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REVIEW PAPER

Diverse roles of ethylene in maize growth and development, and its importance in shaping plant architecture

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Abstract

The gaseous plant hormone ethylene is a key developmental and growth regulator, and a pivotal endogenous response signal to abiotic and biotic interactions, including stress. Much of what is known about ethylene biosynthesis, perception, and signaling comes from decades of research primarily in *Arabidopsis thaliana* and other eudicot model systems. In contrast, detailed knowledge on the ethylene pathway and response to the hormone is markedly limited in maize (*Zea mays* L.), a global cereal crop that is a major source of calories for humans and livestock, as well as a key industrial biofeedstock. Recent reports of forward screens and targeted reverse genetics have provided important insight into conserved and unique differences of the ethylene pathway and downstream responses. Natural and edited allelic variation in the promoter regions and coding sequences of ethylene biosynthesis and signaling genes alters maize shoot and root architectures, and plays a crucial role in biomass and grain yields. This review discusses recent advances in ethylene research in maize, with an emphasis on the role of ethylene in regulating growth and development of the shoot and root systems, and ultimately how this crucial hormone impacts plant architecture and grain yield.

Keywords: Development, ethylene, growth, hormone, inflorescence, leaf, maize, plant architecture, root, yield.

Introduction

The plant hormone ethylene is a simple alkene gas that regulates numerous developmental and growth processes, in addition to having pivotal roles in coordinating responses to biotic and abiotic interactions. Ethylene was first described in the early 1900s as an effector of the triple response in pea plants (Bakshi *et al.*, 2015). The triple response is a set of morphological changes in the response of etiolated seedlings to ethylene, where both roots and hypocotyls grow short and thicker, and have an exaggerated apical hook compared with untreated plants (Guzmán and Ecker, 1990). Other developmental and physiological processes regulated by ethylene include cell division and expansion, growth of the shoot and root, germination, sex determination, senescence, fruit ripening, and fruit shape (reviewed in Iqbal *et al.*, 2017; Dubois *et al.*, 2018; Martínez and Jamilena, 2021). Ethylene is also a key stress hormone that regulates plant responses to abiotic and biotic stress that includes drought, heat, salinity, hypoxia, cold, and pathogen and disease response (reviewed in Broekaert *et al.*, 2006; Van Loon *et al.*, 2006; Tao *et al.*, 2015; Dubois *et al.*, 2018; Hartman *et al.*, 2021; Huang *et al.*, 2023).

Ethylene biosynthesis is linked to methionine recycling metabolism and is composed of three different enzymatic reactions. The first two steps are shared within methionine metabolism: methionine is converted into *S*-adenosyl-L-methionine (SAM) by SAM synthase, and SAM is converted into methylthioadenosine (MTA) and 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) (Fig. 1A; Table 1). As products of the second enzymatic reaction, MTA is recycled back to methionine through the Yang cycle, and ACC is a precursor to ethylene. In the third reaction, ACC oxidase (ACO) enzymes convert ACC into ethylene (Pattyn *et al.*, 2021). As a gas, ethylene diffuses freely inside the cell and to neighboring cells; no transporter has been described to create fluxes of ethylene in the cell or different tissues (Ju and Chang, 2012; Anfang and Shani, 2021). A transporter for ACC has been described: LYSINE HISTIDINE TRANSPORTER1, an amino acid transporter, carries ACC throughout the phloem (Shin *et al.*, 2015; Choi *et al.*, 2019).

A wealth of knowledge on ethylene perception, signal transduction, and response comes from genetic and molecular studies in Arabidopsis (Azhar *et al.*, 2020; Binder, 2020). Ethylene perception occurs inside the cell and is recognized by a family of receptors [ETR1 (ETHYLENE RECEPTOR1), ETR2, ERS1 (ETHYLENE RESPONSE SENSOR1),

