



## TOMATO FRUIT RIPENING: REGULATION OF ETHYLENE PRODUCTION AND ITS RESPONSE

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### SUMMARY

Climacteric ripening behaviour of tomato (*Solanum lycopersicum* L.) fruit has direct implications on its consumption, nutritional status, processing aspects and postharvest losses. Among the category of climacteric fruits, ripening of tomato is recognised as a model system. The plant hormone ethylene is a prime regulator of ripening in tomato fruit. Recent developments in the areas like; factors regulating the endogenous levels of ethylene, interaction of ethylene with other components, sensitivity of tissue towards ethylene and the regulatory mechanisms that operate at perception, signaling and ethylene-mediated response are presented here. The review emphasises the role and regulation of two key enzymes of ethylene production (*i.e.*, ACC-synthase and ACC-oxidase) and system 1 and system 2 of ethylene production at biochemical and molecular levels. The change over from system 1 to system 2 of ethylene production is highlighted in view of newly proposed route that is governed by developmental regulation/factors (independent of ethylene) besides the known route that works *via* system 1 (dependent on ethylene) of ethylene production. Significance of internal microenvironment of fruit (with respect to the levels of O<sub>2</sub>, CO<sub>2</sub> and non-ethylene volatiles such as; acetaldehyde and ethanol) is discussed in view of its regulatory role on biosynthesis and response of ethylene. Various approaches that have been targeted to alter either the endogenous levels of ethylene or its response by following molecular manipulations are presented. Finally, a few front line areas of research have been suggested to get better insight into the regulatory aspects of ethylene for its broader and accurate application in postharvest management.

**Key words:** Climacteric, endogenous volatiles, ethylene, ripening, postharvest, respiration, *Solanum lycopersicum*, system 1, system 2, tomato fruit

### INTRODUCTION

Postharvest physiology, shelf-life and losses due to decay of fruits are inter-linked processes. They all are primarily governed by last phase of fruit's maturation called fruit ripening. Fruit ripening involves various physiological, biochemical and developmental changes that occur in a coordinated and genetically regulated manner (Stepanova and Alonso 2005, Etheridge *et al.* 2006, Barry and Giovannoni 2007). Among the

postharvest metabolic changes, respiratory activity and transpirational loss of water are the two basic processes that determine the storage-life and quality of fruits (Lawes and Prasad 1999). There is correlation between level of ethylene produced by fruit and its shelf-life and postharvest decay (Gussman *et al.* 1993, Zheng and Wolff 2000). Both qualitative and quantitative losses occur in fruits between harvest and consumption. Rapid ripening of tomato (*Solanum lycopersicum* L.) fruits due to its climacteric nature leads to postharvest losses of

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25 to 40 % or even more than this (Verma and Joshi 2000, Sankar *et al.* 2002, Pulamte 2008). So, enhancing the postharvest life of tomato fruit is an important aspect and requires a thorough understanding of the ripening process. In this perspective, this article gives an overview on the recent and till date information related to regulation of ethylene production and its response with respect to tomato fruit ripening.

## IMPORTANCE OF TOMATO

Tomato fruit is the most important vegetable world over, for table purpose as well as for processing. It is available throughout the year and is a rich source of energy, carotenoids, flavonoids, phenolics, mineral nutrients, vitamin C and dietary fibres which are beneficial and serve as protective ingredients for human health (Beecher 1997, Powell and Bennett 2002, Wold *et al.* 2004). Globally the area, production and productivity of tomato during 2008-2009 accounts for 5.26 mha, 130.54 mt and 24.82 t ha<sup>-1</sup>, respectively with the Indian scenario of 0.59 mha, 11.15 mt and 18.60 t ha<sup>-1</sup>, respectively (Kumar *et al.* 2009). India produces about 8.54% of tomato and ranks third after China and USA (Kumar *et al.* 2009). Tomatoes are used for soup, salad, pickles, ketchup, puree, sauces and in many other ways. Versatile health benefits of tomato fruit further emphasise the need and importance of tomato in our daily diet (Gerster 1997, Verhoeyen *et al.* 2002). The shift in food habits both in developed and developing countries is attributed to these health benefits which have led to the increase in the demand for the high quality, fresh and nutritious fruits. This warrants the need for suitable storage technologies to maintain fruits' freshness and to minimise the quantitative as well as qualitative losses. So, tomato fruit ripening has received considerable attention for basic as well as applied research (Theologis 1992, Zeng *et al.* 1995, Zeng *et al.* 1996, Lelievre *et al.* 1997, Hoeberichts *et al.* 2002, Fallik and Aharoni 2004, Giovannoni and El-Rakshy 2005, Brecht *et al.* 2008).

