Molecular Biology of Ethylene, a Review

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Abstract Ethylene is a plant hormone of wide-ranging diverse effects on plants physiology and development. This simple molecule interacts with other hormones and factors with very versatile and fantastic manner for executing growth as well as developmental events in plants. Ethylene has strong impact on myriad developmental events of plants from germination to senescence. Here intent is to focus and highlight the importance, synthesis, regulatory genetic network and signaling integration of this versatile molecule that affects plant growth and development in a myriad ways.

Keywords Ethylene; Biosynthetic pathways; Ethylene signaling; Receptor; Plant physiology; Growth hormones

Introduction

Ethylene is a plant hormone and simplest unsaturated hydrocarbon. It is a gaseous signal molecule and a wide range of metabolic, physiological as well as developmental events in plants is regulated by this biologically active gas (Schaller, 2012). Ethylene has strong impact and effect on climacteric fruits ripening and also on triple responses in etiolated seedling of dicotyledonous plants. Fruit ripening mechanism is a complex and genetically programmed developmental event. Ripening process in fruits is started from its one sector and spreads to adjoining sector. In this process ethylene is diffused cell to cell and thereby is assimilated in whole fruit. This physiological event is culminated in remarkable variations and modification in texture, color, aroma and flavor of fruit flesh (Klee and Giovannoni, 2011).

Ripening mechanism is different in different fruits; therefore on the basis respiration rate and ethylene production, these are grouped into two categories; climacteric and non-climacteric fruits. The former ones show rise in respiratory rate and concomitant ethylene production at high level when they close to maturity and edibility. At this time ethylene burst is achieved. Thus for ripening climacteric fruits have no need to remain attached to tree and they ripen after harvesting such as banana, mango, apple, tomato, papaya etc. Whereas in non-climacteric fruits such as citrus, grapes, strawberries, and pineapple etc, no ethylene burst is achieved at this stage and respiration rate is at its lowest level and is continuously declined until senescence occurs. Hence these fruits only ripen when they are on trees or vines. In climacteric fruits, for operating ethylene regulation, two systems have been proposed i-e., ethylene autoinhibitory and ethylene autocatalytic. During normal vegetative growth, former one is functional and is also involved in the ethylene production at basal level that are noticed in all tissues of climacteric fruits and even in non-climacteric fruits. While later one is operated during climacteric fruit ripening and is also responsible in the senescence of some petals (Alexander and Grierson, 2002).

Pollination stimulates ethylene induction and production in floral organ that leads to growth of ovaries and floral senescence and abscission ((Balbi and Lomax, 2003; Wang et al., 2005). Genes involved in ethylene biosynthetic pathway as well as responsible for its signaling are down regulated after fruit setting (Pandolfini et al., 2007; Stepanova et al., 2008) when plants are pollinated and even treated with gibberellin. The effect of ethylene in fruit setting and development was not under keen consideration of scientists and that’s why had not been studied eagerly until last 1-2 decades. Now research on ethylene
biochemistry, molecular biology and physiology has been flourishing. The extracted knowledge of studies on ethylene synthesis in plants and its role and impact in plant physiology and development has advantageous and beneficial to various agricultural systems and the trend is advancing and expediting.

1 Biosynthetic Pathway of Ethylene

Ethylene biosynthetic pathway is entrenched in higher plants (Bleecker and Kende, 2000). Biosynthetic and signaling pathways of ethylene have been studying extensively for last few decades. These pathways have gained much attention in the area of plant hormone physiology (Kende, 1993). Major breakthrough in this subject came through the identification of S-adenosyl-L-methionine (S-AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC), as ethylene precursors (Yang and Hoffman, 1984). ACC, a non-protein cyclic compound is produced from S-AdoMet in a reaction carried out by a specialized enzyme ACC synthase (ACS) (Kende, 1993). However, S-adenosyl-L-methionine is formed from methionine via S-AdoMet synthetase (SAM synthetase) enzyme at the cost of ATP (Ravanel et al., 1998).

Methionine is not only the essential brick of protein building while about 80% of cellular methionine is also involved in the formation of S-AdoMet (prime methyl donor in plants) (Kevin et al., 2002). In the first committed step of ethylene biosynthetic pathway, besides ACC, another compound 5'-methylthioadenosine (MAT) is also formed that is subsequently converted into methionine via modified methionine cycle (Bleecker and Kende, 2000). As intermediate compound of ethylene synthesis pathway, ACC is then being used as substrate of another enzyme of this route, ACC oxidase (ACO). This enzyme mediates the reaction of ethylene formation from ACC that is the immediate precursor of ethylene (Yang and Hoffman, 1984).

