

REVIEW PAPER

# Role of plant hormones and their interplay in development and ripening of fleshy fruits

Rahul Kumar<sup>1,†</sup>, Ashima Khurana<sup>2</sup> and Arun K. Sharma<sup>1,\*</sup>

<sup>1</sup> Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi 110021, India.

<sup>2</sup> Zakir Husain Delhi College, University of Delhi, New Delhi 110002, India.

<sup>†</sup> Current address: Repository of Tomato Genomics Resources, Department of Plant Sciences, University of Hyderabad, Hyderabad 500046, India.

\* To whom correspondence should be addressed. E-mail: [arun@genomeindia.org](mailto:arun@genomeindia.org)

Received 9 October 2013; Revised 17 April 2014; Accepted 2 June 2014

## Abstract

Plant hormones have been extensively studied for their roles in the regulation of various aspects of plant development. However, in the last decade important new insights have been made into their action during development and ripening, in both dry and fleshy fruits. Emerging evidence suggests that relative functions of plant hormones are not restricted to a particular stage, and a complex network of more than one plant hormone is involved in controlling various aspects of fruit development. Though some areas are extensively covered, considerable gaps in our knowledge and understanding still exist in the control of hormonal networks and crosstalk between different hormones during fruit expansion, maturation, and various other aspects of ripening. Here, we evaluate the new knowledge on their relative roles during tomato fruit development with a view to understand their mechanism of action in fleshy fruits. For a better understanding, pertinent evidences available on hormonal crosstalk during fruit development in other species are also discussed. We envisage that such detailed knowledge will help design new strategies for effective manipulation of fruit ripening.

**Key words:** Auxin, ABA, ethylene, fruit development, phytohormone, ripening, tomato.

## Introduction

In angiosperms, fruiting bodies have evolved to facilitate seed maturation and their subsequent dispersal. A diverse range of fruit types from dry to fleshy forms exists across plant species. Evolutionary studies predict that species that produce dry fruit are the ancestors of species that produce fleshy fruit, and this is the reason for the prevalence of common developmental mechanisms between the two types (Knapp, 2002). In *Arabidopsis*, the model plant with dry fruits, a high-level regulatory network of transcription factors controlling fruit development has been identified.

Similarly, studies on tomato, a model for fleshy fruits, have provided new insights into the networks responsible for the control of ripening (Tomato Genome Consortium, 2012). Together, strong similarities between dry and fleshy as well as within different fleshy fruits in the molecular circuits governing development and maturation indicate that regulatory networks are conserved across a wide spectrum of angiosperm fruit (Seymour *et al.*, 2013). Though information on interplay between hormones at several levels of these steps remains scarce, nonetheless with the advent of “omics”

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate; NCED1, 9-cis-epoxycarotenoid dioxygenase1; ABA, abscisic acid; ACO, ACC oxidase; ACS, ACC synthase; ARF, auxin response factor; BR, brassinosteroids; CNR, colourless non-ripening; ETR, ethylene receptor; ERF, ethylene response factor; IAA, indole-3-acetic acid; PIN, PIN-FORMED; TIR1, transport inhibitor response1; GA, gibberellins; GH3, Gretchen-Hahen3; JA, jasmonic acid; MJ, methyl jasmonate; MT, metric ton; NO, nitric oxide; NOR, non-ripening; PA, polyamine; rin, ripening inhibitor; SA, salicylic acid.

© The Author 2014. Published by Oxford University Press on behalf of the Society for Experimental Biology. All rights reserved.  
For permissions, please email: [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

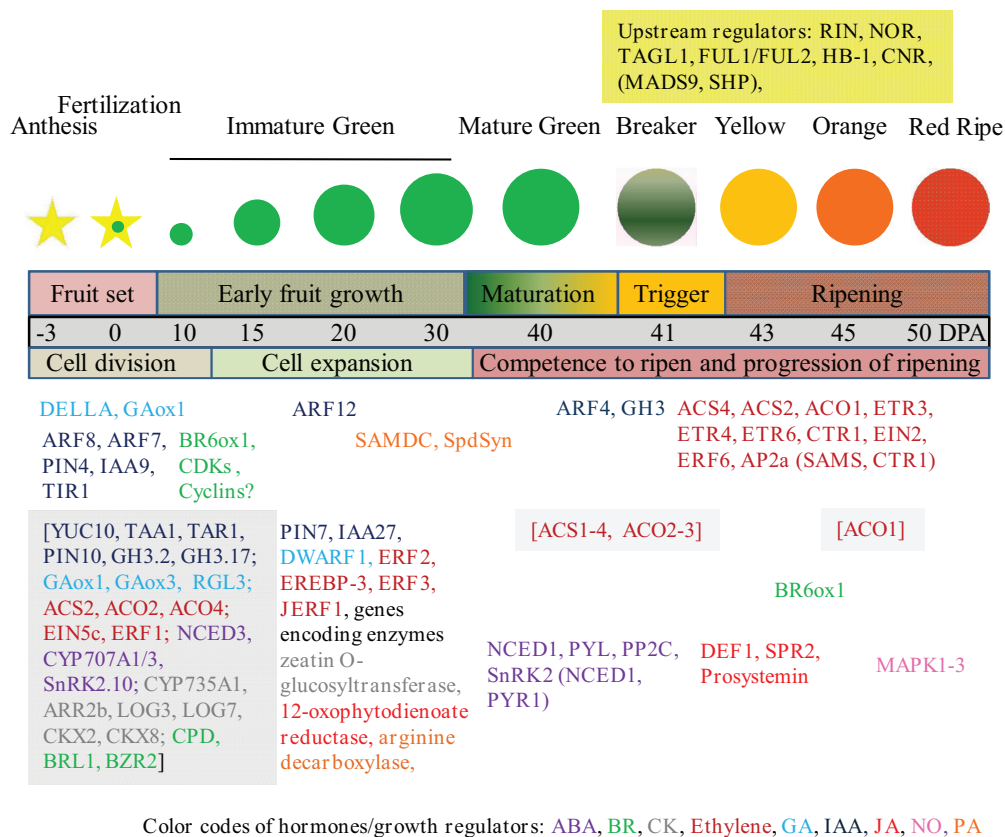
tools, significant progress has been achieved in characterization of hormone responses (Osorio *et al.*, 2011; Rohrmann *et al.*, 2011). These efforts have led to identification of distinct as well as overlapping patterns in the expression of genes associated with hormone action across plant species (Liu *et al.*, 2005; Zhang *et al.*, 2009; Sun *et al.*, 2012a; Gao *et al.*, 2013).

Similar to dry fruits, fleshy fruits are also botanically diverse in the way they develop. Whereas tomato and grape are derived from the ovary, other fruits such as strawberry, pineapple, and apple are derived from the receptacle tissue or from expansion of the sepals. Both dry and fleshy fruits undergo similar developmental steps, including fruit set, fruit growth, maturation, and ripening. Fruit set represents the first stage of the development after the fertilization event. This is followed by an active cell division and later cell expansion phase; both together contribute to the fruit growth phase. The growth phase causes fruits to attain their maximum size. This is followed by a stage where fruits acquire the prerequisite competence to enter into the final developmental stage, i.e. ripening. Ripening signifies a very important phase change and results in conversion of a less palatable green fruit into a highly palatable, nutritionally rich, and coloured fruit. Most of the beneficial pharmacologically active compounds are accumulated in fruits to higher levels during this phase. Further, fleshy fruits are physiologically classified as climacteric and non-climacteric. Climacteric fruits such as tomato, banana, apple, and avocado show a concomitant increase in respiration and ethylene biosynthesis upon initiation of ripening. Non-climacteric fruits such as citrus, grape, and strawberry lack these two attributes at the onset of ripening. However, once commenced, ripening cannot be stalled and generally leads to over-ripening that in turn negatively affects the quality parameters of fruits and leads to products being discarded. Therefore, minimization of postharvest spoilage of fruits remains one of the biggest challenges before plant biologists. Any effort which can reduce such spoilage would mean saving of millions of dollar and ensure the food security to under-nourished populations the world over. Additionally, improvement of fruit-related agronomic traits is essential to alleviate their low consumption related issues, associated with human diet. Plant hormones are long known to be tightly associated with fruit development and ripening (Klee and Giovannoni, 2011; Seymour *et al.*, 2013). Therefore, herein we have surveyed literature published on the role of plant hormones in the context of their influence on various aspects of fruit development and describe the ways in which plant hormones govern these responses, individually or in combination with more than one hormone. Special emphasis has been placed on tomato as it is the most studied model system for molecular genetic analysis of fleshy fruit development and ripening. However, other systems will be mentioned wherever appropriate. Finally, we also highlight the research areas where more efforts are required to improve upon our current understanding on the role of plant hormones during fruit development.

## Hormone regulation during fruit development and ripening

*Auxin, GA, and cytokinin are major regulators of fruit set*

Fruit set represents the very first step of fruit development. In angiosperms, it depends on the successful completion of pollination and a unique double fertilization event where one of the pollen nuclei fertilizes the egg cell, whereas the other fuses with two haploid polar nuclei in the central cell (Raghavan, 2003). These events set the formation of the seed which eventually controls the cell division and fruit growth in a synchronized manner. Current evidence supports that combined action of three hormones, auxin, gibberellins (GAs), and cytokinin, plays a major role in the regulation of fruit set. Individually, any of these hormones can only initiate the fruit development to a certain extent; however, their combined application has been found to induce normal fruit growth even in the absence of fertilization in both dry and fleshy fruits (Nitsch, 1952; Crane, 1964; Gillaspay *et al.*, 1993; Mariotti *et al.*, 2011). Importantly, auxin, GA, and cytokinin levels increase at fruit set and the requirement of their higher levels at fruit set has been already validated by their exogenous treatment, which causes parthenocarpic fruit formation in tomato. Evidence suggests that auxin and GAs also act in a similar way during fruit set in dry fruits. A fertilization-triggered auxin signal is involved in promotion of GA biosynthesis in the ovule, which in turn activates GA signalling in ovules and valves and coordinates silique growth in *Arabidopsis* (Dorcey *et al.*, 2009). Additionally, it has been observed that interaction between auxin and GA signalling pathways is essential for the promotion of fruit set in fleshy fruits (Vivian-Smith and Koltunow, 1999; Srivastava and Handa, 2005; de Jong *et al.*, 2009; Carrera *et al.*, 2012; Ruan *et al.*, 2012). Recently, Kang *et al.* (2013) demonstrated that a number of auxin biosynthesis genes encoding proteins such as YUCCA5, YUCCA11, and tryptophan aminotransferase related1, and GA biosynthesis genes encoding enzymes such as GA 20-oxidase3, and GA 3-oxidase3, 4, 5, and 6 had achene-preferential expression and were largely absent in the receptacle of strawberry. Evidence suggests that auxin promotes fruit set and growth, at least partly, by controlling the GA levels (Fig. 1) (Serrani *et al.*, 2008; Dorcey *et al.*, 2009). Earlier studies at the molecular level had established a role for an Auxin Response Factor (SIARF7) in mediating the crosstalk between auxin and GA. Silencing of this gene caused formation of parthenocarpic fruits with morphological characteristics that seem to be the result of both increased auxin and GA responses, suggesting that SIARF7 also acts as a modifier of the GA response during early stages of fruit development (de Jong *et al.*, 2009; de Jong *et al.*, 2011). Consistent with this observation, a point mutation in a gene encoding DELLA protein was found to be responsible for the constitutive GA responses in *procera* (*pro*) mutant plants (Carrera *et al.*, 2012). Transcriptome analysis suggested that parthenocarpic capacity of *pro* is



