Title Ethylene insensitivity in maize: analysis of ethylene receptors and the ethylene response in maize

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Abstract

The biological role of ethylene, $H_2C=CH_2$, was first identified as a plant hormone responsible for leaf drop from the observation that plants relatively close to gas lamps lost their leaves. Later, it was then known as an important gaseous hormone for climacteric fruit ripening. Further research revealed that ethylene not only regulates entry into several types of plant developmental cell death and senescence programs, but also mediates plant responses to biotic and abiotic stress. For example, ethylene has been implicated in promoting kernel abortion under shading stress in maize. Ethylene production is controlled by the nutritional and stress status of a plant. Despite the broad range of ethylene's effects on development, the primary steps in ethylene action are assumed to be similar in all cases: They all involve the binding of ethylene to a receptor, followed by activation of one or more signal transduction pathways leading to the cellular response. Ultimately, ethylene exerts its effect primarily through alterations in the pattern of gene expression.

Although the hormonal control of root growth and development has been extensively studied, relatively little is known about the role that ethylene plays in cereal root development. To understand how the ethylene biosynthetic machinery is spatially regulated in maize roots and how changes in its expression alter root growth, the expression of ACC synthase (encoded by *ZmACS2, ZmACS6,* and *ZmACS7* in maize) was observed in the root cap and in cortical cells whereas the expression of ACC oxidase (encoded by *ZmACO15, ZmACO20, ZmACO31,* and *ZmACO35* in maize) was detected in the root cap, protophloem sieve elements, and the companion cells associated with metaphloem sieve elements. The results suggest that expression of *ZmACS6* is important in regulating growth of maize roots in response to physical resistance.

To date, many studies on ethylene insensitivity have focused on the function of the Arabidopsis dominant-negative mutant ethylene receptor gene (*etr1-1*) in Arabidopsis or other species. To understand more about the effect of ethylene on cereal crops, maize dominant-negative ethylene receptors (e.g. *Zmetr2* and *Zmers1*) were generated by altering a conserved cysteine residue in one of the transmembrane

domains in the N-terminal region of the receptor. Taking the advantage of its short generation time, *Zmetr2* and *Zmers1* were first studied in Arabidopsis. The results suggest that Cys65 in maize ZmERS1 and ZmETR2 plays the same role that it does for Arabidopsis receptors. Moreover, the results demonstrate that the mutant maize ethylene receptors are functionally dependent on subfamily 1 ethylene receptors in Arabidopsis to exert their dominance, indicating substantial functional conservation between maize and Arabidopsis ethylene receptors despite their sequence divergence.

The *etr1-1* mutant confers a state of ethylene insensitivity constitutively during the lifetime of a plant. Therefore, the effect of *etr1-1* on plant growth and development is limited in that it does not reveal what roles ethylene might play in specific cell types or developmental stages separate from its global influence on the plant. To study the role of ethylene in specific organs or at certain developmental stages in maize, expression of the maize dominant-negative ethylene receptor, *Zmetr2,* was driven by organ-specific promoters, i.e., from PEPC (Phosphoenolpyruvate carboxylase) and RbcS (Rubisco small subunit), for leaf-specific expression, and SH1 (Shunken1), for kernel-specific expression in transgenic maize. Such an approach allows the examination of the effects of creating a state of ethylene insensitivity in specific organs to determine the role of ethylene under normal growth conditions or conditions of stress.