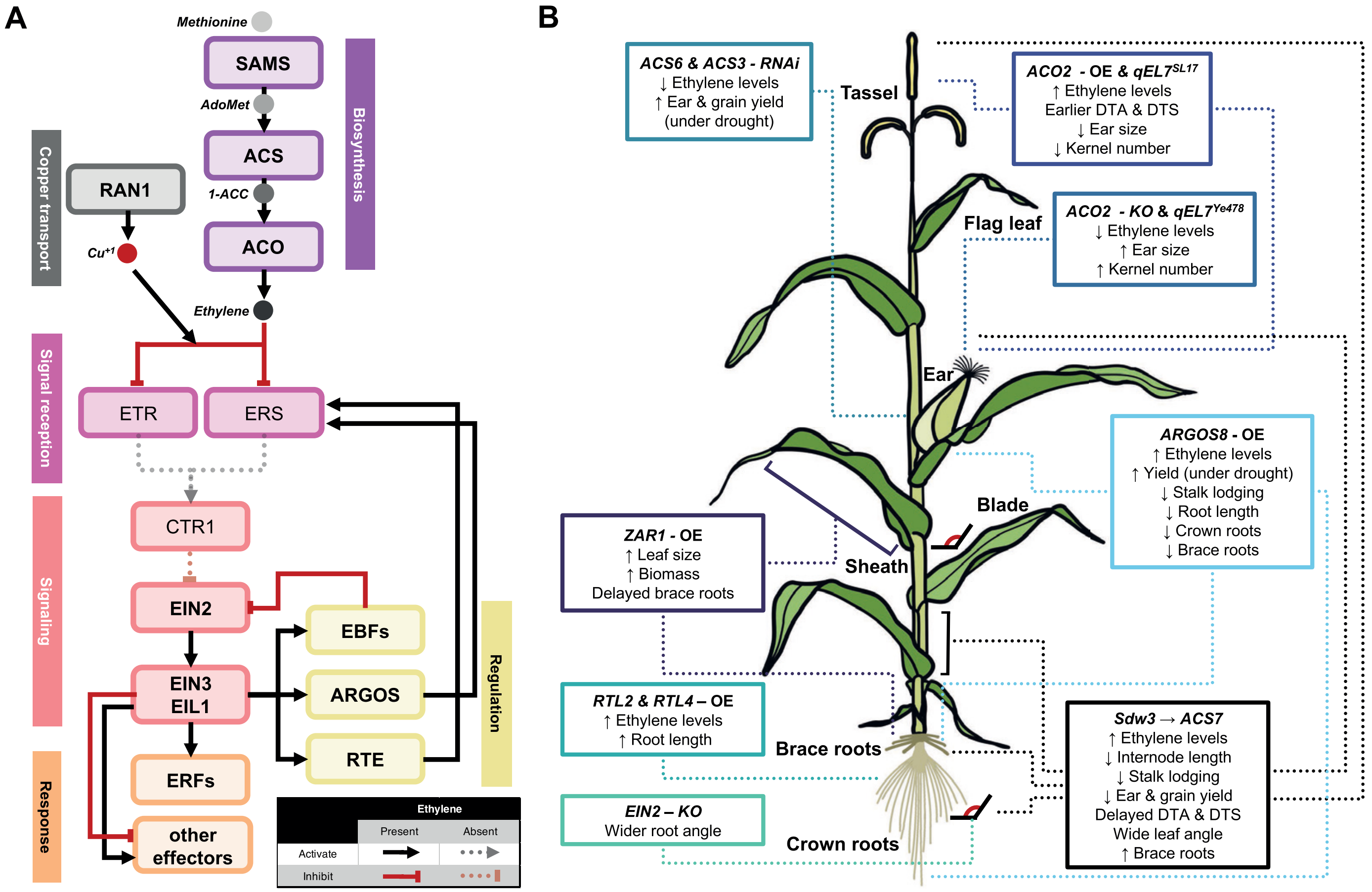

Fig. 1. Ethylene pathway and its contribution to maize growth and development. (A) Schematic representation of known key proteins involved in ethylene biosynthesis, signaling, and response. Ethylene production is mediated by three consecutive enzymes (in purple). Ethylene receptors (in magenta) perceive the hormone at the ER and are functional as dimers. Also, monovalent copper and its transporter RAN1 (in gray) are essential for proper signal perception and three-dimensional conformation of receptors. Ethylene receptors are active in the absence of the hormone and negatively regulate the response by directly interacting with CTR1 (in pink), a Raf-like kinase. CTR1 is a mobile protein that moves in the cytosol to deactivate the signaling pathway by inhibiting EIN2 (in pink), a transmembrane protein also in the ER; by these means, the receptors and CTR1 repress ethylene response. Ethylene binding to the receptors deactivates them, leading to the inactivation of CTR1. This releases the CTR1-mediated inhibition of EIN2 and activates it. When EIN2 is in its active state, the C-terminal part of the protein is autocleaved and released. The EIN2 C-terminal part moves to the nucleus to indirectly stabilize EIN3/EIL1 (in pink). In their active state, the transcription factors EIN3/EIL1 regulate myriad genes, including *ERF* encoding transcription factor genes and other downstream targets (in orange). Negative regulators of ethylene response are also activated by EIN3/EIL1, such as *EBF*, *ARGOS*, and *RTE* genes (in yellow). Those proteins not shown in bold are deactivated in the presence of ethylene. (B) Genetic basis of ethylene in maize development and growth (see text for details). Ethylene biosynthesis genes *ZmACS2/3/6/7* and *ZmACO2* control ethylene levels and play significant roles in vegetative and inflorescence development, yield, heterosis, and root architecture. Negative regulators of ethylene signaling *ZAR1*, *ARGOS8*, and *RTL2/4* function in overall biomass, and seedling and adult root architecture. *EIN2*, a positive regulator of ethylene signaling, regulates root angle in maize seedlings. KO, knockout; OE, overexpression; DTA, days to anthesis; DTS, days to silking.

ERS2, and EIN4 (ETHYLENE INSENSITIVE4] that (co-)localize within the endoplasmic reticulum (ER) membrane (Fig. 1A; Table 1; Chang *et al.*, 1993; Hua and Meyerowitz, 1998; Chen *et al.*, 2002). The receptors work primarily as homodimers, but it has been suggested that they can interact as heterodimers (Grefen *et al.*, 2008; Berleth

Table 1. Genes related to ethylene biosynthesis and signaling in Arabidopsis, rice, and maize[a]