**Fig. 1.** Genes involved in biosynthesis, perception, and signalling of various hormones with their established roles in tomato fruit development and ripening. Evidence suggests modulation in the levels of auxin (IAA), cytokinin (CK), gibberellins (GA), ethylene, polyamines (PA), and brassinosteroid (BR) at fruit set; IAA, GA, and BR at fruit growth; IAA, abscisic acid (ABA), PA during fruit maturation; CK, BR, jasmonic acid (JA), nitric oxide (NO), and salicylic acid (SA) during fruit ripening (for further information see McAtee *et al.*, 2013). Based on published literature, many hormones/growth regulators, such as ABA, BR, ethylene, IAA, GA, and PA have already been characterized and are represented at respective stages. Genes represented in yellow background at upper panel on right side such as RIN (Ripening-Inhibitor), Non-ripening (NOR), CNR (Colourless non-ripening), TAGL1 (Tomato AGAMOUS-Like1), TDR4/FUL1 (FRUITFUL homolog), MBP7/FUL2 (FRUITFUL homolog2), HB-1 (Homeobox domain protein1), and SHP (SHATTERPROOF-LIKE) are the well-characterized regulators of ripening. In addition, other genes of different hormonal pathways which have been found to be involved in the regulation of different aspects of fruit development are represented in the colours matching to their respective hormones. Further, genes presented in italics have been reported to exhibit differential transcript accumulation at the respective stage in tomato (Lemaire-Chamley *et al.*, 2005; Mounet *et al.*, 2009; Liu *et al.*, 2012; Zheng *et al.*, 2014) and those followed by? are differentially expressed genes in cucumber (Fu *et al.*, 2008). Moreover genes present in parentheses represent the already characterized genes with a known role during development and ripening, whereas those present in square brackets with grey background represent putative regulators of the similar stages in strawberry, a non-climacteric fruit. The list includes GAox1 and 3, gibberellin oxidase1 and 3; ARF4, 7, 8, 12, auxin response factor4, 7, 8, and 12; PIN4, 7, 10, PIN-FORMED4, 7, and 10; IAA9, indole-3-acetic acid9; TIR1, transport inhibitor response1; YUC10, YUCCA10; TAA1, TRYPTOPHAN AMIONOTRANSFERASE OF *ARABIDOPSIS*1; TAR1; TRYPTOPHAN AMIONOTRANSFERASE1 RELATED1; GH3.2 and 17, Gretchen Hagen3.2 and 17; SAMDC, S-adenosyl methionine decarboxylase; SpdSyn, spermidine synthase; NCED1 and 3, 9-cis-epoxycarotenoid dioxygenase1 and 3; PYL and PYR, ABA receptors; SIPP2C, Type 2C protein phosphatase; SnRK2, Subfamily 2 of SNF1-related kinases; BR6ox1, BRASSINOSTEROID-6-OXIDASE1; CDKs, cyclin-dependent kinases; CYCs, cyclins; ACC, 1-aminocyclopropane-1-carboxylate; ACS, ACC synthase; ACO, ACC oxidase; ETR3, 4, and 6, ethylene receptor3, 4, and 6; AP2a, AP2 domain containing ethylene response factor; ERF2, 3, 6, Ethylene Response Factor2, 3 and 6; CTR1, constitutive triple response 1; SAMS, S-adenosyl-L-methionine synthetase; EREBP-3, ethylene-responsive element binding protein3; LOG3 and 7, LONELY GUY3 and 7; CKX2 and 8, cytokinin oxidase2 and 3; ARR2b, AHP response regulator2b; BRL1, brassinosteroid LRR-like kinase1; BZR1, brassinazole resistant1; SPR2, suppressor of prosystemin-mediated responses2; DEF1, defenseless1.

mainly associated with changes in the mRNA levels of genes involved in GA and auxin pathways, including *SLARF7* (Carrera *et al.*, 2012). Further, GA-mediated responses are under the tight regulation of growth-repressing DELLA proteins. According to the “relief of restraint” model, any activation of GA signalling requires degradation of DELLA proteins (Harberd, 2003). The involvement of GA-mediated signalling during fruit set has been further substantiated as any reduction in DELLA activity has been found to promote the parthenocarpic fruit growth in both dry and fleshy fruits (Marti *et al.*, 2007; Dorcey *et al.*, 2009). However, a

DELLA-independent pathway also participates during fruit growth in *Arabidopsis*, suggesting additional opportunities for fine-tuning of fruit growth (Fuentes *et al.*, 2012). GA has also been implicated in fruit patterning. GA synthesis is a direct and necessary target of basic helix-loop-helix (bHLH) INDEHISCENT (IND) protein. Another bHLH protein, ALCATRAZ (ALC), which is also required for fruit opening, interacts with DELLA repressors. It has been proposed that interaction between DELLA and bHLH proteins is a versatile regulatory module and is required for tissue patterning in *Arabidopsis* (Arnaud *et al.*, 2010).

ARFs and Aux/IAA proteins also govern the fate of fruit initiation events. Silencing of *SlARF7*, a negative regulator of fruit set, in tomato transgenic lines also results in up-regulation of the *SIGH3* gene. GH3 genes encode IAA-amido synthetases which convert free auxin to its conjugated form and maintains auxin homeostasis inside a cell. The up-regulation of *SIGH3* further indicates that its induction may compensate for excessive auxin in *SlARF7*-silenced plants. Further, differences in cell size between *SlARF7*-silenced lines and the wild type at 6 days post-anthesis, with significant cell size increase in the mesocarp and endocarp layers, suggests that down-regulation of *SlARF7* inhibits cell division and promotes cell expansion (de Jong *et al.*, 2009; de Jong *et al.*, 2011). Nevertheless, *SlARF7* is not the only gene encoding proteins involved in such interaction, as additional auxin regulators such as ARF8, IAA9, SIPIN4 (PIN-FORMED), and SITIR1 (Transport inhibitor response1) have also been implicated in auxin- and GA-induced parthenocarpic fruits (Goetz *et al.*, 2007; Wang *et al.*, 2009; Ren *et al.*, 2011; Mounet *et al.*, 2012). As both ARF8 and IAA9 are implicated in parthenocarpic fruit formation in tomato and *Arabidopsis* and belong to the same signalling cascade, it has been suggested that these proteins may form a transcriptional repressor complex that is destabilized by the aberrant forms of ARF8 to allow transcription of auxin-responsive genes (Goetz *et al.*, 2007). None of these studies mentions if any GA-induced response was observed in the transgenic plants; however down-regulation of *SlARF7* in *SITIR1* over-expressing transgenic tomato plants further supports the involvement of more auxin-related genes in crosstalk between auxin and GA during fruit set.

Besides auxin and GA, cytokinin is also known to induce fruit set in several fruit crops (Matsuo *et al.*, 2012). The endogenous level of cytokinin is directly correlated with the fruit growth, especially in stimulation of cell division. Its external application causes parthenocarpic fruit formation (Gillaspy *et al.*, 1993; Srivastava and Handa, 2005; Mariotti *et al.*, 2011; Matsuo *et al.*, 2012). It was found that cytokinin-treated tomato seedlings mimic the *diogeotropica* (*dgt*) mutant phenotype such as reduced shoot growth, reduced apical dominance, and exhibit auxin inhibited responses (Coenen *et al.*, 2003). In *Arabidopsis*, cytokinin has been implicated in development of the medial region of the gynoecia and formation of valve margins during early fruit development (Marsch-Martinez *et al.*, 2012). Collectively, these results suggest that cytokinin could act via inhibiting auxin responses, at least partially, during fruit set and growth. However, very little information is available on the underlying mechanisms of its action during fruit set.

Ethylene and brassinosteroids (BR) are also believed to play important roles in fruit set (Fu *et al.*, 2008; Serrani *et al.*, 2008; Wang *et al.*, 2009). Ovule lifespan is an important factor in determining the ability to set fruits. GA-induced fruit set is negatively affected by ovule senescence. Recent evidence in *Arabidopsis* suggests that ethylene is involved in both the control of the ovule lifespan and the determination of the pistil/fruit fate. The proposed model suggests that ethylene may modulate the onset of ovule senescence and, consequently, the window of GA fruit set responsiveness by altering GA

perception and signalling. Though an actual mechanism remains unidentified, it is suggested that the ethylene produced in ovules would directly prevent the GA response, for example, by stabilizing the DELLAs via CTR1 (Carbonell-Bejerano *et al.*, 2011). Evidence indicates there is a transient increase in ethylene production in tomato pistils after pollination, which eventually decreases after fertilization. The ethylene-associated changes are also reflected at the mRNA levels of several ethylene related genes such as *1-aminocyclopropane-1-carboxylate (ACC) synthase2* and *ACC oxidase1 (ACO1)*, *Ethylene-insensitive3-related (EIL3)*. Many genes encoding ethylene response factors (ERFs) also show a marked shift in their expression during flower to fruit transition (Vriezen *et al.*, 2008; Pascual *et al.*, 2009). In cucumber, exogenous application of BR induces parthenocarpic fruit formation, by inducing cell division, whereas its inhibitor abolishes the natural parthenocarpic capacity in a parthenocarpic cucumber. However, this effect seems to be limited to a few fruit crops only as no such effect of BR application could be repeated in tomato (Marti *et al.*, 2007).

Additionally, the plant hormones/growth regulators abscisic acid (ABA) and polyamines (PAs) are implicated in fruit development, but knowledge of their precise role and mode of action remains sketchy (Gillaspy *et al.*, 1993; Nitsch *et al.*, 2009). ABA levels show a decrease at fruit set and this decline is associated with the down-regulation of ABA biosynthesis genes encoding enzymes such as 9-cis-epoxycarotenoid dioxygenase 1 (NCED1) and neoxanthin synthase (NSY) and up-regulation of ABA degradation gene encoding protein; namely, ABA-8'-hydroxylase CYP707A, after pollination (Vriezen *et al.*, 2008). ABA has also been found to abolish GA-induced changes during fruit set in pea (García-Martínez and Carbonell, 1980).

System-level investigation of molecular events underlying fruit set and early-stage fruit development in strawberry further confirmed the major role of auxin and GA during this phase. However, differential regulation of many genes related to biosynthesis and signalling of other phytohormones, including ethylene, ABA, cytokinin, and brassinosteroid further suggested that besides auxin and GA, these hormones are also important during fruit set and early fruit development stages (Kang *et al.*, 2013). Taken together, these findings indicate involvement of several plant hormones in the regulation of fruit set and suggest that a precise balance between their biosynthesis and responses is of fundamental importance. It involves the concerted action of auxin and/or GA and/or cytokinin or BR (depending on the plant species) through their biosynthesis and/or signalling which regulates the activation of core cell cycle genes during early fruit development stages. Evidence also suggests that ABA and ethylene play an antagonistic role in fruit set, but the underlying mechanism of their action remains unidentified and requires further investigation (Fig. 1).

*Fruit growth is mainly dependent on auxin and cytokinin*

Seed and fruit development are intimately connected and synchronized processes. It is now well established that seeds

are rich source of hormones, particularly auxins, GA, and cytokinin, which are involved in stimulating growth of surrounding tissues and even determine the fruit size (Crane, 1964; Ozga *et al.*, 2003). It has been observed that auxin and cytokinin levels increase in the seed during its development, concomitant with fruit growth stages where cell division is followed by a cell-expansion phase (Blumenfeld and Gazit, 1970; Devoghalare *et al.*, 2012; Yang *et al.*, 2002). Seed removal has also been found to cause reduced GA biosynthesis in the pericarp of pea (Ozga *et al.*, 1992). Furthermore, the role of seed-originated auxin and GA has also been established in the regulation of GA levels in pea pericarp. The proposed mechanism shows that two hormones control the conversion of GA<sub>19</sub> to GA<sub>20</sub> in pea pericarp at least partly by regulating the transcript levels of a GA-oxidase gene (Van Huizen *et al.*, 1997). These observations indicate that interplay between these hormones is necessary for fruit growth. The established role of auxin is in the regulation of cell expansion and it seems to be the most important hormone during this developmental phase. In addition, recent evidence revealed the presence of an internal-to-external IAA gradient during the cell-expansion phase in tomato fruits and suggested that auxin, present in the outer layer of placental cells, promotes the placental expansion to surround the seeds and fill the locular cavity (Pattison and Catala, 2012). Literature accumulated on similar developmental events in tissues other than fruits suggests that once the cell division stage is over, auxin and GA become the main regulators of the cell expansion phase. In fact, several studies have already demonstrated the potential role of ARFs, the foremost regulators of auxin responses, in the regulation of cell division during early growth stages in fleshy fruits, suggesting that molecular mechanisms underlying the regulation of fruit size might have a common point of origin (Kumar *et al.*, 2011; Devoghalare *et al.*, 2012). In addition, ABA has also been implicated in regulation of expansion phase in tomato as fruits of ABA-deficient mutants are smaller in size (Gillaspy *et al.*, 1993; Nitsch *et al.*, 2012). The evidence indicates that these hormones are localized mostly in seeds and then transported to the surrounding tissues but, except for the auxin, our knowledge on this aspect remains strikingly limited and we propose that more efforts in this direction are required to unravel the underlying molecular circuits.