## TOMATO FRUIT – A MODEL FOR CLIMACTERIC FRUIT RIPENING

The term climacteric was initially proposed to indicate the drastic increase in respiration (rise in the production level of CO<sub>2</sub>) of fruit during ripening. Thereby, fruits have

been classified as climacteric or non-climacteric according to their respiratory pattern (Biale 1964). Response of fruits towards the exogenous ethylene treatment has indicated that it could also distinguish climacteric and non-climacteric fruits (McMurchie *et al.* 1972, Bufler 1986). The process of ripening in climacteric fruits can be triggered and also enhanced by exogenous ethylene treatment (Tucker 1993). Application of propylene (a substitute of ethylene) was reported to initiate an increase in respiration in climacteric and non-climacteric fruits, but propylene mediated induction or rise in the endogenous level of ethylene was stimulated only in climacteric fruits and not in non-climacteric fruits (McMurchie *et al.* 1972, Yamane *et al.* 2007). In climacteric fruits, a substantial proportion of rise in the rate of respiration is reported to be contributed by cyanide-insensitive or cyanide-resistant respiration in fruits like; banana, mango and tomato (Kumar and Sinha 1992, Pandey *et al.* 1995, Reddy and Srivastava 1999). In contrast to climacteric fruits, cyanide-resistant respiration is present only to a limited extent in non-climacteric fruits. In these fruits, the upsurge in respiration and ethylene is either not observed or it is only transitory even after the application of exogenous ethylene (Lurie and Klein 1989, Kays and Paull 2004). However, a definite ethylene-mediated stimulation of respiration was noticed in the ripening of peel in citrus fruits (Goldschmidt 1997). Today, the use of ethylene production along with the rise in CO<sub>2</sub> level are being accepted as physiological criteria to identify climacteric fruits (Abeles *et al.* 1992, Atta-Aly *et al.* 2000). Since the basis of colour, composition, flavour, aroma and textural changes occurring in a regulated and coordinated manner during ripening of the fruits is well understood, tomato is considered as a model system of ripening in climacteric fruits (Fraser *et al.* 1994).

## ETHYLENE AS THE MAIN REGULATOR OF RIPENING IN TOMATO FRUIT

Ethylene is a natural plant growth regulator having numerous effects on the growth, development and storage life of many fruits. It plays a major role in the ripening process of climacteric fruits (Fig. 1) (Theologis *et al.* 1992, Yang 1995, Nagata *et al.* 1995, Lelievre *et al.* 1997, Saltveit 1999, Barry *et al.* 2000, Atta-Aly *et al.* 2000, Klee 2002, Alexander and Grierson 2002,

REGULATION OF ETHYLENE PRODUCTION AND ITS RESPONSE

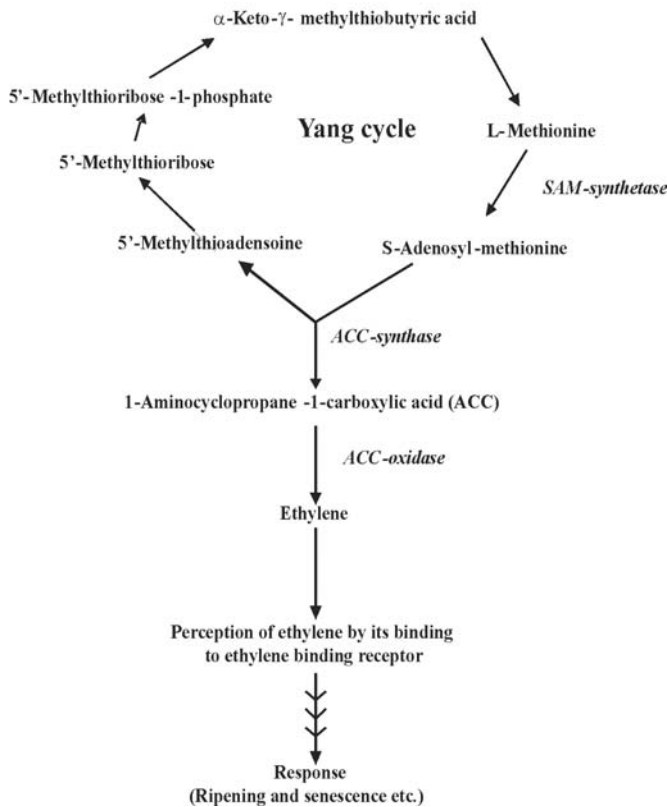


Fig. 1. Ethylene biosynthesis, perception and response

Hoeberichts *et al.* 2002). It has been suggested by Theologis (1992) that ethylene act as a rheostat rather than as a trigger for fruit ripening, which implies that ethylene must be present continuously in order to maintain transcription of the necessary genes. Later, Hoeberichts *et al.* (2002) reported that ethylene perception is required for the expression of tomato ripening related genes and associated physiological changes even at advanced stages of ripening. Clear-cut role of ethylene in the tomato fruit ripening was elucidated by developing transgenic plants with altered levels of ethylene production (Hamilton *et al.* 1990, Klee *et al.* 1991, Oeller *et al.* 1991). Chemical inhibitors of ethylene perception, particularly with 1-MCP, also proved the same (Fig. 2) (Beyer 1976, Sisler and Yang 1984, Sisler and Blankenship 1993, Sisler and Serek 1997). The production of aroma also depends strongly on the levels and action of ethylene (Golding *et al.* 1998, 1999, Rupasinghe *et al.* 2000, Alexander and Grierson 2002, Flores *et al.* 2002). In a review by Zhu *et al.* (2005) role of ethylene in the biosynthetic pathways of aroma

