ETHYLENE RESPONSE FACTOR6, a central regulator of plant growth in response to stress

Ting Li,[1,2,3] Zhen Peng,[1] Kangxi Du,[4] Dirk Inzé,[2,3] and Marieke Dubois,[2,3,*]

[1] State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Sichuan Agricultural University, 611130 Chengdu, China.

[2] Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Gent, Belgium.

[3] Center for Plant Systems Biology, VIB, 9052 Gent, Belgium.

[4] State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Rice Research Institute, Sichuan Agricultural University, 611130 Chengdu, Sichuan, China.

Correspondence: Marieke.dubois@psb.vib-ugent.be

ABSTRACT

ETHYLENE RESPONSE FACTOR6 (ERF6) has emerged as a central player in stress-induced plant growth inhibition. It orchestrates complex pathways that enable plants to acclimate and thrive in challenging environments. In response to various abiotic and biotic stresses, ERF6 is promptly activated through both ethylene-dependent and -independent pathways, and contributes to enhanced stress tolerance mechanisms by activating a broad spectrum of genes at various developmental stages. Despite the crucial role of ERF6, there is currently a lack of published comprehensive insights into its function in plant growth and stress response. In this respect, based on the tight connection between ethylene and ERF6, we review the latest research findings on how ethylene regulates stress responses and the mechanisms involved. In addition, we summarize the trends and advances in ERF6-mediated plant performance under optimal and stressful conditions. Finally, we also highlight key questions and suggest potential paths to unravel the ERF6 regulon in future research.

KEYWORDS: ERF6, ethylene, plant growth, biotic stress response, abiotic stress response, signaling network

1 | INTRODUCTION

Being sessile organisms, plants need to adapt to a wide range of (a)biotic stresses (Zhu 2016; Brenya *et al.* 2022). To properly react to these environmental changes, multiple sophisticated mechanisms, like hormone signal transduction and kinase cascade activation, are induced, enabling plants to complete their life cycle under suboptimal growth conditions (Yu *et al.* 2020; Waadt *et al.* 2022; Zhang & Zhang 2022). Members of the APETALA2/ETHYLENE RESPONSIVE FACTOR (AP2/ERF) transcription factor family rapidly respond to environmental stimuli and alter downstream target gene expression to enable survival under stress conditions (Feng *et al.* 2020; Shoji & Yuan 2021). The ERF subfamily consists of 65 members in *Arabidopsis* (Nakano *et al.* 2006), all featuring a conserved AP2 domain that enables them to bind to target gene promoters and regulate gene expression, by acting either as activators or repressors (Wessler 2005). Other protein domains are distinct in different ERFs, which allow classification into several sub-groups.

ERF6, a transcriptional activator, belongs to the ERF group IX subfamily, which is further categorized into three subgroups based on the conserved motifs (CMIX) found in the proteins (Figure 1a) (Nakano *et al.* 2006). For instance, both ERF14 and ERF15 are classified under group IX-c due to the presence of the CMIX-1 motif, whereas the three osmotic stress-induced *ERF1A* and *ERF2* and *ERF13* belong to group IX-a as they share one CMIX-3 motif, a putative transcription activation domain. By contrast, ERF6 and its closest homolog ERF5 are classified within group IX-b, due to the presence of the CMIX-2 motif in their N-terminus, along with an additional putative MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) phosphorylation motif, designated as CMIX-5, located in the C-terminus. Mutations in these domains would significantly impact the stability and function of ERF proteins. For example, a single nucleotide substitution in the AP2 domain can lead to the loss of ERF6 function (Li *et al.* 2019). Alternatively, the Ser residues in the ERF6 CMIX-5 are crucial for ERF6 phosphorylation and stability: MPK3/MPK6 phosphorylate these residues, promoting ERF6 stabilization (Meng *et al.* 2013; Wang *et al.* 2013). In concert, compared to *35S::ERF6*^{WT}, a higher level of ERF6 protein is detected in the *35S::ERF6*^{4D} line, where these Ser residues were altered to mimic phosphorylation (Meng *et al*. 2013). Notably, inhibition of the 26S proteasome pathway still enhanced the ERF6 protein level in the *35S::ERF6*^{4D} line, suggesting that ERF6 is also subjected to an ubiquitin-proteasome degradation pathway independent of the MPK3/MPK6-mediated phosphorylation.

In contrast to group IX-c proteins, the roles of group IX-a and IX-b proteins in plant growth and stress response have been extensively investigated (Skirycz *et al.* 2011; Van den Broeck *et al.* 2017; Yu *et al.* 2024b). ERF6 serves as the core member in subgroup IX-b when it comes to studying stress-related phenotypes, as either *ERF6* loss- or gain-of-function mutants display clear sensitivity or tolerance to various stresses (Nakano *et al.* 2006). Interestingly, the role of ERF6 in stress responses was not restricted to Arabidopsis, but the peanut (*Arachis hypogaea*), grapevine (*Vitis vinifera*) and longan (*Dimocarpus longan*) *ERF6* orthologs were also associated to growth and stress response (Du *et al.* 2023; Zhang *et al.* 2023). Additionally, multiple stress conditions, including cold, hypoxia, osmotic and high light stress, and also fungal or bacterial attacks, are able to rapidly induce *ERF6* expression and stabilize ERF6 proteins, whereas cadmium represses *ERF6* (Figure 1b)(Hruz *et al.* 2008; Dubois *et al.* 2013; Meng *et al.* 2013; Vogel *et al.* 2014; Chen *et al.* 2024). Interestingly, the *ERF6* homolog *ERF5* is also induced by these stress conditions, suggesting that the upregulation of both *ERF6* and *ERF5* under multiple stresses may be subjected to similar transcriptional regulatory mechanisms (Moffat *et al.* 2012; Pan *et al.* 2012; Son *et al.* 2012; Wang *et al.* 2018; Illgen *et al.* 2020). Given the presence of MYB and WRKY binding motifs in both the *ERF6* and *ERF5* promoters, it is possible that these groups of transcription factors are involved in coordinating the expression of *ERF6* and *ERF5*. Notably, *ERF5* orthologs were also found to be stress-responsive in apple (*Malus domestica*), tomato (*Solanum lycopersicum*) and tobacco (*Nicotiana tabacum*) (Zhu *et al.* 2018; Ji *et al.* 2022; Wang *et al.* 2022a). Downstream, upon activation, ERF6 regulates downstream targets involved in signal transduction, lactoperoxidase activity and glutathione binding, contributing to the coordination of various biological processes such as leaf growth, detoxification and defense responses against pathogens. Moreover, being the stress-responsive hormone, ethylene plays a crucial role in the transcriptional activation of *ERF6* (Skirycz *et al.* 2011). Given the pivotal role of ethylene in the regulation of ERF6 activity, we begin this review with a brief summary of the ethylene biosynthesis and signaling pathway and its involvement in plant growth regulation during environmental stress. More importantly, we highlight that ERF6 acts as a key regulator in stress signaling and growth.

2 | OVERVIEW OF ETHYLENE BIOSYNTHESIS AND SIGNAL TRANSDUCTION IN *ARABIDOPSIS*

The biosynthesis of ethylene consists of three simple steps, with the first step being the conversion of the amino acid methionine (Met) into S-adenosyl-methionine (SAM) by SAM synthases (Lieberman & Kunishi 1965; Yang & Hoffman 1984; Wang *et al.* 2002). Subsequently, 1-aminocyclopropane-1-carboxylic acid (ACC)-SYNTHASEs (ACS) catalyze the rate-limiting step of ethylene biosynthesis by converting SAM to ACC (Figure 2a). ACC is then converted into ethylene, through further processing by ACC-OXIDASE (ACO). As the rate of ethylene biosynthesis is limited by the ACS level, regulation of *ACS* gene expression is a key mechanism to control ethylene production (Chae & Kieber 2005; Park *et al.* 2021). This is done post-translationally, for example by MPK3/MPK6 and CASEIN KINASE 1.8 (CK1.8), phosphorylating ACS2 and ACS5, respectively, leading to increased ethylene levels under stress conditions (Tan & Xue 2014; Wang *et al.* 2022b).