*Fruit maturation involves auxin, but the contribution of other hormones remains largely undetermined*

Fruit maturation signifies the preparedness of a fruit to undergo the ripening process. Auxin and cytokinin seem to be primary hormones involved in control of fruit maturation as levels of both of these hormones are higher in a ripening inhibitor (*rin*) mutant at breaker stage compared with wild-type fruits (Davey and Van Staden, 1978). This observation found support in recent findings where transgenic apples with suppressed transcripts of a *rin*-like MADS-box gene (*MADS8/9*) caused maintenance of high levels of auxin during maturation and prevented the initiation of ripening (Ireland *et al.*, 2013; Schaffer *et al.*, 2013). Moreover, in fruit

types where ripening traits are not strictly associated with ethylene, auxin treatment is known to delay ripening (Jones *et al.*, 2002). For instance, in strawberry, removal of achenes causes precocious ripening of receptacles, whereas exogenous application of auxin can stall this phenotype (Given *et al.*, 1988). Importantly, auxin levels are higher in seeds as compared with their surrounding fruit tissue. It has been suggested that for seeds to become dormant, auxin biosynthesis or transport to the rest of the fruit is inhibited, which in turn allows mature fruits to undergo ripening (Devoghalare *et al.*, 2012). Reduction in auxin levels has been observed in a number of fruit crops and it seems that this reduction is a prerequisite for ripening to commence (Given *et al.*, 1988; Zaharah *et al.*, 2012).

Physiologically active concentrations of auxins are maintained by GH3 class of proteins which are required for auxin conjugation. These proteins have been found at high levels at fruit maturity in several fruit-bearing species (Bottcher *et al.*, 2010). In a striking example of their involvement in fruit development, over-expression of a capsicum *GH3* gene was found to reduce auxin levels in tomato fruits and eventually this reduction was thought to be responsible for the early fruit-ripening phenotype of these transgenic tomatoes (Liu *et al.*, 2005). This example highlighted the complexity of auxin action, where any change in its concentration can lead to a different physiological response. Presence of two ripening-associated *GH3* genes in tomato further supports the hypothesis that low auxin is required for the initiation of ripening (Devoghalare *et al.*, 2012; Kumar *et al.*, 2012a). Additionally, display of non-synchronous ripening phenotype by a cytokinin-deficient mutant of *Arabidopsis* and decrease in free levels of this hormone before ripening initiation in orange and grapes suggests that cytokinin plays some role in the fruit maturation (Werner *et al.*, 2003; Bottcher *et al.*, 2011). Likewise, evidence suggests that ABA plays an important role as an inducer of ripening along with ethylene (Zhang *et al.*, 2009). Notwithstanding with the investigations where role of these hormones in fruit set and ripening has been extensively explored, very few reports on their role during fruit maturation are available. Therefore, one of the main challenges for future work remains to have the complete understanding of the molecular circuits underlying fruit maturation and interaction between hormones, as it is expected that events taking place at this step could be of vital importance and might have far reaching consequences on ripening and postharvest biology of fruits.

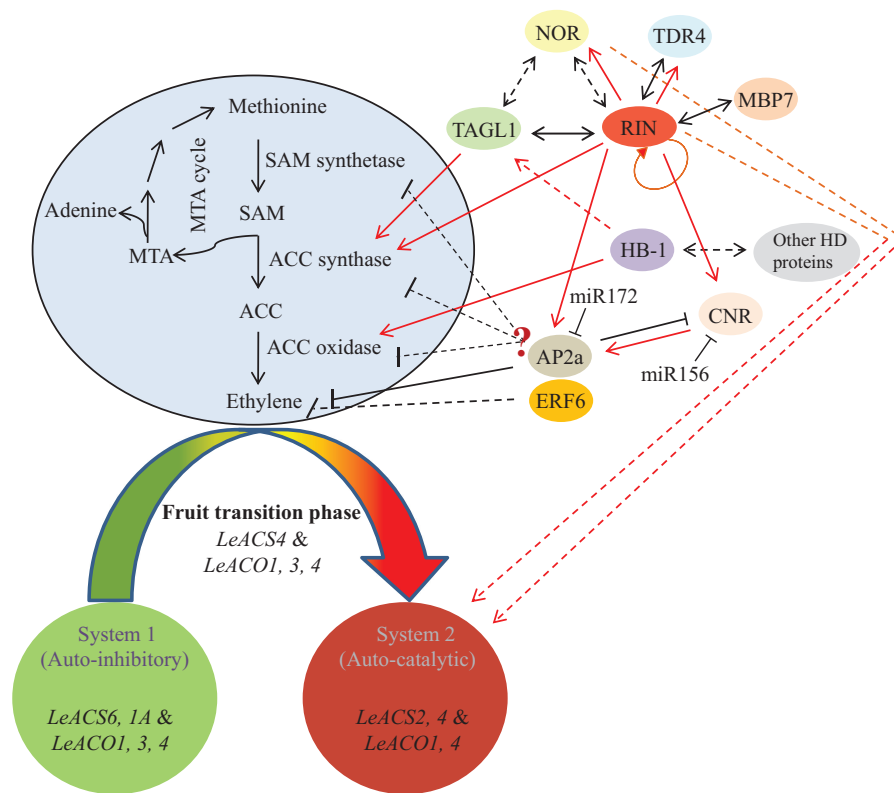
*Fruit ripening: Ethylene and ABA are the major contributors whereas other growth regulators are required for fine tuning of the process*

Fruit ripening involves well-orchestrated coordination of several regulatory steps, which brings about subtle changes to the metabolic and physiological traits in ripening fruits. With its progression, the colour of fruits change owing to accumulation of pigments. Complex carbohydrates are converted to the sugars, the acidity of fruits decreases with the accumulation of sugars, the flavour and aroma compounds

accumulate, and cell wall dynamics change, leading to either a dehiscence or a softening (Klee and Giovannoni, 2011; Seymour et al., 2013). The above process involves initiation of multiple genetic and biochemical pathways. However, the molecular hierarchy of their regulators remains to be ascertained. As these changes have been observed in the context of response of various hormones, the major ripening control seems to be achieved predominantly by ethylene and ABA (Giovannoni, 2004; McAtee et al., 2013). Owing to its predominant role in ripening of the climacteric fruits, ethylene remains the most explored hormone (Bapat et al., 2010). Two systems of ethylene biosynthesis operate during fruit development and ripening in climacteric fruits. Although ethylene is produced at basal level in system 1 and is auto-inhibitory, ethylene production markedly increases in system 2, during ripening, in an autocatalytic manner, and is regulated in both ethylene-dependent (autocatalytic) and ethylene-independent fashion (Van de Poel et al., 2012). The ethylene production in these two systems is controlled via differential regulation of different ACC synthase (ACS) and ACC oxidase (ACO) genes (Fig. 2) (Barry et al., 2000). In climacteric fruits, several lines of evidence suggest that both ethylene and indole-3-acetic acid (IAA) are involved

in crosstalk with each other during ripening. This notion is supported by the observations that (i) concomitant increase of IAA with that of ethylene production is observed in tomato and peach fruits and (ii) genes for ethylene biosynthesis (*ACS2*, *ACS4* and *ACO1* etc.) and signalling (*ETRs* and *ERFs* etc.) are up-regulated by auxin and vice versa, in fruits of both species (Gillaspy et al., 1993; Jones et al., 2002; Trainotti et al., 2007). However, auxin action during this crosstalk seems to be intricate in nature, as, firstly, high auxin concentration during early ripening phase is mainly attributed to high levels of the hormone in seed, as auxin is very low or undetectable in the pericarp or locular tissue, and secondly, ripening-associated *GH3* genes are supposed to decrease the free IAA concentration and the low auxin levels in the remaining fruit tissues may be an effect of their action. Moreover, up-regulation of many *GH3* genes, including *GH3.1*, *GH3.5*, *GH3.9*, and *GH3.17* in the strawberry seed tissues with high auxin levels indicated that even IAA biosynthesis may induce IAA conjugation by promoting the expression of members of this gene family (Kang et al., 2013).

In non-climacteric fruits where no burst in ethylene production during ripening is observed, ABA seems to have



**Fig. 2.** Regulation of ethylene biosynthesis during fruit development and ripening. Upper part of the figure shows the regulation of various ethylene biosynthesis steps by different developmental regulators during ripening in tomato. The lower part of the figure shows the transition phase from green to red tomato fruits and participation of ACS and ACO genes in different phases of fruit development. Dotted lines/arrows represent the proposed but not yet experimentally confirmed interactions among the genes/proteins. Solid lines/arrows show the interactions supported by some experimental findings. Bidirectional black arrows indicate protein-protein interactions between the two ripening regulators. Solid red arrows denote the interactions between ripening regulator proteins and their target promoters. Red dotted arrows indicate that RIN and NOR regulate fruit ripening primarily via controlling the ethylene biosynthesis aspect during system 2. (⊥) sign denotes negative regulation of these genes by their regulators. Abbreviations: SAM, S-adenosyl-methionine; SAMS, SAM synthase; ACC, 1-aminocyclopropane-1-carboxylate; ACS, ACC synthase; ACO, ACC oxidase; SAMDC, SAM decarboxylase; NOR, non-ripening; TAGL1, tomato AGAMOUS-like 1; RIN, ripening-inhibitor; TDR4 and MBP7, tomato FRUITFULL homologs; HB-1, homeobox protein-1; CNR, colourless non-ripening; AP2a, AP2 domain containing ethylene response factor (ERF); and miR156 and 172, microRNA 156 and 172, respectively.

stronger role during ripening (McAtee *et al.*, 2013). Indeed, there is an increase in ABA content during ripening in fleshy fruits and any treatment which delays this increase has been found to delay the induction of ripening (Zhang *et al.*, 2009). In tomato and peach fruits, the maximum ABA content precedes the climacteric ethylene production. It has been shown that ABA promotes ripening by promoting ethylene biosynthesis through up-regulation of ethylene biosynthesis genes (Sun *et al.*, 2012a). Likewise, concomitant increase of ABA in maturing siliques has been linked with ethylene-mediated promotion of dehiscence in *Arabidopsis* (Kanno *et al.*, 2010). In addition to ABA, GA has also been found to delay fruit ripening in many other fruits such as tomatoes, peach mango, sapota etc. (Dostal and Leopold, 1967; Martínez-Romero *et al.*, 2000; Singh *et al.*, 2007; Sudha *et al.*, 2007). Besides these hormones, endogenous level of methyl jasmonate (MJ) has been found to increase with the progression of ripening in apple, mangoes, pears and tomatoes (Fan *et al.*, 1998).

Overall, the ripening phase is constituted by several seemingly independent physiological aspects. Initial investigations on fruit ripening were mainly focused at ethylene, colour changes, and cell wall dynamics (Giovannoni, 2004; Saladie *et al.*, 2007), whereas other ripening characteristics received less attention. There is evidence that individual ripening processes themselves may be under specific hormonal control (McAtee *et al.*, 2013). Therefore, we further survey the literature available on relative roles of these hormones on different ripening traits independent of each other.

