Title：Seedling's strategy to overcome a soil barrier.

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ABSTRACT:
The impact of the plant hormone ethylene on seedling development has long been recognized; however, its eco-physiological relevance is unexplored. Three recent studies demonstrate that ethylene is a critical endogenous integrator of various environmental signals including mechanical stress, light and oxygen availability during seedlings germination and growth through the soil.

Key words: light, mechanical stress, hypoxia, ethylene, germination.

Main text:

How do germinating seedlings cope with heterogeneity of their surrounding environment?

By nature, seeds mostly germinate in the subterranean environment. As germinating seedlings penetrate the soil and grow towards the soil surface mechanical forces gradually weaken, and simultaneously the amount of light and oxygen increases. Seedlings must sense all these changes and adequately adjust their development to prevent potential damage. The understanding of the molecular mechanisms of how plants perceive and integrate these external signals to precisely adjust their developmental pattern is one of the main challenges in plant research nowadays.

Three recent reports bring novel insights into mechanisms that underlie the communication between germinating seedlings and their surrounding environment [1-3]. A set of elegant assays to monitor the growth of seedlings through the soil with diverse depth and texture has demonstrated that ethylene which is a phytohormone with well-established function in seedling development [4], is a critical endogenous factor that coordinates this process [1-3].

Ethylene is perceived by a family of endoplasmic reticulum (ER) membrane-localized receptors that act as negative regulators in the ethylene-signaling pathway. In the absence of ethylene, the receptors activate CTR1, a Ser/Thr kinase that restrains the ethylene response [5]. Upon ethylene binding, the receptors inactivate CTR1. As a result, ETHYLENE INSENSITIVE2 (EIN2) is post-translationally processed such that its C-terminal domain is released to migrate to the nucleus [6]. In the nucleus, EIN2 activates the transcription factors ETHYLENE INSENSITIVE3 (EIN3) and EIN3 like1 (EIL1) to trigger the early transcription and the following secondary transcriptional response to ethylene [7, 8]. The EIN3 protein level is tightly regulated by the 26S-proteasome-mediated degradation pathway through two F box proteins, EBF1 and EBF2 (EBF1/2) [6-10] (Figure 1).

Through monitoring of the ethylene production and responses during seedling germination, Zhong and co-authors revealed that soil overlay effectively activates ethylene production which leads to the accumulation of the master-regulator of ethylene signaling EIN3, and to transcriptional induction of the second-tier ethylene transcription factor *ETHYLENE RESPONSE FACTOR* (*ERF1*), thereby inhibits hypocotyl elongation [1, 7, 8]. The stunted hypocotyl phenotype resulting from enhanced ethylene pathway contributes to the developmental adaptation that facilitates seedlings penetration through the soil. With decreasing distance from the surface the mechanical stress imposed by overlaying soil gradually diminishes. Simultaneously, seedlings are exposed to increasing amount of light that triggers their transition from dark (skoto-) to light (photo-) morphogenic developmental program [11].

To prevent photo oxidative damage that might be caused by sudden exposure to light, a timely onset of the chlorophyll biosynthesis and cotyledon greening is essential. Ethylene regulates the expression of *PHYTOCHROME INTERACTING FACTOR3* (*PIF3*) which is a direct target of EIN3 and thereby controls this process in an *EIN3/ETHYLENE INSENSITIVE like* (*EIL*)-dependent manner [12]. In mutants lacking either EIN3/EIL or PIF3 activity the expression of chlorophyll biosynthesis genes such as *HEMA1*, *GUN4* and *GUN5* is deregulated causing the accumulation of Pchlide and hypersensitivity to photo-oxidative damage [1]. Although the *pif3* mutant suffers from photo-oxidative damage in cotyledons, it exhibits normal hypocotyl growth in response to ethylene, indicating that hypocotyl elongation and chlorophyll biosynthesis downstream of *EIN3* are controlled through two independent pathways [1]. Thus ethylene through *PIF3* and *ERF1* transcriptional circuitry synchronizes elongation growth of seedlings with the onset of the chlorophyll biosynthesis to prevent damage by photo-oxidation after initial exposure to the light when penetrating through the soil [1] (Figure 1).