Upon ethylene accumulation, the ETHYLENE RESPONSE1 (ETR1) receptor inhibits the kinase CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) from phosphorylating ETHYLENE-INSENSITIVE2 (EIN2), resulting in the cleavage of its C-terminal end (EIN2-CEND) (Figure 2a)(Kieber *et al.* 1993; Alonso *et al.* 1999; Hall *et al.* 2000; Zhao *et al.* 2021a). Consequently, EIN2-CEND translocates to the nucleus, protecting EIN3 and EIN3-LIKE1 (EIL1) from degradation by EIN3-BINDING F-BOX PROTEIN (EBF1/2) (An *et al.* 2010; Qiao *et al.* 2012; Li *et al.* 2015). Downstream, the ERFs are activated for the ethylene response (Solano & Ecker 1998; Binder 2020; Feng *et al.* 2020). Interestingly, despite CTR1 being generally considered as a negative regulator of ethylene signaling, a recent study found that the CTR1 protein still partially translocates to the nucleus and triggers a second ethylene response in an EIN2-independent manner (Figure 2a) (Park *et al.* 2023). Moreover, the nuclear localization of EIN2 is also modulated by the glucose-activated TARGET OF RAPAMYCIN (TOR) kinase, suggesting a role of EIN2 in response to metabolic signals (Fu *et al.* 2021). In addition to the canonical CTR1-mediated ethylene signaling pathway, ethylene signal transduction can circumvent the CTR1 kinase. This non-canonical transduction might be achieved by the interaction of ETR1 and the cytokinin-positive regulator HISTIDINE-CONTAINING PHOSPHOTRANSMITTER (AHP) (Scharein *et al.* 2008; Zdarska *et al.*

2019). Overall, these diverse regulatory mechanisms illustrate the need for a precise control of ethylene synthesis and signaling transduction in plants (Figure 2a).

3 | THE MULTIPLE ROLES OF ETHYLENE IN STRESS RESPONSES

Although the chemical structure of ethylene is simple, ethylene broadly affects a large variety of pivotal biological processes (Bleecker & Kende 2000; Dubois *et al.* 2018; Hartman *et al.* 2021; Huang *et al.* 2023). Multiple stress conditions, such as wounding, osmotic stress and cold, alter *ACS* expression or ACS protein stability, inducing ethylene synthesis (Figure 2b) (Dong *et al.* 2011; Catalá *et al.* 2014; Li *et al.* 2018). Further downstream, a wide array of proteins acts to connect the ethylene accumulation to a proper stress response, depending on which type of stress is perceived. As such, the core component of the SALT OVERLY SENSITIVE (SOS) pathway, SOS2, interacts with CTR1 to induce EIN2-CEND cleavage, initiating the activation of salt-responsive genes and enhancing plant resilience to salt stress (Li *et al.* 2024). Ethylene is involved in temperature stress resistance as well (Bolt *et al.* 2017; Antonietta *et al.* 2023; Huang *et al.* 2023), as the ethylene-insensitive mutants *etr1-1* and *ein2-5* show heat sensitivity and freezing tolerance (Figure 2b) (Larkindale *et al*., 2005; Shi *et al*., 2012). Recent findings revealed that moderate increases in temperature disrupt EIN3 protein proteolysis by targeting the degradation of EBF1/2 through SALT- AND DROUGHT-INDUCED RING FINGER1 (SDRF1), as such modulating the ethylene response during changing temperature (Hao *et al.* 2021). Ethylene also serves as the primary signal in response to flooding stress (Hartman *et al.* 2019; Hartman *et al.* 2021). The intrinsic ethylene levels rise rapidly in waterlogged root tips and further induce EIN2-dependent core hypoxia genes transcription when O_2 is depleted (Hinz *et al.* 2010; Licausi *et al.* 2011; Yang *et al.* 2011). In addition, ethylene also contributes to flooding acclimation by promoting the phosphorylation of the initiation factor 2α (eIF2α) by GENERAL CONTROL NON-DEREPRESSIBLE 2 (GCN2) (Lageix *et al.* 2008). This phosphorylation inhibits overall protein translation, while stimulating the translation of hypoxia-related mRNAs (Cho *et al.* 2022). Finally, with the increase of ethylene production upon osmotic stress, several *ERFs*, including *ERF1*, *ERF2*, *ERF5*, *ERF6* and *ERF11* are rapidly induced in growing leaves (Skirycz *et al.* 2011). Interestingly, this type of induction is still observed in the absence of EIN3 and EIL1, suggesting that an EIN3 and EIL1-independent pathway is established for the regulation of *ERFs* by osmotic stress. Possibly, ERFs that can be activated

post-translationally (by MPK3/MPK6, Figure 1b) and that can activate their own expression, such as ERF6, could be one of these pathways. In conclusion, ethylene plays a complex role in the interaction of plants with environmental stress (Figure 2b).

4 | ERF6 CONNECTS ETHYLENE AND GIBBERELLIN SIGNALING IN GROWTH REGULATION

Growing evidence indicates that ERF6 functions as a central molecular hub to fine-tune plant growth and defense tradeoffs. In *Arabidopsis*, ERF6 inhibits cell division and expansion, ultimately resulting in the suppression of leaf growth (Dubois *et al*. 2013). Therefore, *ERF6* overexpression results in dark green and dwarf plants, while *erf5erf6*, double loss-of-function mutants, and the double mutant of *erf6* with another ERF, *erf13*, are larger compared to wild-type plants (Dubois *et al.* 2013; Meng *et al.* 2013; Chen *et al.* 2024). Among the early induced ERF6 target genes, *GIBBERELLIN 2-β-DIOXYGENASE 6* (*GA2OX6*) was identified. The oxygenase GA2OX6 is responsible for inactivating gibberellin (GA), a crucial phytohormone promoting growth. GA blocks root and leaf growth through the degradation of the GA repressor protein DELLA (Yamaguchi 2008; Davière & Achard 2013). During the vegetative stage, DELLA proteins orchestrate cell division and cell expansion through the promotion of genes encoding cell cycle inhibitors like *KIP-RELATED PROTEIN 2* (*KRP2*) and *SHOOT MERISTEMLESS* (*STM*) and the repression of *EXPANSIN8* (Achard *et al.* 2009; Serrano-Mislata *et al.* 2017). Interestingly, ERF6 stabilizes DELLA proteins, most likely as a consequence of the induction of *GA2OX6* (Figure 1c). Furthermore, overexpressing *GA20-OX* in the *35S::ERF6-GR* line, in which ERF6 is tagged with a rat glucocorticoid receptor domain to control its activation, reverses the dwarf phenotype caused by ERF6, highlighting the essential role of the GA-DELLA module in ERF6-mediated growth inhibition. In addition, the upregulation of *ERF6* also induces its downstream target *ERF11* (Figure 1c). In turn, ERF11, a transcriptional inhibitor, competes with ERF6 for the regulation of downstream target genes, such as *MYB51*, *GA2OX6*, in a dose-dependent manner, leading to the downregulation of *GA2OX6* and partial abolishment of the dwarfism caused by elevated *ERF6* expression (Dubois *et al.* 2015). On top of its negative effect on *GA2OX6* expression, ERF11 enhances the GA response in the internode by stimulating the production of bioactive GA_4 and

physically antagonizing DELLA proteins (Figure 1c). As a result, it facilitates cell expansion and promotes increased plant height (Zhou *et al.* 2016).

Overall, these studies demonstrate that ERF6 connects ethylene and GA signaling in a dual manner (Figure 1b). On one front, ERF6 inhibits GA signaling by enhancing *GA2OX6* expression, thereby reducing the levels of bioactive GAs. Conversely, ERF6 induces *ERF11*, encoding a protein that promotes the GA response in the internode. Although these two pathways seemingly counteract each other, it suggests that the regulation of plant growth by ethylene and GA does not occur in a linear manner but likely involves feedback loops or tissue-specific regulation to finely modulate plant growth.

5 | ERF6 TRANSMITS OSMOTIC STRESS SIGNALS TO DOWNSTREAM STRESS DEFENSE GENES

For decades, in-plate osmotic stress has been utilized to mimic drought stress *in vitro* since both conditions lead to turgor loss in plants. However, unlike drought stress, osmotic stress generated by mannitol, sorbitol, sucrose or NaCl triggers plasmolysis and, thus, a distinct downstream molecular response compared to in soil-applied drought stress (Yu *et al.* 2024a). Notably, *ERF6* gene expression is quickly induced by osmotic stress but not by drought stress (Clauw *et al.* 2015; Dubois *et al.* 2017). Upon short-term osmotic stress treatment, a series of molecular events takes places (reviewed in Yu *et al.* (2024a)). Changes in membrane tension activate the hyperosmolarity-gated calcium channel OSCA1 protein, initiating the influx of cytosolic free Ca^{2+} into the cytoplasm (Yuan *et al.* 2014; Pei *et al.* 2022; Han *et al.* 2024). These calcium ions are subsequently taken up by the chloroplast with the help of CHLOROPLAST-LOCALIZED MITOCHONDRIAL CALCIUM UNIPORTER (cMCU). Further downstream, histidine kinases (HKs) and receptor-like kinases (RLKs) directly perceive the physiological alterations (Hoang *et al.* 2021). The ethylene receptor ETR1, which is one of the HK kinases in play, interacts with the RAF-like kinase ARK to transfer the signal to the subclass III-type sucrose-non-fermenting-1(SNF1)-related protein kinase 2 (SnRK2), which in turn induces an osmotic stress response (Lin *et al.* 2020; Soma *et al.* 2020).

