germination rates of nonstratified batch C seeds of Col-0, *hy5*, and *hy5-215* on  $0.5 \times$  MS plates without and with  $1 \mu\text{M}$  ABA counted up to 5 d after imbibition. H, Germination rates of Col-0, *hy5*, and *hy5-215*

**Figure 2.** HY5 acts downstream of COP1 to regulate ABA-mediated inhibition of post-germination seedling establishment. A and B, Representative images (A) and seedling establishment rates (B) of 4-d-old Col-0, *cop1-4*, *hy5-215*, and *cop1-4 hy5-215* on 0.5× Murashige and Skoog plates complemented with and without 1  $\mu\text{M}$  ABA. Scale bars = 5 mm. Plates were kept in long days (16 h of light/8 h of dark) with 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  white light after stratification. Seedling establishment was marked by complete opening and greening of cotyledons. Values represent means  $\pm$  SE of three experiments with 50 or more seeds used in each experiment. Lowercase letters above the bars indicate statistical groups as determined by one-way ANOVA followed by Tukey's posthoc test ( $P \leq 0.05$ ). C, Model showing the regulation of ABA-mediated inhibition of early seedling development by the COP1-HY5 regulatory module.



However, the *hy5-ks50* mutant showed significantly less seedling establishment in the presence of ABA as compared with the *Ws* wild type (Fig. 1, A and B). The seedling establishment of *hy5-ks50* in ABA was greater than that of *hy5* (SALK\_096651) and *hy5-215*, indicating that the postgermination ABA sensitivity is generally weaker in the *Ws* genetic background (Fig. 1, A and B). All the *hy5* mutant alleles tested showed varying extents of ABA-hypersensitive seedling establishment, underlining that HY5 might be a negative regulator of ABA-mediated postgermination seedling growth arrest, contrary to what has been known.

Seed age can affect dormancy, thereby modulating germination and postgermination phenotypes of seedlings both in the presence and absence of ABA (Weitbrecht et al., 2011; Shu et al., 2016). All the seeds used in the experiment mentioned above (Fig. 1, A and B) were freshly harvested. To verify if seedling establishment in Col-0 and *hy5* mutants varies between different seed batches, we compared three seed batches: batch A (freshly harvested), batch B (harvested 6 months ago), and batch C (harvested 1 year ago; Fig. 1C). Sensitivity to ABA decreased in both Col-0 and *hy5* mutants with seed age (Fig. 1C). However, in all the batches, *hy5* and *hy5-215* showed enhanced sensitivity to ABA compared with Col-0 (Fig. 1C). In our

experiments, seeds were stratified for a period of 3 d, whereas the protocol followed by Chen et al. (2008) is slightly ambiguous, as the figure 2 legend therein mentions stratification while the methods section refers to a previous article that does not include stratification (Xiong et al., 2001). To investigate if stratification modulates seedling establishment, we compared the establishment of stratified and nonstratified batch C seeds (Fig. 1D). *hy5* mutants showed ABA-hypersensitive seedling establishment phenotypes irrespective of the stratification treatment, although bypassing the stratification caused a stronger inhibition (Fig. 1D). Taking these data into consideration, we suspect that the difference in seed age and dormancy levels between Col-0 and *hy5* seeds used by Chen et al. (2008) could possibly have contributed to the reduced ABA sensitivity of *hy5* during seedling establishment seen before.

We further asked whether the ABA hypersensitivity of *hy5* mutants is confined to the postgermination development of seedlings or if it starts from the germination process itself. To test this, we monitored the germination rates of these mutants in the presence and absence of ABA (Fig. 1, E–G). In the absence of ABA, freshly harvested Col-0, *hy5* (SALK\_096651), and *hy5-215* seeds germinated at similar rates, whereas in the presence