Function	Module	Plant species				
		Arabidopsis thaliana[b]		***Oryza sativa***	***Zea mays***[c]	
Biosynthesis	SAMS	*SAMS1*	*AT1G02500*	*LOC_Os01g18860*	*SAMS1*	*Zm00001eb354640*
		SAMS2	*AT4G01850*	*LOC_Os01g22010*	*SAMS2*	*Zm00001eb340450*
		METK3	*AT2G36880*	*LOC_Os05g04510*	*SAMS3*	*Zm00001eb417370*
		METK4	*AT3G17390*	*LOC_Os07g29440*	*SAMS4*	*Zm00001eb130870*
	ACS	*ACS1*	*AT3G61510*	*LOC_Os01g09700*	*ACS1*	*Zm00001eb121190*
		ACS2	*AT1G01480*	*LOC_Os03g51740*	*ACS2*	*Zm00001eb073290*
		ACS4	*AT2G22810*	*LOC_Os04g48850*	*ACS3*	*Zm00001eb378300*
		ACS5	*AT5G65800*	*LOC_Os05g10780*	*ACS6*	*Zm00001eb055950*
		ACS6	*AT4G11280*	*LOC_Os05g25490*	*ACS7/SDW1*	*Zm00001eb428490*
		ACS7	*AT4G26200*	*LOC_Os06g03990*		
		ACS8	*AT4G37770*			
		ACS9	*AT3G49700*			
		ACS10	*AT1G62960*			
		ACS11	*AT4G08040*			
		ACS12	*AT5G51690*			
	ACO	*ACO1*	*AT2G19590*	*LOC_Os01g39860*	*ACCO1*	*Zm00001eb277290*
		ACO2	*AT1G62380*	*LOC_Os02g53180*	*ZmACO2/ACCO2/qEL7*	*Zm00001eb314610*
		ACO3	*AT1G12010*	*LOC_Os05g05670*	*ACCO3/ACCO4*	*Zm00001eb418130*
		ACO4	*AT1G05010*	*LOC_Os05g05680*	*ACCO5*	*Zm00001eb357070*
		ACO5	*AT1G77330*	*LOC_Os06g37590*	*ACCO6*	*Zm00001eb091080*
				LOC_Os09g27750	*ACCO7*	*Zm00001eb201390*
				LOC_Os09g27820	*ZmACO15/ACCO15*	*Zm00001eb418140*
				LOC_Os11g08380	*ZmACO20/ACCO20*	*Zm00001eb193360*
					ZmACO31/ACCO31	*Zm00001eb418090*
					ZmACO35/ACCO35	*Zm00001eb256320*
Copper transport	RAN1	*RAN1*	*AT5G44790*	*LOC_Os02g07630*	*ZmRAN1/HMA9*	*Zm00001eb389830*
				LOC_Os06g45500	*ZmRAN2/HMA11*	*Zm00001eb207090*
Signal reception	ETR ERS EIN4	*EIN4*	*AT3G04580*	*LOC_Os03g49500*	*ETR2*	*Zm00001eb419810*
		ERS1	*AT2G40940*	*LOC_Os04g08740*	*ETR3*	*Zm00001eb282800*
		ERS2	*AT1G04310*	*LOC_Os05g06320*	*ETR4*	*Zm00001eb323290*
		ETR1	*AT1G66340*		*ETR5*	*Zm00001eb205280*
		ETR2	*AT3G23150*		*ETR6*	*Zm00001eb191130*
					ERS14	*Zm00001eb216810*
					ERS25	*Zm00001eb054170*
					ERS40	*Zm00001eb088180*
Signaling	CTR1	*CTR1*	*AT5G03730*	*LOC_Os02g32610*	*CTR1*	*Zm00001eb242490*
				LOC_Os09g39320	*CTR2*	*Zm00001eb071130*
					CTR3	*Zm00001eb096080*
	EIN2	*EIN2*	*AT5G03280*	*LOC_Os03g49400*	*EIN1/ZmEIN2*	*Zm00001eb054060*
				LOC_Os07g06130	*EIN2*	*Zm00001eb119690*
					EIN2/UFG27	*Zm00001eb216860*
	EIN3 EIL1	*EIN3*	*AT3G20770*	*LOC_Os02g36510*	*ZmEIL1*[d]	*Zm00001eb015440*
		EIL1	*AT2G27050*	*LOC_Os03g20780*	*ZmEIL2*[d]	*Zm00001eb111680*
		EIL2	*AT5G21120*	*LOC_Os03g20790*	*ZmEIL5*[d]	*Zm00001eb182890*
		EIL4	*AT5G10120*	*LOC_Os04g38400*	*ZmEIL6*[d]	*Zm00001eb331080*
		EIL5	*AT5G65100*	*LOC_Os07g48630*	*ZmEIL7*[d]	*Zm00001eb081010*
					ZmEIL8[d]	*Zm00001eb244830*
					ZmEIL9[d]	*Zm00001eb395480*

Table 1. Continued

Function	**Module**	**Plant species**				
		Arabidopsis thaliana[b]		***Oryza sativa***	***Zea mays***[c]	
Regulation	EBF	*EBF1*	*AT2G25490*	*LOC_Os02g10700*	*EBF1*	*Zm00001eb276590*
		EBF2	*AT5G25350*	*LOC_Os06g40360*	*EBF2*	*Zm00001eb235810*
					EBF3	*Zm00001eb386390*
					EBF4	*Zm00001eb205780*
	ARGOS	*ARGOS*	*AT3G59900*	*LOC_Os01g66530*	*ZAR1/ZmARGOS1*	*Zm00001eb423180*
		ARL	*AT2G44080*	*LOC_Os04g36670*	*ZAR3/ZmARGOS3*	*Zm00001eb112430*
		OSR1	*AT2G41230*	*LOC_Os05g34530*	*ZAR4/ZmARGOS4*	*Zm00001eb288210*
		OSR2	*AT2G41225*	*LOC_Os08g02180*	*ZAR5/ZmARGOS5*	*Zm00001eb260800*
				LOC_Os11g14190	*ZAR6/ZmARGOS6*	*Zm00001eb140590*
				LOC_Os12g10750	*ZAR7/ZmARGOS7*	*Zm00001eb244140*
					ZAR8/ZmARGOS8	*Zm00001eb287000*
					ZAR9/ZmARGOS9	*Zm00001eb138320*
	RTE	*RTE1*	*AT2G26070*	*LOC_Os01g51430*	*RTL1/ZmRTL1*	*Zm00001eb361330*
		RTH	*AT3G51040*	*LOC_Os03g58520*	*RTL2/ZmRTL2*	*Zm00001eb155080*
				LOC_Os05g46240	*RTL3/ZmRTL3*	*Zm00001eb294260*
					RTL4/ZmRTL4	*Zm00001eb061120*

[a] Please see the text for maize genes that have been functionally characterized for ethylene response.
Colored boxes in the 'Function' column match the color designation in Fig. 1A.
[b] Gene names and IDs are taken from TAIR (https://www.arabidopsis.org).
[c] Gene names and IDs are taken from https://www.maizegdb.org from Zm-B73-Reference-NAM 5.0.
[d] Maize transcription factor nomenclature according to Grassius (https://grassius.org). Note that *ZmEIL3* (Zm00001eb034980) and *ZmEIL4* (Zm00001eb316730) are not listed in the table as these belong to a clade outside of the *EIN3/EIL1* clade. Whether *ZmEIL5*, *ZmEIL7*, or *ZmEIL8* has a role in ethylene response remains to be determined.

et al., 2019). Each functional unit detects ethylene from the N-terminal part of the proteins facing the lumen of the ER. Once the signal is received, it passes through the transmembrane domain inside the ER membrane and then to the C-terminal part of the protein, located in the cytosol (Schaller *et al.*, 1995; Hall *et al.*, 2007; Grefen *et al.*, 2008). One molecule of ethylene is needed to elicit the response in each receptor. During this process, a monovalent copper (Cu^{1+}) ion is an essential cofactor for the high-affinity binding of ethylene in the receptors (Rodríguez *et al.*, 1999; McDaniel and Binder, 2012). Copper ions are transported within the cell by RESPONSIVE TO ANTAGONIST1 (RAN1), an ATP-dependent copper transporter (Woeste and Kieber, 2000). Mutations in *RAN1* inhibit ethylene signaling by reducing proper binding of ethylene molecules to the receptor due to the lack of copper. In addition, the presence of RAN1 and copper has been suggested to be important to the proper formation of ethylene receptors (Hirayama *et al.*, 1999; Binder *et al.*, 2010).

