

Abscisic acid biosynthesis and catabolism and their regulation roles in fruit ripening

La biosíntesis y el catabolismo del ácido abscísico y sus funciones de regulación en la maduración del fruto

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Abstract. Abscisic acid (ABA) plays a series of significant physiology roles in higher plants including but not limited to promote bud and seed dormancy, accelerate foliage fall, induce stomatal closure, inhibit growth and enhance resistance. Recently, it has been revealed that ABA also has an important regulator role in the growth, development and ripening of fruit. In higher plants ABA is produced from an indirect pathway from the cleavage products of carotenoids. The accumulation of endogenous ABA levels in plants is a dynamic balance controlled by the processes of biosynthesis and catabolism, through the regulation of key ABA biosynthetic gene and enzyme activities. It has been hypothesized that ABA levels could be part of the signal that trigger fruit ripening, and that ABA may play an important role in the regulation of ripening and senescence of both non-climacteric and climacteric fruit. The expensive costs of natural ABA and labile active ABA for its chemical synthesis limit its application in scientific research and agricultural production. These findings that ABA has various of important roles in the regulation of growth and development, quality formation, coloring and softening, ripening and senescence of fruit, are providing opportunities and challenges for Horticultural Science. This is to elucidate the specific mechanism of response and biosynthesis, signal transduction, and receptor recognition of ABA in fruit, employing comprehensive research methods, such as molecular biology, plant physiology and molecular genetics. Further and more in-depth research about ABA has a great, realistic significance for knowing the mechanisms behind the process of fruit ripening.

Keywords: Abscisic acid (ABA); Biosynthesis; Catabolism; Regulation; Fruit; Ripening.

Resumen. El ácido abscísico (ABA) desempeña una serie de funciones importantes en la fisiología de las plantas superiores, incluyendo pero no limitado a promover la dormancia de yemas y semillas, acelerar la caída de follaje, inducir el cierre de los estomas, inhibir el crecimiento y mejorar la resistencia. Recientemente, se ha revelado que el ABA también tiene un papel regulador importante en el crecimiento, desarrollo y maduración de la fruta. En las plantas superiores ABA se produce a partir de una vía indirecta a partir de los productos de excisión de carotenoides. La acumulación de los niveles endógenos de ABA en las plantas es un equilibrio dinámico controlado por los procesos de biosíntesis y catabolismo, a través de la regulación de las actividades clave de enzimas y genes relacionados con la biosíntesis de ABA. Se ha planteado la hipótesis de que los niveles de ABA podrían ser parte de la señal que desencadena la maduración del fruto, y que ABA puede desempeñar un papel importante en la regulación de la maduración y senescencia de la fruta tanto no climatérica como climatérica. Los altos precios de ABA natural y lábil activo de la síntesis de ABA químico limitan su aplicación en investigaciones científicas y la producción agrícola. El hecho de que ABA tiene una variedad de roles importantes en la regulación del crecimiento y desarrollo, y la determinación de la calidad, color, ablandamiento, maduración y senescencia de la fruta, están proporcionando oportunidades y desafíos en el campo de las Ciencias Hortícolas. Estas investigaciones son para dilucidar el mecanismo específico de respuesta y biosíntesis, transducción de señales, y reconocimiento del receptor de ABA en la fruta, empleando métodos de investigación integrales, tales como la biología molecular, la fisiología vegetal y la genética molecular. Se necesita investigación adicional y en profundidad sobre ABA, la que tiene una gran importancia realista para conocer los mecanismos involucrados en la maduración de la fruta.

Palabras clave: Ácido abscísico; Biosíntesis; Catabolismo; Regulación; Fruta; Maduración.