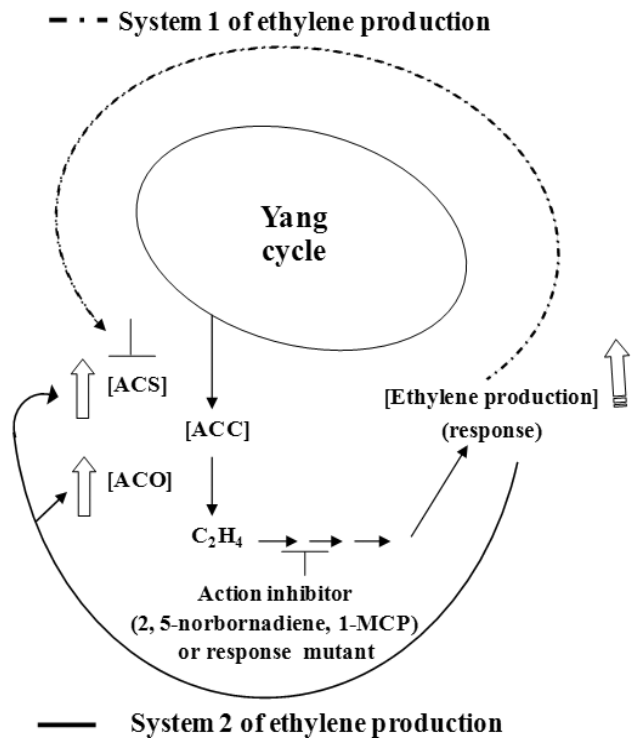


Fig. 2. Simplified pathway of ethylene biosynthesis in plants showing auto-inhibition (inhibiting its own production) and auto-induction of ethylene (inducing its own production). These systems are referred as system 1 and system 2 of ethylene production respectively. In system 1, ethylene inhibits its own production by inhibiting (⊥) ACS (ACC-synthase) expression/activity. It may be noted that the ACO (ACC-oxidase) activity is enhanced during system 1 but due to the absence of any enhancement in the activity of ACS there is no auto-induction. In system 2, ethylene induces more of its own production by stimulating (↑) the expression/activity of both of the enzymes (ACS and ACO) simultaneously and this enhances the ethylene production (↑). 2, 4-norbornadiene and 1-MCP (1-methylcyclopropene), which are action inhibitors of ethylene, and response mutants of ethylene block (⊥) the action/response of the ethylene and thereby inhibit the system 2 of ethylene production. (Source: Adapted and modified from Srivastava 2001).

volatiles in climacteric fruits such as tomato were updated and at the same time, it was also made clear that production of aroma volatiles may or may not totally dependent on ethylene. Besides this, low levels of ethylene are involved in wound healing and responses to various infections in some fruits of either climacteric or non-climacteric nature (Pech *et al.* 2003, Saltveit 1999, Van Loon *et al.* 2006). Other hormones and

regulators such as ABA (Zhang *et al.* 2009a) and nitric oxide (NO) (Eum *et al.* 2009) also affect the ripening process in tomato however their actions are mediated through ethylene only.

## REGULATION OF ETHYLENE PRODUCTION

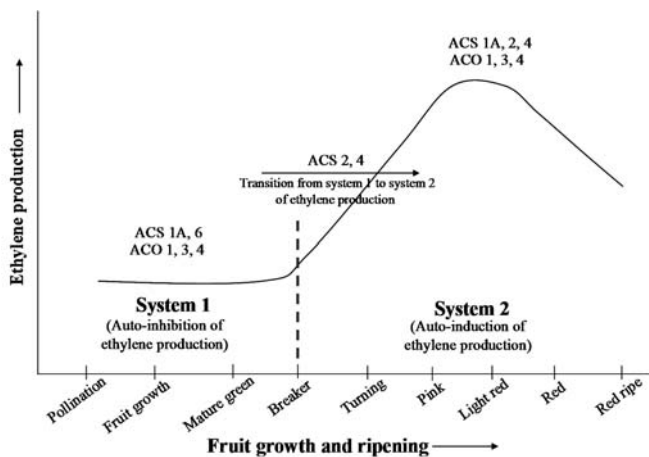
Rate of ethylene production in fruit during the course of ripening is controlled by the ability of the tissue to synthesise 1-aminocyclopropane-1-carboxylic acid (ACC) and to convert it in to ethylene (Fig. 1). The two key enzymatic controls are at the expression and activity levels of ACC-synthase (ACS) and ACC-oxidase (ACO) (Tucker 1993) (Fig. 1). These enzymes are encoded by multigene families in various plants and regulated by number of factors like; endogenous levels of O<sub>2</sub>, CO<sub>2</sub> and ethylene itself, degree and type of stress (wounding, water stress disease), developmental/maturity stage, temperature and concentrations of S-adenosyl-L-methionine (SAM), 1-aminocyclopropane-1-carboxylic acid (ACC), pyridoxal-5-phosphate, ascorbate, spermidine, spermine, L-canaline, IAA, Fe<sup>2+</sup>, Co<sup>2+</sup> and Ni<sup>2+</sup> at cellular level (Smith *et al.* 1992, Abeles *et al.* 1992, McKeon *et al.* 1995, Fluhr and Mattoo 1996, Lelievre *et al.* 1997, Nakatsuka *et al.* 1998, Saltveit 1999, Barry *et al.* 2000, Alexander and Grierson 2002, Nakano *et al.* 2003, Genard and Gouble 2005, Barry and Giovannoni 2007, Yokotani *et al.* 2009). Ethylene (which is final product of the above reactions) itself also strongly regulates the expression and activity of ACS and ACO (Lelievre *et al.* 1997).

