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

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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”
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 responsible for the constitutive GA responses in Arabidopsis (Dorcy et al., 2009). 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.

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; Gillaspy 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 (Dorcy 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; Dorcy 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
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 DELLAs proteins. According to the “relief of restraint” model, any activation of GA signalling requires degradation of DELLAs proteins (Harbord, 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 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 SIARF7, 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 SIARF7-silenced plants. Further, differences in cell size between SIARF7-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 SIARF7 inhibits cell division and promotes cell expansion (de Jong et al., 2009; de Jong et al., 2011). Nevertheless, SIARF7 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 SIARF7 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 gynoeceum 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-aminoacyclopropane-1-carboxylate (ACC) synthase 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 (Garcia-Martinez and Carbonell, 1980).

System-level investigation of molecular events underlining 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; Devoghalaere 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$_{19}$ to GA$_{20}$ 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; Devoghalaere 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 (Devoghalaere 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 (Devoghalaere 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...
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; Martinez-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 SlERF6, 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 (fle) and sittens (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 β-carotene accumulation in transgenic tomato fruits where SINCED1 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 hydrolases 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; Guillou 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 acetyl-esterases 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 SINCED1 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, 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. SlAREB1, 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 SlAREB1 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 crosstalk 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, PIN1, 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;
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...
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,

![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. (┴) denotes the possible link between the ethylene biosynthesis inhibition, whereas (→) 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 (↔), during ripening.](https://academic.oup.com/jxb/article-abstract/65/16/4561/2877436)
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 changes 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 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 ripening and also their links to ripening 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 changes and small RNAs play in these interactions is yet to be discovered.

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Interplay between plant hormones ensures fidelity in fruit development programme


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