ERF VII transcription factors maintain lateral root growth at hypoxic conditions in Arabidopsis

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Introduction

Flooding is a major abiotic stress that can cause severe damage to plants. *Arabidopsis thaliana* is a widely employed model plant to study the molecular mechanisms that mediate plant adaptation to flooding. ERF (ethylene response factor) transcription factors of group VII (ERFVIIs) that includes RAP2.2, RAP2.3, RAP2.12, HRE2, and HRE1, have identified as key regulators of the metabolic low oxygen response (Paul *et al*, 2016). ERFVIIs regulate hypoxia core genes, many of which encode metabolic enzymes such as alcohol dehydrogenase. In addition, ERFVIIs have recently emerged as developmental regulators during hypoxia. Since roots are the first to suffer from flooding we hypothesized that the root system adapts to these conditions and that ERFVIIs play a role in this process. Our study provides evidence that ERFVIIs promote lateral root growth during oxygen deprivation by down regulating abscisic acid (ABA) levels.

Results

ERFVII TFs maintain lateral root growth upon hypoxia

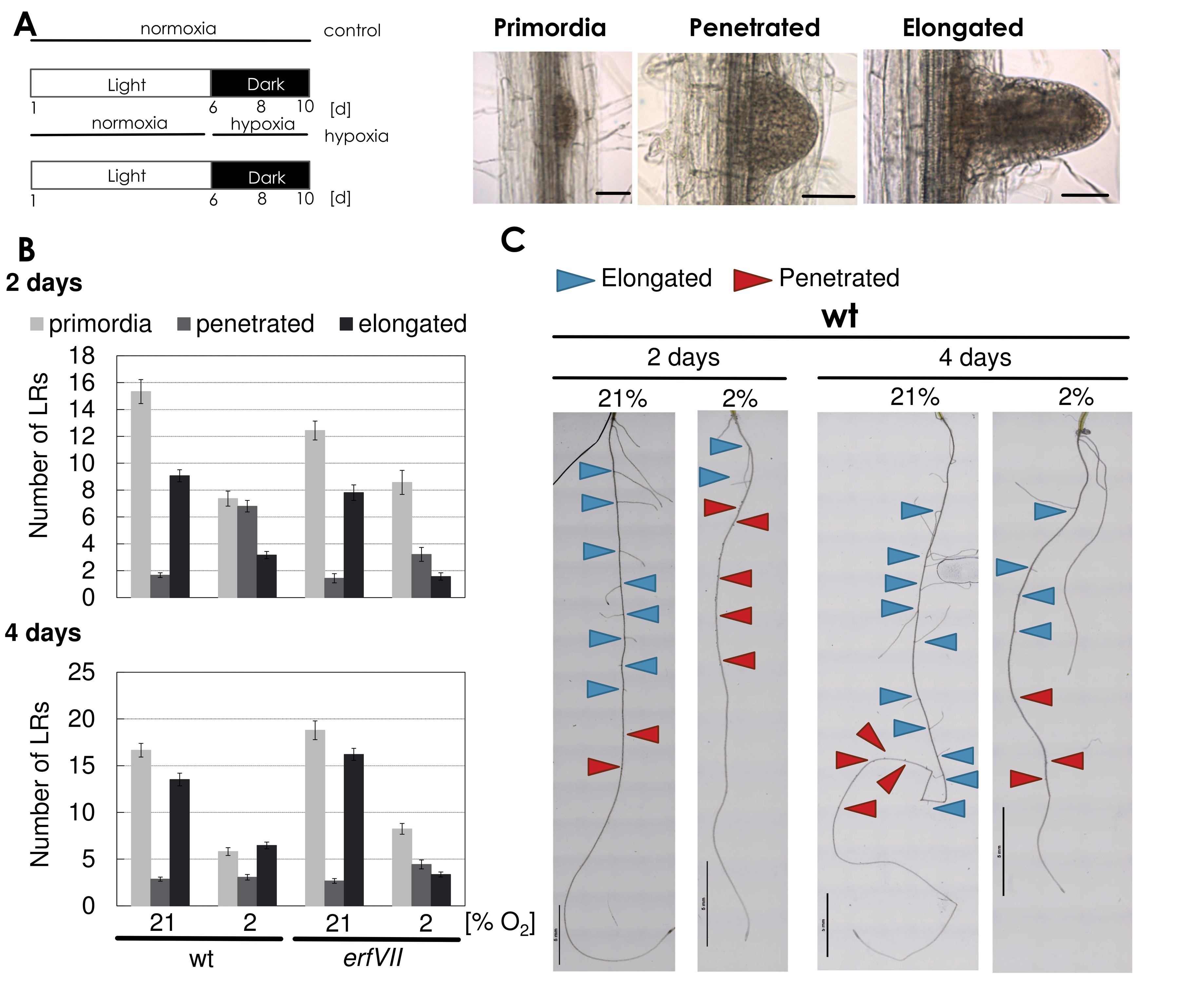

Figure 1. Hypoxia results reduced LR growth positively regulated by ERFVII TFs.
(A) Left: scheme indicating the treatment protocols. Right: stages of lateral root development.
(B) The average number of LRs categorized in primordia, penetrated and elongated LRs after 2 or 4 days of hypoxia in wild-type and *erfVII* pentuple (Abbas *et al*, 2014) seedlings.
(C) Representative wt seedlings under normoxic and hypoxic conditions with the indicated time. Red arrows show LRs in penetrated stage, blue arrows in elongated stage.

Inhibition of LR growth by low O_2 results from lowered cell division activity.