The receptors function as negative regulators of the ethylene response by keeping CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) active in the absence of the hormone (Fig. 1A; Table 1). CTR1 is a Raf-like kinase that directly interacts as a dimer with the C-terminal part of ethylene receptors in the cytosol (Kieber *et al.*, 1993; Raz and Fluhr, 1993; Clark *et al.*, 1998; Hua *et al.*, 1998; Wang *et al.*, 2007). CTR1 is activated by phosphorylation in its kinase domain, and then it moves through the cytosol to inactivate EIN2. While CTR1 does not contain any transmembrane domains, EIN2 does and is anchored to the ER membrane. Phosphorylation of EIN2 induces its degradation, preventing signal transduction to the nucleus, thereby suppressing any transcriptional response (Stepanova and Alonso, 2009; Merchante *et al.*, 2013).

When ethylene is present, it binds to and deactivates the receptors (Fig. 1A). This produces a structural change in the receptors that keeps CTR1 monomers separated and inactive (Kieber *et al.*, 1993; Mayerhofer *et al.*, 2012). With CTR1 inactive, it can no longer phosphorylate EIN2. The unphosphorylated state of EIN2 promotes cleavage of its C-terminal part, which translocates to the nucleus. In the nucleus, the C-terminal domain of EIN2 indirectly activates a first wave of transcription factors belonging to the EIN3/EIN3-LIKE (EIL) family (Alonso *et al.*, 1999; An *et al.*, 2010; Ju *et al.*, 2012; Qiao *et al.*, 2012; Wen *et al.*, 2012). EIN3/EIL transcription factors bind directly to DNA and trigger the transcriptional regulation to ethylene acting as positive activators of the hormone response. EIN3 and some EILs promote and inhibit a great number of ethylene-responsive genes, including the expression of *ETHYLENE RESPONSE FACTOR* (*ERF*) transcription factor genes and other regulators of the ethylene response [e.g. *ARGOS* (*AUXIN REGULATED GENE INVOLVED IN ORGAN SIZE*), *EBF* (*EIN3-BINDING F-BOX PROTEIN*), and *RTE*

(*REVERSION-TO-ETHYLENE SENSITIVITY*)]. EIN3/EIL1 are localized in the nucleus and function as dimers when they bind ethylene response elements (Table 1) (Chao *et al.*, 1997; Solano *et al.*, 1998; Chang *et al.*, 2013).

The ethylene signaling pathway exhibits multiple levels of regulation by a diverse group of proteins in different modules (Fig. 1A). Here, we mention a few examples of regulation at the receptors and the EIN3/EIL1 transcription factors. Without ethylene, EBF1 and EBF2 mark the EIN3/EIL1 proteins for proteasomal degradation. In the presence of the hormone, the EIN2 C-terminal domain binds to EBFs to stabilize EIN3/EIL1 by preventing their protein turnover. Nevertheless, the ethylene response forms a feedback loop where EIN3 regulates itself by binding directly to promoter sequences in *EBF* genes (Konishi and Yanagisawa, 2008; Chang *et al.*, 2013). RTE and its family members [RTE-LIKE (RTL)] are other negative regulators of the ethylene response. These transmembrane proteins directly interact with ethylene receptors, at least with ETR1 on the ethylene-binding domain. Not only are they localized in the ER, but they also found in the Golgi apparatus (Resnick *et al.*, 2006; Dong *et al.*, 2008, 2010). Experiments have shown that *RTE* is also a target of EIN3, suggesting the possible activation of *RTE* expression during the ethylene response that would allow the desensitization of ethylene receptors (Chang *et al.*, 2013). Lastly, the *ARGOS* genes are another family induced by ethylene and have an important role in negatively regulating the ethylene response. By intersecting with the receptor complex via RTE, ARGOS also functions to desensitize ethylene responses. Interestingly, *ARGOS* genes have been characterized as modulators of organ size and plant biomass (Feng *et al.*, 2011; Rai *et al.*, 2015; Shi *et al.*, 2015, 2016).

The diverse functions of ethylene have been characterized in many plant species and, within this exciting field, new roles for this hormone are still being discovered. Whereas much of the ethylene pathway and response have been extensively studied in eudicots, similarly concentrated research in monocots remains disproportionate. Growing work on ethylene signaling in rice (*Oryza sativa*) has found largely conserved mechanisms; however, through cloning and characterization of ethylene response rice mutants, new regulators of the pathway have shed light on diverged aspects of ethylene signaling (Yang *et al.*, 2015; Zhao et al., 2021). While the mechanisms that regulate ethylene signaling in rice have greatly expanded our view of ethylene response across plants with diverse life histories and adaptive challenges, studies in this semi-aquatic cereal provide just a snapshot of ethylene biology in monocots. Expanding our understanding of the ethylene pathway to include cereals that have been domesticated and selected to grow in more temperate or arid environments may advance agronomic improvement efforts in other crops. Here, we review past and present research on ethylene biosynthesis, signaling, and response pathways in maize, and highlight conserved and diverged functions of the pathway (Table 1). We underscore the impact of ethylene on maize architecture and grain yield, and the importance of this pathway for further improvement of agronomic traits.