#### *Fruit colour and pigmentation are controlled by multiple hormones in both ethylene-dependent and ethylene-independent manner*

Colour change in fruits during ripening is achieved by chlorophyll degradation (degreening) and production of colour metabolites such as carotenoids and anthocyanins. Several genes of carotenoid pathway, including *PSY1* are known to be induced by ethylene, indicating that production of secondary metabolites, responsible for various colours is tightly regulated by ethylene. Further, manipulation of genes related to ethylene biosynthesis such as *LeACS2* and signalling, such as *SLAP2a* and *SIERF6*, has been found to affect fruit pigmentation in tomatoes (Karlova *et al.*, 2011; Lee *et al.*, 2012). In apple, no direct association of ethylene with degreening of fruits is proven. However, it has been reported that ethylene causes acceleration of this process (Johnston *et al.*, 2009). Similarly, degreening of fruit skin of citrus and melon also requires ethylene to proceed effectively. Besides ethylene, an increase of ABA has also been found to be associated with colour change in fruits. In tomato over-pigmentation mutants such as *higher pigment3* (*hp3*), *flacca* (*flc*) and *sitiens* (*sit*), a lower level of ABA is believed to be directly linked with over-pigmentation in mature fruits (Galpaz *et al.*, 2008). This observation is further supported by higher levels of lycopene and  $\beta$ -carotene accumulation in transgenic tomato fruits where *SINCE1* had been silenced (Sun *et al.*, 2012b). Further, ABA has also been implicated in colour change in grapes and strawberry (Jia *et al.*, 2011). The ABA action

related to colour change seems dependent on ethylene as application of 1-methylcyclopropene, an inhibitor of ethylene responses, was found to delay this process (Chervin *et al.*, 2004). Additionally, positive effects of other phytohormones such as GA in banana and kakis fruits (Rossetto *et al.*, 2003; Payasi *et al.*, 2004), BRs in tomato, grape, and strawberry fruits (Vardhini and Rao, 2002; Symons *et al.*, 2006; Chai *et al.*, 2013), jasmonic acid (JA) in tomato and strawberry (Perez *et al.*, 1997; Liu *et al.*, 2012) and negative effects of NO in apple, longan and banana (Pristijono *et al.*, 2006; Duan *et al.*, 2007; Cheng *et al.*, 2009) have already been established.

#### *Cell wall dynamics and fruit softening*

Cell wall metabolism during ripening is an important aspect and has been explored extensively in the past, but without any conclusive evidence as to the precise relationship between these changes and softening. Although the cell wall changes in fleshy fruits involve softening of flesh tissue, it is manifested by the formation of a dehiscence zone in dry fruits. Ethylene in general and its biosynthetic genes in particular have been implicated in the regulation of fruit softening and maintenance of shelf-life in several fleshy fruits (Xiong *et al.*, 2005; Nishiyama *et al.*, 2007; Lopez-Gomez *et al.*, 2009). Both ethylene-dependent and ethylene-independent softening, as demonstrated by the differential regulation of cell wall-related genes, has been observed in melon and apple. In the dry fruits of *Arabidopsis*, combinatorial action of ethylene, ABA, and JA is involved in the promotion of normal floral organ abscission through the manipulation of genes encoding cell wall hydrolysis enzymes such as polygalacturonase (PG) (Ogawa *et al.*, 2009). The suppression of PG1 or pectate lyase encoding genes in transgenic strawberry plants caused altered pectin solubility and extended fruit firmness during ripening (Quesada *et al.*, 2009). In tomato, auxin, through SIARF4, has been found to control fruit firmness by regulating the fine pectin structure and tissue architecture, although the mechanism is unknown (Jones *et al.*, 2002; Guillon *et al.*, 2008). Moreover, down-regulation of this gene in *SLAP2a*-suppressed plants suggests that some of the ethylene-mediated responses are performed through auxin action, at least in part, during ripening (Karlova *et al.*, 2011). However, the cell wall metabolism and fruit softening is a complex trait as *SLAP2a* itself is a direct target of a master regulator of ripening, ripening-inhibitor (RIN). Additionally, RIN is linked to the alteration of the expression of many genes encoding cell wall-degrading enzymes and proteins involved in modification of cell wall architecture. Indeed, differential regulation of more than 50 such genes, including members of xyloglucan transglucosylase/hydrolases, pectin acetyltransferases etc. substantiated the complex nature of cell wall metabolism and fruit softening during fruit development and ripening (Tomato Genome Consortium, 2012). ABA also determines fruit firmness in tomato and promotes softening, synergistically with ethylene, in banana (Lohani *et al.*, 2004). Inhibition of the expression of *SINCE1* has been found to cause enhanced fruit firmness and increased shelf-life (Sun *et al.*, 2012a, 2012b). In addition, other reports have further

implicated role of ABA, SA, GA, cytokinin, PAs, MJ, and NO in fruit softening in several fruits crops, such as banana (Srivastava and Dwivedi, 2000; Cheng *et al.*, 2009), peach (Martínez-Romero *et al.*, 2000; Bregoli *et al.*, 2002), sapota (Sudha *et al.*, 2007), sweet cherry (Kondo *et al.*, 2000), and tomato (Eum *et al.*, 2009).

#### *Relative roles of hormones in accumulation of sugars and acids remain poorly studied*

Despite the availability of overwhelming evidence regarding the involvement of plant hormones in fruit development, currently there is very little information available on their role in starch to sucrose conversion during fruit ripening. Although a number of studies have described the metabolic changes associated with fruit maturation and ripening, the information on hormonal control of metabolite accumulation remains inadequate (Carrari *et al.*, 2006; Fait *et al.*, 2008; Osorio *et al.*, 2011; Lee *et al.*, 2012). Ethylene is known to bring marked changes in climacteric fruits, but its role in starch hydrolysis is not well studied. Exogenous application of ABA to grape at veraison stage leads to enhanced accumulation of several metabolites, including sugars (Deluc *et al.*, 2007). An ABA-response element binding factor encoding gene, i.e. *SlAREBI*, has been linked to the control of fruit quality in tomato. Several metabolites such as citric acid, malic acid, glutamic acid, glucose, and fructose are accumulated at higher concentration in tomato fruits over-expressing *SlAREBI* gene in comparison with antisense suppression lines at red ripe stage, implicating ABA in controlling fruit quality (Bastias *et al.*, 2011). Furthermore, ABA is involved in promotion of starch hydrolysis in melon (Sun *et al.*, 2012c). In all these studies, it is difficult to establish whether ABA acts directly or via altering ethylene levels. GA application has also been found to delay starch degradation in mango fruits (Singh *et al.*, 2007). More investigations are required to delineate the exact mechanism of these hormones as well as to identify role of other hormones in fruit quality.

#### *Flavour and aroma production becomes another important area where the relative roles of plant hormones need exploration*

Ethylene positively regulates aroma production in melon by controlling the level of alcohol dehydrogenases (ADH), as melon fruits treated with 1-MCP and transgenic fruits with suppressed expression of the gene encoding ACC oxidase showed inhibited ADH activity (Manriquez *et al.*, 2006). Several other lines of independent evidences also confirm the involvement of ethylene in production of aroma in fruits (Flores *et al.*, 2002; Botondi *et al.*, 2003). Transgenic apple fruits with low endogenous ethylene level exhibit enhanced production of volatiles in the presence of exogenous ethylene (Schaffer *et al.*, 2007). Jasmonates have also been shown to induce aroma production in apples which is expected to be mediated by ethylene (Kondo *et al.*, 2000). ABA is involved in the regulation of flavonoid biosynthesis in highbush blueberry fruits (Zifkin *et al.*, 2012). Furthermore, transgenic

tomato lines with severely reduced MJ levels accumulate lower levels of polyamines in their fruits, indicating that intracellular MJ is important in regulating overall primary metabolism, especially amino acids and polyamines (Kausch *et al.*, 2011). Although ethylene has been implicated in the production of flavour and aroma compounds in fruits, very little literature is available on the effects of other hormones on aroma production.

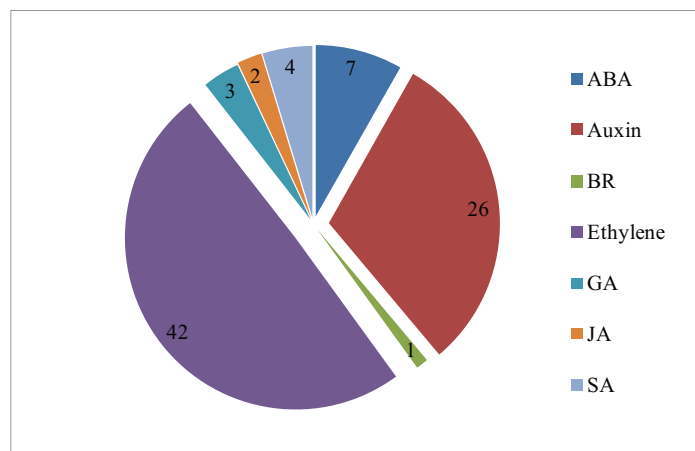
### **Plant hormones primarily act through tweaking the ethylene action during fruit ripening**

Ethylene and other phytohormones have been suggested to crosstalk to each other in the regulation of various aspects of plant development (Lin *et al.*, 2008a; Santisree *et al.*, 2011). The involvement of several plant growth regulators in fruit development and ripening indicates the possibility of an intricate hormonal co-action module (Osorio *et al.*, 2011). Our study on comparison of fruit transcriptomes at five ripening stages between wild type and *rin* mutant fruits has revealed that next to ethylene, auxin-related genes were the most represented in hormone response category (Fig. 3). The final outcome of this crosstalk is believed to determine the fate of development, ripening, and postharvest quality in fleshy fruits. In brief, published evidence suggests that ethylene and auxin start crosstalking to each other even at the ethylene receptor level.

A novel TPR (tetratricopeptide repeat) protein interacts with the ethylene receptors NR and LeETR1 in tomato (Lin *et al.*, 2008a). At the molecular level, its over-expression caused alteration in the expression of early auxin responsive genes such as *LeIAA9* and *SISAUR-like*. As ethylene negatively regulates the expression of *LeIAA9*, this study suggested that SITPR1 is involved in crosstalk between ethylene and auxin signalling in tomato (Lin *et al.*, 2008a). Up-regulation of *ACO1* and *ACS1* by auxin, whereas induction of the auxin transporter gene, *PINI*, by ethylene and requirement of high auxin levels to produce large amount of system 2 ethylene in peaches further validate the earlier observation (Trainotti *et al.*, 2007; Tatsuki *et al.*, 2013). Another piece of evidence for ethylene–auxin crosstalk can be drawn from the fact that an auxin-related *GH3* gene is induced by ethylene in capsicum fruits (Liu *et al.*, 2005). Likewise, induction of a *GH3* gene by ABA and ethylene during ripening in grape and by several other phytohormones in tomato, suggests that along with ethylene, auxin can crosstalk with ABA and other hormones (Bottcher *et al.*, 2010; Kumar *et al.*, 2012a, b). Furthermore, in fleshy fruits ABA promotes ripening by promoting ethylene biosynthesis (Jiang *et al.*, 2000; Gambetta *et al.*, 2010). Exogenous application of ABA increased the transcript levels of *ACS2*, *ACS4*, and *ACO1* genes, whereas their inhibition by fluridone (ABA inhibitor) indicates that ABA and ethylene signalling pathways crosstalk primarily through controlling the ethylene biosynthesis pathway and *vice versa* (Chernys and Zeevaart, 2000; Zhang *et al.*, 2009).

It is believed that PAs can act as cellular signals in the complex crosstalk between hormonal pathways, including ethylene, auxin and ABA (Alcazar *et al.*, 2010; Cui *et al.*, 2010;





**Fig. 3.** Comparative representation of differentially expressed genes, involved in biosynthesis/signalling of various phytohormones/growth regulators during fruit ripening in tomato. Number in the pie chart represents the number of genes related to a particular phytohormone/growth regulator. Fruit transcriptomes of five stages, including mature green, breaker, breaker+3, breaker+5 and breaker+20 of wild type were included in the study. The Gene Expression Omnibus database series accession number of the raw data for microarray analysis is GSE20720. ABA, abscisic acid; BR, brassinosteroids; GA, gibberellins; JA, jasmonic acid; SA, salicylic acid.