### *System 1 and system 2 of ethylene production*

Two systems of ethylene production have been defined in plants namely system 1 and system 2 (McMurchie *et al.* 1972). The system 1 operates and functions during normal growth and development besides in response to various stresses. It is responsible for the production of basal level of ethylene in vegetative tissues and unripe fruit (Fig. 2). It is not auto-inductive in nature *i.e.*, even in presence of exogenous ethylene there is no triggered production of ethylene (Fig. 2 and 3). The system 2 operates during fruit ripening (Fig. 2 and 3). This system represents a massive increase in ethylene production associated with fruit ripening. During ripening in climacteric fruits, C<sub>2</sub>H<sub>4</sub> promotes its own synthesis via

a positive feedback mechanism (*i.e.*, auto-inductive C<sub>2</sub>H<sub>4</sub> production) (Fig. 3) (Theologis *et al.* 1992, Yang 1995, Nagata *et al.* 1995, Lelievre *et al.* 1997, Saltveit 1999, Barry *et al.* 2000, Atta-Aly *et al.* 2000, Klee 2002, Alexander and Grierson 2002). It is regulated in an auto-inductive manner (Oetiker and Yang 1995, Lelievre *et al.* 1997, Nakatsuka *et al.* 1998, Inaba, 2007) (Fig. 3) which means that exogenous ethylene application on climacteric fruits (at mature stage) could stimulate ethylene biosynthesis and speed up fruit ripening. Only in the presence of any action inhibitor of ethylene that the auto-inductive rise in the ethylene production is suppressed or blocked (Fig. 2).

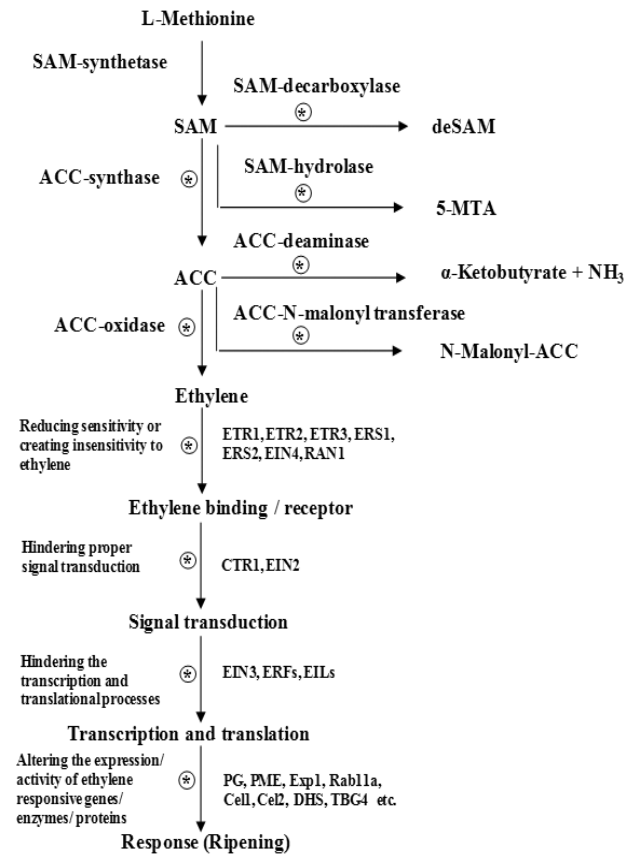
Both the key enzymes *i.e.*, ACC-synthase and ACC-oxidase are encoded by multigene families in various plants. These two enzymes are regulated by a number of regulating factors including the ethylene itself (already described earlier). The genes for these two enzymes are differentially expressed in various tissues at different stages of development and in response to internal and external stimuli such as ripening, senescence, wounding and auxin (Alexander and Grierson 2002). Expression of these genes in mature green tomato fruit is rapidly induced and/or enhanced by treatment with ethylene (Barry *et al.* 2000, Alexander and Grierson, 2002). Tomato possesses at least nine ACS (*LeACS1A*, *LeACS1B*, and *LeACS2-8*) and five ACO (*LeACO1-5*) genes (Fig. 3) (Zarem-binski and Theologis 1994, Barry *et al.* 1996, Oetiker *et al.* 1997, Nakatsuka *et al.* 1998, Van-der-Hoeven *et al.* 2002). Expression analysis studies have revealed that at least four ACS (*LeACS1A*, *LeACS2*, *LeACS4*, *LeACS6*) and three ACO (*LeACO1*, *LeACO3*, *LeACO4*) genes are differentially expressed in tomato fruit (Rottmann *et al.* 1991, Nakatsuka *et al.* 1998, Barry *et al.* 1996, 2000, Barry and Giovannoni 2007) (Fig. 3). *LeACO1*, *LeACO3*, and *LeACO4* are expressed at low levels in green fruit representing system 1 of ethylene synthesis. Transcripts of each of these genes were found to increase at the onset of ripening when the fruit shows transition from system 1 to system 2 of ethylene production. During ripening, *LeACO1* and *LeACO4* are sustained in expression, whereas the increase in expression of *LeACO3* is transient (Barry *et al.* 1996, Nakatsuka *et al.* 1998). Studies on the regulation of ACS gene expression during fruit ripening by Barry *et al.* (2000)