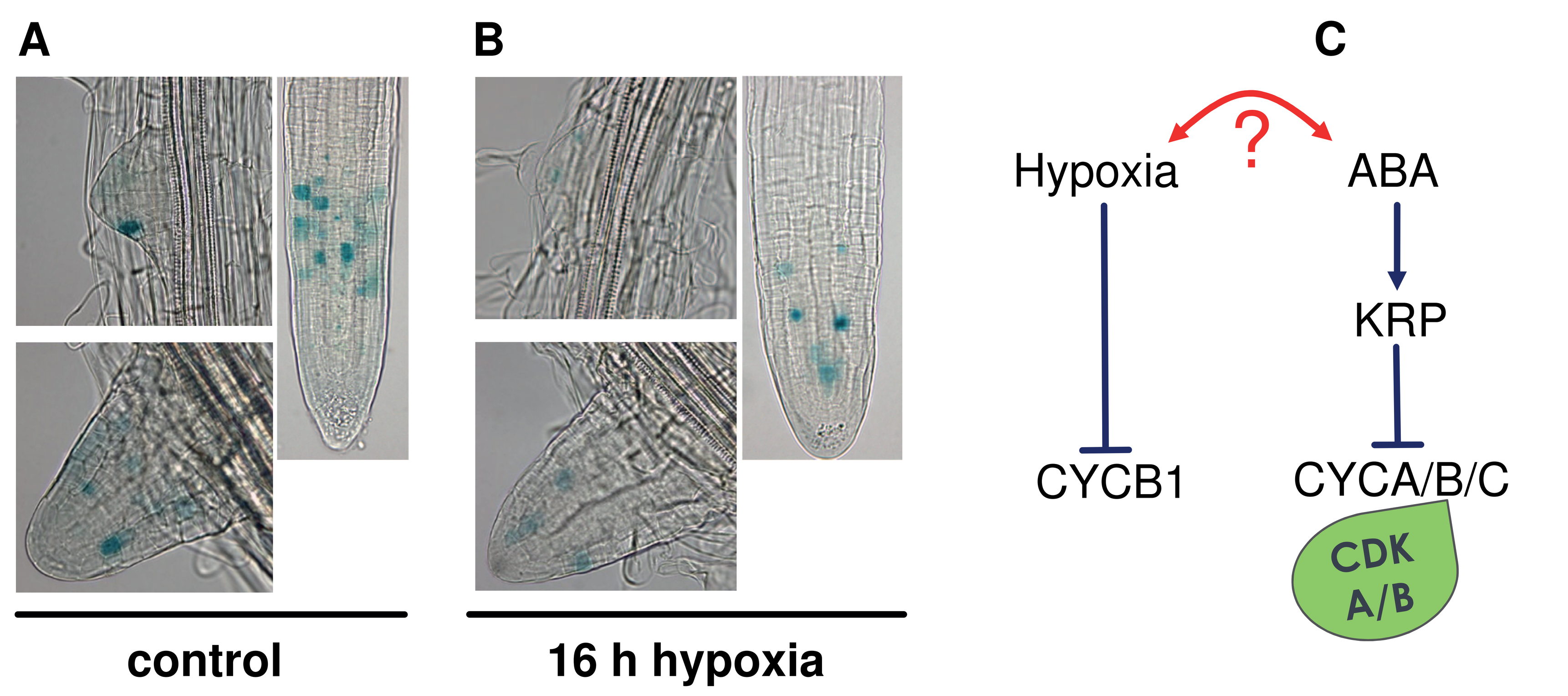

Figure 2. Hypoxia inhibits meristematic activity during LR growth.
Histocheimcal analysis of *CyclinB1;1::GUS* in developing LRs of 10-d-old light-grown seedlings exposed to dark at **(A)** control (21% O_2) or **(B)** hypoxic (2% O_2) conditions for 16 hours. **(C)** Model of ABA mediated inhibition of cell division (Kalve *et al*, 2014).

ABA enhances hypoxia response during LR development.