Modified methionine cycle is a type of salvage pathway that keeps and save methylthio group from every round and for another cycle of ethylene production at the expense of one ATP molecule. Thereby ethylene can be synthesized even at high rate, when there is scarcity of methionine in a cell (Bleecker, 2000 and Kevin et al., 2002). In addition to ethylene, cyanide and CO2, are also the end products of this biological route. As cyanide is toxic, thus cell has to flush out this compound, as sakes of its survival. Therefore by the activity of β-cyanalanine synthase (CAS) that detoxifies cyanide and converts it into β-cyanalanine, a non-toxic compound, cell is prevented to toxicity (Kevin et al., 2002) (Figure 1).

2 Regulation of Ethylene Biosynthesis Related Genes

In plants, progress in ripening stage had proved to be linked with the induction and upregulation of various genes. Hence involvement of ethylene dependent and ethylene independent gene regulations have been observed using molecular and expression analyses of ripening related genes by developing mutants and transgenic plants (Picton et al., 1993 and Theologis et al., 1993). Impact of positive as well as negative feedback regulation on ethylene biosynthases is also well established (Barry et al., 2000). Genes encoding ACC synthase and ACC oxidase belong to small multigene families. The differential expression of these enzymes is regulated through, hormonal, environmental and developmental stimuli or signals (Zarembinski and Theologis, 1994 and Barry et al., 2000). A complex regulatory network comprising environmental and developmental signals is existed for regulating the expression of genes involved in biosynthetic pathway of ethylene (Johnson and Ecker, 1998). Regulation of ACC synthase gene is based on the expression of this gene in response to internal and external cues. Various isoforms of ACS is present such as in tomato, Le-ACS2, Le-ACS4 and Le-ACS6. However their feedback regulation is different when ethylene is synthesized during fruit ripening thereby former two are positively regulated whereas last one is negatively regulated by ethylene synthesis at this stage (Nakatsuka et al., 1998). The only common feature of these isoforms of ACS gene is their spatial and temporal regulation by various endogenous as well as exogenous stimuli or signals.

3 Ethylene Regulated Genes

The mechanism of ethylene-regulated genes has unraveled by molecular characterization of the promoter regions of ripening-related genes and thereby an ethylene-responsive element containing 8 bp motifs (A (A/T) TTCATAA) has been identified (Montgomery et al., 1993 and Itzhaki et al., 1994).
Similarly, two ethylene regulated genes, E4 and ER69 have been identified (Zegzouti et al., 1999) and these both genes are involved also in methionine cycle, encoding methionine sulphoxide reductase protein and cobalamine-independent methionine synthase respectively (Montgomery et al., 1993 and Zegzouti et al., 1999). In addition to E4, another gene E8 is also ethylene dependent for its induction (Lincoln et al., 1987). Although expression of E4 gene is induced in leaves in response to ethylene while it is not in case of E8 gene, predicting that their ethylene regulation is tissue specific and even developmentally regulated (Lincoln and Fischer, 1988).

4 Effect of Ethylene on Plant Physiology
Ethylene has crucial impact on the growth and development of plant. This simple, gaseous plant hormone affects and regulates the cell division, cell size and cell differentiation; thereby it has major effect on plant physiology. Plant growth and developmental changes are synchronized and under hormonal control. Therefore any mutation in ethylene synthesis related genes as well as ethylene signaling related genes would affect and alter the timing of plant developmental processes, thus this type of mutation is heterochronic mutation (Tsuchisaka and Theologis, 2004).

Ethylene modulates and alters developmental processes and growth dynamics such as seed germination, growth and development of plants organs, ripening and maturing as well as organs senescence and abscission (Schaller and Kieber, 2002). Ethylene induces and control root induction and formation, root nodules formation in leguminous crops, restricts the development of bulbs and tubers, stimulates the
formation of female part instead of male part in family cucurbitaceae, similarly in some plants species ethylene promote flower induction while in others, it’s otherwise.

In plants, all biotic and abiotic factors are elicited ethylene synthesis. Regulation of ethylene synthesis is highly influenced by various developmental signals and hormones including auxin, cytokinins, and gibberellin etc (Figure 2). This is also intensively enhanced by environmental and biological factors such as drought, salinity, cold, pathogens as well as insect pests etc (Bleecker and Kende, 2000). This shows the key role of ethylene in internal growth coordination and even in defense mechanism for survival against environmental confronts. Up-and-coming evidences have unraveled that ethylene sensitivity varies in diverse tissues and divergent developmental stages in consequence of signaling interactions with other plant hormones, metabolites and environmental cues (Klee, 2004; Alonso and Stepanova, 2004). Current studies in Arabidopsis thaliana by developing its ethylene mutants have also revealed an interconnection between early ovule abortion and silique size (Guo and Ecker, 2004).

