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***Review Article***

# TRANSCRIPTIONAL CONTROL OF ETHYLENE RESPONSIVE GENES IN RIPENING OF CLIMACTERIC FRUITS: AN OVERVIEW

***S. K. Sinha**
**Department of Botany, Victoria Institution (College),*
*78B, A.P.C. Road, Kolkata-700009, India*
**Author for Correspondence*

**ABSTRACT**
Ethylene controls most of the events in ripening of the climacteric fruits. ACC synthase and ACC oxidase involve in the biosynthesis of ethylene and also responsible for autocatalytic ethylene production on the onset of ripening. Both ACC synthase and ACC oxidase along with E4 and E8 genes are well known as ripening specific genes as well as ethylene inducible genes, too. Promoter analysis of all these genes reveals presence of one or more ethylene responsive regions (ERE; the 8 bp motif A(A/T)TTCAAA). Apart from this, other *cis*-acting region like stress-related motifs, anaerobiosis-responsive elements etc. are also notable.

***Key Words:*** *ACC Synthase, ACC Oxidase, Ehtylene, Climacteric Fruits, Cis-Acting Element, Ethylene Responsive Regions*

## INTRODUCTION

One of the major goals in plant molecular biology is to analyze the molecular changes that regulate gene expression during different stages of growth and differentiation in plant life. Advancements in modern technologies, *viz*. recombinant DNA technology, transgenic, mutant production and analysis, protein engineering etc., are helpful for the scientists to identify the factors involved in flowering, fertilization, fruit ripening, development, differentiation of vegetative organs. Among these different physiological phenomenons, fruit ripening process draw an immense importance and major thrust area for research activity because of the specificity of this developmental process to plant biology and the practical importance of ripening to the human and animal diets. Tomato (*Lycopersicon esculentum* Mill.) is a model plant for analysis of ripening originally due to its significance as a food source and diverse germplasm, and more recently, high density physical map, large number of EST collection, the availability of molecular tools, ongoing genome sequencing project and efficient transformation procedure. The present article was aimed to summarize in a comprehensive manner towards the understanding of ethylene inducible promoter regions of ripening specific genes in climacteric fruits, particularly tomato.

## ETHYLENE AND FRUIT RIPENING

Post harvest physiological, biochemical and molecular changes of edible fruits, which are responsible for ripening; have been studied in great details from long before. It is well established fact that either developmental program alone or developmental program along with additional events induced by ethylene, the only gaseous plant hormone, triggers the ripening process of fruits. Depending on the production of ethylene and associated carbon dioxide during ripening process, fruits with different ripening mechanisms can be divided into two groups; climacteric, in which ripening is accompanied by a peak of respiration and a concomitant burst of ethylene; and non-climacteric, in which respiration shows no dramatic change and ethylene production remains at a very low level. It might be so that the increase in respiration rate is necessary to fuel the changes that occur during ripening of climacteric fruit. It is found that this respiratory climacteric is associated with a similar pattern of increase or burst of ethylene production during ripening (McMurchie *et al.,* 1972). Climacteric fruits at mature green stage can be induced to ripen by treatment with exogenous ethylene, and inhibition or removal of ethylene from the

environment of mature green fruit delays the onset of ripening (Giovannoni, 2001). Once ripening is induced, the endogenous ethylene production rises automatically. The sharp increase in ethylene production at the onset of ripening process is considered as a factor for controlling the initiation of changes in colour, aroma, texture, flavor and other biochemical and physiological attributes.

Ethylene mediates its ripening effect via regulated gene expression. The differential expression of mRNA transcripts and proteins can be evaluated under conditions of exogenous ethylene addition or inhibition in wild-type fruits, natural ripening mutants and transgenic plants, which are altered in expression of ethylene synthesis. The examples of best characterized ethylene regulated fruit genes are listed in Table 1. To understand the mechanisms that control the expression of ethylene responsive genes during tomato ripening, the promoter regions of several genes were isolated and analyzed with the aim to identify functional regulatory motifs.

## TRANSCRIPTIONAL REGULATION OF ACC OXIDASE AND ACC SYNTHASE GENE

The structure of the SlACO1 gene promoter is well characterized (Blume and Grierson, 1997). The -1855 to -396 region of the promoter confers ethylene dependent expression. It contains two repeat regions (RPT) with homology to ethylene responsive promoters of the ripening-specific genes 2A11 and E4. Several ethylene responsive regions (ERE; the 8 base pair motif A (A/T) TTCAAA) and stress-related motifs (TCA; the 10 base pair motif TCATCTTCTT) are also present in this promoter region. In contrast, the -396 region confers ethylene-independent expression.

Specific regulatory elements controlling the expression of ACS2 and ACS4 genes during tomato development and ripening were reported (Lin *et al.,* 2007). It was shown that both promoters share a wound response element and the SlACS4 promoter contains a sequence with similarities to an anaerobiosis-responsive element (ARE) found in the alcohol dehydrogenase genes of maize. An analysis of the SlACS6 promoter has been recently reported (Lin *et al.,* 2007). The aim of this study was to identify the *cis*-elements responsible for the negative feedback control of ethylene at the transition from System 1 to System 2 during fruit ripening. The results localized putative *cis*-elements required for negative ethylene-response between -347 and -266 upstream from the translation start. Several SlACS6::GUS stable lines containing internal deletion of this region showed loss of response of the promoter to exogenous ethylene and provide a molecular explanation for the System 1 repression phenotype of this gene. Further analyses of the *cis*-elements and the proteins that interact with them are needed to better understand the transcriptional regulation by ethylene of this gene.