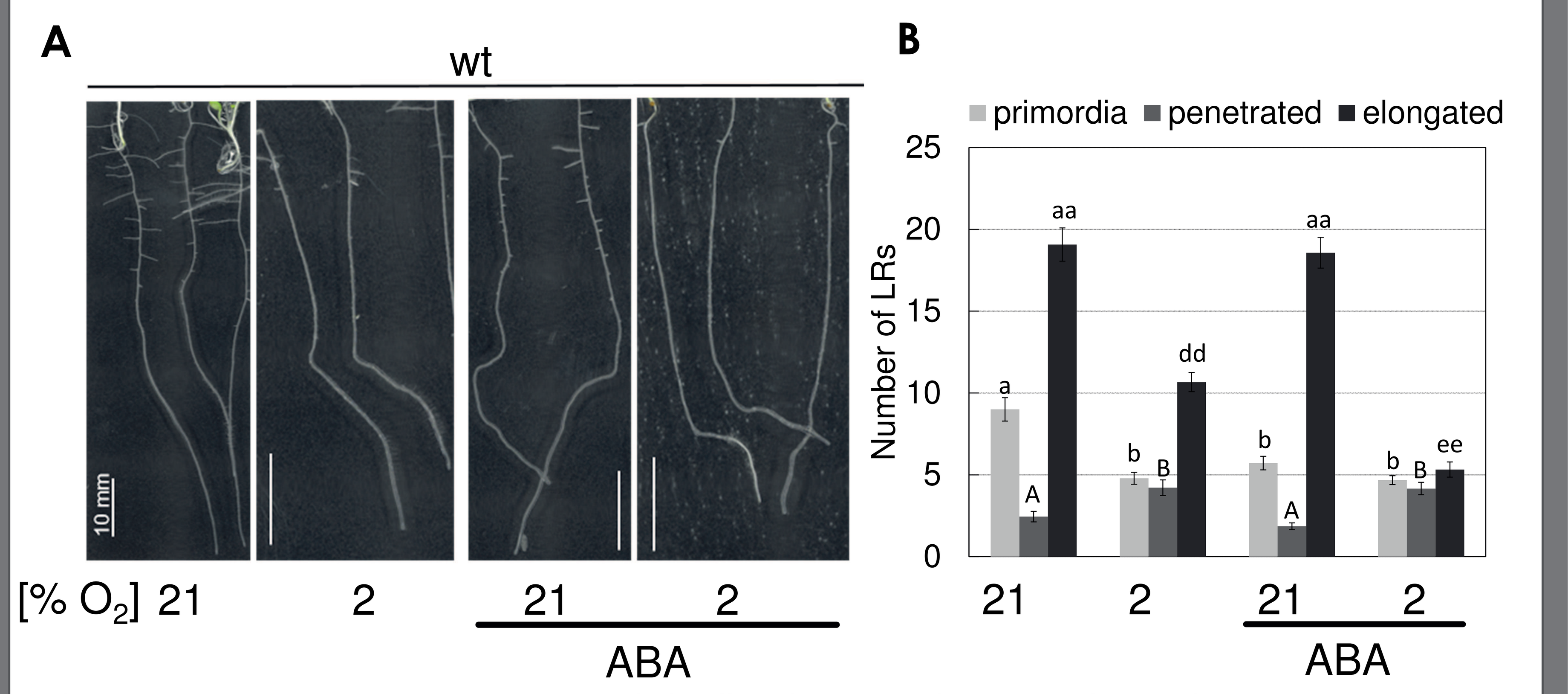

Figure 3. Exogenous ABA acts synergistically with hypoxia on LR elongation.
(A) Representative 6-d-old wild-type seedlings that were transferred in the dark to control (21% O_2) or hypoxic (2% O_2) conditions with or without 5 µM ABA for 4 days.
(B) Average number of wild-type LRs in defined stages (±SE) at the conditions indicated. Different minor or capital, single or double letters indicate significantly different values between treatments at defined LR stages (Kruskal-Wallis, $P<0.05$, n=29-31).