**Fig. 3: Differential expression of ACC-synthase (ACS) and ACC-oxidase (ACO) genes associated with system 1 and system 2 ethylene synthesis during development and ripening of tomato fruit. Auto-inhibition of ethylene synthesis during system 1 ethylene production is mediated by a reduction in *LeACS1A* and 6 (genes of ACS) expression. Auto-inductive ethylene biosynthesis (at the onset of fruit ripening) is mediated through ethylene-stimulated expression of *LeACS2* and 4 (genes of ACS) and *LeACO1* and 4 (genes of ACO). See text for more details (Source: Adapted and modified from Barry and Giovannoni 2007)**

and Barry and Giovannoni (2007) (Fig. 3) revealed the following information; 1. *LeACS6* is expressed in wild-type green fruit but the expression declines rapidly at the onset of ripening during the transition (of ethylene synthesis) from system 1 to system 2. 2. *LeACS6* transcripts persist throughout development and ripening in the *rin* mutant. 3. *LeACS6* is responsible for low-level of ethylene production in preclimacteric fruit. 4. *LeACS1A* gene may be important in regulating the transition from system 1 to 2. 5. *LeACS4* is not expressed in green fruit but is induced at the onset of ripening. 6. *LeACS2* expression is induced at the onset of ripening, this induction requires ethylene. 7. It seems that *LeACS1A* and *LeACS4* are responsible for initiating system 2 of ethylene synthesis and is maintained by a combination of *LeACS2* and *LeACS4* (Fig. 3). 8. Auto-inhibition of ethylene synthesis during system 1 of ethylene production is mediated by a reduction in expression of *LeACS1A* and 6 genes of ACS (Fig. 3) and 9. Auto-induction of ethylene synthesis at the onset of fruit ripening is mediated through ethylene-stimulated

expression of *LeACS2* and 4 and *LeACO1* and 4 (Fig. 3). In order to alter the biosynthesis of ethylene, various enzymes that have been targeted at molecular levels are presented in Fig. 4.



**Fig. 4: Ethylene biosynthesis and its signaling. The symbol (\*) denotes the sites that have been targeted through genetic engineering to suppress/inhibit the production, sensitivity or response of ethylene. Genetic manipulation can reduce the sensitivity towards ethylene if ethylene receptors (ETR1, ETR2, ERS1, ERS2 and EIN4) are targeted. After the perception of ethylene, the response related changes can be hindered by targeting the components of signal transduction (CTR and EIN 2) and transcription factors (EIN3, ERFs and EILs). Further, antisense or co-suppression approaches are also being used successfully to reduce the expression of ethylene responsive genes coding for enzymes/proteins associated with the process of ripening such as polygalacturonase (PG), pectin methylesterase (PME), expansin (Exp1), RabGTPase (Rab11a), cellulases (Cell1, Cel2), deoxyhypusine synthase (DHS), β-galactosidase (TBG4) etc. SAM: S-Adenosyl-L-methionine, ACC: 1-Aminocyclopropane-1-carboxylic acid, 5-MTA: 5'-Methyl thioadenosine and deSAM: Decarboxylated SAM**