Interestingly, within 10 min, the stromal Ca^{2+} signal also initiates the activation of MPK3/MPK6 and the expression of *ERF6* (Teardo *et al.* 2019). Accordingly, in the absence of cMCU, plants fail to induce *ERF6* expression, enhancing resistance towards mannitol. Confirming this finding, our lab previously observed that treating plants with mannitol causes a rapid increase in ACC levels, together with a fast elevation of *ERF5* and *ERF6* gene expression in actively growing young leaves (Skirycz *et al.* 2011). The fast induction of *ERF5* and *ERF6* by osmotic stress may be attributed to elevated ethylene levels, but given the completion of this signal transduction within 10 min, it is plausible that the induction of *ERF6* expression does not occur via the canonical ethylene signaling pathway. An alternative could be the earlier-discussed MPK3/MPK6 cascade that is activated by ethylene. Upon ERF6 activation by osmotic stress, ERF6 in turn regulates the expression of its targets. When comparing genes that are activated by osmotic stress and by ERF6, we observed not only stress-responsive genes, such as *SALT TOLERANCE ZINC FINGER* (*STZ*) and *MYB51*, but also many genes associated with growth (Figure 1c) (Skirycz *et al.* 2011). Accordingly, mutation of *ERF5* and *ERF6* allows plants to grow better than wild-type plants when exposed to mild osmotic stress, suggesting a growth-repressive function for ERF5 and ERF6 under osmotic stress.

While there is currently no clear evidence in the literature that drought stress triggers *ERF5* and *ERF6* expression, one study reported that *ERF5* and *ERF6* might participate in the regulation of the drought response (Arjmand *et al.* 2023). In addition, two studies performed on tomato observed that increasing the expression of the paralog *SlERF5* could enhance the survival rate of tomato plants subjected to severe drought (Pan *et al.* 2012; Zhu *et al.* 2018). However, when overlapping the genes that are upregulated by both drought stress and ERF6, only 12 genes were identified, indicating that ERF6 may not serve as the primary regulator in the drought response (Figure 1c). Additionally, it remains unclear whether *ERF5* and *ERF6* overexpression lines exhibit a more tolerant phenotype under severe drought stress in *Arabidopsis*.

6 | ERF6 PROTECTS PLANTS UNDER OXIDATIVE AND HIGH LIGHT STRESS

Rapid induction of the *ERF6* gene is also triggered by high light and oxidative stress (Vermeirssen *et al.* 2014). Under high light and oxidative stress, reactive

oxygen species (ROS) like superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) are produced, leading to cellular membrane damage, protein structure alterations and even cell death (König *et al.* 2018; Shi *et al.* 2022; Wu *et al.* 2023). In response to ROS, plants produce ROS scavenger-like SUPEROXIDE DISMUTASES (SODs) that convert $O_2^{\bullet -}$ to H_2O_2, which is subsequently eliminated by CATALASES (CATs) and PEROXIDASES (PRXs) (Waszczak *et al.* 2018; Abuelsoud *et al.* 2020). In contrast, low-level ROS function as signaling molecules to efficiently modulate various cellular processes, like cell proliferation and differentiation, by activating protein cascades and regulating gene expression (Mittler 2017; Qi *et al.* 2018).

ERF6 is considered as a ROS-responsive gene, with its expression being strongly and rapidly stimulated either by exogenous ROS application from the herbicide paraquat or by subjecting young seedlings to high light conditions (Figure 1b) (Sewelam *et al.* 2013; Wang *et al.* 2013; König *et al.* 2018; Roeber *et al.* 2021). The mechanism by which the ROS signal activates ERF6 is not fully elucidated. One possible pathway could involve ethylene, as ROS has been shown to induce *ACS* genes in rice and *Arabidopsis* (Wi *et al.* 2012; Martin *et al.* 2022). Furthermore, ROS activate MPK3/MPK6, leading to the stabilization of the ERF6 protein (Meng *et al.* 2013; Wang *et al.* 2013). Additionally, in the *triose phosphate translocator* (*TPT*) mutant, where the export of triose phosphate from the chloroplast to the cytosol is compromised, early *ERF6* induction by high light is absent, suggesting a role for metabolic signals in regulating early ERF6 responses (Vogel *et al.* 2014).

Similarly to the osmotic stress-responsive genes, a significant portion of the ROS-responsive genes can be regulated by ERF6. Approximately 15% of genes induced by ROS are upregulated in *35S::ERF6^{4D}* young seedlings and/or by *35S::ERF6-GR* (Figure 1c) (Dubois *et al.* 2013; Meng *et al.* 2013; Sewelam *et al.* 2013; Wang *et al.* 2013). Moreover, ERF6 is able to bind to a specific element called ROS7/GCC in the promoter regions of target genes (Wang *et al.* 2013). Genes such as *WRKY33*, which are upregulated by ROS and ERF6, have one or more ROS7/GCC boxes in their promoter regions, indicating potential direct binding of ERF6 during the ROS response. Although no ROS7/GCC box was identified in the promoters of *MYB51* and *DARK INDUCIBLE11* (*DIN11*), these two genes are also promoted by ROS and ERF6, but potentially through another binding site. Interestingly, some genes commonly upregulated in the *35S::ERF6-GR* and *35S::ERF6^{4D}* plants, like *PATHOGENESIS-RELATED* (*PR5*) and *PROTODERMAL FACTOR1.2* (*PDF1.2*), are not induced by short-term H_2O_2 in the wild type but can be further induced by ERF6

upon exogenous H_2O_2 application. Hence, these genes could also be classified as ROS-responsive genes possibly directly regulated by ERF6. Conversely, evidence suggests that ERF6 alters the H_2O_2 level in plants, as an increased H_2O_2 content was observed in the *erf6* mutant. Accordingly, *erf6* displays a more sensitive phenotype in response to oxidative stress. This may be achieved by the strong downregulation of ROS-induced *CATALASE3* (*CAT3*) and *MONODEHYDROASCORBATE REDUCTASE3* (*MDAR3*), two enzymes responsible for ROS-detoxification, in *erf6* (Sewelam *et al.* 2013).

Altogether, these published studies support a role for ERF6 in the oxidative stress response. ERF6 is transcriptionally induced by ROS and stabilized at the protein level (Wang *et al.* 2013). Subsequently, ERF6 participates in the ROS response to protect plants by activating oxidative stress-defense genes and by, likely indirectly, regulating ROS-detoxification enzymes to maintain the ROS levels at a low, harmless level.

7 | ERF6 IS REQUIRED FOR THE BIOTIC STRESS RESPONSE

Plants frequently encounter biotic stress, including attacks from bacteria, viruses, fungi or oomycetes. In response to these pathogens, plants reprogram multiple hormone signaling pathways to combat the invaders. Different hormones play distinct roles toward specific pathogens (Bastías *et al.* 2022). Upon necrotrophic pathogen attack, jasmonic acid (JA) levels are quickly elevated and promote the expression of the downstream *ERF1* and *ORA*/*ERF59* genes, and these ERFs further induce *PDF1.2* expression (Berrocal-Lobo *et al.* 2002; Zander *et al.* 2014; Song *et al.* 2022). Ethylene also accumulates during necrotrophic pathogen infections and triggers the expression of *ERF1*, *ORA*/*ERF59* and *PDF1.2* (Kim *et al.* 2018; Yang *et al.* 2021). In contrast, biotrophic pathogens activate the salicylic acid (SA) pathway, stimulating SA synthesis and subsequently inducing the expression of a group of WRKY transcription factors and *Pathogenesis-Related* (*PR*) genes (van Verk *et al.* 2011; Han *et al.* 2022).

Whereas ethylene might play a less prominent role in the biotic stress response compared to JA or SA, studies have demonstrated significant functions for ERF6 in regulating genes responsive to biotic stresses (Figure 1b-c). Upon necrotrophic pathogen attack, *ERF5* and *ERF6* are strongly induced in infected leaves (Moffat *et al.* 2012). Transcriptome analysis in *35S::ERF6*^{4D} and *35S::ERF6-GR* lines identified a substantial number of genes belonging to the *GLUTATHIONE S-TRANSFERASE*

(*GST*), *PR*, and *PDF* families that are typically highly induced by pathogens. An overlap analysis of genes induced by the necrotrophic pathogen *Botrytis cinerea* and the ERF6-responsive genes shows that approximately 58% and 31% of pathogen-responsive genes are upregulated in *35S::ERF6^{4D}* and *35S::ERF6-GR* plants, respectively (Sham *et al.* 2014) (Figure 1c). Among these genes, *PDF1.1* and *PDF1.2* are of particular interest: following *B. cinerea* inoculation, both genes are strongly induced in *35S::ERF6^{4D}* but not in *35S::ERF6-EAR* plants, where the ERF6 function is repressed by the ERF-associated amphiphilic repression (EAR) motif. This indicates a critical role for ERF6 in regulating the expression of genes involved in the defense response against necrotrophic pathogens. Moreover, ERF6 promotes the biosynthesis of 4-methoxyindol-3-ylmethylglucosinolate (4MI3G) by directly triggering the genes encoding key enzymes in this pathway, such as *CYP81F2* and *INDOLE GLUCOSINOLATE O-METHYLTRANSFERASE* (Xu *et al.* 2016). 4MI3G is a natural product derived from aliphatic glucosinolates that increases plant innate immunity against various fungal and oomycetic pathogens (Bednarek *et al.* 2009; Tao *et al.* 2022). Supporting this, the *ERF6*-overexpressing plants exhibit an increased resistance against the necrotrophic *B. cinerea*, while *B. cinerea* causes more damage to *35S::ERF6-EAR* plants (Xu *et al.* 2016). Furthermore, the *PDF1.2* promoter contains a ROS/GCC-box *cis*-element, which can be bound by ERF6. Strikingly, overexpressing *ER6F^{4D}* in the constitutive ethylene response mutant *etr1-1* and ethylene insensitive mutant *ein2* does not influence the positive impact of ERF6 on *PDF1.2* expression, demonstrating that alternative signaling pathways, rather than ethylene signaling, are involved in this context (Meng *et al.* 2013). However, it is worth noting that another study presented contradictory results, where overexpression of *ERF5* causes hypersensitivity to necrotrophic fungi *Alternaria brassicicola*, while the *erf5erf6* double mutant exhibited enhanced tolerance against this pathogen (Son *et al.* 2012).