INTRODUCTION

Abscisic acid (ABA) was first discovered and identified as a substance controlling higher plant physiological processes in the 1960s by several research groups. Thereafter, articles and reviews of its discovery, structure, chemistry properties and biological functions were published. To date, ABA, indole acetic acid (IAA), gibberellins (GA), cytokinins (CTK) and ethylene (ETH) are recognized as five classic plant hormones which regulate a multitude of significant plant physiological processes. These processes include, but are not limited to abscission, dormancy, germination, growth, ripening, root geotropism, and stomatal functions (Taiz & Zeiger, 2006; Hopkins & Hüner, 2008). ABA is widely distributed in higher plants, and a variety of young and aging organs and tissues. It plays an important role in plant growth and development, such as the promotion of organ loss, bud and seed dormancy, stomatal closure and increased plant resistance (Xiong et al., 2002; Taiz & Zeiger, 2006; Hopkins & Hüner, 2008). In recent years, several studies have found that ABA is a key plant hormone in the regulation of fruit ripening and senescence, and the controlling mechanisms of such processes are being revealed gradually in detail (Rodrigo et al., 2006; Lund et al., 2008; Jia et al., 2011; Li et al., 2011). More and more researchers believe that ABA plays an important role in the growth and development, quality formation, coloring and softening, ripening and senescence processes of fruit (Zhang et al., 2009a; Zhang et al., 2009b; Chai et al., 2011; Symons et al., 2012). Generally, in the processes of growth, development, ripening and senescence of fruit, endogenous ABA level gradually increases from the lower contents, reaches a peak and then slowly decline (Karppinen et al., 2013). Apparently, endogenous ABA accumulation in the fruit depends primarily on the ABA biosynthesis and catabolism (Nambara & Marion-Poll, 2005; Schwartz & Zeevaart, 2010). When the ABA contents accumulated to a certain extent, it will regulate a series of physiological activities, such as growth and maturation of the fruit through a complex signal transduction pathway (Rodrigo et al., 2003). With these further investigations, the key regulatory role of ABA in the growth and ripening process in fruit, especially in non-climacteric fruit, is gradually being revealed (Rodrigo et al., 2006; Lund et al., 2008; Chai et al., 2011; Jia et al., 2011; Sun et al., 2011; Liu et al., 2013; Sun et al., 2013; Wang et al., 2013; Zaharah et al., 2013).

ABA biosynthesis and catabolism

Abscisic acid biosynthesis process was studied by the specific steps of the lack of mutants blocked (Pla et al., 1991; Walton & Li, 1995; Taylor et al., 2000). ABA biosynthesis and catabolism had been brilliantly revealed and studied excellently, but the complete, exact pathways are not understood clearly and fully.

Abscisic acid, a kind of C15 sesquiterpene acid could be synthesized via two pathways in higher plants, either direct-

ly from three C15 precursors or indirectly from those C40 carotenoids. Both direct and indirect pathways begin with Mevalonic acid (MVA) which is converted to Isopentenyl diphosphate (IPP) or Dimethylallyl diphosphate (DMAPP) and Farnesyl pyrophosphate (FPP) by a series of biochemical reactions and changes (Walton & Li, 1995). At this point, three FPPs can form ABA by cyclization and oxidation in the direct pathway. However, in the indirect pathway, FPP continues synthesis to Geranylgeranyl pyrophosphate (GGPP) and C40 carotenoids (e.g., phytoene, ζ -carotene, lycopene and β -carotene) until appears the important precursor-Xanthoxin. In higher plants, the sesquiterpenoid ABA is produced from an indirect pathway from the cleavage products of C40 carotenoids (Schwartz et al., 2003; Galpaz et al., 2008).

Thus, there are two ABA biosynthesis pathways in nature (Seo & Koshiba, 2002; Nambara & Marion-Poll, 2005; Finkelstein, 2013). One is the C15 direct pathway. Three isopentenyl polymerize into C15 precursors-FPP, by the cyclization and oxidation FPP formed directly C15 ABA (Milborrow, 2001). The other is the C40 indirect pathway in higher plants. Further studies have identified the probable biosynthesis pathways as cleaving from C40 carotenoids. First, MVA polymerizes into C40 carotenoids in the plastid (Walton & Li, 1995; Rodrigo et al., 2013; Ohmiya, 2013). Second, C40 carotenoids (phytoene, ζ -carotene, lycopene and β -carotene) transform into Zeaxanthin, and Zeaxanthin create some epoxy-carotenoids by cyclization, such as 9-*cis*-Violaxanthin, 9-*cis*-Neoxanthin, and then 9-*cis*-Violaxanthin or 9-*cis*-Neoxanthin will cleave into Xanthoxin in the plastid (Seo & Koshiba, 2002; Cutler et al., 2010). And finally, Xanthoxin as a C15 framework forms ABA by a series of changes in the cytosol (Seo & Koshiba, 2002; Schwartz et al., 2003; Nambara & Marion-Poll, 2005). Three possible pathways are proposed in the cytosol for the formation of ABA (Nambara & Marion-Poll, 2005; Finkelstein, 2013). Most enzymes (indicated in grey colour) and compound names discussed in this article are shown in Figure 1.