Ethylene regulates growth and development of the maize vegetative shoot

Germination is a crucial event in a plant's life cycle, where uniformity and full seedling emergence are important agronomic traits. Ethylene plays a key role in germination in Arabidopsis and other eudicot species (Linkies and Leubner-Metzger, 2012). Natural variation in ethylene production correlates with different germination rates in maize, and lines that produce more ethylene during germination tend to have a faster germination rate (Kong *et al.*, 2020). Maize kernels germinated in the presence of the ethylene precursor ACC have higher germination rates compared with ethylene antagonists that decrease germination rates (Kong *et al.*, 2020). The underground germinating maize shoot consists of the coleoptile and mesocotyl, both of which are sensitive to ACC and exogenous ethylene treatments. Elongation of the mesocotyl is inhibited in some dark-grown maize inbred lines upon treatment with ACC or ethephon (Liu *et al.*, 2020), and its girth increases when exposed to ethylene (Camp and Wickliff, 1981). Similarly, treatment with ACC or ethylene inhibits coleoptile growth during germination in some maize inbred lines, in both dark and light conditions (Yang *et al.*, 2015; Yin *et al.*, 2023). Once the coleoptile emerges from the soil, it ceases growth and dehisces to expose seedling leaves and allow growth of the shoot.

The three-dimensional organization of the vegetative shoot, which includes internode length and diameter, plant height, degree of tillering and lateral branching, and shape, size, and arrangement of leaves, collectively impacts cultivation and harvest (Wang and Li, 2008; Strable, 2021). Early studies in maize revealed that the plant growth regulator ethrel could influence internode length, plant height, and leaf area (Earley and Slife, 1969). The active component of ethrel, ethephon (2-chloroethylphosphonic acid), promotes ethylene emission (Warner and Leopold, 1967; Cooke and Randall, 1968; McMurray and Miller, 1968). Similarly, treating maize directly with ethephon suppresses plant height, reduces ear height, and increases brace root emergence (Langan and Oplinger, 1987; Cox and Andrade, 1988; Konsler and Grabau, 1989). Norberg *et al.* (1989) found that ethephon could decrease internode length, and increase internode diameter and weight per unit length. Maize stem lodging, the inability of the shoot to remain upright, is a key agronomic trait largely influenced by stalk strength (Tuberosa and Salvi, 2009) and anchorage support provided by brace roots (Ennos *et al.*, 1993). The effects of ethephon on internode traits is correlated with reduced stalk lodging, as well as plant and ear height in hybrid maize (Norberg *et al.*, 1989).

Characterization of maize *Semidwarf3* (*Sdw3*), a semi-dominant mutant, and subsequent positional cloning of the candidate gene *ZmACS7* provide insight into the genetic basis for ethylene's role in regulating shoot architecture (Li *et al.*, 2020). Semi-dominant *Sdw3* mutants have shoots of short stature, due to shorter internodes attributed to reduced parenchyma cell elongation (Fig. 1B). Importantly, *Sdw3* mutant plants are less prone to stalk lodging. ZmACS7 is an ethylene biosynthesis enzyme that converts SAM into the ethylene precursor ACC (Fig. 1A; Table 1). ACS enzymes catalyze the key rate-limiting step of ethylene biosynthesis (Yu *et al.*, 1979), and *ACS* genes represent a multigene family in land plants (Li *et al.*, 2022). *Sdw3* mutant plants have elevated levels of ACC and emit more ethylene compared with non-mutant plants due to insertion in the fourth exon of *ZmACS7* that alters the encoded C-terminus of the enzyme. Li *et al.* (2020) phenocopied *Sdw3*, including increased resistance to stalk lodging, by CRISPR/Cas9 [clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein 9]-directed editing of the *ZmACS7* coding sequence to modify its C-terminus and in transgenic plants that overexpressed *ZmACS7* or its paralog *ZmACS2*.

Maize leaves are a significant component of the shoot and have a strap-like blade connected by a pair of wedge-shaped auricles to a cylindrical sheath that wraps around the stem. Auricle size and morphology at the blade–sheath boundary are key factors that influence how much the blade tilts away from the plant axis (Kong *et al.*, 2017; Wang *et al.*, 2024). Leaf angle is a key factor in shaping canopy architecture and, historically, is an important breeding trait to increase maize productivity (Duvick *et al.*, 2003; Cao *et al.*, 2022). *Sdw3* mutant leaves produce wider blade angles, resulting in a more open leaf canopy (Fig. 1B) (Li *et al.*, 2020). Interestingly, the auricle margin that connects the blade and sheath margins is longer in *Sdw3* leaves compared with the wild type, largely due to increased cell elongation in the auricle. The auricle region at the blade–sheath boundary in *Sdw3* mutants accumulates more ACC and emits more ethylene, consistent with similar measurements from internode tissue. Surprisingly, knocking out *SDW3* function does not result in more upright leaves (Li *et al.*, 2020). Given that overproduction of ethylene in *Sdw3* mutant plants inhibits cell elongation in the internode and promotes cell elongation in the auricle, this suggests that growth responses to ethylene are tissue specific. In support of these observations, profiling the transcriptomes of developing internode and auricle tissues of *Swd3* mutants revealed that members of the kinesin gene family, which are essential for division and elongation of cells (Li *et al.*, 2011), are up-regulated in developing auricles and down-regulated in developing internodes (Li *et al.*, 2020).

Plant hormones have important roles in biomass production (Demura and Ye, 2010). Members of the *ARGOS* gene family increase overall biomass by regulating cell proliferation and/or cell expansion to increase growth of lateral organs (Hu *et al.*, 2003). Exogenous ethylene induces transcription of *ARGOS* family members to a greater magnitude than does auxin application (Rai *et al.*, 2015). *ARGOS* genes function as negative regulators of ethylene responses in both Arabidopsis and maize by regulating ethylene sensitivity as part of a negative feedback loop (Fig. 1A) (Rai *et al.*, 2015; Shi *et al.*, 2015). In paired ChIP sequencing (ChIP-seq) and transcriptomic (RNA-seq) analyses, *ARGOS* genes were identified as bound and modulated targets of EIN3, the major transcriptional regulator of ethylene response (Chao *et al.*, 1997; Chang *et al.*, 2013). These collective data indicate that a key output of ethylene response is the desensitization of ethylene perception through a negative feedback loop.