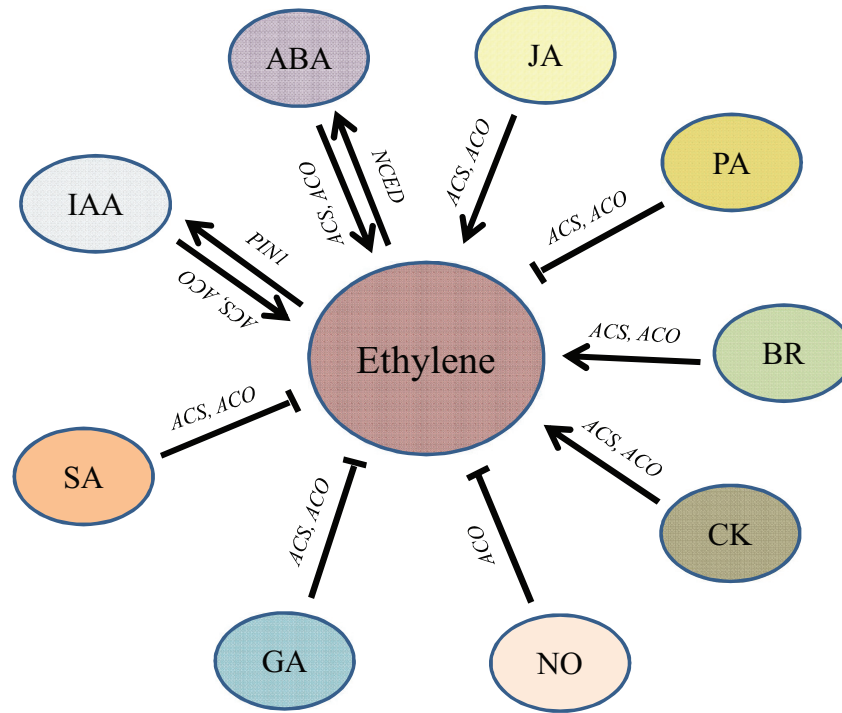
Parra-Lobato and Gomez-Jimenez, 2011; Torrigiani *et al.*, 2012). Ethylene and PAs are known to have antagonistic effects during fruit maturation. PAs along with SA have been shown to repress the expression of *ACS* gene in tomato fruit (Li *et al.*, 1992). Exogenous application of SA has been found to either repress the expression of *ACS* and *ACO* genes or results in the reduced activity of the enzymes encoded by these genes, thus inhibiting ethylene biosynthesis and delaying the ripening in several fruits (Li *et al.*, 1992; Fan *et al.*, 1996; Xu *et al.*, 2000). Moreover, PA application induces NO production in olive fruit as well as in *Arabidopsis* (He *et al.*, 2004; Parra-Lobato and Gomez-Jimenez, 2011). Antagonistic interplay between NO and ethylene in determination of postharvest fruit quality is well established (Manjunatha *et al.*, 2012). Published evidence suggests that NO crosstalks with SA and JA to control postharvest attributes in some fleshy fruits; however, the underlying mechanism still remains unknown (Ziosi *et al.*, 2008). SIARF7-mediated auxin-GA crosstalk in the regulation of fruit set has already been covered earlier.

It has been observed that ethylene delays GA-mediated responses either by reducing the bioactive GA levels or by stabilizing the DELLA proteins during various developmental processes in *Arabidopsis* (Achard *et al.*, 2007). Cytokinin and BRs are known to induce ethylene production by increasing ACS protein stability; however, no such evidence is available, which suggests that these two hormones regulate expression of *ACS* genes or stabilize their proteins in fleshy fruits (Abel *et al.*, 1995; Yamagami *et al.*, 2003). In summary, auxin, ABA, JA, BR, and cytokinins are known to activate, whereas GA, SA, NO, and PAs inhibit the expression of *ACS* and *ACO* genes of ethylene biosynthetic pathway (Fig. 4).

### Genetic and epigenetic regulation of hormonal networks during fruit ripening

In previous sections, we reviewed the published literature on involvement of plant hormones in fruit development;

however, the role of other ripening regulators in mediating these responses has been less studied. Ethylene controls initiation of tomato fruit ripening and any reduction either in its synthesis or interference with its perception inhibits this process. Though obligatory to ripening, two facts suggest involvement of additional regulatory constraint(s) that are involved in developing competence in fruits to respond to ethylene and ripen. The first is that the effects of ethylene are not restricted only to ripening and the second is the inability of ethylene to induce ripening in immature tomato fruits whose seeds are not viable. Several ripening regulators have already been identified but information on the “missing developmental cue” of ripening still remains elusive. Recent evidence indicates that the fruit epigenome is not in a static state and changes during various stages of fruit development and ripening. Manning *et al.* (2006) for the first time demonstrated that the epigenome could govern ripening in tomato and more recently it was revealed that changes in methylation are likely to be involved in transition to the ripening phase. This indicates that it could be one of a missing link, whereas development stage-specific fine tuning between plant hormones could be another factor that might contribute to the competence of fruits to ripen (Manning *et al.*, 2006; Zhong *et al.*, 2013). Moreover, interplay between hormones itself seems to be regulated by transcription factors. Several non-ripening mutants of tomato have helped in gaining initial insights into the molecular and biochemical basis of fruit ripening. As a result, various ripening regulators, including RIN, SIMADS1, Non-ripening (NOR), Colourless non-ripening (CNR), Tomato AGAMOUS-Like1 (TAGL1), FRUITFUL homolog1 (TDR4/FUL1), FRUITFUL homolog2 (MBP7/FUL2), APETALA2a (SIAP2a), Ethylene Response Factor6 (SIERF6), and Homeobox domain protein1 (HB-1) have been identified in tomato (Thompson *et al.*, 1999; Vrebalov *et al.*, 2002, 2009; Lin *et al.*, 2008b; Chung *et al.*, 2010; Bemer *et al.*, 2012; Lee *et al.*, 2012; Dong *et al.*, 2013; Fujisawa *et al.*, 2014). RIN, a MADS-box transcriptional activator, acts as a master regulator of tomato fruit ripening and its presence



**Fig. 4.** Ethylene and its plausible relations/interactions with other growth regulators, abscisic acid (ABA), auxin (Aux), brassinosteroids (BR), cytokinins (CK), gibberellic acid (GA), jasmonic acid (JA), nitric oxide (NO), polyamines (PA), and salicylic acid (SA) during ripening. ( $\perp$ ) denotes the possible link between the ethylene biosynthesis inhibition, whereas ( $\rightarrow$ ) denotes the ethylene biosynthesis activation by the other growth regulator through the regulation of *ACC synthase* (*ACS*) and *ACC oxidase* (*ACO*) genes. In case of ABA and Aux, ethylene reciprocally activates the expression of a few genes such as *NCED* of ABA and *PIN1* of aux signalling pathways, denoted by ( $\leftrightarrow$ ), during ripening.

in other fruit crops, including non-climacteric ones, further supports the conservation of regulatory mechanisms during ripening (Vrebalov et al., 2002; Jaakola et al., 2010; Seymour et al., 2011; McAtee et al., 2013; Schaffer et al., 2013). It is very clear that *RIN* regulates ethylene biosynthesis during ripening; however, equally significant is the fact that in the *rin* mutant the fruits do not ripen, even in response to external application of ethylene, suggesting that this gene is also important for developing competence to respond to ethylene. In climacteric fruits, it is believed that *RIN* achieves diverse functions through direct binding to the promoters of its target genes. Over 240 *RIN* target genes were found to be involved in almost all the aspects of fruit ripening, including ethylene biosynthesis and responses, carotenoid accumulation, cell wall hydrolysis, aroma production, and transcriptional regulation of ripening-related genes encoding transcription factors in tomato, including *NOR*, *CNR*, *SIAP2a*, *TDR4*, and *HB-1* (Martel et al., 2011; Qin et al., 2012; Fujisawa et al., 2013). The diversity of *RIN* function, including ethylene-dependent and ethylene-independent responses where some of its targets show up-regulation whereas others do not undergo any change in transcript levels upon *RIN* binding, is also achieved, at least partly, by its interaction with other MADS-box proteins involved in ripening as well as its dependence on other proteins such as *CNR* for its binding to the target promoters (Martel et al., 2011; Bemer et al., 2012). In brief, *RIN* directly activates the expression of *LeACS2* and *LeACS4* genes and causes elevated ethylene production during fruit ripening. Additionally, *RIN* induces a check mechanism for ethylene production through activation of a negative

regulator of ripening, i.e. *SIAP2a*. The *Cnr* mutant was discovered to be due to an epigenetic lesion in its promoter (Manning et al., 2006) and transcript levels of both *SIAP2a* and *CNR* are also controlled by miR172 and miR156, respectively, suggesting that multi-level regulatory mechanisms are involved in precise control of their expression during fruit ripening (Fig. 2) (Karlova et al., 2013). This study added a new dimension to the current repertoire of control mechanisms involved in regulation of ripening-related transcription networks. In addition to *RIN*, another MADS-box protein, *TAGL1* binds to the *ACS2* promoter and regulates ethylene biosynthesis (Vrebalov et al., 2009). All these evidences place *RIN* at the centre of ethylene-mediated aspects of fruit ripening. Interestingly, ethylene has also been found to regulate the expression of *RIN* and its target TFs, which indicates the presence of a robust interaction mechanism between *RIN* and ethylene signalling (Fujisawa et al., 2013). Furthermore, a *RIN*-homologue of apple, *MADS8/9*, has been found to regulate fruit ripening by directly controlling the auxin levels. Mature *MADS8/9*-suppressed apples demonstrated reduced expression of *GH3*, the auxin-conjugating enzymes, and exhibited a higher concentration of free IAA (Schaffer et al., 2013). In addition, auxin and ABA have been found to affect the expression of *SHATTERPROOF*-like gene (*FaSHP*) during fruit ripening in strawberries, suggesting that the MADS-box may impact the ripening of fleshy structures with fruit function independently of their anatomical origin (Daminato et al., 2013). In tomato, identification of at least two ripening-associated *GH3* genes and several ARFs, which showed reduced accumulation in *rin* mutant fruit during ripening,

further suggests that though still unidentified, similar mechanisms would be operating in tomato as well (Kumar *et al.*, 2011, 2012a).

In summary, it is clear that RIN acts as a master regulator of ripening and it not only controls the ethylene signalling but may also regulates auxin responses. The apparent conservation of RIN-like MADS-box genes in ripening control before the evolutionary split of monocots and dicots suggests a conserved ripening function in this important family of floral development regulators (Klee and Giovannoni, 2011). Control of RIN and other transcriptional regulators by ethylene and RIN itself, and involvement of RIN in the regulation of auxin content in fruits during ripening suggests that the full complexity of these regulatory mechanisms between ripening regulators and hormones is still unknown and what role, if any, epigenomic machinery and small RNAs play in these interactions is yet to be discovered.

## Conclusions and perspectives

It is clear that several plant hormones are involved in the regulation of fruit development and ripening across fleshy and dry fruits. Auxin, GA, and cytokinin have been actively implicated in the control of fruit set and a few potential candidates that facilitate crosstalk between auxin and GA have been identified in the past decade, but the detailed knowledge of underlying molecular mechanisms remains elusive. The molecular characterization of the crosstalk between cytokinin and auxin and/or GA during fruit set needs to be undertaken. Although there are areas that have been explored extensively, such as the role of ethylene in the initiation of ripening, our understanding of combined as well as individual relative roles of specific hormones during fruit expansion, maturation, starch hydrolysis, and aroma production is very limited. It is essential to understand how hormone networks behave and what kind of changes they undergo during ripening; also their links to ripening regulators have to be established. Given the growing importance of interrelationships between hormone networks vis-a-vis their coexistence with regulators of fruit development and ripening, we anticipate that future models integrating both the aspects will allow far greater understanding of the complex dynamics underlying the firmly synchronized biological processes.

## Acknowledgements

We sincerely thank Prof. Akhilesh Kumar Tyagi, University of Delhi and Director, National Institute of Plant Genome Research, India, for critical reading of the manuscript and useful comments. The research work in our lab is supported by the Department of Biotechnology, Government of India. RK acknowledges Council of Scientific and Industrial Research and Department of Science and Technology, Government of India, for the fellowships while AK acknowledges University Grant Commission, Government of India for the financial support.

## References

Abel S, Nguyen MD, Chow W, Theologis A. 1995. ACS4, a primary indoleacetic acid-responsive gene encoding 1-aminocyclopropane-1-carboxylate

synthase in *Arabidopsis thaliana*. Structural characterization, expression in *Escherichia coli*, and expression characteristics in response to auxin. *Journal of Biological Chemistry* **270**, 19093–19099.

Achard P, Baghour M, Chapple A, Hedden P, Van Der Straeten D, Genschik P, Moritz T, Harberd NP. 2007. The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. *Proceedings of the National Academy of Sciences, USA* **104**, 6484–6489.

Alcazar R, Altabella T, Marco F, Bortolotti C, Reymond M, Koncz C, Carrasco P, Tiburcio AF. 2010. Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. *Planta* **231**, 1237–1249.

Arnaud N, Girin T, Sorefan K, Fuentes S, Wood TA, Lawrenson T, Sablowski R, Ostergaard L. 2010. Gibberellins control fruit patterning in *Arabidopsis thaliana*. *Genes and Development* **24**, 2127–2132.

