# Recent Developments on the Role of Ethylene in the Ripening of Climacteric Fruit

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## Abstract

**It has long been recognised that ethylene plays a major role in the ripening process of climacteric fruit. A more thorough analysis, however, has revealed that a number of biochemical and molecular processes associated with climacteric fruit ripening are ethylene-independent. One of the crucial steps of the onset of ripening is the induction of autocatalytic ethylene production. In ethylene-suppressed melons, ACC synthase activity is induced at the same time as in control melons, indicating that ACC biosynthesis during the early stages of ripening seems to be a developmentally-regulated (ethylene-independent) process. The various ripening events exhibit differential sensitivity to ethylene. For instance, the threshold level for degreening of the rind is 1ppm, while 2.5 ppm are required to trigger some components of the softening process. The saturating level of ethylene producing maximum effects is less than 5 ppm, which is by far lower than the internal ethylene concentrations found in the fruit at the climacteric peak (over 100 ppm). In many fruit chilling temperatures hasten ethylene production and ripening and in some late season pear varieties, exposure to chilling temperatures is even absolutely required for the attainment of the capacity to synthesize autocatalytic ethylene. This is correlated with the stimulation of expression of ACC oxidase and of members of the ACC synthase gene family. Ethylene operates via a perception and transduction pathway to induce the expression of genes responsible for the biochemical and physiological changes observed during ripening. However, only a few genes induced via the ethylene transduction pathway have been described so far. We have used a differential display method to isolate novel ethylene-reponsive (ER) cDNA clones of tomato that potentially play a role in propagating the ethylene response and in regulating fruit ripening. Collectively, these data permit a general scheme of the molecular mechanisms of fruit ripening to be proposed.**

## INTRODUCTION

For the horticulturist, the ripening of fleshy fruit involves a series of organoleptic changes that make the fruit attractive to the consumer. For the physiologist, the ripening process corresponds to a developmental stage during which biochemical and physiological events are initiated that lead to changes in texture, aroma, color, etc. With the development of molecular biology, fruit ripening is viewed as a genetically programmed event involving the regulated expression of specific genes. Although these processes vary from one type of fruit to the next, fruit can be divided into two broad groups, known as climacteric and non climacteric. The classification into one group or the other depends on whether or not a fruit exhibits a peak in respiration and ethylene production during ripening. Autocatalytic ethylene production is a major feature of climacteric fruit (McMurchie et al., 1972) as compared to non climacteric, where ethylene is not considered as essential. However, the generation of ethylene-suppressed fruit has revealed that ethylene-independent processes also occur during the ripening of climacteric fruit. Recent progress have been made in this area that will be briefly reviewed in this report.

It has long been claimed that the various ripening events do not exhibit the same sensitivity to ethylene. Experimental evidence will be given for such an assumption.

The mechanisms by which climacteric fruit develop the capacity to synthesize ethylene are poorly understood. In many fruit, chilling temperatures hasten the onset of the climacteric (Jobling et al., 1991; Knee et al., 1983) ). In some late pear varieties a chilling treatment is absolutely required for proper ripening (Blankenship and Richardson 1985; Morin et al. 1985; Knee 1987). We will report on the differential expression of members of the ACC synthase gene family during the low temperature-induced capacity to ripen.

Great progress has been made in recent years in the understanding of the ethylene perception and transduction pathway. However, the number of genes induced through this pathway that have been isolated so far is low, in relation to the large set of events controlled by the hormone. Progress in the search for novel ethylene-responsive genes will be described.

## THE RIPENING PROCESS OF CLIMACTERIC FRUIT IS PRIMARILY REGULATED BY ETHYLENE, BUT IT ALSO INVOLVES ETHYLENE-INDEPENDENT EVENTS