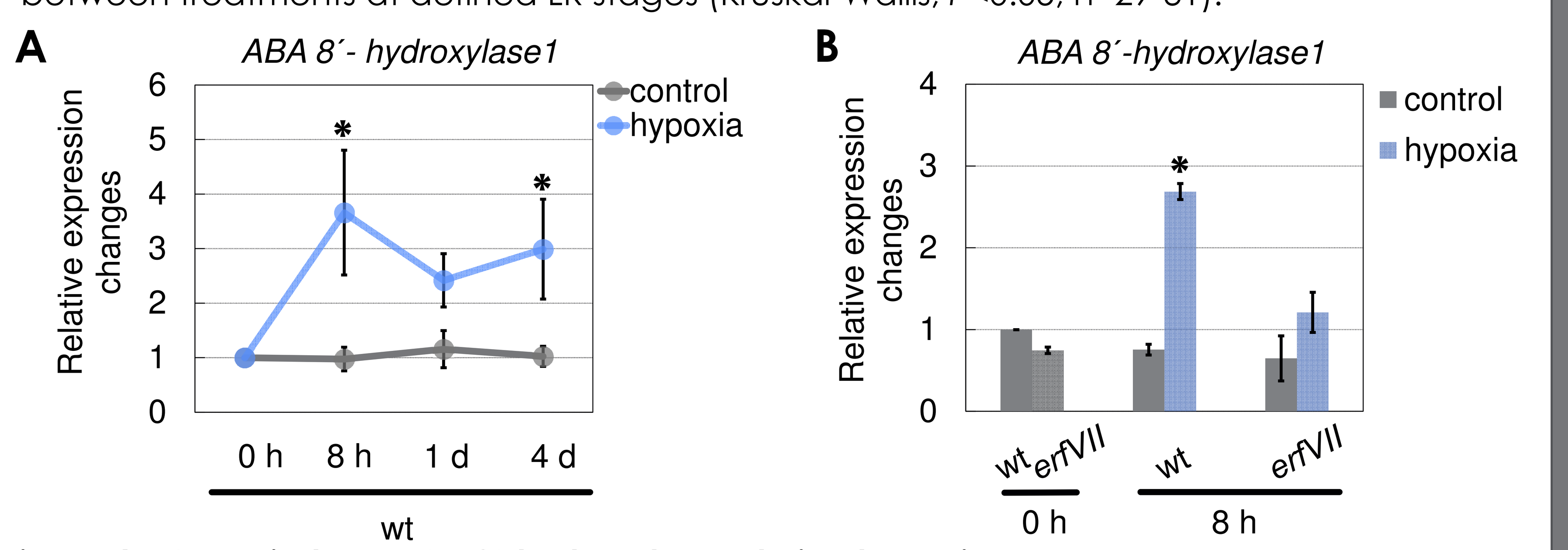

Figure 4. ERFVIIs induce *ABA 8'-hydroxylase1* during hypoxia.
(A) Kinetics of *ABA 8´-hydroxylase1* expression in wt roots (LR zone only) during hypoxia analyzed by qRT-PCR. Values represent averages (±SE) in 3 biological replicates and stars indicat significantly different values compared to untreated wt (One-way ANOVA with Tukey`s test, $P<0.05$, n=3)
(B) *ABA 8´-hydroxylase1* expression at normoxic or hypoxic conditions in wt and *erfVII* analyzed by qRT-PCR. Values were normalized to untreated wt roots. Values represent averages (±SE) in 3 biological replicates and star indicats significantly different value between treatments (Two-Sample T-Test, $P<0.05$, n=3).

Summary

- Hypoxia inhibits the number of initiated and elongated LR.
- ABA-hypoxia crosstalk regulate LR growth at hypoxic conditions.