Cross-talk between light and ethylene signaling pathways orchestrates emergence of seedlings through the soil.

The newly revealed regulatory role of ethylene in the precise onset of the photo-morphogenesis implies that both ethylene and light signaling pathways are tightly interconnected. The recent study of Shi et al. brings important insights into molecular basis of the cross-talk between these two signaling pathways during germination [2] (Figure 1). This study reveals that both pathways converge at *CONSTITUTIVE PHOTOMORPHOGENIC 1* (*COP1*), the central repressor of the plant photo-morphogenic developmental program, to tightly control seedling emergence [2]. Severe defects of *cop1* seedlings in penetrating soil were partially rescued by overexpression of *EIN3*, which pointed at the regulatory link between light and ethylene pathways [2]. Detailed exploration of this interaction using genetic and biochemical tools revealed that COP1 promotes ubiquitination and 26-proteosome degradation of two F-box proteins, the EIN3 Binding F-box 1 (EBF1) and EBF2, previously found to target EIN3 for degradation [2, 9]. Noteworthy, although both COP1 and ethylene increase the EIN3 stability through the negative regulation of EBF1/2, they act independently and ethylene mediated EIN3 stabilization still occurs in the *cop1* mutant [2].

Altogether these findings shed light on the molecular strategies that plants have evolved to sense external signals of different quality and strength, and translate them into optimal growth and developmental responses. Growing seedlings are exposed to inverse gradients of mechanical forces and light that are translated into ethylene activity gradient. Whereas increasing mechanical forces promote ethylene biosynthesis and activity, increasing amount of light attenuates ethylene signaling through the COP1-pathway (Figure 1).

Ethylene pathway is involved in sensing of oxygen availability

Besides the light and mechanical stress, buried seedlings may also encounter fluctuations in oxygen content [3] (Figure 1). Abbas et al. have recently reported that germinating seedlings delicately respond to oxygen availability [3]. Under low oxygen conditions the apical hook, which protects the fragile apical shoot meristem from damage during penetration of soil, remains closed and its opening is repressed [3]. Furthermore, hypoxia conditions limit chlorophyll biosynthesis and` greening of cotyledons. Similarly to light and mechanical stress the ethylene pathway is also involved in sensing of the oxygen availability. At the molecular level oxygen promotes the degradation of the ethylene response factors of group VII (ERF-VII) and thus attenuates the ethylene pathway [3]. Remarkably, unlike ethylene-mediated responses to either light or mechanical stress that have an impact on apical hook angle and hypocotyl growth, the response of seedlings to oxygen availability is manifested exclusively in the apical hooks. Hence, a specific branch of the ethylene pathway involving ERF-VII factors is activated and translated into distinct developmental output in response to oxygen availability [3].

Concluding remarks

Rapid sensing and responding to constant changes in surrounding environment is one of the most important strategies for plant survival. Plants recognize fluctuations in various environmental factors such as nutrient, water, light, oxygen availability, or temperature and respond with amazing sensitivity by adapting their growth and development. However, how do plants sense these qualitatively and different signals? Recent reports indicate that despite different nature of these external inputs their sensing converges at the ethylene signaling pathway. Seedlings exposed to mechanical forces imposed by overlaying soil, light and oxygen gradients employ ethylene pathway to integrate and process these environmental stimuli into plant developmental program. The combinatorial effects of light, oxygen and mechanical stimuli act in concord with each other and other environmental factors to fine-tune seedling germination and penetration through soil. Interestingly, although ethylene is used as universal and rapid switch to react to different outer signals, the specific regulatory circuits are activated to achieve distinct developmental responses.