By contrast, ERF6 contributes a negative role during infection with biotrophic pathogens, like *Golovinomyces cichoracearum*. For example, ERF6 indirectly suppresses the expression of *RESISTANCE TO POWDERY MILDEW 8.1* (*RPW8.1*), which encodes an atypical resistance protein involved in broad-spectrum resistance against powdery mildew pathogens (Zhao *et al.* 2021b). Therefore, the *erf6* mutant shows increased immunity against powdery mildew pathogens. Comparative analysis of genes upregulated in *35S::ERF6^{4D}* and *35S::ERF6-GR* plants with those responsive to the pathogen *Pseudomonas syringae* (*Pst*) reveals

a significant overlap. Because the *erf6* mutant displays increased susceptibility to *Pst* DC3000 infection, these data suggest that ERF6 also participates in defense against *P. syringae* (Son *et al.* 2012; Gupta & Senthil-Kumar 2017). One of the critical genes involved in defense against *P. syringae* is SA-induced *PR1*, which has been shown to be induced upon *ERF5* or *ERF6* overexpression (Son *et al.* 2012). In accordance, overexpressing *ERF5* or *ERF6* has been associated with an increased resistance to *P. syringae*, although this could not be confirmed in another study (Moffat *et al.* 2012). Discrepancies observed in these studies might be attributed to variations in time points or methods of pathogen invasion.

Besides fungi and bacteria, other pathogens, like the root-knot nematode *Meloidogyne incognita*, also trigger ERF6 function (Warmerdam *et al.* 2019). A genome-wide association analysis of genome loci linked to invasion by this nematode highlights ERF6 for modulating defense processes against these destructive pathogens. After *M. incognita* infection, 327 genes that are enriched for nucleotide metabolism, photosynthesis and hormone metabolism processes, are differentially expressed in the hypersusceptible *erf6* mutant, but not in the wild type, underscoring the control of ERF6 over this response and its contribution to the plant's tolerance. Interestingly, the ethylene synthesis genes *ACO2* and *ACO3* are repressed in nematode-infected *erf6* roots, suggesting that ERF6-dependent ethylene synthesis may be required for the resistance to *M. incognita* (Warmerdam *et al.* 2019). Taken together, these findings underline the complex and context-dependent roles of ERF6 in modulating plant responses to different types of pathogens, highlighting the need for further research to elucidate the intricate signaling pathways involved in plant–microbe interactions.

8 | CONCLUDING REMARKS AND PERSPECTIVES

Although emerging evidence highlights the role of ERF6 in the regulation of plant growth and diverse stress responses, several unresolved questions deserve to be explored in future studies. First, apart from the stress conditions discussed earlier, it is worth noting that other environmental stresses, like virus infection, hypoxia and cold stress, can also trigger the expression of *ERF6* (Dubois *et al.* 2013; Illgen *et al.* 2020). Given that ERF5, a close homolog of ERF6, has been implicated in plant resistance against viruses, it is interesting to address the questions of whether ERF6 plays a similar role in viral response regulation and whether these regulatory mechanisms overlap.

Second, in response to many abiotic stresses, both ethylene and *ERF6* expression are induced within a very short time, raising the question of how ERF6 transcription is regulated so fast. Presently, EIN3 and EIL1 are the sole known transcription factors partially involved in *ERF6* expression regulation. An alternative is that ethylene-induced MPK3/MPK6 activity leads to increased phosphorylation of ERF6 (Meng *et al.* 2013). This more stable form of ERF6 might initiate a positive feedback loop that promotes *ERF6* expression. Furthermore, it has been observed that *ERF6* transcripts are unstable, as evidenced by the detection of ERF6-derived siRNAs in the *35S::ERF6-GR* line (Li *et al.* 2019). Therefore, it is possible that *ERF6* transcripts rapidly accumulate by modulating the siRNA-mediated post-transcriptional gene silencing pathway during early stress responses (Wu *et al*. 2020), and this novel hypothesis requires exploration.

Third, the current understanding of the regulation of ERF6 by post-translational modifications is still incomplete. Actually, only phosphorylation of ERF6 by MPK3/MPK6 at specific sites has been studied (Meng *et al.* 2013; Wang *et al.* 2013). However, *in silico* analysis predicts other potential phosphorylation sites that remain to be investigated (Netphos 3.1, Blom *et al.* 2004), and it is known that the interplay between different phosphorylations is vital for fine-tuning protein activities (Fu *et al.* 2021; Bilbrough *et al.* 2022). Additionally, a prior study confirmed that the phosphorylated form of ERF6 can be stabilized by proteasome inhibition, suggesting the possibility of ERF6 being ubiquitinated. Therefore, deciphering novel phosphorylation and ubiquitination sites and revealing the regulatory processes controlling them could provide valuable insights into the regulation of the stability or function of the ERF6 protein.

Fourth, we are currently still lacking insights into how exactly ERF6 regulates its targets. A comparison of two transcriptome datasets from *35S::ERF6-GR* (in very young leaves) and *35S::ERF6^{4D}* (in 12-day-old seedlings) revealed that 50% of genes upregulated by ERF6^{4D} are also induced by ERF6-GR proteins (Figure 1c). These common genes, including for example *PDF1.2* and *DIN11*, can be considered as robust ERF6 targets, induced independently of the developmental context. However, not all target gene promoters contain the ROS7/GCC box, suggesting that additional, yet to be discovered, binding sites may be necessary for ERF6-mediated transcriptional regulation. Alternatively, a previous study proposed that ERF6 might be intricately associated with chromatin (Meng *et al.* 2013). This suggests that ERF6 acts as a pioneering transcription factor to initiate gene

transcription through chromatin remodeling, but more concrete analyses are required to further confirm this and other ERF6 modes of action.

Fifth, given the strong phenotype caused by *ERF6-GR* and *ERF6^{4D}* overexpression, it appears unlikely that the downregulation of the GA response by ERF6 is solely responsible for this phenotype. It is essential to dive deeper into the interplay between ERF6 and a broader array of growth-related genes to gain a comprehensive understanding of how ERF6 regulates plant growth.

Finally, as ERF6 is a central positive regulator of stress responses, it will be exciting to investigate this pathway in crops and explore its potential in contributing to breeding towards stress tolerance. However, because ERF6 is an unstable protein and inhibits leaf growth in *Arabidopsis*, simply knocking-out or overexpressing *ERF6* in crops ~~is~~ would not be sufficient to increase yield. Therefore, an alternative strategy could be to drive *ERF6* expression with a specific promoter that triggers *ERF6* expression in some specific organs or cell types. This method could help mitigate the biomass loss typically associated with growing tissues affected by ERF6 while still preserving the potential for stress resistance. Moreover, most of previous ERF6-related research was performed in *Arabidopsis* and the function of ERF6 and its homolog protein may not be completely conserved in crops. Gaining information from *Arabidopsis* and investigating ERF6 function in crops is therefore necessary to make the knowledge more applicable.

Overall, addressing these outstanding questions will not only deepen our comprehension of the diverse roles executed by ERF6 in plant development and stress responses but will also facilitate the exploration of innovative strategies to enhance crop resilience and productivity in challenging environments.

ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation of Sichuan Province (23NSFSC0765), Ghent University ('Bijzonder Onderzoeksfonds Methusalem Project' no. BOF08/01M00408) and China Postdoctoral Science Foundation (2023M732505). Marieke Dubois is a post-doctoral fellow of Flanders Research Foundation (FWO no. 12Q7923N). We thank Dr. Nathalie Gonzalez and Dr. Annick Bleys for critically reading and improving the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORCID

Ting Li: https://orcid.org/0000-0001-9503-1675
Zhen Peng: https://orcid.org/0009-0001-7895-0191
Kangxi Du: https://orcid.org/0000-0002-7985-7861
Dirk Inzé: https://orcid.org/0000-0002-3217-8407
Marieke Dubois: https://orcid.org/0000-0002-5190-2130

REFERENCES

Abuelsoud W., Cortleven A. & Schmülling T. (2020) Photoperiod stress induces an oxidative burst-like response and is associated with increased apoplastic peroxidase and decreased catalase activities. *J Plant Physiol* **253,** 153252.