**Figure 1.** (Continued.)

seeds (batch C) on filter paper soaked with sterile water without or with 1  $\mu\text{M}$  ABA counted up to 5 d after stratification treatment. I, Seedling establishment rates of batch C seeds of Col-0, *hy5*, and *hy5-215* germinated on water-soaked filter paper after stratification and transferred on day 2 to 0.5× MS plates without or with 5  $\mu\text{M}$  ABA and grown for another 2 d. The 0.5× MS plates containing 1% (w/v) agar and no added Suc were used in the experiments. Plates were kept in long days (16 h of light/8 h of dark) with 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  white light. Seeds were scored as germinated upon the emergence of radicle out of the testa and endosperm. Seedling establishment was marked by complete opening and greening of cotyledons. Values represent means  $\pm$  SE of three experiments with 50 or more seeds used in each experiment. Lowercase letters above the bars in B and I indicate statistical groups as determined by one-way ANOVA followed by Tukey's posthoc test ( $P \leq 0.05$ ).

of ABA, the germination rates of *hy5* mutants were marginally faster than that of Col-0 (Fig. 1E), which is in agreement with previous reports (Chen et al., 2008; Xu et al., 2014; Fernando and Schroeder, 2015; Yang et al., 2018). When we performed the same experiment with the 1-year-old seed batch, *hy5* mutants showed slower germination rates compared with the wild type, reiterating the role of dormancy or seed age in modulating the sensitivity of *hy5* mutants to ABA (Fig. 1F). Next, we investigated the role of stratification in regulating the germination of *hy5* mutants in the presence and absence of ABA. In 1-year-old seeds in the absence of stratification, *hy5* mutants germinated faster than Col-0 (Fig. 1G), which is opposite to their hypersensitive response when stratified (Fig. 1F). This emphasizes the role of stratification in regulating germination under stress.

Since Chen et al. (2008) performed their germination assays on filter paper, we also verified the germination of 1-year-old seeds on filter paper in the presence and absence of ABA and found that *hy5* mutants retain the ABA-hypersensitive germination phenotype in this condition as well (Fig. 1H). When the seeds germinated on water-soaked filter paper were transferred to ABA-containing plates for further growth, *hy5* mutants continued to show ABA-hypersensitive responses during seedling establishment, indicating that the postgermination ABA hypersensitivity of *hy5* mutants is not a consequence of its delayed germination in ABA (Fig. 1I). Together, these results indicate that the ABA sensitivity of *hy5* mutants during early development is highly influenced by levels of dormancy in different seed batches and stratification of the seeds, which might have been overlooked in some of the previous studies.

According to our observations, while *hy5* mutants show ABA hypersensitivity during postgermination development, *cop1* mutants show ABA hyposensitivity (Yadukrishnan et al., 2020). Since HY5 acts downstream of COP1 in light signaling and during germination, we asked if COP1 and HY5 act in a similar module to regulate postgermination ABA sensitivity. To test this, we grew *cop1-4 hy5-215* double mutants (Rolauuffs et al., 2012) in the presence and absence of ABA and studied their postgermination ABA sensitivity (Fig. 2, A and B). In the absence of ABA, all lines achieved 100% seedling establishment by 4 d (Fig. 2, A and B). In 1  $\mu$ M ABA, *cop1-4* and *hy5-215* exhibited ABA-hyposensitive and -hypersensitive seedling establishment, respectively. The *cop1-4 hy5-215* double mutant showed ABA sensitivity similar to *hy5-215* (Fig. 2, A and B). The epistatic phenotype of *hy5-215* over *cop1-4* suggests that HY5 is necessary for the ABA-hyposensitive seedling establishment phenotype of *cop1-4*, and HY5 acts downstream of COP1 to regulate ABA-mediated inhibition of postgermination development (Fig. 2C).

To conclude, sensing light cues from the environment and integrating them with the ABA pathway might be crucial for making the decision to switch to autotrophic growth. Under stress conditions, ABA dictates the seedling to remain in a prolonged postgermination quiescent state, whereas light favors photomorphogenic growth

and autotrophic establishment of the seedling. The interaction of HY5, COP1, and possibly other regulators of light signaling with the ABA pathway might be decisive in determining the right timing for seedling establishment.

#### Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers: *HY5* (AT5G11260); *COP1* (AT2G32950); and *ABI5* (AT2G36270).

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