One of the most important questions in the physiology of fruit ripening is about the mechanism that initially induces system 2 of ethylene synthesis. As per Klee (2004), one explanation may be that the cumulative effects of system 1 (even if the level is low) reaches a certain limit and induces system 2. The second explanation is that there is a change in the sensitivity of fruit to ethylene (already reported earlier by Biale and Young 1981, McGlason 1985). Fruit might become more sensitive to system 1 of ethylene as its development progresses. So, the transition from system 1 to system 2 is caused by a change in ethylene sensitivity due to continuous exposure of fruit to ethylene from system 1 (Barry *et al.* 2000). The involvement of system 1 in the transition process is supported by the observation that the treatment of an immature fruit with ethylene for a short time which did not induce system 2 immediately, rather, shortens the period preceding or required for the onset of system 2 (Yang 1987). Stresses such as wounding, water stress and disease during fruit development induce ethylene production which shortens the period required for the onset of fruit ripening (Abeles *et al.* 1992, Nakano *et al.* 2003). Kevany *et al.* (2007) demonstrated that the shortened period of ripening by exogenous ethylene is closely related to the level of the ethylene receptor protein (a negative regulator of ethylene signaling). These observations indicate that during development, both exogenous and endogenous ethylene increase the physiological age of fruit and also sensitise the fruit towards ethylene. So, it has been proposed that the level of the ethylene receptors modulates the timing of the onset of fruit ripening by measuring and memorising ethylene exposure along with the role of system 1 of ethylene production in initiation of system 2 of ethylene production (Kevany *et al.* 2007, 2008). However, the physiological and molecular pathways that act to initiate the transition from system 1 to system 2 mode of ethylene synthesis, at the onset of ripening, are largely unknown (Barry and Giovannoni 2007, Cara and Giovannoni 2008).

#### ***Internal gaseous composition of tomato fruit and its role in regulation of ripening***

There are three major routes through which gaseous exchange takes place across the surface of harvested fruit (1) outermost layers (cuticle and periderm) (2)

apertures (stomates and/or lenticels) and (3) stem scar region, (Solomos 1987, Ben-Yehoshua and Rodav 2003). In this way, there is an anatomical basis for gaseous exchange across the harvested fruits including tomato. The extent of diffusibility of gases across the fruit boundaries determines the internal microenvironment of the fruit (Nuevo *et al.* 1984, Ben-Yehoshua *et al.* 1985, Solomos 1987, Kader 1988, Banks and Nicholson 2000, Kader and Saltveit 2003a, 2003b, Paul and Srivastava 2006, Paul *et al.* 2010a). For tomato fruits, the stem scar region (the place where the pedicel along with sepals connects the fruits to the stem) is the predominant site for gaseous exchange (Burg and Burg 1965, Cameron and Yang 1982). It was demonstrated by de Vries *et al.* (1995) that 85-90 % of ethylene exchange takes place through the stem scar region of the tomato fruit.

Saltveit (1999) reported that once ripening of climacteric fruits has started, the internal ethylene concentration increases quickly to higher level (reaching up to  $100 \mu\text{l l}^{-1}$ ) attributed to the stronger diffusion resistance, especially during later stages of fruit development (Bargel and Neinhuis 2005, Paul and Srivastava 2006). The role of surface morphology in determining the ripening behaviour, rate of respiration and water loss by attached and detached tomato fruits was emphasised and there was variety dependent increase in the density of lenticels with the progress of ripening (Paul and Srivastava 2006). Further, for tomato fruit, stem scar region and the lenticels (on the peel's surface) were reported to show profound effect in controlling the respiration and ripening by determining exchange of gases depending upon the developmental stage/ripening status and variety (Paul and Srivastava 2006).

Ethylene production was found to be reduced by either low oxygen or high carbon dioxide or both, and the effects are additive (Zagory and Kader 1988). Earlier, Burg and Burg (1967) showed that the amount of  $\text{CO}_2$  in the intercellular spaces of fruits at pre-climacteric stage is low but this may approach to higher levels of around 10 % during ripening and post-climacteric phase. This higher endogenous level of  $\text{CO}_2$  probably raises the threshold for ethylene action in fruits to higher levels. Short period of anaerobiosis prior to storage reported to retard the ripening process in tomato fruit (Pesis and Marinansky 1993). It has been

demonstrated that elevated carbon dioxide inhibited accumulation of ACC in tomato besides decreasing the activity of ACC-oxidase (Mathooko *et al.* 1995). Hypoxia was further reported to decrease synthesis as well as the action of ethylene (Gorny and Kader 1996, Mathooko 1996). In addition to this, hypoxia condition in the microenvironment of the tomato fruit was also reported to increase the activity of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) enzymes (Chen and Chase 1993, Longhurst *et al.* 1994) and as a result the production of acetaldehyde and ethanol was triggered in tomato (Longhurst *et al.* 1994, Speirs *et al.* 1998). Ethanol and acetaldehyde have already been reported to delay ripening in tomato and other fruits (Beaulieu *et al.* 1997, Pesis 2005, 2006). Likewise, for another non-ethylene volatile compound *i.e.*, nitric oxide (NO) was identified as natural senescence-delaying plant growth substance acting by down-regulating ethylene production (Leshem *et al.* 2000). NO influences the ethylene production through its direct regulatory effect on ACC-synthase or ACC-oxidase enzymes as well as on genes (Eum *et al.* 2009).