Achard P., Gusti A., Cheminant S., Alioua M., Dhondt S., Coppens F., . . . Genschik P. (2009) Gibberellin signaling controls cell proliferation rate in Arabidopsis. *Curr Biol* **19,** 1188-1193.

Alonso J.M., Hirayama T., Roman G., Nourizadeh S. & Ecker J.R. (1999) EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. *Science* **284,** 2148-2152.

An F., Zhao Q., Ji Y., Li W., Jiang Z., Yu X., . . . Guo H. (2010) Ethylene-induced stabilization of ETHYLENE INSENSITIVE3 and EIN3-LIKE1 is mediated by proteasomal degradation of EIN3 binding F-box 1 and 2 that requires EIN2 in Arabidopsis. *Plant Cell* **22,** 2384-2401.

Antonietta M., de Felipe M., Rothwell S.A., Williams T.B., Skilleter P., Albacete A., . . . Dodd I.C. (2023) Prolonged low temperature exposure de-sensitises ABA-induced stomatal closure in soybean, involving an ethylene-dependent process. *Plant Cell Environ* **46,** 2128-2141.

Arjmand M.P., Lahiji H.S., Golfazani M.M. & Biglouei M.H. (2023) New insights on the regulatory network of drought-responsive key genes in *Arabidopsis thaliana*. *Genetica* **151,** 29-45.

Bastías D.A., Balestrini R., Pollmann S. & Gundel P.E. (2022) Environmental interference of plant-microbe interactions. *Plant Cell Environ* **45,** 3387-3398.

Bednarek P., Pislewska-Bednarek M., Svatos A., Schneider B., Doubsky J., Mansurova M., . . . Schulze-Lefert P. (2009) A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. *Science* **323,** 101-106.

Berrocal-Lobo M., Molina A. & Solano R. (2002) Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in Arabidopsis confers resistance to several necrotrophic fungi. *Plant J* **29,** 23-32.

Bilbrough T., Piemontese E. & Seitz O. (2022) Dissecting the role of protein phosphorylation: a chemical biology toolbox. *Chem Soc Rev* **51,** 5691-5730.

Binder B.M. (2020) Ethylene signaling in plants. *J Biol Chem* **295,** 7710-7725.

Bleecker A.B. & Kende H. (2000) Ethylene: a gaseous signal molecule in plants. *Annu Rev Cell Dev Biol* **16,** 1-18.

Blom N., Sicheritz-Pontén T., Gupta R., Gammeltoft S. & Brunak S. (2004) Prediction of post-translational glycosylation and phosphorylation of proteins from the amino acid sequence. *Proteomics* **4,** 1633-1649.

Bolt S., Zuther E., Zintl S., Hincha D.K. & Schmülling T. (2017) ERF105 is a transcription factor gene of Arabidopsis thaliana required for freezing tolerance and cold acclimation. *Plant Cell Environ* **40,** 108-120.

Brenya E., Pervin M., Chen Z.H., Tissue D.T., Johnson S., Braam J. & Cazzonelli C.I. (2022) Mechanical stress acclimation in plants: Linking hormones and somatic memory to thigmomorphogenesis. *Plant Cell Environ* **45,** 989-1010.

Catalá R., López-Cobollo R., Mar Castellano M., Angosto T., Alonso J.M., Ecker J.R. & Salinas J. (2014) The Arabidopsis 14-3-3 protein RARE COLD INDUCIBLE 1A links low-temperature response and ethylene biosynthesis to regulate freezing tolerance and cold acclimation. *Plant Cell* **26,** 3326-3342.

Chae H.S. & Kieber J.J. (2005) Eto Brute? Role of ACS turnover in regulating ethylene biosynthesis. *Trends Plant Sci* **10,** 291-296.

Chen W., Shi Y., Wang C. & Qi X. (2024) AtERF13 and AtERF6 double knockout fine-tunes growth and the transcriptome to promote cadmium tolerance in Arabidopsis. *Gene* **911,** 148348.

Cho H.Y., Chou M.Y., Ho H.Y., Chen W.C. & Shih M.C. (2022) Ethylene modulates translation dynamics in Arabidopsis under submergence via GCN2 and EIN2. *Sci Adv* **8,** eabm7863.

Clauw P., Coppens F., De Beuf K., Dhondt S., Van Daele T., Maleux K., . . . Inzé D. (2015) Leaf responses to mild drought stress in natural variants of Arabidopsis. *Plant Physiol* **167,** 800-816.

Davière J.M. & Achard P. (2013) Gibberellin signaling in plants. *Development* **140,** 1147-1151.

Dong H., Zhen Z., Peng J., Chang L., Gong Q. & Wang N.N. (2011) Loss of ACS7 confers abiotic stress tolerance by modulating ABA sensitivity and accumulation in Arabidopsis. *J Exp Bot* **62,** 4875-4887.

Du P., Deng Q., Wang W., Garg V., Lu Q., Huang L., . . . Liu H. (2023) scRNA-seq Reveals the Mechanism of Fatty Acid Desaturase 2 Mutation to Repress Leaf Growth in Peanut (Arachis hypogaea L.). *Cells* **12**.

Dubois M., Claeys H., Van den Broeck L. & Inzé D. (2017) Time of day determines Arabidopsis transcriptome and growth dynamics under mild drought. *Plant Cell Environ* **40,** 180-189.

Dubois M., Skirycz A., Claeys H., Maleux K., Dhondt S., De Bodt S., . . . Inzé D. (2013) Ethylene response factor 6 acts as a central regulator of leaf growth under water-limiting conditions in Arabidopsis. *Plant Physiol* **162,** 319-332.

Dubois M., Van den Broeck L., Claeys H., Van Vlierberghe K., Matsui M. & Inzé D. (2015) The ETHYLENE RESPONSE FACTORs ERF6 and ERF11 antagonistically regulate mannitol-induced growth inhibition in Arabidopsis. *Plant Physiol* **169,** 166-179.

Dubois M., Van den Broeck L. & Inzé D. (2018) The pivotal role of ethylene in plant growth. *Trends Plant Sci* **23,** 311-323.

Feng K., Hou X.L., Xing G.M., Liu J.X., Duan A.Q., Xu Z.S., . . . Xiong A.S. (2020) Advances in AP2/ERF super-family transcription factors in plant. *Crit Rev Biotechnol* **40,** 750-776.

Fu L., Liu Y., Qin G., Wu P., Zi H., Xu Z., . . . Xiong Y. (2021) The TOR-EIN2 axis mediates nuclear signalling to modulate plant growth. *Nature* **591,** 288-292.

Gupta A. & Senthil-Kumar M. (2017) Transcriptome changes in Arabidopsis thaliana infected with Pseudomonas syringae during drought recovery. *Sci Rep* **7,** 9124.

Hall A.E., Findell J.L., Schaller G.E., Sisler E.C. & Bleecker A.B. (2000) Ethylene perception by the ERS1 protein in Arabidopsis. *Plant Physiol* **123,** 1449-1458.

Han Q., Tan W., Zhao Y., Yang F., Yao X., Lin H. & Zhang D. (2022) Salicylic acid-activated BIN2 phosphorylation of TGA3 promotes Arabidopsis PR gene expression and disease resistance. *Embo J* **41,** e110682.

Han Y., Zhou Z., Jin R., Dai F., Ge Y., Ju X., . . . Zhang Y. (2024) Mechanical activation opens a lipid-lined pore in OSCA ion channels. *Nature* **628,** 910-918.

Hao D., Jin L., Wen X., Yu F., Xie Q. & Guo H. (2021) The RING E3 ligase SDIR1 destabilizes EBF1/EBF2 and modulates the ethylene response to ambient temperature fluctuations in Arabidopsis. *Proc Natl Acad Sci U S A* **118,** e2024592118.

Hartman S., Liu Z., van Veen H., Vicente J., Reinen E., Martopawiro S., . . . Voesenek L. (2019) Ethylene-mediated nitric oxide depletion pre-adapts plants to hypoxia stress. *Nat Commun* **10,** 4020.

Hartman S., Sasidharan R. & Voesenek L. (2021) The role of ethylene in metabolic acclimations to low oxygen. *New Phytol* **229,** 64-70.

Hinz M., Wilson I.W., Yang J., Buerstenbinder K., Llewellyn D., Dennis E.S., . . . Dolferus R. (2010) Arabidopsis RAP2.2: an ethylene response transcription factor that is important for hypoxia survival. *Plant Physiol* **153,** 757-772.