Enzymes involved in the synthesis of ABA mainly are Deoxyxylulose-5-phosphate synthase (DXS), 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR), Farnesyl diphosphate synthase (FPS), Phytoene Synthase (PSY), Phytoene desaturase (PDS), Zeaxanthin epoxidase (ZEP), Neoxanthin synthase (NXS), Xanthophyll isomerase (XISO) (predicted), 9-*cis*-epoxycarotenoid dioxygenase (NCED), Short chain dehydrogenase (SDR), Aldehyde oxidase (AO) (Yoshioka et al., 1998; Taylor et al., 2000; Sauter et al., 2001). Biochemistry and genetics experimental results show that the process that 9-*cis*-Violaxanthin or 9-*cis*-Neoxanthin cleave into Xanthoxin is the rate-limiting step of ABA biosynthesis in higher plants, so NCED is a key enzyme which catalyzes this reaction in the ABA biosynthesis pathways (Tan et al., 1997; Iuchi et al., 2001; Messing et al., 2010). Nordihydroguaiaretic acid (NDGA) is an inhibitor of NCED and

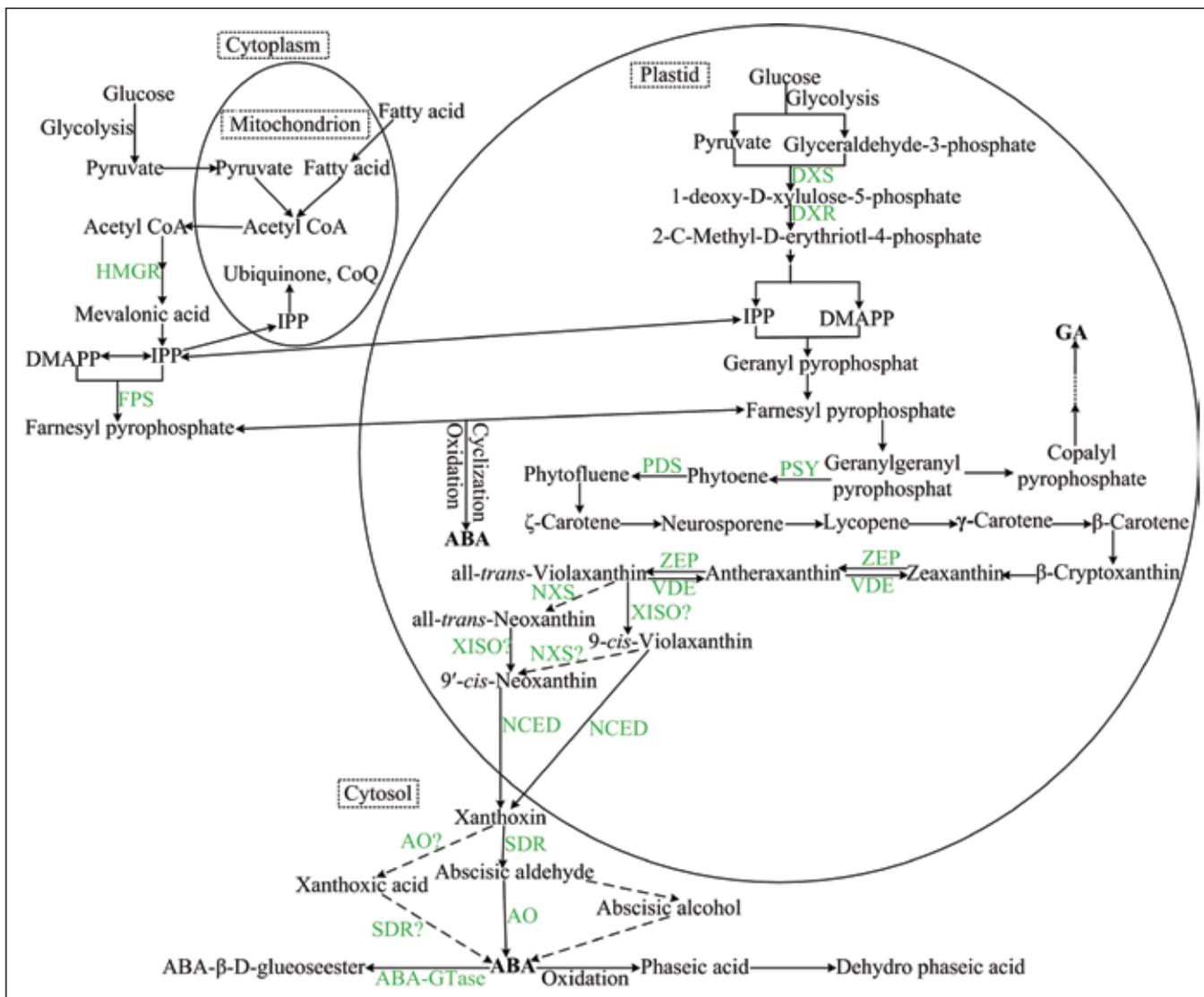


Fig. 1. The biosynthesis and catabolism pathways of ABA in higher plants. Green letters represent enzymes, arrows indicate the order of ABA biosynthesis, dashed-line arrows mark possible biosynthetic pathways, ellipses represent the organelles, and dotted boxes represent cellular components.

Fig. 1. Las vías de la biosíntesis y catabolismo de ABA en las plantas superiores. Letras verdes representan las enzimas, las flechas indican el orden de la biosíntesis de ABA, flechas de líneas cortadas marcan posibles rutas biosintéticas, elipses representan los orgánulos, y cajas punteadas representan componentes celulares.

could effectively inhibit the biosynthesis pathway of ABA (Sun et al., 2012). Fluridone is an inhibitor of phytoene desaturase, which converts phytoene to phytofluene in the pathway of carotenoids biosynthesis (Bartels & Watson, 1978). Due to C40 carotenoids are the main precursors of ABA in higher plants, the inhibition of carotenogenesis should also prevent ABA biosynthesis (Yoshioka et al., 1998).