Ethylene is routinely reported as a major regulator of the ripening of climacteric fruit. However, other hormonal and/or developmental factors are also involved, but the nature of these factors remains largely unknown. The availability of ethylene-inhibited transgenic tomatoes (Murray et al., 1993; Picton et al., 1993; Theologis et al., 1993) and melons (Ayub et al., 1996; Guis et al., 1997) has allowed researchers to determine among the ripening events those that could proceed in the absence of the hormone. Colour changes can be either ethylene dependent or independent according to the type of pigments involved and the fruit species. In ethylene-suppressed tomatoes, the accumulation of lycopene is strongly impaired (Oeller et al., 1991; Murray et al., 1993) while the synthesis of cartenoids is ethylene-independent in the melon (Guis et al., 1997). Chlorophyll degradation is totally prevented in ethylene-suppressed fruit, consistent with the stimulation by ethylene of chlorophyllase gene expression (Jacob-Wilk et al., 1999). Sugar and acids accumulation is unaffected by ethylene suppression. Some other ripening events appear to depend partially on ethylene. For instance, softening and membrane deterioration comprise both ethylene-dependent and –independent components. This observation is consistent with the involvement of a complex set of differentially regulated genes. Among the polygalacturonase gene family of melon, some members are ethylene regulated others not (Guis et al., 1999). Aroma volatiles also exhibit great dependence upon ethylene in the tomato and the melon (Baldwin et al., 2000; Bauchot et al., 1998) although some biosynthetic pathways seem to escape ethylene regulation (Flores et al., 2001a). Because the upsurge of ACC syntheses activity occurs simultaneously in ethylene-suppressed and wild type fruit, it was concluded that the onset of autocatlytic ethylene production is ethylene-independent. These data support the concept that although ethylene plays a major role in the ripening of climacteric fruit, both ethylene-dependent and -independent pathways co-exist in climacteric fruit (Figure 1). On the other hand, non-climacteric fruit comprise ethylene-dependent events (Goldsmith, 1997).

## THE VARIOUS EVENTS OF THE RIPENING PROCESS EXHIBIT DIFFERENTIAL SENSITIVITY TO ETHYLENE

It has previously been suggested that the various components of the ripening process exhibit differential sensitivity to ethylene, but few experimental data are available in support of the concept (Wang and Hansen, 1970). One of the major difficulties in studying the role of ethylene regulation of individual ripening processes is that effects of a pre-climacteric ethylene treatment are masked by the initiation of autocatalytic ethylene production. In this respect, ethylene-suppressed transgenic fruit represent a unique material for ‘titrating’ the sensitivity to ethylene of the different ripening-associated pathways. By applying various concentrations of ethylene to antisense ACC oxidase melons, we were able to show that the threshold level of ethylene capable of physiological activity varied from 1 ppm for degreening of the rind to 2.5 ppm for softening, membrane deterioration and cell separation in the peduncular abscission zone (Flores et al., 2001b). The saturating levels producing maximum effect varied from 2.5 ppm for flesh softening and membrane deterioration to 5 ppm for the abscission zone detachment and degreening of the rind. These levels are by far lower than the internal ethylene concentration found in the fruit at the climacteric peak (over 100 ppm).

**CHILLING TREATMENT AFFECTS THE INITIATION OF AUTOCATALYTIC ETHYLENE PRODUCTION**

In winter pear varieties such as D'Anjou, Beurre Bosc and Passe Crassane, postharvest exposure to chilling temperatures is absolutely required for the induction of autocatalytic ethylene production (Blankenship and Richardson 1985; Morin et al. 1985; Knee 1987). A cold treatment, although not absolutely required, is also capable of hastening and synchronizing the onset of the climacteric rise of ethylene production and ripening of Bartlett pears, both on the tree (Wang et al., 1971) or detached (Looney, 1972). Similar effects have also been reported in Conference pears (Knee, 1987), apple varieties including Granny Smith (Jobling et al., 1991) and Golden Delicious (Knee et al., 1983). In Granny Smith apples, cold treatment induces ACO activity (Jobling et al., 1991; Larrigaudière and Vendrell, 1993) and ACO protein accumulation (Lelièvre et al., 1995). In Passe Crassane pears a 3-month chilling treatment at 0°C strongly stimulated ACC oxidase activity and to a lower extent ACC synthase activity (Lelièvre et al. 1997). At the same time, the levels of mRNAs hybridizing to ACC synthase and ACC oxidase non gene-specific probes increased dramatically. Fruit stored at 18°C immediately after harvest did not exhibit any of these changes. We have isolated and fully sequenced four members of the ACC synthase family that are expressed in the fruit. These genes exhibit differential expression in the absence or not of chilling treatment (Figure 2). The expression of the ACS1 gene is strongly stimulated during cold storage, indicating a probable participation in the development of competence to ripen. ACS 3 gene expression at the mRNA level is significant at harvest and in the absence of chilling treatment, conditions where ACC synthase activity cannot be detected. The other 2 genes, ACS 4 and ACS 5, are essentially related to the climacteric peak of ethylene production. When the ripening process is initiated, all 4 genes exhibit high levels of expression. Further analysis of the regulatory elements of the ACS1 gene may give information on the molecular mechanisms involved in the induction of the capacity to synthesize autocatalytic ethylene.