Hoang X.L.T., Prerostova S., Thu N.B.A., Thao N.P., Vankova R. & Tran L.P. (2021) Histidine Kinases: Diverse Functions in Plant Development and Responses to Environmental Conditions. *Annu Rev Plant Biol* **72,** 297-323.

Hruz T., Laule O., Szabo G., Wessendorp F., Bleuler S., Oertle L., . . . Zimmermann P. (2008) Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Adv Bioinformatics* **2008,** 420747.

Huang J., Zhao X., Bürger M., Chory J. & Wang X. (2023) The role of ethylene in plant temperature stress response. *Trends Plant Sci* **28,** 808-824.

Illgen S., Zintl S., Zuther E., Hincha D.K. & Schmülling T. (2020) Characterisation of the ERF102 to ERF105 genes of Arabidopsis thaliana and their role in the response to cold stress. *Plant Mol Biol* **103,** 303-320.

Ji Y., Xu M., Liu Z., Yuan H., Lv T., Li H., . . . Wang A. (2022) NUCLEOCYTOPLASMIC shuttling of ETHYLENE RESPONSE FACTOR 5 mediated by nitric oxide suppresses ethylene biosynthesis in apple fruit. *New Phytol* **234,** 1714-1734.

Kieber J.J., Rothenberg M., Roman G., Feldmann K.A. & Ecker J.R. (1993) CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the raf family of protein kinases. *Cell* **72,** 427-441.

Kim N.Y., Jang Y.J. & Park O.K. (2018) AP2/ERF family transcription factors ORA59 and RAP2.3 interact in the nucleus and function together in ethylene responses. *Front Plant Sci* **9,** 1675.

König K., Vaseghi M.J., Dreyer A. & Dietz K.J. (2018) The significance of glutathione and ascorbate in modulating the retrograde high light response in Arabidopsis thaliana leaves. *Physiol Plant* **162,** 262-273.

Lageix S., Lanet E., Pouch-Pélissier M.N., Espagnol M.C., Robaglia C., Deragon J.M. & Pélissier T. (2008) Arabidopsis eIF2alpha kinase GCN2 is essential for growth in stress conditions and is activated by wounding. *BMC Plant Biol* **8,** 134.

Li Q., Fu H., Yu X., Wen X., Guo H., Guo Y. & Li J. (2024) The SALT OVERLY SENSITIVE 2-CONSTITUTIVE TRIPLE RESPONSE1 module coordinates plant growth and salt tolerance in Arabidopsis. *J Exp Bot* **75,** 391-404.

Li S., Han X., Yang L., Deng X., Wu H., Zhang M., . . . Xu J. (2018) Mitogen-activated protein kinases and calcium-dependent protein kinases are involved in wounding-induced ethylene biosynthesis in Arabidopsis thaliana. *Plant Cell Environ* **41,** 134-147.

Li T., Natran A., Chen Y., Vercruysse J., Wang K., Gonzalez N., . . . Inzé D. (2019) A genetics screen highlights emerging roles for CPL3, RST1 and URT1 in RNA metabolism and silencing. *Nat Plants* **5,** 539-550.

Li W., Ma M., Feng Y., Li H., Wang Y., Ma Y., . . . Guo H. (2015) EIN2-directed translational regulation of ethylene signaling in Arabidopsis. *Cell* **163,** 670-683.

Licausi F., Kosmacz M., Weits D.A., Giuntoli B., Giorgi F.M., Voesenek L.A., . . . van Dongen J.T. (2011) Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. *Nature* **479,** 419-422.

Lieberman M. & Kunishi A.T. (1965) Ethylene production from methionine. *Biochem J* **97,** 449-459.

Lin Z., Li Y., Zhang Z., Liu X., Hsu C.C., Du Y., . . . Wang P. (2020) A RAF-SnRK2 kinase cascade mediates early osmotic stress signaling in higher plants. *Nat Commun* **11,** 613.

Martin R.E., Marzol E., Estevez J.M. & Muday G.K. (2022) Ethylene signaling increases reactive oxygen species accumulation to drive root hair initiation in *Arabidopsis*. *Development* **149,** dev200487.

Meng X., Xu J., He Y., Yang K.Y., Mordorski B., Liu Y. & Zhang S. (2013) Phosphorylation of an ERF transcription factor by Arabidopsis MPK3/MPK6 regulates plant defense gene induction and fungal resistance. *Plant Cell* **25,** 1126-1142.

Mittler R. (2017) ROS are good. *Trends Plant Sci* **22,** 11-19.

Moffat C.S., Ingle R.A., Wathugala D.L., Saunders N.J., Knight H. & Knight M.R. (2012) ERF5 and ERF6 play redundant roles as positive regulators of JA/Et-mediated defense against *Botrytis cinerea* in Arabidopsis. *PLoS ONE* **7,** e35995.

Nakano T., Suzuki K., Fujimura T. & Shinshi H. (2006) Genome-wide analysis of the ERF gene family in Arabidopsis and rice. *Plant Physiol* **140,** 411-432.

Pan Y., Seymour G.B., Lu C., Hu Z., Chen X. & Chen G. (2012) An ethylene response factor (ERF5) promoting adaptation to drought and salt tolerance in tomato. *Plant Cell Rep* **31,** 349-360.

Park C., Lee H.Y. & Yoon G.M. (2021) The regulation of ACC synthase protein turnover: a rapid route for modulating plant development and stress responses. *Curr Opin Plant Biol* **63,** 102046.

Park H.L., Seo D.H., Lee H.Y., Bakshi A., Park C., Chien Y.C., . . . Yoon G.M. (2023) Ethylene-triggered subcellular trafficking of CTR1 enhances the response to ethylene gas. *Nat Commun* **14,** 365.

Pei S., Liu Y., Li W., Krichilsky B., Dai S., Wang Y., . . . Yuan F. (2022) OSCA1 is an osmotic specific sensor: a method to distinguish Ca(2+) -mediated osmotic and ionic perception. *New Phytol* **235,** 1665-1678.

Qi J., Song C.P., Wang B., Zhou J., Kangasjärvi J., Zhu J.K. & Gong Z. (2018) Reactive oxygen species signaling and stomatal movement in plant responses to drought stress and pathogen attack. *J Integr Plant Biol* **60,** 805-826.

Qiao H., Shen Z., Huang S.S., Schmitz R.J., Urich M.A., Briggs S.P. & Ecker J.R. (2012) Processing and subcellular trafficking of ER-tethered EIN2 control response to ethylene gas. *Science* **338,** 390-393.

Roeber V.M., Bajaj I., Rohde M., Schmülling T. & Cortleven A. (2021) Light acts as a stressor and influences abiotic and biotic stress responses in plants. *Plant Cell Environ* **44,** 645-664.

Scharein B., Voet-van-Vormizeele J., Harter K. & Groth G. (2008) Ethylene signaling: identification of a putative ETR1-AHP1 phosphorelay complex by fluorescence spectroscopy. *Anal Biochem* **377,** 72-76.

Serrano-Mislata A., Bencivenga S., Bush M., Schiessl K., Boden S. & Sablowski R. (2017) DELLA genes restrict inflorescence meristem function independently of plant height. *Nat Plants* **3,** 749-754.

Sewelam N., Kazan K., Thomas-Hall S.R., Kidd B.N., Manners J.M. & Schenk P.M. (2013) Ethylene response factor 6 is a regulator of reactive oxygen species signaling in *Arabidopsis*. *PLoS ONE* **8,** e70289.

Sham A., Al-Azzawi A., Al-Ameri S., Al-Mahmoud B., Awwad F., Al-Rawashdeh A., . . . AbuQamar S. (2014) Transcriptome analysis reveals genes commonly induced by *Botrytis cinerea* infection, cold, drought and oxidative stresses in *Arabidopsis*. *PLoS ONE* **9,** e113718.

Shi Y., Ke X., Yang X., Liu Y. & Hou X. (2022) Plants response to light stress. *J Genet Genomics* **49,** 735-747.

Shoji T. & Yuan L. (2021) ERF gene clusters: working together to regulate metabolism. *Trends Plant Sci* **26,** 23-32.

Skirycz A., Claeys H., De Bodt S., Oikawa A., Shinoda S., Andriankaja M., . . . Inzé D. (2011) Pause-and-stop: the effects of osmotic stress on cell proliferation during early leaf development in Arabidopsis and a role for ethylene signaling in cell cycle arrest. *Plant Cell* **23,** 1876-1888.

Solano R. & Ecker J.R. (1998) Ethylene gas: perception, signaling and response. *Curr Opin Plant Biol* **1,** 393-398.

Soma F., Takahashi F., Suzuki T., Shinozaki K. & Yamaguchi-Shinozaki K. (2020) Plant Raf-like kinases regulate the mRNA population upstream of ABA-unresponsive SnRK2 kinases under drought stress. *Nat Commun* **11,** 1373.

Son G.H., Wan J., Kim H.J., Nguyen X.C., Chung W.S., Hong J.C. & Stacey G. (2012) Ethylene-responsive element-binding factor 5, ERF5, is involved in chitin-induced innate immunity response. *Mol Plant Microbe Interact* **25,** 48-60.