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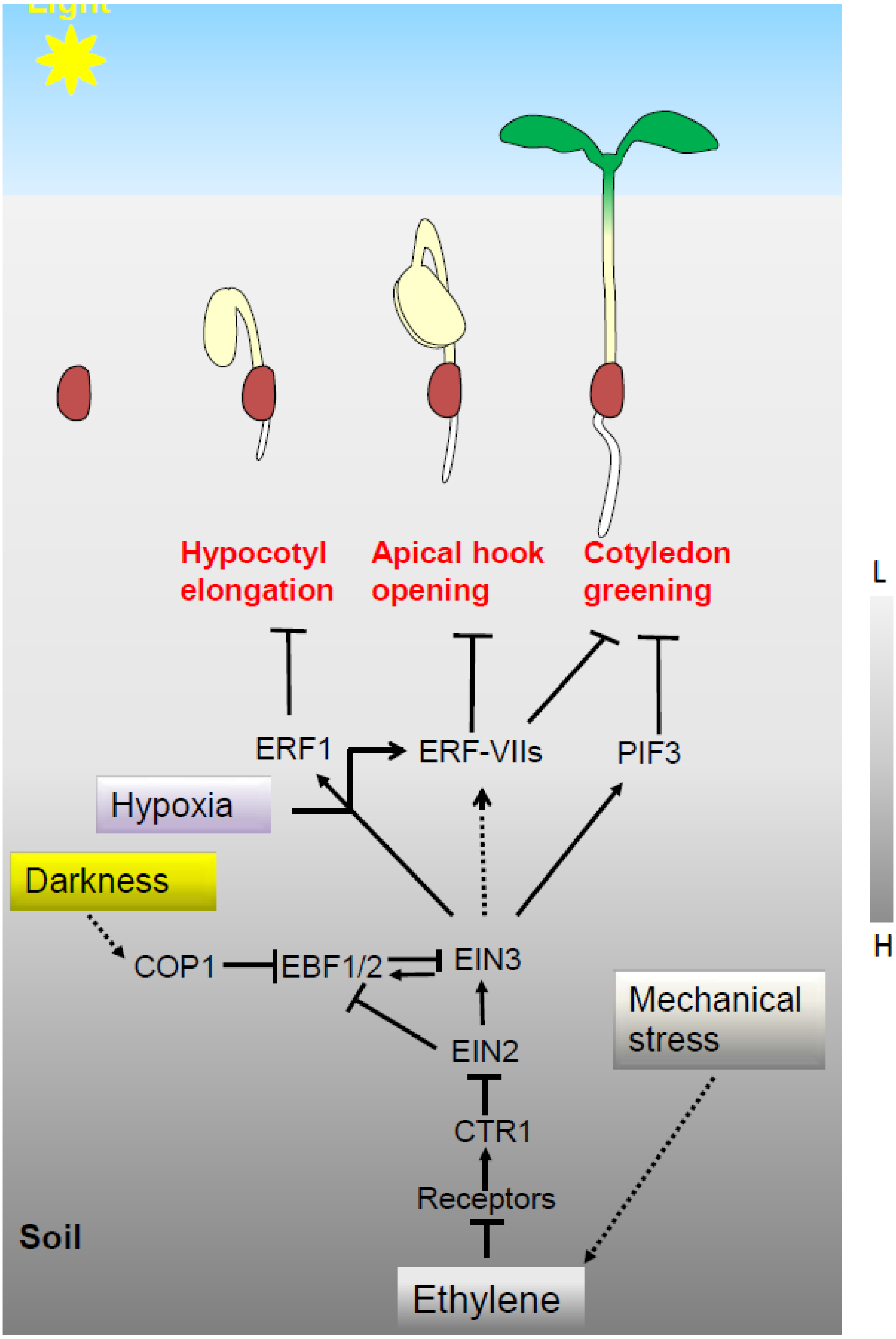

Figure 1. Ethylene pathway is involved in sensing mechanical stress, light and oxygen availability during seedlings germination.

Germinating seedlings are exposed to mechanical forces and light gradients that are translated into ethylene activity gradient. Whereas increasing mechanical forces promote ethylene biosynthesis and activity, increasing amount light attenuates ethylene signaling through

COP1-pathway to orchestrate the hypocotyl growth with greening of cotyledons. In addition, the ethylene pathway is involved in sensing of oxygen availability. Under hypoxia conditions the ethylene response factors of group VII (ERF-VII) are stabilized which enhances activity of a specific branch of the ethylene pathway controlling the apical hook development and greening of cotyledons. Colored rectangles indicate light/darkness, oxygen availability and mechanical stress gradients, respectively. High (H) to Low (L). (See text for details).