Bapat VA, Trivedi PK, Ghosh A, Sane VA, Ganapathi TR, Nath P. 2010. Ripening of fleshy fruit: molecular insight and the role of ethylene. *Biotechnology Advances* **28**, 94–107.

Barry CS, Llop-Tous MI, Grierson D. 2000. The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. *Plant Physiology* **123**, 979–986.

Bastias A, Lopez-Climent M, Valcarcel M, Rosello S, Gomez-Cadenas A, Casaretto JA. 2011. Modulation of organic acids and sugar content in tomato fruits by an abscisic acid-regulated transcription factor. *Physiologia Plantarum* **141**, 215–226.

Bemer M, Karlova R, Ballester AR, Tikunov YM, Bovy AG, Wolters-Arts M, Rossetto Pde B, Angenent GC, de Maagd RA. 2012. The Tomato FRUITFULL homologs TDR4/FUL1 and MBP7/FUL2 regulate ethylene-independent aspects of fruit ripening. *Plant Cell* **24**, 4437–4451.

Blumenfeld A, Gazit S. 1970. Cytokinin activity in avocado seeds during fruit development. *Plant Physiology* **46**, 331–333.

Botondi R, DeSantis D, Bellincontro A, Vizovitis K, Mencarelli F. 2003. Influence of ethylene inhibition by 1-methylcyclopropene on apricot quality, volatile production, and glycosidase activity of low- and high-aroma varieties of apricots. *Journal of Agricultural and Food Chemistry* **51**, 1189–1200.

Bottcher C, Boss PK, Davies C. 2011. Acyl substrate preferences of an IAA-amido synthetase account for variations in grape (*Vitis vinifera* L.) berry ripening caused by different auxinic compounds indicating the importance of auxin conjugation in plant development. *Journal of Experimental Botany* **62**, 4267–4280.

Bottcher C, Keyzers RA, Boss PK, Davies C. 2010. Sequestration of auxin by the indole-3-acetic acid-amido synthetase GH3-1 in grape berry (*Vitis vinifera* L.) and the proposed role of auxin conjugation during ripening. *Journal of Experimental Botany* **61**, 3615–3625.

Bregoli AM, Scaramagli S, Costa G, Sabatini E, Ziosi V, Biondi S, Torrigiani P. 2002. Peach (*Prunus persica*) fruit ripening: aminoethoxyvinylglycine (AVG) and exogenous polyamines affect ethylene emission and flesh firmness. *Physiologia Plantarum* **114**, 472–481.

Carbonell-Bejerano P, Urbez C, Granell A, Carbonell J, Perez-Amador MA. 2011. Ethylene is involved in pistil fate by modulating the onset of ovule senescence and the GA-mediated fruit set in *Arabidopsis*. *BMC Plant Biology* **11**, 84.

Carrari F, Baxter C, Usadel B *et al.* 2006. Integrated analysis of metabolite and transcript levels reveals the metabolic shifts that underlie tomato fruit development and highlight regulatory aspects of metabolic network behavior. *Plant Physiology* **142**, 1380–1396.

Carrera E, Ruiz-Rivero O, Peres LE, Atares A, Garcia-Martinez JL. 2012. Characterization of the *procera* tomato mutant shows novel functions of the SIDELLA protein in the control of flower morphology, cell division and expansion, and the auxin-signaling pathway during fruit-set and development. *Plant Physiology* **160**, 1581–1596.

Chai Y-M, Jia H-F, Li C-L, Dong Q-H, Shen Y-Y. 2013. FaPYR1 is involved in strawberry fruit ripening. *Journal of Experimental Botany* **62**, 5079–5089.

Cheng G, Yang E, Lu W, Jia Y, Jiang Y, Duan X. 2009. Effect of nitric oxide on ethylene synthesis and softening of banana fruit slice during ripening. *Journal of Agricultural and Food Chemistry* **57**, 5799–5804.

- Chernys JT, Zeevaert JA.** 2000. Characterization of the 9-cis-epoxycarotenoid dioxygenase gene family and the regulation of abscisic acid biosynthesis in avocado. *Plant Physiology* **124**, 343–353.
- Chervin C, El-Kereamy A, Roustan JP, Latche A, Lamon J, Bouzayen M.** 2004. Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit. *Plant Science* **167**, 1301–1305.
- Chung MY, Vrebalov J, Alba R, Lee J, McQuinn R, Chung JD, Klein P, Giovannoni J.** 2010. A tomato (*Solanum lycopersicum*) *APETALA2/ERF* gene, *SIAP2a*, is a negative regulator of fruit ripening. *The Plant Journal* **64**, 936–947.
- Coenen C, Christian M, Luthen H, Lomax TL.** 2003. Cytokinin inhibits a subset of diageotropica-dependent primary auxin responses in tomato. *Plant Physiology* **131**, 1692–1704.
- Crane JC.** 1964. Growth substances in fruit setting and development. *Annual Review of Plant Physiology* **15**, 303–326.
- Cui X, Ge C, Wang R, Wang H, Chen W, Fu Z, Jiang X, Li J, Wang Y.** 2010. The *BUD2* mutation affects plant architecture through altering cytokinin and auxin responses in *Arabidopsis*. *Cell Research* **20**, 576–586.
- Daminato M, Guzzo F, Casadoro G.** 2013. A *SHATTERPROOF*-like gene controls ripening in non-climacteric strawberries, and auxin and abscisic acid antagonistically affect its expression. *Journal of Experimental Botany* **64**, 3775–3786.
- Davey JE, Van Staden J.** 1978. Endogenous cytokinins in the fruits of ripening and non-ripening tomatoes. *Plant Science Letters* **11**, 359–364.
- de Jong M, Wolters-Arts M, Feron R, Mariani C, Vriezen WH.** 2009. The *Solanum lycopersicum* auxin response factor 7 (*SIARF7*) regulates auxin signaling during tomato fruit set and development. *The Plant Journal* **57**, 160–170.
- de Jong M, Wolters-Arts M, Garcia-Martinez JL, Mariani C, Vriezen WH.** 2011. The *Solanum lycopersicum* AUXIN RESPONSE FACTOR 7 (*SIARF7*) mediates cross-talk between auxin and gibberellin signalling during tomato fruit set and development. *Journal of Experimental Botany* **62**, 617–626.
- Deluc LG, Grimplet J, Wheatley MD, Tillett RL, Quilici DR, Osborne C, Schooley DA, Schlauch KA, Cushman JC, Cramer GR.** 2007. Transcriptomic and metabolite analyses of Cabernet Sauvignon grape berry development. *BMC Genomics* **8**, 429–429.
- Devoghalaere F, Doucen T, Guittton B et al.** 2012. A genomics approach to understanding the role of auxin in apple (*Malus x domestica*) fruit size control. **12**, 7.
- Dong T, Hu Z, Deng L, Wang Y, Zhu M, Zhang J, Chen G.** 2013. A tomato MADS-box transcription factor, *SIMADS1*, acts as a negative regulator of fruit ripening. *Plant Physiology* **163**, 1026–1036.
- Dorcey E, Urbez C, Blazquez MA, Carbonell J, Perez-Amador MA.** 2009. Fertilization-dependent auxin response in ovules triggers fruit development through the modulation of gibberellin metabolism in *Arabidopsis*. *The Plant Journal* **58**, 318–332.
- Dostal HC, Leopold AC.** 1967. Gibberellin delays ripening of tomatoes. *Science* **158**, 1579–1580.
- Duan X, Su X, You Y, Qu H, Li Y, Jiang Y.** 2007. Effect of nitric oxide on pericarp browning of harvested longan fruit in relation to phenolic metabolism. *Food Chemistry* **104**, 571–576.
- Eum HL, Kim HB, Choi SB, Lee SK.** 2009. Regulation of ethylene biosynthesis by nitric oxide in tomato (*Solanum lycopersicum* L.) fruit harvested at different ripening stages. *European Food Research and Technology* **228**, 331–338.
- Fait A, Hanhineva K, Beleggia R, Dai N, Rogachev I, Nikiforova VJ, Fernie AR, Aharoni A.** 2008. Reconfiguration of the achene and receptacle metabolic networks during strawberry fruit development. *Plant Physiology* **148**, 730–750.
- Fan X, Matches JP, Fellowman JK.** 1996. Inhibition of apple fruit 1-aminocyclopropane-1-carboxylic acid oxidase activity and respiration by acetylsalicylic acid. *Journal of Plant Physiology* **149**, 469–471.
- Fan X, Mattheis JP, Fellman JK.** 1998. A role for jasmonates in climacteric fruit ripening. *Planta* **204**, 444–449.
- Flores F, El Yahyaoui F, de Billerbeck G, Romojaro F, Latche A, Bouzayen M, Pech JC, Ambid C.** 2002. Role of ethylene in the biosynthetic pathway of aliphatic ester aroma volatiles in Charentais Cantaloupe melons. *Journal of Experimental Botany* **53**, 201–206.
- Fu FQ, Mao WH, Shi K, Zhou YH, Asami T, Yu JQ.** 2008. A role of brassinosteroids in early fruit development in cucumber. *Journal of Experimental Botany* **59**, 2299–2308.
- Fuentes S, Ljung K, Sorefan K, Alvey E, Harberd NP, Ostergaard L.** 2012. Fruit growth in *Arabidopsis* occurs via DELLA-dependent and DELLA-independent gibberellin responses. *Plant Cell* **24**, 3982–3996.
- Fujisawa M, Nakano T, Shima Y, Ito Y.** 2013. A large-scale identification of direct targets of the tomato MADS box transcription factor RIPENING INHIBITOR reveals the regulation of fruit ripening. *Plant Cell* **25**, 371–386.
- Fujisawa M, Shima Y, Nakagawa H, Kitagawa M, Kimbara J, Nakano T, Kasumi T, Ito Y.** 2014. Transcriptional regulation of fruit ripening by tomato FRUITFULL homologs and associated MADS box proteins. *Plant Cell* doi: <http://dx.doi.org/10.1105/tpc.113.119453>.
- Galpaz N, Wang Q, Menda N, Zamir D, Hirschberg J.** 2008. Abscisic acid deficiency in the tomato mutant *high-pigment 3* leading to increased plastid number and higher fruit lycopene content. *The Plant Journal* **53**, 717–730.
- Gambetta GA, Matthews MA, Shaghasi TH, McElrone AJ, Castellarin SD.** 2010. Sugar and abscisic acid signaling orthologs are activated at the onset of ripening in grape. *Planta* **232**, 219–234.
- Gao C, Ju Z, Li S, Zuo J, Fu D, Tian H, Luo Y, Zhu B.** 2013. Deciphering ascorbic acid regulatory pathways in ripening tomato fruit using a weighted gene correlation network analysis approach. *Journal of Integrative Plant Biology* **55**, 1080–1091.
- García-Martínez JL, Carbonell J.** 1980. Fruit-set of unpollinated ovaries of *Pisum sativum* L. Influence of plant-growth regulators. *Planta* **147**, 451–456.
- Gillaspy G, Ben-David H, Gruissem W.** 1993. Fruits: a developmental perspective. *Plant Cell* **5**, 1439–1451.
- Giovannoni JJ.** 2004. Genetic regulation of fruit development and ripening. *Plant Cell* **16** Suppl, S170–180.
- Given NK, Venis MA, Grierson D.** 1988. Hormonal regulation of ripening in the strawberry, a non-climacteric fruit. *Planta* **174**, 402–406.
- Goetz M, Hooper LC, Johnson SD, Rodrigues JC, Vivian-Smith A, Koltunow AM.** 2007. Expression of aberrant forms of AUXIN RESPONSE FACTOR8 stimulates parthenocarp in *Arabidopsis* and tomato. *Plant Physiology* **145**, 351–366.
- Guillon F, Philippe S, Bouchet B, Devaux M-F, Frasse P, Jones B, Bouzayen M, Lahaye M.** 2008. Down-regulation of an auxin response factor in the tomato induces modification of fine pectin structure and tissue architecture. *Journal of Experimental Botany* **59**, 273–288.
- Harberd NP.** 2003. Botany. Relieving DELLA restraint. *Science* **299**, 1853–1854.
- He Y, Tang RH, Hao Y et al.** 2004. Nitric oxide represses the *Arabidopsis* floral transition. *Science* **305**, 1968–1971.
- Ireland HS, Yao JL, Tomes S, Sutherland PW, Nieuwenhuizen N, Gunaseelan K, Winz RA, David KM, Schaffer RJ.** 2013. Apple *SEPALLATA1/2*-like genes control fruit flesh development and ripening. *The Plant Journal* **73**, 1044–1056.
- Jaakola L, Poole M, Jones MO et al.** 2010. A SQUAMOSA MADS box gene involved in the regulation of anthocyanin accumulation in bilberry fruits. *Plant Physiology* **153**, 1619–1629.
- Jia H-F, Chai Y-M, Li C-L, Lu D, Luo J-J, Qin L, Shen Y-Y.** 2011. Abscisic acid plays an important role in the regulation of strawberry fruit ripening. *Plant Physiology* **157**, 188–199.
- Jiang Y, Joyce DC, Macnish AJ.** 2000. Effect of abscisic acid on banana fruit ripening in relation to the role of ethylene. *Journal of Plant Growth Regulation* **19**, 106–111.
- Johnston JW, Gunaseelan K, Pidakala P, Wang M, Schaffer RJ.** 2009. Co-ordination of early and late ripening events in apples is regulated through differential sensitivities to ethylene. *Journal of Experimental Botany* **60**, 2689–2699.
- Jones B, Frasse P, Olmos E, Zegzouti H, Li ZG, Latche A, Pech JC, Bouzayen M.** 2002. Down-regulation of DR12, an auxin-response-factor homolog, in the tomato results in a pleiotropic phenotype including dark green and blotchy ripening fruit. *The Plant Journal* **32**, 603–613.
- Kang C, Darwish O, Geretz A, Shahan R, Alkharouf N, Liu Z.** 2013. Genome-scale transcriptomic insights into early-stage fruit development in woodland strawberry *Fragaria vesca*. *Plant Cell* **25**, 1960–1978.