## TRANSCRIPTIONAL REGULATION OF E4 AND E8 GENE IN TOMATO

The contrasting expression profiles of the E4 and E8 genes in response to ethylene make them attractive for analysis. Ethylene stimulates the transcription of the E4 gene in tomato fruit in response to both System 1 and System 2 ethylene. Indeed, every tissue analyzed for E4 expression results in expression upon exposure to ethylene and in virtually all tissues producing exogenous ethylene suggesting this gene is responsive to ethylene irrespective of tissue and developmental stage. In contrast, E8 is only induced in mature fruit (System 2- specific), indicating that ethylene regulation of this gene is both tissue-specific and developmentally regulated (Lincoln *et al.,* 1987 and Lincoln and Fischer, 1988). Analysis of the E4 promoter has shown that ethylene responsiveness of this gene requires a minimum of two co-operative *cis*-elements, an upstream regulatory element between -150 and -121 bp and a downstream regulatory element between -40 and +65 (Xu *et al.,* 1996). An ERF (Ethylene Responsive Factor, also known as Ethylene Responsive Element-Binding Protein or EREBP) interacts with E4 and also the E8 promoter in a region that is necessary and sufficient for ethylene response in fruit from 1528 to 1100 as defined by promoter deletion studies of the E8 gene. This ERF is present in unripe fruit and its DNA-binding activity is reduced when treated with ethylene. This suggests that this ERF plays a repressor role in transcription. Another DNA-binding protein, E4/E8BP, has been identified that interacts with the downstream element of the E4 promoter and with a region regulating fruit-specific expression in the E8 promoter (from -1088

to -863 bp). A cDNA encoding a similar DNA-binding specificity was cloned and the encoded protein was named E4/E8BP-1. E4/E8BP-1 expression rates were found to be higher in fruit and increased during ripening, suggesting that E4/E8BP-1 plays a positive role on expression during ripening. The E8 gene promoter also contains a sequence from -409 to -263 required for expression during ripening but no binding element has been reported to date. In addition, enhancer elements that are active in leaves, anthers and pollen and other uncharacterized positive regulatory elements are present in the E8 promoter (Deikman *et al.,* 1998). Taken together, these results imply that E4 and E8 may respond to a common mechanism for ethylene responsive gene expression but additional control systems limit E8 expression to the System 2 ripening fruit. Polygalacturonase is only expressed in maturing tomato fruit tissue and its transcription is specifically activated during ripening. This characteristic expression pattern of the gene made the PG promoter very attractive for characterization with respect to fruit and ripening-specific regulation (Montgomery *et al.,* 1993b and Nicholass *et al.,* 1995). The different regions observed in the promoter and the 3'-flanking region showed complex interactions between positive and negative regulatory elements that tightly control gene expression. Whereas the -4822 to -1412 promoter region in conjunction with the 1.8 kb 3' flanking region controls ripening-specific expression, the -1412 to -150 promoter region contains elements that direct spatial expression of the gene in the inner and outer pericarp. With recent development of genomics tools for tomato and other species (Alba *et al.,* 2005 and Rose *et al.,* 2004) and associated bioinformatics approaches for large scale data analysis and integration (Fei *et al.,* 2006), it is becoming easier to analyze the expression profile of thousands of genes and metabolites in the same sample, opening the door to discovery of new regulatory networks not accessible with prior technologies. Almost 869 genes were identified which differentially expressed in tomato pericarp during ripening and 37% of them were shown to be under ethylene regulation as defined by differential expression in the *Nr* mutant. 72 of these genes were annotated as being related to signal transduction or transcriptional control.

**Table 1: The ethylene-enhanced genes involved in ripening of tomato fruit**

| Gene | Homology | Function |
|---|---|---|
| SlACS2 | ACC synthasse | Catalyses system 2 ACC formation |
| SlACS6 | ACC synthasse | Catalyses system 1 ACC formation |
| SlACO1.6 | Dioxygenase, ACC oxidase | Catalyses $C_2H_4$ formation |
| E4 | Methionine sulphoxide reductase | Unknown |
| E8 | Dioxygenase | Unknown |
| ER24 | Transcriptional co activator MBF1 | Link EREBPs to TATA box binding protein |
| ER49 | Translation elongation factor EF-Ts | Post-transcriptional regulation |
| ER68 | RNA helicase DBP2 | Post-transcriptional regulation |
| ER50 | *Arabidopsis* CTR1 | Negative regulator of ethylene signal transduction |
| SlRab11a | Rab GTPase | Trafficking of cell-wall modifying enzymes |
| TomloxA, B, C | Lipoxygenase | Hydroperoxidation of polyunsaturated fatty acid |
| PG | Polygalacturonase | Depolymerizes cell wall pectins |
| PME | Pectin methylesterase | Maintains tissue integrity in senescent fruit |
| SlEXP1 | Expansin | Disrupts hydrogen bonds in wall matrix |
| SlPSY | Phytoene synthase | Catalyze formation of phytoene |

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