5 Role of Ethylene in Biotic Stresses in Plants

Ethylene significant role in plant defense depicts its association in multiple physiological processes by stimulating necrosis and establishing hypersensitive response in plants (Lund et al., 1998 and Ciardi et al., 2001). Plant defense related dynamics including phytoalexins induction, production pathogenesis related proteins as well as cell wall modulations are activated by ethylene (Torner et al., 1997 and Fan et al., 2000). Hence ethylene has been an object of intensive study regarding resistance pathways and mechanisms from last few decades. The role of ethylene in resistance against pathogens had been studied by Hoffman et al., (1999) by developing soybean ethylene insensitive mutants. They observed that ethylene helps in restricting the pathogen invasion by leaf abscission when disease is aggravated. Similar studies had been done on Arabidopsis thaliana and Nicotiana tabacum (Knoester et al., 1998 and Thomma et al., 1999) by developing their mutants to explore the versatile role of ethylene in plant defense against pathogens.

6 Ethylene Perception and Signaling Pathway

Plants have diverse mechanisms for modulating themselves and showing elasticity against altering internal and external environmental conditions. Among these, ethylene signaling pathway is at imperative status that plants have espoused for regulating stress responses. The foremost elements in ethylene signaling pathway comprise the ethylene receptors, the Raf-like serine/threonine kinase, CTR1, EIN2 (transmembrane protein), and the EIN3-like (EIL) family of transcription factors (Alonso et al., 1999).
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Figure 2 Ethylene synthesis and signaling are influenced by multiple factors including Abiotic and biotic factors, developmental cues and plant hormones that alter plant processes from germination to senescence. (Idea taken from Yoo SD, Cho Y, Sheen J. 2009. Emerging connections in the ethylene signaling network. Trends Plant Sci. 14(5):270-9. doi: 10.1016/j.tplants.2009.02.007. Epub 2009 Apr 15.)

A linear ethylene signal pathway has been recognized based on genetic and molecular analyses of ethylene response mutants starting from hormone perception at the membrane to transcriptional regulation in the nucleus (Bleecker and Kende, 2000; Chang and Studler, 2001). Ethylene is sensed by transmembrane receptors. CTR1 as a negative regulator of this pathway is present downstream to receptors. Downstream of CTR1, ethylene receptors EIN2 and EIN3, EIN5, and EIN6 are present as positive regulators while EIN3 intercede transcriptional cascade of ethylene-regulated genes (Chen et al., 2005; Guo and Ecker, 2004). There are various families of ethylene induced genes that are present downstream of EIN3 like transcription factors (EIL) or proteins including ethylene response factor (ERF) and ethylene response DNA-binding factor (EDF) that signifying and demonstrating the transcription cascade that works downstream of EIL proteins (Figure 3). On the basis sequence and structural similarities, ethylene receptors present in different plant species are categorized into two groups. Group I receptors consists of ETR1 and ERSI whereas ETR2, EIN4, and ERS2 are the members of group II receptors in arabidopsis. Ethylene on binding to receptors leads to the inactivation of these receptors (Hua and Meyerowitz, 1998). Thus absence of ethylene supposed to be the basis of the activation of CTR1 that is the negative regulator of ethylene signaling pathway (Kieber et al., 1993). Binding of ethylene to its receptors is allied with its co-factor that is copper, which is carried by the copper transporter RAN (Tuteja, 2007).

Although clear functional attributes of EIN2, EIN5, and EIN6 at molecular level has not been cleared yet. However EIN2 is an integral membrane protein while EIN3 is a nuclear protein and as a transcription factor...
it regulates the expression of its immediate target genes like Ethylene Response Factor1 (ERF1) (Alonso et al., 1999; Chao et al., 1997 and Solano et al., 1998). This factor belongs to a large family of transcription factors containing APETALA2 domain. Transcription factors of this family GCC box, present in ethylene inducible gene’s promoters (Hao et al., 1998). Thus, a transcriptional cascade that is interceded by EIN3/EIN3-like (EIL) and Ethylene Response Factor1 escorts the ethylene controlled gene expression regulation.

By using molecular genetics and bioinformatics tools prime signaling components of ethylene pathway has been identified in Arabidopsis thaliana that are multiple membrane receptors, nuclear transcription factor families, an intracellular signaling protein kinase (PK), a membrane transporter-like regulator and F-box proteins (Johnson and Ecker, 1998; Chang et al., 1993 and Solano et al., 1998). The existence and identifications of these key ethylene related regulators in about all plant species has allude to highly conserved nature of this ethylene signaling pathway that have developed for functioning in sundry lifestyles and developmental programs.

7 Outlook
Ethylene as a gaseous phytohormone has been attaining great interest of plant scientists for few decades. Ethylene signaling, perception and integration of identified genetic components and regulators into mechanistic proceedings and events at genetic as well as molecular level are the current challenges. For elucidating and explicit the ethylene functional role in signaling pathway, the direct and specific regulatory components of ethylene linked networks should be distinguished from indirect, shared or general regulators as well as mediators. Understanding of molecular interaction of ethylene with other plant hormones is a novel aspect for plant physiologists that unravel unique underlying mechanisms. This would explicit ethylene related responses that drive coordination and interaction of endogenous developmental routes as well as internal programs with external environmental signals or stimuli for modulating and adapting plant’s physiology for its survival and growth.

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