- Kanno Y, Jikumaru Y, Hanada A, Nambara E, Abrams SR, Kamiya Y.** 2010. Comprehensive hormone profiling in developing *Arabidopsis* seeds: examination of the site of ABA biosynthesis, ABA transport and hormone interactions. *Plant Cell Physiology* **51**, 1988–2001.
- Karlova R, Rosin FM, Busscher-Lange J, Parapunova V, Do PT, Fernie AR, Fraser PD, Baxter C, Angenent GC, de Maagd RA.** 2011. Transcriptome and metabolite profiling show that APETALA2a is a major regulator of tomato fruit ripening. *Plant Cell* **23**, 923–941.
- Karlova R, van Haarst JC, Maliepaard C, van de Geest H, Bovy AG, Lammers M, Angenent GC, de Maagd RA.** 2013. Identification of microRNA targets in tomato fruit development using high-throughput sequencing and degradome analysis. *Journal of Experimental Botany* **64**, 1863–1878.
- Kausch KD, Sobolev AP, Goyal RK, Fatima T, Laila-Beevi R, Saftner RA, Handa AK, Mattoo AK.** 2011. Methyl jasmonate deficiency alters cellular metabolome, including the aminome of tomato (*Solanum lycopersicum* L.) fruit. *Amino Acids* **42**, 843–856.
- Klee HJ, Giovannoni JJ.** 2011. Genetics and control of tomato fruit ripening and quality attributes. *Annual Review of Genetics* **45**, 41–59.
- Knapp S.** 2002. Tobacco to tomatoes: a phylogenetic perspective on fruit diversity in the Solanaceae. *Journal of Experimental Botany* **53**, 2001–2022.
- Kondo S, Tomyiama A, Seto H.** 2000. Changes of endogenous jasmonic acid and methyl jasmonate in apples and sweet cherries during fruit development. *Journal of the American Society for Horticultural Science* **125**, 282–287.
- Kumar R, Agarwal P, Tyagi AK, Sharma AK.** 2012a. Genome-wide investigation and expression analysis suggest diverse roles of auxin-responsive GH3 genes during development and response to different stimuli in tomato (*Solanum lycopersicum*). *Molecular Genetics and Genomics* **287**, 221–235.
- Kumar R, Sharma MK, Kapoor S, Tyagi AK, Sharma AK.** 2012b. Transcriptome analysis of rin mutant fruit and *in silico* analysis of promoters of differentially regulated genes provides insight into LeMADS-RIN-regulated ethylene-dependent as well as ethylene-independent aspects of ripening in tomato. *Molecular Genetics and Genomics* **287**, 189–203.
- Kumar R, Tyagi AK, Sharma AK.** 2011. Genome-wide analysis of auxin response factor (ARF) gene family from tomato and analysis of their role in flower and fruit development. *Molecular Genetics and Genomics* **285**, 245–260.
- Lee JM, Joung JG, McQuinn R, Chung MY, Fei Z, Tieman D, Klee H, Giovannoni J.** 2012. Combined transcriptome, genetic diversity and metabolite profiling in tomato fruit reveals that the ethylene response factor SIERF6 plays an important role in ripening and carotenoid accumulation. *The Plant Journal* **70**, 191–204.
- Lemaire-Chamley M, Petit J, Garcia V, Just D, Baldet P, Germain V, Fagard M, Mouassite M, Cheniclet C, Rothan C.** 2005. Changes in transcriptional profiles are associated with early fruit tissue specialization in tomato. *Plant Physiology* **139**, 750–769.
- Li N, Parsons BL, Liu DR, Mattoo AK.** 1992. Accumulation of wound-inducible ACC synthase transcript in tomato fruit is inhibited by salicylic acid and polyamines. *Plant Molecular Biology* **18**, 477–487.
- Lin Z, Arciga-Reyes L, Zhong S, Alexander L, Hackett R, Wilson I, Grierson D.** 2008a. SITPR1, a tomato tetratricopeptide repeat protein, interacts with the ethylene receptors NR and LeETR1, modulating ethylene and auxin responses and development. *Journal of Experimental Botany* **59**, 4271–4287.
- Lin Z, Hong Y, Yin M, Li C, Zhang K, Grierson D.** 2008b. A tomato HD-Zip homeobox protein, LeHB-1, plays an important role in floral organogenesis and ripening. *The Plant Journal* **55**, 301–310.
- Liu K, Kang BC, Jiang H, Moore SL, Li H, Watkins CB, Setter TL, Jahn MM.** 2005. A GH3-like gene, *CcGH3*, isolated from *Capsicum chinense* L. fruit is regulated by auxin and ethylene. *Plant Molecular Biology* **58**, 447–464.
- Liu L, Wei J, Zhang M, Zhang L, Li C, Wang Q.** 2012. Ethylene independent induction of lycopene biosynthesis in tomato fruits by jasmonates. *Journal of Experimental Botany* **63**, 5751–5761.
- Lohani S, Trivedi PK, Nath P.** 2004. Changes in activities of cell wall hydrolases during ethylene-induced ripening in banana: effect of 1-MCP, ABA and IAA. *Postharvest Biology and Technology* **31**, 119–126.
- Lopez-Gomez R, Cabrera-Ponce JL, Saucedo-Arias LJ, Carreto-Montoya L, Villanueva-Arce R, Diaz-Perez JC, Gomez-Lim MA, Herrera-Estrella L.** 2009. Ripening in papaya fruit is altered by ACC oxidase cosuppression. *Transgenic Research* **18**, 89–97.
- Manjunatha G, Gupta KJ, Lokesh V, Mur LA, Neelwarne B.** 2012. Nitric oxide counters ethylene effects on ripening fruits. *Plant Signaling and Behavior* **7**, 476–483.
- Manning K, Tor M, Poole M, Hong Y, Thompson AJ, King GJ, Giovannoni JJ, Seymour GB.** 2006. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nature Genetics* **38**, 948–952.
- Manriquez D, El-Sharkawy I, Flores FB, El-Yahyaoui F, Regad F, Bouzayen M, Latche A, Pech JC.** 2006. Two highly divergent alcohol dehydrogenases of melon exhibit fruit ripening-specific expression and distinct biochemical characteristics. *Plant Molecular Biology* **61**, 675–685.
- Mariotti L, Picciarelli P, Lombardi L, Ceccarelli N.** 2011. Fruit-set and early fruit growth in tomato are associated with increases in indoleacetic acid, cytokinin, and bioactive gibberellin contents. *Journal of Plant Growth Regulation* **30**, 405–415.
- Marsch-Martinez N, Reyes-Olalde JI, Ramos-Cruz D, Lozano-Sotomayor P, Zuniga-Mayo VM, de Folter S.** 2012. Hormones talking: does hormonal cross-talk shape the *Arabidopsis* gynoceium? *Plant Signaling and Behavior* **7**, 1698–1701.
- Martel C, Vrebalov J, Tafelmeyer P, Giovannoni JJ.** 2011. The tomato MADS-box transcription factor RIPENING INHIBITOR interacts with promoters involved in numerous ripening processes in a COLOURLESS NONRIPENING-dependent manner. *Plant Physiology* **157**, 1568–1579.
- Marti C, Orzaez D, Ellul P, Moreno V, Carbonell J, Granell A.** 2007. Silencing of DELLA induces facultative parthenocarpy in tomato fruits. *The Plant Journal* **52**, 865–876.
- Martinez-Romero D, Valero D, Serrano M, Burló F, Carbonell A, Burgos L, Riquelme F.** 2000. Exogenous polyamines and gibberellic acid effects on peach (*Prunus persica* L.) storability improvement. *Journal of Food Science* **65**, 288–294.
- Matsuo S, Kikuchi K, Fukuda M, Honda I, Imanishi S.** 2012. Roles and regulation of cytokinins in tomato fruit development. *Journal of Experimental Botany* **63**, 5569–5579.
- McAtee P, Karim S, Schaffer R, David K.** 2013. A dynamic interplay between phytohormones is required for fruit development, maturation, and ripening. *Front Plant Science* **4**, 79.
- Mounet F, Moing A, Garcia V et al.** 2009. Gene and metabolite regulatory network analysis of early developing fruit tissues highlights new candidate genes for the control of tomato fruit composition and development. *Plant Physiology* **149**, 1505–1528.
- Mounet F, Moing A, Kowalczyk M et al.** 2012. Down-regulation of a single auxin efflux transport protein in tomato induces precocious fruit development. *Journal of Experimental Botany* **63**, 4901–4917.
- Nishiyama K, Guis M, Rose JK et al.** 2007. Ethylene regulation of fruit softening and cell wall disassembly in Charentais melon. *Journal of Experimental Botany* **58**, 1281–1290.
- Nitsch JP.** 1952. Plant hormones in the development of fruits. *The Quarterly Review of Biology* **27**, 33–57.
- Nitsch L, Kohlen W, Oplaat C et al.** 2012. ABA-deficiency results in reduced plant and fruit size in tomato. *Journal of Plant Physiology* **169**, 878–883.
- Nitsch LM, Oplaat C, Feron R, Ma Q, Wolters-Arts M, Hedden P, Mariani C, Vriezen WH.** 2009. Abscisic acid levels in tomato ovaries are regulated by LeNCED1 and SICYP707A1. *Planta* **229**, 1335–1346.
- Osawa M, Kay P, Wilson S, Swain SM.** 2009. ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE1 (ADPG1), ADPG2, and QUARTET2 are Polygalacturonases required for cell separation during reproductive development in *Arabidopsis*. *Plant Cell* **21**, 216–233.
- Osorio S, Alba R, Damasceno CM et al.** 2011. Systems biology of tomato fruit development: combined transcript, protein, and metabolite analysis of tomato transcription factor (*nor*, *rin*) and ethylene receptor (*Nr*) mutants reveals novel regulatory interactions. *Plant Physiology* **157**, 405–425.
- Ozga JA, Brenner ML, Reinecke DM.** 1992. Seed effects on gibberellin metabolism in pea pericarp. *Plant Physiology* **100**, 88–94.
- Ozga JA, Yu J, Reinecke DM.** 2003. Pollination-, development-, and auxin-specific regulation of gibberellin 3beta-hydroxylase gene expression in pea fruit and seeds. *Plant Physiology* **131**, 1137–1146.