**NOVEL ETHYLENE-RESPONSIVE GENES: THE LARGE DIVERSITY OF GENE TYPES IS REPRESENTATIVE OF THE DIVERSITY OF EVENTS AFFECTED BY ETHYLENE DURING FRUIT RIPENING**

The ethylene receptor genes and components of the ethylene transduction pathway have been discovered in recent years (Giovanonni, 2001). However the number of genes that have been demonstrated to be induced through this pathway is low (Lincoln et al., 1987) in relation to the variety of physiological responses of plants to ethylene. We have used 'Differential display' to isolate early ethylene-regulated (ER) genes from late immature green tomato fruit in order to obtain a broader understanding of the molecular basis by which ethylene co-ordinates the ripening process (Zegzouti et al., 1999). A large set of clones have been isolated that show homology to genes involved in (i) transcriptional and post-transcriptional regulation, (ii) signal transduction components (iii) stress-related proteins, and (iv) primary metabolism. A number of these ER clones have so far no assigned function. We are currently using a reverse genetics approach to investigate the function of these genes and address their role in the ripening process. The latest data indicated that: ER50 is a CTR-like clone potentially involved in the ethylene transduction pathway; ER24 is homologous to a multi-bridging factor involved in transcriptional activation and ER49, a putative mitochondrial translational elongation factor that could be involved in the stimulation by ethylene of mitochondrial activity during the climacteric rise of respiration.

## CONCLUSIONS

The ripening of climacteric fruit is a complex developmental process in which ethylene plays a major role in association with other hormones and developmental factors. The ripening of climacteric fruit includes ethylene-independent events and, vice-versa, the ripening of non climacteric fruit comprises ethylene-dependent pathways. It is now well established that individual ripening events show differential sensitivity to ethylene. However, the level of ethylene present in ripening climacteric fruit is more than sufficient for inducing the major ripening-associated events such as softening and colour changes. The development of competence to synthesize autocatalytic ethylene appears to be an ethylene-independent process. In late season pear fruit, this competence is associated with the stimulation, by chilling temperatures, of the expression of a specific ACC synthase gene. A number of novel ethylene-responsive regulatory genes have been isolated, that may explain the diversity of ethylene responses and the regulation of a wide range of ripening events.