Mechanistically, Arabidopsis and maize ARGOS proteins physically interact with RTE1 and RTL ethylene receptor-interacting proteins (Shi *et al.*, 2016) that, in Arabidopsis, stabilize signaling of the ETR1 receptor (Dong *et al.*, 2010). Among the eight *ARGOS* family members in maize (Shi *et al.*, 2015), also known as *ZAR* genes (i.e. *Zea mays ARGOS*; Table 1) (Guo *et al.*, 2014), *ARGOS8* physically associates with the ethylene receptor complex and regulates ethylene perception (Shi *et al.*, 2016). Moreover, overexpression of *ARGOS8*, or maize *RTL2* (*ZmRTL2*) or *ZmRTL4*, in transgenic Arabidopsis or maize plants reduces ethylene sensitivity (Shi *et al.*, 2015, 2016). Transgenic maize plants that overexpress *ARGOS8* or other *ARGOS* family members, such as *ZAR1*, have larger leaves and increased biomass, and are taller compared with non-transgenic control plants (Fig. 1B) (Guo *et al.*, 2014; Shi *et al.*, 2015). Plant height increases slightly in maize lines that constitutively express moderate levels of *ARGOS8* through CRISPR/Cas9 editing of its promoter (Shi *et al.*, 2017).

Increased biomass and improved performance are often observed in F_1 hybrid progeny compared with its two inbred parental lines, a phenomenon known as heterosis (Springer and Stupar, 2007). In a survey of Dupont Pioneer's germplasm, Guo *et al.* (2014) found that two founder alleles of *ZAR1* are retained in elite germplasm, with one allele in female and the other in male heterotic groups. Transgenic plants that co-express the native *ZAR1* allelic variant from each heterotic group outperform transgenic plants that expressed either *ZAR1* allele separately, suggesting a role for *ZAR1* in heterosis (Guo *et al.*, 2014). Similar observations for a role of *ZAR1* role in heterosis have been reported for the contribution of *ZAR1* natural variants in F_1 hybrid progeny from other founder inbred lines (Wang *et al.*, 2023). However, the direct effects of *ZAR1* on ethylene levels or ethylene sensitivity in relation to heterosis in maize have not been reported. Interestingly, reduced ethylene production in Arabidopsis F_1 hybrids, through diurnal down-regulation of *ACS* biosynthesis genes, increases biomass associated with heterosis (Song *et al.*, 2018). A similar observation that maize F_1 hybrid progeny have reduced accumulation of ethylene biosynthesis gene products compared with either parent suggests a conserved role for ethylene in heterosis (Birdseye *et al.*, 2021). Arabidopsis *acs* mutants with reduced ethylene levels have an increase in biomass relative to wild-type plants (Song *et al.*, 2018) and, similarly, the proteome of maize *acs2;acs6* double mutant plants phenocopies that of the F_1 hybrid progeny (Fig. 1B) (Birdseye *et al.*, 2021). In addition, natural variation in the promoter region of the maize ethylene biosynthesis gene *ZmACO2* contributes to heterosis by increasing yield in F_1 hybrid progeny for some combinations of inbred parental lines (Wang *et al.*, 2023). Expression differences due to natural variation in the *ZmACO2* promoter, as well as transgenic overexpression or knocking out its function, alters ethylene production and influences ear traits (Ning *et al.*, 2021).

Ethylene influences the transition from vegetative to reproductive development

The transition from vegetative to reproductive development is a crucial event in the life cycle of a plant and the production of grain and seeds. In flowering plants, the timing of floral transition is tightly regulated by environmental and endogenous cues (Freytes *et al.*, 2021; Izawa, 2021). Ethylene is among the several phytohormones that influence when a plant ceases vegetative growth and commences production of inflorescences and flowers (Hall and Bleecker, 2003; Ogawara *et al.*, 2003). For example, ethephon was first reported to inhibit flowering in pineapple (Cooke and Randall, 1968), and ethylene gas and the ethylene precursor ACC were shown to delay flowering in Arabidopsis (Achard *et al.*, 2006, 2007). To date, few studies have directly examined the effects of ethylene on flowering time in maize, namely days to anthesis (DTA) and days to silk (DTS). Overexpression of maize *ARGOS8* in wild-type Arabidopsis delayed flowering by up to 10 d (Shi *et al.*, 2015); however, effects of overexpressing *ARGOS8* on flowering time have not been reported in transgenic maize. Interestingly, transgenic maize that overexpress *ZAR1* display faster, but not extended, growth and maturation rates, compared with non-transgenic control plants (Guo *et al.*, 2014), but to what degree DTA and/or DTS are affected has not been reported.

Sdw3 mutant plants that overproduce ethylene have delayed DTA and DTS by ~2 d and 6 d, respectively (Fig. 1B) (Li *et al.*, 2020). Interestingly, transcriptomic analysis of developing internodes and the leaf ligular region (i.e. tissues of the blade–sheath boundary that contains developing ligule and auricle) revealed up-regulation of *ZmRAP2.7* and down-regulation of *Z. mays MADS-BOX4* (*ZmMADS4*/*ZMM4*) and *ZmMADS67* (Li *et al.*, 2020). *ZmRAP2.7* is a negative regulator of maize floral transition; transgenic maize that overexpress *ZmRAP2.7* have more leaves and delayed floral transition (Salvi *et al.*, 2007). *ZmMADS4* and *ZmMADS67* are positive regulators of floral transition; CRISPR/Cas9 knockout alleles of each gene display increased leaf number and delayed floral transition (Sun *et al.*, 2020). As maize floral induction signals are produced in young adult leaves and perceived at the vegetative shoot apex (Meng *et al.*, 2011), it will be important to understand the mechanism by which ethylene regulates floral transition.