- ERFVIIs maintain LR growth at hypoxic conditions by inducing *ABA´8 hydroxylase1* responsible for ABA degradation.
- Outlook:
  - Determine ABA metabolite levels.
  - Identify the ERFVII(s) responsible for LR growth promotion.
  - Verify ABA 8´ hydroxylase1 as a direct target of ERFVII(s).

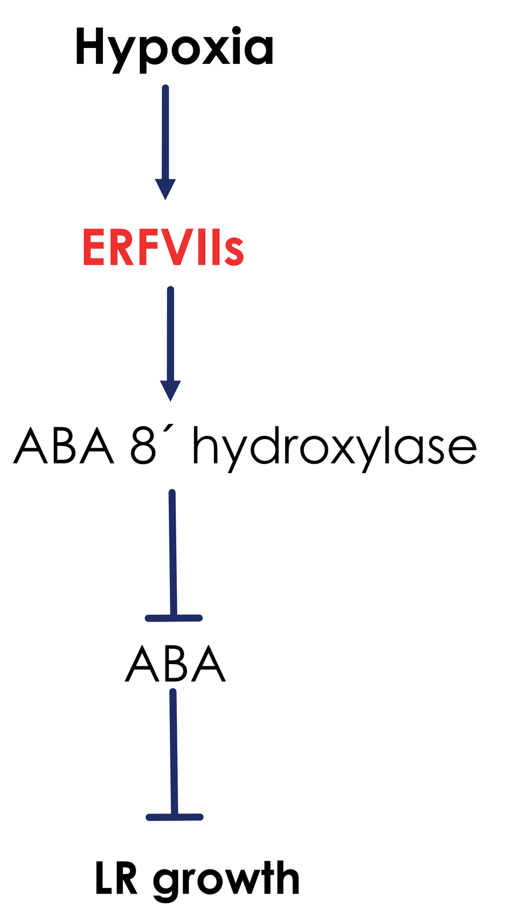

Figure 5. Model summarizing the molecular mechanism that maintains LR growth during hypoxia.

References
Paul MV, *et al* (2016) Oxygen sensing via the ethylene response transcription factor RAP2.12 affects plant metabolism and performance under both normoxia and hypoxia. *Plant Physiol.* 172(1):141-53.
Kalve S, De Vos D, Beemster G T. S. (2014) Leaf development: a cellular perspective. *Frontiers in Plant Science* 5(362):1-25.
Abbas M, *et al* (2015) Oxygen sensing coordinates photomorphogenesis to facilitate seedling survival. *Curr Biol.* 25: 1483-1488.

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