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**Figures**

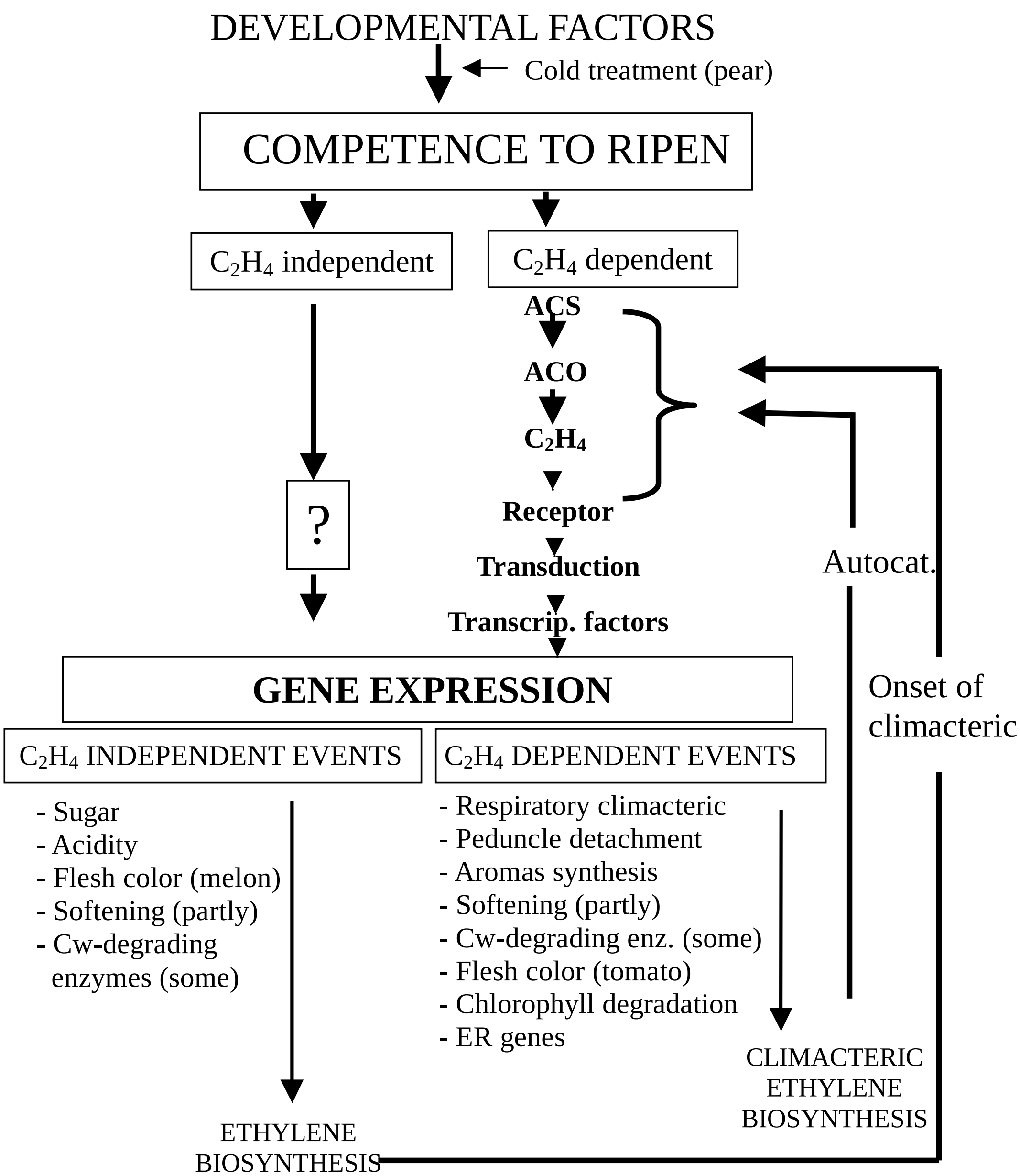


Fig. 1. Model scheme showing that the ripening process of a climacteric fruit includes ethylene-dependent (climacteric type) and -independent (non-climacteric type) physiological and molecular events.

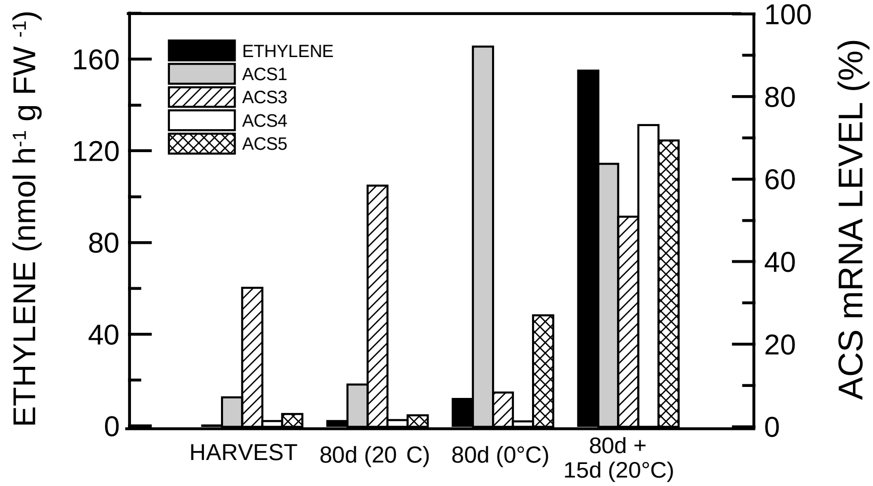


Fig. 2. Ethylene production and level of mRNA expression of four ACC synthase genes expressed in Passe-Crassane fruit at harvest, after exposure at 20°C after harvest, after chilling at 0°C for 80 days and after chilling followed by ripening at 20°C.