Ethylene impacts yield traits in maize

Maize is the most produced and highest yielding cereal worldwide according to available data (FAOSTAT, https://www.fao.org/faostat/). The maize ear and each of the hundreds of kernels it will produce contribute to grain yield. Per ear yield considers the circumference and length of the ear (ear row number and ear length), the length of the ear that bears kernels (kernel set length), and kernel weight, among other ear-related traits (Liu *et al.*, 2021; Xu *et al.*, 2021). Early reports suggested that ethephon has a negative effect on maize grain yield (Langan and Oplinger, 1987; Cox and Andrade, 1988). Similarly, *Sdw3* mutants, as well as transgenic variants of *ZmACS7* with a stabilized C-terminus and transgenic plants that overexpressed *ZmACS7* have reduced ear traits and lower grain yield compared with control plants (Li *et al.*, 2020).

Maize ear development and related yield traits are sensitive to abiotic stresses, especially drought (Boyer and Westgate, 2004; Danilevskaya *et al.*, 2019). Ethylene plays a pivotal role in the abiotic stress response, in addition to its role in plant growth and development (Dubois *et al.*, 2018; Chen *et al.*, 2021; Huang *et al.*, 2023). The results of many studies suggest that ethylene negatively impacts maize grain yield, especially under stress conditions. Several key studies report utilizing biotechnological approaches to reduce ethylene levels and/or a plant's sensitivity to ethylene by leveraging negative regulators of ethylene response (Guo *et al.*, 2014; Habben *et al.*, 2014; Shi *et al.*, 2015, 2017). By using RNAi to knock down transcript levels of *ACS6* and related *ACS3*, ethylene biosynthesis genes, field-grown transgenic plants had reduced ethylene emission (by ~50%) and improved grain yield compared with non-transgenic plants under controlled drought stress conditions (Habben *et al.*, 2014). Similarly, decreasing ethylene sensitivity by overexpressing *ARGOS*/*ZAR* genes resulted in transgenic maize plants with enhanced drought tolerance and increased yield under applied drought stress (Guo *et al.*, 2014; Shi *et al.*, 2015, 2017).

Progeny from two contrasting inbred lines for ear length, SL17 with shorter ears relative to Ye478, were screened for quantitative trait loci that affect ear length (*qEL*; Zhou *et al.*, 2015). Subsequent fine mapping using near isogenic lines $qEL7^{SL17}$ and $qEL7^{Ye47}$ uncovered sequence variation in the promoter of *ZmACO2* (Table 1) (Ning *et al.*, 2021). Line $qEL7^{SL17}$ had higher expression of *ZmACO2* in developing ears that correlated with higher ethylene produced compared with line $qEL7^{Ye478}$. CRISPR/Cas9 null alleles of *ZmACO2*, or transgenic maize that overexpressed *ZmACO2*, produced longer and shorter ears, respectively, that phenocopied the respective $qEL7^{Ye478}$ and $qEL7^{SL17}$ lines (Fig. 1B). Interestingly, ears of $qEL7^{SL17}$ lines had more unfilled (i.e. more unfertilized) florets compared with ears of $qEL7^{Ye478}$ lines. This was largely due to a high frequency of pistillate florets developing into staminate florets, which otherwise would be found in the tassel (Ning *et al.*, 2021). Transcriptomes of developing ears from

$qEL7^{SL17}$ and $qEL7^{Ye478}$ suggest that phytohormone pathways, in addition to ethylene, are altered in the two lines, which may, in part, explain the pleiotropy of *ZmACO2* on ear traits. For example, transcripts from genes that encode enzymes for jasmonic acid (JA) biosynthesis, including *TS1* (Acosta *et al.*, 2009) and *TS2* (DeLong *et al.*, 1993), accumulate to lower levels in ears from the $qEL7^{Ye478}$ line, which also has a lower concentration of JA and of JA conjugates and precursors (Ning *et al.*, 2021). Mutations in *TS1* or *TS2* result in the development of pistillate florets in the tassel (Emerson, 1920; Nickerson and Dale, 1955), whereas in normal tassels carpel primordia abort, giving rise to staminate florets (Cheng *et al.*, 1983). However, to our knowledge, it remains to be reported whether *ts1* or *ts2* mutant ears have staminate tips. Because ears from $qEL7^{SL17}$ and $qEL7^{Ye478}$ lines, or ears from transgenic plants with loss of *ZmACO2* function or ectopic *ZmACO2* activity, do not display pistillate florets in tassels (Ning *et al.*, 2021), more work is needed to understand the potential crosstalk between ethylene and JA pathways during inflorescence development and the specification of floral identity. Perhaps more compelling support for the appearance of staminate florets at the tip of ears from $qEL7^{SL17}$ lines is that *TASSELS REPLACE UPPER EARS1* (*TRU1*) expression is lower in developing ears in this line (Ning *et al.*, 2021). Mutations in *TRU1* cause upper ear primordia to be replaced by long axillary branches that terminate with a tassel (Dong *et al.*, 2017), whereas secondary ear primordia in *tru1* mutants have mostly pistillate florets with staminate florets developing at the ear tip (Li, 2012). Ethylene biosynthesis and signaling plays a crucial role in floral organ identity in cucurbits (Martínez and Jamilena, 2021). Future research will help elucidate mechanisms and potential crosstalk between ethylene and other hormone pathways during maize floral development.

Ethylene shapes maize root architecture

The maize root system anchors the shoot and provides it with water and nutrients from the soil. Development and growth of this complex three-dimensional system (Martínez and Jamilena, 2021) are regulated by endogenous and exogenous cues (Hochholdinger and Tuberosa, 2009). Maize seedling roots consist primarily of embryonic primary and seminal roots (i.e. small roots that form during embryogenesis around the scutellar node; Perkins and Lynch, 2021) and the lateral roots they initiate. The adult maize roots are mostly whorls of shoot-borne roots that consist of underground crown roots and above-ground brace roots (Feldman, 1994; Hochholdinger, 2009). It has been known for decades that exogenous ethylene inhibits growth of primary roots in various cereals (Smith and Robertson, 1971). Maize primary, seminal, and brace roots react similarly to ethylene and to the ethylene precursor ACC by showing significant reduction in overall length compared with untreated roots (Jackson *et al.*, 1981; Whalen and Feldman, 1988; Kim and Mulkey, 1997; Alarcon *et al.*, 2009; Shi *et al.*, 2015, 2016). Interestingly, removing the root cap abolishes the inhibitory effects of ethylene on primary root elongation, while regeneration of the root cap restores ethylene sensitivity to the primary root (Hahn *et al.*, 2008). Transcripts of maize *ACS* (*ACS2/6/7*) and *ACO* (*ACO15/20/31/35*) genes accumulate predominantly in the root cap (Gallie *et al.*, 2009; Geisler-Lee *et al.*, 2010). Additionally, transgenic maize that overexpress *ZmRTL2* or *ZmRTL4* are less sensitive to the inhibitory effects of exogenous ACC on root elongation (Table 1; Shi *et al.*, 2016).