A theory of ethylene emission by tomato fruit was developed as the base to formulate simulation model called 'ETHY' by Genard and Gouble (2005). There are various factors that affect the production and release of ethylene by the fruit. This model was found to be highly sensitive to the factors like permeability of skin surface, internal concentration of O<sub>2</sub>, CO<sub>2</sub> and ACC, change in fruit growth and temperature, activities of ACC-oxidase and ACC-synthase, concentration of ethylene itself and production status of ATP. Similar model has been proposed for gas exchange in pear fruit (Ho *et al.* 2008). Significance of the available surface area of stem scar region in determining the rate of ripening and extent of climacteric rise in tomato fruit was reported by Paul *et al.* (2010a). Differences in the endogenous levels of ethylene and other gases such as; O<sub>2</sub> and CO<sub>2</sub> were shown to regulate the ripening and ripening-related changes in tomato fruits of different varieties (Paul *et al.* 2010a, 2010b). Role of fruit's internal microenvironment was described in relation to ripening and storability of different fruits by Paul and Pandey (2011). They discussed various reasons for the variations in the endogenous levels of ethylene and non-ethylene gases/volatiles among different species and

cultivars along with the regulatory interactions of non-ethylene components with the production and response of ethylene. Extent of the 1-MCP mediated delay in ripening depends on the endogenous level of ethylene in the tomato fruits (Zhang *et al.* 2009b). This evinces that the cultivar dependent variations in the ripening behaviour of fruits can also be due to the differences in their internal gaseous environment.

### ***Perception, signaling and response of ethylene***

Once the auto-inductive (also referred as auto-catalytic) system 2 of ethylene production starts, it triggers a cascade of events leading to many interactive signaling and metabolic pathways for the progress of ripening in climacteric fruits (Stepanova and Alonso 2005, Etheridge *et al.* 2006, Barry and Giovannoni 2007). On the basis of mutant analysis of *Arabidopsis*, ethylene signaling pathway (Fig. 4) has been proposed (Alexander and Grierson 2002, Klee 2004, Kendrick and Chang 2008). Various sites of ethylene signaling that have been targeted at molecular levels to suppress or inhibit the ethylene signaling and thereby ethylene mediated responses (including fruit ripening) are also shown in Fig. 4.

Ethylene is perceived by receptors [ETHYLENE RESISTANCE1 (ETR1)] and related proteins (Chang *et al.* 1993, Hua and Meyerowitz 1998). Based on phylogenetic analysis and the presence of conserved sequences (H, N, G, F, and G) in the histidine kinase domain, the two subfamilies of ethylene receptors are - I (ETR1 and ERS1) and II (ETR 2, ERS 2 and EIN 4). The ethylene-binding domain consists of three transmembrane domains. Subfamily II receptors have an additional putative signal sequence preceding the transmembrane domains. All five members of the ethylene receptor family have a GAF domain of unknown function (Etheridge *et al.* 2006). Ethylene is perceived by receptors [ETHYLENE RESISTANCE1 (ETR1)] and related proteins (Chang *et al.* 1993, Hua and Meyerowitz 1998). The ethylene signal is transduced to messengers, such as ETHYLENE INSENSITIVE3 (EIN3) through CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) (Kieber *et al.* 1993) and ETHYLENE INSENSITIVE2 (EIN2) (Alonso *et al.* 1999) (Fig. 4). EIN3 is a transcription factor that plays

a crucial role in regulation of expression of ethylene responsive genes (Chao *et al.* 1997, Solano *et al.* 1998). In the absence of ethylene, EIN3 protein is quickly degraded through a ubiquitin–proteasome pathway mediated by two F-box proteins [EIN3-binding F box protein 1 and 2 (EBF1 and EBF2)] whereas EIN3 protein is stabilised by ethylene itself (Guo and Ecker 2003, Potuschak *et al.* 2003, Gagne *et al.* 2004). Also, the receptors activate CTR1, a negative regulator that suppresses downstream signaling. CTR1 is related to the MAPKKs, but it is unclear whether CTR1 functions as the first step in a MAP kinase cascade. Downstream of CTR1, EIN3 levels are reduced by proteasome-mediated degradation, involving action of the E3 complex components EBF1 and EBF2. Perception of ethylene results in the inactivation of CTR1 and prevents EIN3 degradation, thus activating the ethylene-signaling pathway. Activation of the EIN3/EIL family of transcription factors induces a transcriptional cascade to establish ethylene responses (Fig. 4). In tomato, four *EIN3*-like (*EIL*) genes, say *LeEIL1–LeEIL4* have been isolated (Tieman *et al.* 2001, Yokotani *et al.* 2003). Reduced expression of tomato *LeEIL* genes by antisense technology modulate ethylene responses, including leaf epinasty, flower senescence and fruit ripening (Tieman *et al.* 2001).