Song S., Liu B., Song J., Pang S., Song T., Gao S., . . . Qi T. (2022) A molecular framework for signaling crosstalk between jasmonate and ethylene in anthocyanin biosynthesis, trichome development, and defenses against insect herbivores in Arabidopsis. *J Integr Plant Biol* **64,** 1770-1788.

Tan S.T. & Xue H.W. (2014) Casein kinase 1 regulates ethylene synthesis by phosphorylating and promoting the turnover of ACS5. *Cell Rep* **9,** 1692-1702.

Tao H., Miao H., Chen L., Wang M., Xia C., Zeng W., . . . Wang Q. (2022) WRKY33-mediated indolic glucosinolate metabolic pathway confers resistance against Alternaria brassicicola in Arabidopsis and Brassica crops. *J Integr Plant Biol* **64,** 1007-1019.

Teardo E., Carraretto L., Moscatiello R., Cortese E., Vicario M., Festa M., . . . Szabo I. (2019) A chloroplast-localized mitochondrial calcium uniporter transduces osmotic stress in Arabidopsis. *Nat Plants* **5,** 581-588.

Van den Broeck L., Dubois M., Vermeersch M., Storme V., Matsui M. & Inzé D. (2017) From network to phenotype: the dynamic wiring of an Arabidopsis transcriptional network induced by osmotic stress. *Mol Syst Biol* **13,** 961.

van Verk M.C., Bol J.F. & Linthorst H.J. (2011) WRKY transcription factors involved in activation of SA biosynthesis genes. *BMC Plant Biol* **11,** 89.

Vermeirssen V., De Clercq I., Van Parys T., Van Breusegem F. & Van de Peer Y. (2014) Arabidopsis ensemble reverse-engineered gene regulatory network discloses interconnected transcription factors in oxidative stress. *Plant Cell* **26,** 4656-4679.

Vogel M.O., Moore M., König K., Pecher P., Alsharafa K., Lee J. & Dietz K.J. (2014) Fast retrograde signaling in response to high light involves metabolite export, MITOGEN-ACTIVATED PROTEIN KINASE6, and AP2/ERF transcription factors in Arabidopsis. *Plant Cell* **26,** 1151-1165.

Waadt R., Seller C.A., Hsu P.K., Takahashi Y., Munemasa S. & Schroeder J.I. (2022) Plant hormone regulation of abiotic stress responses. *Nat Rev Mol Cell Biol* **23,** 680-694.

Wang J., Zou A., Xiang S., Liu C., Peng H., Wen Y., . . . Sun X. (2022a) Transcriptome analysis reveals the mechanism of zinc ion-mediated plant resistance to TMV in Nicotiana benthamiana. *Pestic Biochem Physiol* **184,** 105100.

Wang K.L., Li H. & Ecker J.R. (2002) Ethylene biosynthesis and signaling networks. *Plant Cell* **14 Suppl,** S131-151.

Wang L., Waters M.T. & Smith S.M. (2018) Karrikin-KAI2 signalling provides Arabidopsis seeds with tolerance to abiotic stress and inhibits germination under conditions unfavourable to seedling establishment. *New Phytol* **219,** 605-618.

Wang P., Du Y., Zhao X., Miao Y. & Song C.P. (2013) The MPK6-ERF6-ROS-responsive cis-acting Element7/GCC box complex modulates oxidative gene transcription and the oxidative response in Arabidopsis. *Plant Physiol* **161,** 1392-1408.

Wang X., Meng H., Tang Y., Zhang Y., He Y., Zhou J. & Meng X. (2022b) Phosphorylation of an ethylene response factor by MPK3/MPK6 mediates negative feedback regulation of pathogen-induced ethylene biosynthesis in Arabidopsis. *J Genet Genomics* **49,** 810-822.

Warmerdam S., Sterken M.G., Van Schaik C., Oortwijn M.E.P., Lozano-Torres J.L., Bakker J., . . . Smant G. (2019) Mediator of tolerance to abiotic stress ERF6 regulates susceptibility of Arabidopsis to Meloidogyne incognita. *Mol Plant Pathol* **20,** 137-152.

Waszczak C., Carmody M. & Kangasjärvi J. (2018) Reactive oxygen species in plant signaling. *Annu Rev Plant Biol* **69,** 209-236.

Wessler S.R. (2005) Homing into the origin of the AP2 DNA binding domain. *Trends Plant Sci* **10,** 54-56.

Wi S.J., Ji N.R. & Park K.Y. (2012) Synergistic biosynthesis of biphasic ethylene and reactive oxygen species in response to hemibiotrophic Phytophthora parasitica in tobacco plants. *Plant Physiol* **159,** 251-265.

Wu A., Allu A.D., Garapati P., Siddiqui H., Dortay H., Zanor M.I., . . . Balazadeh S. (2012) JUNGBRUNNEN1, a reactive oxygen species-responsive NAC transcription factor, regulates longevity in Arabidopsis. *Plant Cell* **24,** 482-506.

Wu B., Qi F. & Liang Y. (2023) Fuels for ROS signaling in plant immunity. *Trends Plant Sci* **28,** 1124-1131.

Wu H., Li B., Iwakawa H.O., Pan Y., Tang X., Ling-Hu Q., . . . Guo H. (2020) Plant 22-nt siRNAs mediate translational repression and stress adaptation. *Nature* **581,** 89-93.

Xu J., Meng J., Meng X., Zhao Y., Liu J., Sun T., . . . Zhang S. (2016) Pathogen-responsive MPK3 and MPK6 reprogram the biosynthesis of indole glucosinolates and their derivatives in Arabidopsis immunity. *Plant Cell* **28,** 1144-1162.

Yamaguchi S. (2008) Gibberellin metabolism and its regulation. *Annu Rev Plant Biol* **59,** 225-251.

Yang C.Y., Hsu F.C., Li J.P., Wang N.N. & Shih M.C. (2011) The AP2/ERF transcription factor AtERF73/HRE1 modulates ethylene responses during hypoxia in Arabidopsis. *Plant Physiol* **156,** 202-212.

Yang S.F. & Hoffman N.E. (1984) Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Biology* **35,** 155-189.

Yang Y.N., Kim Y., Kim H., Kim S.J., Cho K.M., Kim Y., . . . Park O.K. (2021) The transcription factor ORA59 exhibits dual DNA binding specificity that differentially regulates ethylene- and jasmonic acid-induced genes in plant immunity. *Plant Physiol* **187,** 2763-2784.

Yu B., Chao D.Y. & Zhao Y. (2024a) How plants sense and respond to osmotic stress. *J Integr Plant Biol* **66,** 394-423.

Yu Z., Duan X., Luo L., Dai S., Ding Z. & Xia G. (2020) How plant hormones mediate salt stress responses. *Trends Plant Sci* **25,** 1117-1130.

Yu Z., Qu X., Lv B., Li X., Sui J., Yu Q. & Ding Z. (2024b) MAC3A and MAC3B mediate degradation of the transcription factor ERF13 and thus promote lateral root emergence. *Plant Cell*.

Yuan F., Yang H., Xue Y., Kong D., Ye R., Li C., . . . Pei Z.M. (2014) OSCA1 mediates osmotic-stress-evoked Ca2+ increases vital for osmosensing in Arabidopsis. *Nature* **514,** 367-371.

Zander M., Thurow C. & Gatz C. (2014) TGA transcription factors activate the salicylic acid-suppressible branch of the ethylene-induced defense program by regulating ora59 expression. *Plant Physiol* **165,** 1671-1683.

Zdarska M., Cuyacot A.R., Tarr P.T., Yamoune A., Szmitkowska A., Hrdinová V., . . . Hejátko J. (2019) ETR1 integrates response to ethylene and cytokinins into a single multistep phosphorelay pathway to control root growth. *Mol Plant* **12,** 1338-1352.

Zhang M. & Zhang S. (2022) Mitogen-activated protein kinase cascades in plant signaling. *J Integr Plant Biol* **64,** 301-341.

Zhang S., Zhu C., Zhang X., Liu M., Xue X., Lai C., . . . Lin Y. (2023) Single-cell RNA sequencing analysis of the embryogenic callus clarifies the spatiotemporal developmental trajectories of the early somatic embryo in Dimocarpus longan. *Plant J* **115,** 1277-1297.

Zhao H., Yin C.C., Ma B., Chen S.Y. & Zhang J.S. (2021a) Ethylene signaling in rice and Arabidopsis: New regulators and mechanisms. *J Integr Plant Biol* **63,** 102-125.

Zhao Z.X., Feng Q., Liu P.Q., He X.R., Zhao J.H., Xu Y.J., . . . Wang W.M. (2021b) RPW8.1 enhances the ethylene-signaling pathway to feedback-attenuate its mediated cell death and disease resistance in Arabidopsis. *New Phytol* **229,** 516-531.