Treatment of maize with ethephon, the ethylene precursor ACC, or exogenous ethylene promotes initiation and/or emergence of seminal, crown, and brace roots (Jackson *et al.*, 1981; Langan and Oplinger, 1987; Cox and Andrade, 1988; Shi *et al.*, 2019). In support of these observations, *Sdw3* mutant plants, or transgenic plants with perturbed or overexpressed *ZmACS7*, genotypes that all have increased ethylene production, display more nodes with brace roots (Li *et al.*, 2020). In contrast, reducing ethylene sensitivity by attenuating ethylene signaling in transgenic maize plants that overexpress *ARGOS8* delays emergence of brace roots, but does not alter the formation of brace root primordia (Fig. 1B; Shi *et al.*, 2019). Maize *ARGOS8* overexpression transgenic lines have increased root lodging in the field compared with non-transgenic control plants, suggesting that delaying brace root growth negatively impacts lodging in maize (Shi *et al.*, 2019). Interestingly, hybrid transgenic maize that restrict *ARGOS8* expression to adult plant tissues, namely those that do not ectopically express *ARGOS8* in juvenile nodal tissues, display increased yield with no significant difference in lodging compared with control plants (Shi *et al.*, 2019). While brace roots that enter the soil increase resistance to root lodging, those that do not bring high metabolic and maintenance costs to the plant (Lynch, 2013). Reducing the number of brace roots improves shoot biomass by promoting the elaboration of the root system in the soil that can capture more nitrogen (Guo and York, 2019). Whether maize transgenic plants that overexpress *ARGOS8* (Shi *et al.*, 2019) have improved nitrogen uptake, or have changes in seminal or crown root architecture remains to be reported.

The angles at which seedling lateral and seminal roots, and adult crown and brace roots emerge and grow relative to the primary root or stem axes, respectively, provide crucial anchorage as well as establishing an architecture that enables the root network to interact with various soil layers for uptake of water and nutrients (Kirschner *et al.*, 2024). *Sdw3* mutant plants, along with transgenic plants that mimic the increased ethylene production of the mutation in *ZmACS7*, have brace roots with steeper (i.e. smaller) angles compared with non-mutant plants (Li *et al.*, 2020). In contrast, CRISPR/Cas9 knockout alleles of maize *EIN2* (*ZmEIN2*) result in seedlings with wider seminal root angles compared with unedited control plants (Fig. 1B; Kong *et al.*, 2024). Exogenous ACC decreases seminal root angle in wild-type maize seedlings, whereas *Zmein2* mutants are insensitive and continue to display increased seminal root angles (Kong *et al.*, 2024).

Conclusions and perspectives

To date, the role of ethylene role in plant growth, development, and physiology is known largely due to decades of research conducted primarily in eudicot model systems, namely in Arabidopsis (Azhar *et al.*, 2020; Binder, 2020), and to a lesser, but significant, extent in the monocot rice (Zhao *et al.*, 2021). By comparison, advancements in our understanding of the genetic and molecular underpinnings of ethylene function in maize are markedly limited. Key studies highlighted in this review emphasize central conserved and diverged roles of ethylene during germination, development, and growth of the vegetative shoot and reproductive inflorescences, and root system, as well as its pivotal contributions to heterosis, grain yield, and mitigating the effects of stress. Ethylene regulates the development of structures unique to grasses (i.e. ligule, auricle, and brace roots) and maize (i.e. tassel and ear), suggesting that maize, and related grasses, might have specific features in the ethylene signaling pathway and/or its regulation.

A clear role for ethylene has emerged in regulating several key agronomic traits (Fig. 1). Future research that considers genetic screens for suppressor/enhancer loci, natural variation, and targeted CRISPR/Cas9 perturbation of ethylene responses will be likely to identify genes and genomic factors with novel functions in ethylene signaling. Major targets for breeders across decades have been, and continue to be, tolerance to abiotic and biotic stresses (Cooper *et al.*, 2014). Previous studies utilizing transgenic maize with altered ethylene biosynthesis or signaling have proven to be successful in maintaining or increasing grain yield in elite hybrids during field-conditioned drought stress (Guo et al., 2014; Habben *et al.* 2014; Shi *et al.*, 2016; Simmons *et al.*, 2021). In general, addressing the challenges of inducing stable transgene-free edits will be an important step in broadening the benefits of more climate-resilient maize and related crops (Kocsisova and Coneva, 2023); such approaches could be considered to modulate the ethylene pathway for tailored responses. In addition, leveraging single-cell and spatial transcriptomic analyses (Satterlee *et al.*, 2020; Laureyns *et al.*, 2022) affords the ability to evaluate spatiotemporal tissue- and cellular-level heterogeneity of ethylene response during maize development and/or in response to various environmental conditions. Additionally, proteomic studies of maize ethylene response will undoubtedly be informative, especially when compared with transcriptomic studies. Overall, significant insights on ethylene roles in maize expand our understanding of the pathway, and research in this field has revealed developmental and growth responses, in leaves, ears, and brace roots, that are distinct from Arabidopsis and rice. Continued elucidation of the ethylene pathway in maize carries the potential for the discovery of new targets that may accelerate improvement of maize and related cereal crops.

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Conflict of interest

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