- Parra-Lobato MC, Gomez-Jimenez MC.** 2011. Polyamine-induced modulation of genes involved in ethylene biosynthesis and signalling pathways and nitric oxide production during olive mature fruit abscission. *Journal of Experimental Botany* **62**, 4447–4465.
- Pascual L, Blanca JM, Canizares J, Nuez F.** 2009. Transcriptomic analysis of tomato carpel development reveals alterations in ethylene and gibberellin synthesis during *pat3/pat4* parthenocarpic fruit set. *BMC Plant Biology* **9**, 67.
- Pattison RJ, Catala C.** 2012. Evaluating auxin distribution in tomato (*Solanum lycopersicum*) through an analysis of the PIN and AUX/LAX gene families. *The Plant Journal* **70**, 585–598.
- Payasi A, Misra PC, Sanwal GG.** 2004. Effect of phytohormones on pectate lyase activity in ripening *Musa acuminata*. *Plant Physiology and Biochemistry* **42**, 861–865.
- Perez AG, Sanz C, Olias R, Olias JM.** 1997. Effect of methyl jasmonate on in vitro strawberry ripening. *Journal of Agricultural and Food Chemistry* **45**, 3733–3737.
- Pristijono P, Wills R, Golding J.** 2006. Inhibition of browning on the surface of apple slices by short term exposure to nitric oxide (NO) gas. *Postharvest Biology and Technology* **42**, 256–259.
- Qin G, Wang Y, Cao B, Wang W, Tian S.** 2012. Unraveling the regulatory network of the MADS box transcription factor RIN in fruit ripening. *The Plant Journal* **70**, 243–255.
- Quesada MA, Blanco-Portales R, Pose S et al.** 2009. Antisense down-regulation of the FaPG1 gene reveals an unexpected central role for polygalacturonase in strawberry fruit softening. *Plant Physiology* **150**, 1022–1032.
- Raghavan V.** 2003. Some reflections on double fertilization, from its discovery to the present. *New Phytologist* **159**, 565–583.
- Ren Z, Li Z, Miao Q, Yang Y, Deng W, Hao Y.** 2011. The auxin receptor homologue in *Solanum lycopersicum* stimulates tomato fruit set and leaf morphogenesis. *Journal of Experimental Botany* **62**, 2815–2826.
- Rohrmann J, Tohge T, Alba R et al.** 2011. Combined transcription factor profiling, microarray analysis and metabolite profiling reveals the transcriptional control of metabolic shifts occurring during tomato fruit development. *The Plant Journal* **68**, 999–1013.
- Rossetto MRM, Purgatto E, Do Nascimento JRO, Lajolo FM, Cordenunsi BR.** 2003. Effects of gibberellin acid on sucrose accumulation and sucrose biosynthesizing enzymes activity during banana ripening. *Plant Growth Regulation* **31**, 207–214.
- Ruan YL, Patrick JW, Bouzayen M, Osorio S, Fernie AR.** 2012. Molecular regulation of seed and fruit set. *Trends Plant Science* **17**, 656–665.
- Saladie M, Matas AJ, Isaacson T et al.** 2007. A reevaluation of the key factors that influence tomato fruit softening and integrity. *Plant Physiology* **144**, 1012–1028.
- Santisree P, Nongmaithem S, Vasuki H, Sreelakshmi Y, Ivanchenko MG, Sharma R.** 2011. Tomato root penetration in soil requires a coaction between ethylene and auxin signaling. *Plant Physiology* **156**, 1424–1438.
- Schaffer RJ, Friel EN, Souleyre EJ et al.** 2007. A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. *Plant Physiology* **144**, 1899–1912.
- Schaffer RJ, Ireland HS, Ross JJ, Ling TJ, David KM.** 2013. SEPALLATA1/2-suppressed mature apples have low ethylene, high auxin and reduced transcription of ripening-related genes. *AoB PLANTS* **5**, pls047.
- Serrani JC, Ruiz-Rivero O, Fos M, Garcia-Martinez JL.** 2008. Auxin-induced fruit-set in tomato is mediated in part by gibberellins. *The Plant Journal* **56**, 922–934.
- Seymour GB, Ostergaard L, Chapman NH, Knapp S, Martin C.** 2013. Fruit development and ripening. *Annual Review of Plant Biology* **64**, 219–241.
- Seymour GB, Ryder CD, Cevik V, Hammond JP, Popovich A, King GJ, Vrebalov J, Giovannoni JJ, Manning K.** 2011. A *SEPALLATA* gene is involved in the development and ripening of strawberry (*Fragaria x ananassa* Duch.) fruit, a non-climacteric tissue. *Journal of Experimental Botany* **62**, 1179–1188.
- Singh R, Singh P, Pathak N, Singh VK, Dwivedi UN.** 2007. Modulation of mango ripening by chemicals: Physiological and biochemical aspects. *Plant Growth Regulation* **53**, 137–145.
- Srivastava A, Handa AK.** 2005. Hormonal regulation of tomato fruit development: a molecular perspective. *Journal of Plant Growth Regulation* **24**, 67–82.
- Srivastava MK, Dwivedi UN.** 2000. Delayed ripening of banana fruit by salicylic acid. *Plant Science* **158**, 87–96.
- Sudha RR, Amutha S, Muthulakshmi W, Baby R, Indira K, Mareeswari P.** 2007. Influence of pre and post harvest chemical treatments on physical characteristics of sapota (*Achras sapota* L.) var. PKM 1. *Journal of Agricultural and Biological Science* **3**, 450–452.
- Sun L, Sun Y, Zhang M et al.** 2012a. Suppression of 9-cis-epoxycarotenoid dioxygenase, which encodes a key enzyme in abscisic acid biosynthesis, alters fruit texture in transgenic tomato. *Plant Physiology* **158**, 283–298.
- Sun L, Yuan B, Zhang M, Wang L, Cui M, Wang Q, Leng P.** 2012b. Fruit-specific RNAi-mediated suppression of SINCE1 increases both lycopene and beta-carotene contents in tomato fruit. *Journal of Experimental Botany* **63**, 3097–3108.
- Sun Y, Chen P, Duan C et al.** 2012c. Transcriptional regulation of genes encoding key enzymes of abscisic acid metabolism during melon (*Cucumis melo* L.) fruit development and ripening. *Journal of Plant Growth Regulation* **32**, 233–244.
- Symons GM, Davies C, Shavrukov Y, Dry IB, Reid JB, Thomas MR.** 2006. Grapes on steroids. Brassinosteroids are involved in grape berry ripening. *Plant Physiology* **140**, 150–158.
- Tatsuki M, Nakajima N, Fujii H, Shimada T, Nakano M, Hayashi K, Hayama H, Yoshioka H, Nakamura Y.** 2013. Increased levels of IAA are required for system 2 ethylene synthesis causing fruit softening in peach (*Prunus persica* L. Batsch). *Journal of Experimental Botany* **64**, 1049–1059.
- Thompson AJ, Tor M, Barry CS, Vrebalov J, Orfila C, Jarvis MC, Giovannoni JJ, Grierson D, Seymour GB.** 1999. Molecular and genetic characterization of a novel pleiotropic tomato-ripening mutant. *Plant Physiology* **120**, 383–390.
- Tomato Genome Consortium.** 2012. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* **485**, 635–641.
- Torrighiani P, Bressanin D, Beatriz Ruiz K, Tadiello A, Trainotti L, Bonghi C, Ziosi V, Costa G.** 2012. Spermidine application to young developing peach fruits leads to a slowing down of ripening by impairing ripening-related ethylene and auxin metabolism and signaling. *Physiologia Plantarum* **146**, 86–98.
- Trainotti L, Tadiello A, Casadoro G.** 2007. The involvement of auxin in the ripening of climacteric fruits comes of age: the hormone plays a role of its own and has an intense interplay with ethylene in ripening peaches. *Journal of Experimental Botany* **58**, 3299–3308.
- Van de Poel B, Bulens I, Markoula A et al.** 2012. Targeted systems biology profiling of tomato fruit reveals coordination of the yang cycle and a distinct regulation of ethylene biosynthesis during postclimacteric ripening. *Plant Physiology* **160**, 1498–1514.
- Van Huizen R, Ozga JA, Reinecke DM.** 1997. Seed and hormonal regulation of gibberellin 20-oxidase expression in *Pea* pericarp. *Plant Physiology* **115**, 123–128.
- Vardhini VB, Rao SS.** 2002. Acceleration of ripening of tomato pericarp discs by brassinosteroids. *Phytochemistry* **61**, 843–847.
- Vivian-Smith A, Koltunow AM.** 1999. Genetic analysis of growth-regulator-induced parthenocarp in *Arabidopsis*. *Plant Physiology* **121**, 437–451.
- Vrebalov J, Pan IL, Arroyo AJM et al.** 2009. Fleshy fruit expansion and ripening are regulated by the Tomato SHATTERPROOF gene *TAGL1*. *Plant Cell* **21**, 3041–3062.
- Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R, Schuch W, Giovannoni J.** 2002. A MADS-box gene necessary for fruit ripening at the tomato *ripening-inhibitor (rin)* locus. *Science* **296**, 343–346.
- Vriezen WH, Feron R, Maretto F, Keijman J, Mariani C.** 2008. Changes in tomato ovary transcriptome demonstrate complex hormonal regulation of fruit set. *New Phytologist* **177**, 60–76.
- Wang H, Schauer N, Usadel B, Frasse P, Zouine M, Hernould M, Latche A, Pech JC, Fernie AR, Bouzayen M.** 2009. Regulatory features underlying pollination-dependent and -independent tomato fruit set revealed by transcript and primary metabolite profiling. *Plant Cell* **21**, 1428–1452.
- Werner T, Hanus J, Holub J, Schmullig T, Van Onckelen H, Strnad M.** 2003. New cytokinin metabolites in IPT transgenic *Arabidopsis thaliana* plants. *Physiologia Plantarum* **118**, 127–137.

- Xiong AS, Yao QH, Peng RH, Li X, Han PL, Fan HQ.** 2005. Different effects on ACC oxidase gene silencing triggered by RNA interference in transgenic tomato. *Plant Cell Reports* **23**, 639–646.
- Xu WP, Chen KS, Li F, Zhang SL.** 2000. Regulation of lipoxygenase on jasmonic acid biosynthesis in ripening kiwifruit. *Acta Phytophysiologica Sinica* **26**, 507–514.
- Yamagami T, Tsuchisaka A, Yamada K, Haddon WF, Harden LA, Theologis A.** 2003. Biochemical diversity among the 1-amino-cyclopropane-1-carboxylate synthase isozymes encoded by the *Arabidopsis* gene family. *Journal of Biological Chemistry* **278**, 49102–49112.
- Yang J, Zhang J, Huang Z, Wang Z, Zhu Q, Liu L.** 2002. Correlation of cytokinin levels in the endosperms and roots with cell number and cell division activity during endosperm development in rice. *Annals of Botany* **90**, 369–377.
- Zaharah S, Singh Z, Symons G, J. R.** 2012. Role of brassinosteroids, ethylene, abscisic acid, and indole-3-acetic acid in mango fruit ripening. *Journal of Plant Growth Regulation* **31**, 363–372.
- Zhang M, Yuan B, Leng P.** 2009. The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. *Journal of Experimental Botany* **60**, 1579–1588.
- Zheng Y, Hong H, Chen L, Li J, Sheng J, Shen L.** 2014. LeMAPK1, LeMAPK2 and LeMAPK3 are associated with nitric oxide-induced defense response against *Botrytis cinerea* in the *Lycopersicon esculentum* fruit. *Journal of Agricultural and Food Chemistry* DOI: 10.1021/jf404870d.
- Zhong S, Fei Z, Chen YR, Zheng Y, Huang M, Vrebalov J, McQuinn R, Gapper N, Liu B, Xiang J, Shao Y, Giovannoni JJ.** 2013. Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. *Nature Biotechnology* **31**, 154–159.
- Zifkin M, Jin A, Ozga JA, Zaharia LI, Scherthner JP, Gesell A, Abrams SR, Kennedy JA, Constabel CP.** 2012. Gene expression and metabolite profiling of developing highbush blueberry fruit indicates transcriptional regulation of flavonoid metabolism and activation of abscisic acid metabolism. *Plant Physiology* **158**, 200–224.
- Ziosi V, Bonghi C, Bregoli AM, Trainotti L, Biondi S, Sutthiwal S, Kondo S, Costa G, Torrigiani P.** 2008. Jasmonate-induced transcriptional changes suggest a negative interference with the ripening syndrome in peach fruit. *Journal of Experimental Botany* **59**, 563–573.