Zhou X., Zhang Z.L., Park J., Tyler L., Yusuke J., Qiu K., . . . Sun T.P. (2016) The ERF11 transcription factor promotes internode elongation by activating gibberellin biosynthesis and signaling. *Plant Physiol* **171,** 2760-2770.

Zhu J.K. (2016) Abiotic stress signaling and responses in plants. *Cell* **167,** 313-324.

Zhu T., Zou L., Li Y., Yao X., Xu F., Deng X., . . . Lin H. (2018) Mitochondrial alternative oxidase-dependent autophagy involved in ethylene-mediated drought tolerance in Solanum lycopersicum. *Plant Biotechnol J* **16,** 2063-2076.

FIGURES

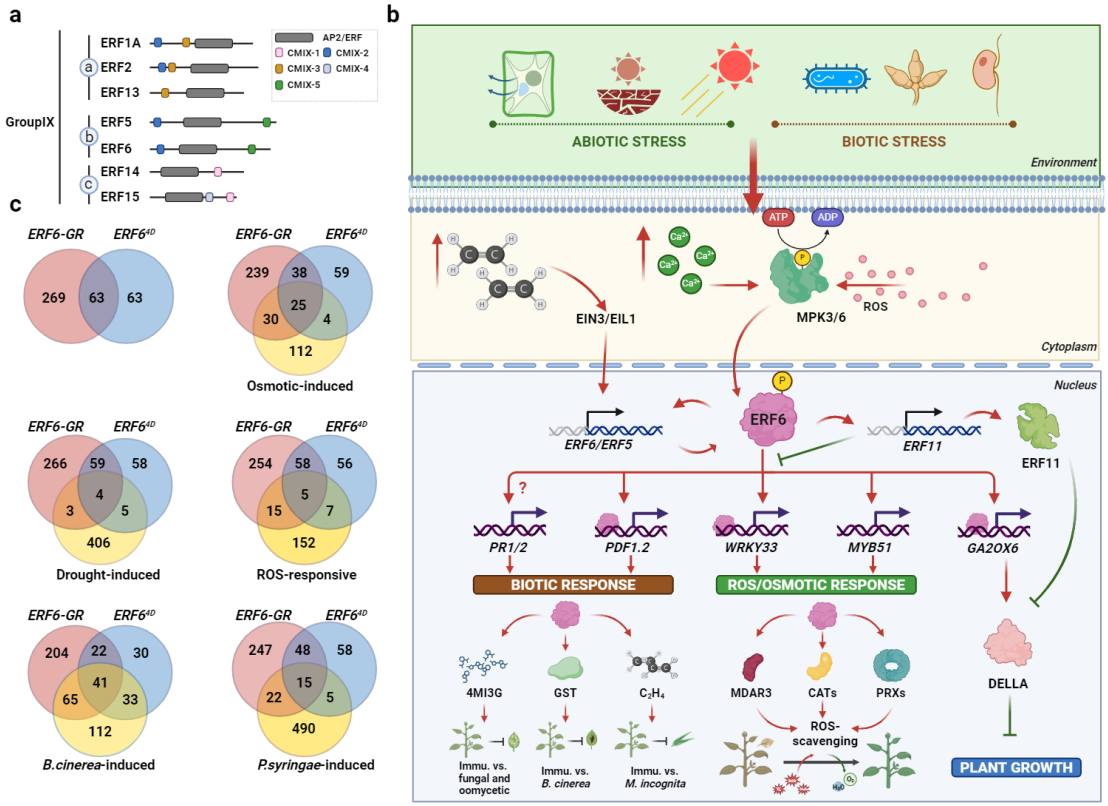

Figure 1 | Overview of the role of ERF6 in plant growth and stress responses. (a) Overview of the ERF group IX members. According to the conserved motifs of group IX (CMIX), the group IX is divided into three sub-groups, including group IX-a, group IX-b, and group IX-c. In group IX-b, ERF5 is the closest homolog of ERF6. (b) Recent advances regarding ERF6's function in plant growth and stress responses. See text for more details. (c) Comparison of genes induced by ERF6-GR or ERF6^{4D} and genes triggered by osmotic stress, reactive oxygen species (ROS), drought stress, *Botrytis cinerea* or *Pseudomonas syringae* based on published microarray or RNA-sequencing data (Wu *et al.* 2012; Dubois *et al.* 2013; Meng *et al.* 2013; Sham *et al.* 2014; Gupta & Senthil-Kumar 2017). The *35S::ERF^{4D}* line was generated by substituting Ser-266 and Ser-299 of the ERF6 protein with Asp to mimic the phosphorylation at these specific sites. The figure is created with BioRender.com (https://www.biorender.com/).

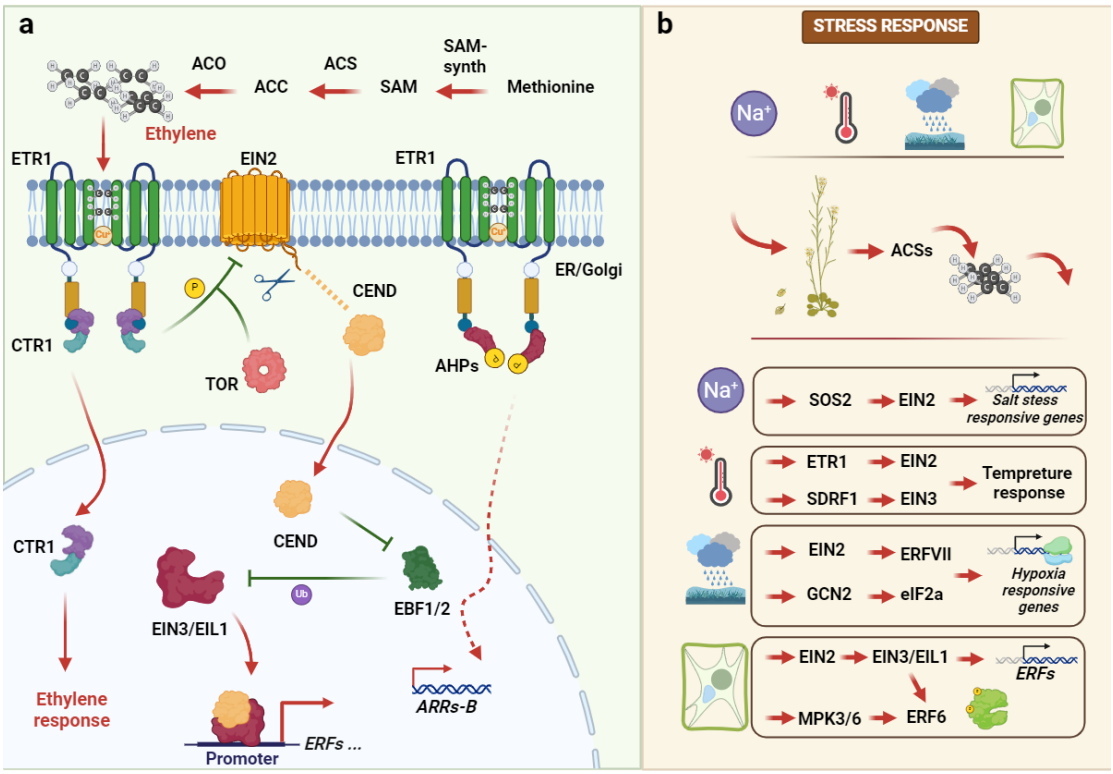

Figure 2 | Ethylene biosynthesis and signaling, and its role in stress responses. (a) The biosynthesis and signal transduction of ethylene. See text for more details. (b) The involvement of ethylene in stress responses. Upon exposure to different stresses, like wounding, pathogens, cold, etc., the genes encoding ACSs are trans-activated and ACSs accumulate to a high level, promoting ethylene biosynthesis. High ethylene levels trigger positive regulators of ethylene responses such as EIN2, EIN3, and ERFs, which in turn activate downstream stress responses. ACC, 1-aminocyclopropane-1-carboxylic acid; ACO, ACC-OXIDASE; ACS, ACC-SYNTHASE; AHP, HISTIDINE-CONTAINING PHOSPHOTRANSMITTER; ARRs-B, type-B *ARABIDOPSIS* RESPONSE REGULATOR; CTR1, CONSTITUTIVE TRIPLE RESPONSE1; EBF1/2, EIN3-BINDING F-BOX PROTEIN; eIF2α, initiation factor 2α; EIL1, EIN3-LIKE; EIN2-CEND, C-terminal domain of ETHYLENE INSENSITIVE2; ERFs, ETHYLENE RESPONSE FACTORs; ETR1, ETHYLENE RESPONSE1; GCN2, GENERAL CONTROL NON-DEREPRESSIBLE 2; Met, methionine; SOS2, SALT OVERLY SENSITIVE 2; SRDF1, SALT- AND DROUGHT-INDUCED RING FINGER1, SAM, S-adenosyl-l-methionine. The figure is created with BioRender.com (https://www.biorender.com/).