Ito *et al.* (2008) has demonstrated that ripening inhibitor (RIN protein) exhibits transactivator activity because it binds to the promoter region of *LeACS2*. Thus, part of the gradual increase in ethylene production as observed in tomato fruit lines where *LeEIL* genes were suppressed by RNA interference engineering (*RiEIL*) may be due to the direct up-regulation of the *LeACS2* gene by RIN. However, it is unclear whether system 1 alone is enough to have this effect or not. Yokotani *et al.* (2009) attempted to identify if system 2 is completely regulated by an auto-induced system only or partly by any other mechanism. For this, they used RNA silencing (hairpin RNA-induced gene silencing technique) of *LeEIL* genes to suppress the total expression of *LeEIL1–LeEIL4* to trace levels) along with the application of 1-MCP [1-MCP binds with ETR1 protein (a receptor of ethylene) and inhibits the ethylene signal and its response]. The obtained results revealed that ripening-associated ethylene biosynthesis is regulated

by both *i.e.*, auto-induced system of ethylene and ethylene-independent developmental factors. So, fruit can initiate system 2 of ethylene production even without the cumulative effects of system 1 of ethylene production. Suppression of ethylene production would be expected in 1-MCP-treated transgenic fruit if leaky *LeEIL* mRNA is involved in ethylene production. However, the pattern and level of ethylene production in 1-MCP-treated transgenic fruit were almost identical to those in non-treated transgenic and 1-MCP-treated wild-type fruit. Despite the double block in the ethylene signal, the residual ethylene was detected, which indicated that the ethylene production is probably not due to leaky ethylene sensitivity, but rather due to an ethylene-independent developmental factor. Thus, it was proved that ripening ethylene (system 2) in wild-type tomato fruit consists of two parts: a large part that occurs under auto-induced regulation and a minor part regulated by an ethylene-independent developmental system (Yokotani *et al.* 2009).

Based on the outcome on molecular switches and signal transduction pathway studies, a model was proposed by Yokotani *et al.* (2009) to explain the transition from system 1 to system 2 (Fig. 5). According to this model, ethylene in system 1 is produced *via* *LeACS1A* and *LeACS6*, which in turn is regulated by a negative feedback regulation (Nakatsuka *et al.* 1998, Barry *et al.* 2000, Alexander and Grierson 2002). Transition of system 1 to system 2, under natural conditions *i.e.*, in absence of exogenous ethylene and stress, occurs mainly *via* limited expression of *LeACS2* and *LeACS4*. This transition occurs even if the effect of system 1 of ethylene is eliminated. In this way, fruit can initiate system 2 of ethylene production leading to fruit ripening. This transition can be controlled by developmental factor(s) independent of ethylene besides the involvement of ethylene. So, shifting towards system 2 of ethylene production in tomato fruits consists of both, ethylene-dependent and ethylene-independent mechanisms. Besides this, changes in the levels of receptors and/or tissue sensitivity towards ethylene with development and ripening have been reported in *Arabidopsis* plant (Yoo *et al.* 2009) and climacteric fruits like tomato (Kevany *et al.* 2007, 2008).



**RESEARCHABLE AREAS/ISSUES**

In the recent past lot of progress and understanding has been made on the changes associated with ripening at physiological, biochemical and molecular levels. This has contributed immensely in deciphering the mechanisms and signaling pathways involved and linked with fruit ripening. It has been demonstrated very clearly that besides many other regulatory controls there also exists developmental control on the production of system 2 of ethylene. This thereby can have a strong control not only in triggering the process of ripening but also in the progress of ripening.

More effective strategies for postharvest management can be developed with the better understanding of following areas/issues related to ethylene and its regulatory aspects.

- How exactly does the transition occur from system 1 to system 2 of ethylene synthesis at the onset of ripening? How is system 2 of ethylene synthesis perpetuated?
- To document and harness the available natural variation in the ripening behaviour for generating new varieties with broad consumer appeal and enhanced storage capability.
- What are the functions of the individual ethylene signaling components and how is this network of proteins assemble *in vivo*?
- What are the downstream targets of the EIN 3 like transcriptional factors (EILs) and ethylene responsive factors (ERFs) in ripening fruits and how do these transcription factors activate or repress specific ripening-related pathways?
- What is the exact reason and specific role of rise in respiration (climacteric rise) during ripening of climacteric fruits?
- How is ethylene linked to respiratory metabolism at biochemical and molecular levels? Alternate respiration also needs the attention in terms of its

role and significance in climacteric fruit ripening. Can the ripening and ripening-related changes be altered by manipulating alternate respiration?

- To integrate genetically identified regulatory components into mechanistic actions at the biochemical, molecular and cellular levels for better understanding of ethylene signaling and its functions.

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