The biosynthesis pathways of ABA in higher plants are relatively well established (Nambara & Marion-Poll, 2005; North et al., 2007). The first critical step in ABA biosynthetic

indirect pathway is a two-step epoxidation reaction which catalyzes Zeaxanthin to Violaxanthin by ZEP in plastids (Marin et al., 1996). The conversion reactions of all-trans-Violaxanthin or 9-cis-Violaxanthin to all-trans-Neoxanthin or 9-cis-Neoxanthin in ABA biosynthetic pathways are not completely clear, but these reactions have been shown to be catalyzed by the transcriptional expression products of the *ABA4* gene in *Arabidopsis* (North et al., 2007). This is believed to encode for the NXS probably. In higher plants, Xanthoxin (which forms 9-cis-Neoxanthin and 9-cis-Violaxanthin) is the

important direct C15 precursor of ABA (Tan et al., 1997; Iuchi et al., 2001). Thereafter, Xanthoxin is transported to the cytosol and soon afterwards transformed into abscisic aldehyde by the SDR, which encoded by the related *AtABA2/AtGIN1* genes in *Arabidopsis* in the main biosynthesis pathway (Cheng et al., 2002; González-Guzmán et al., 2002). Subsequently, abscisic aldehyde is oxidated by AO to generate ABA (Seo et al., 2000; Harrison et al., 2011).

While ABA metabolism is rapid in higher plants, especially under stress conditions, some studies reported that ABA metabolic rate is exceedingly variable, ranging from hours to days (Gowing et al., 1993). And the metabolism of ABA in higher plants mainly via the two pathways of hydroxylation and glycosylation (Cutler & Krochko, 1999; Kushiro et al., 2004) that is oxidative degradation and combined inactivation. In most higher plant tissues, the critical process of ABA metabolic inactivation is the hydroxylation by monooxygenase catalysis of ABA 8'-methyl group. This is catalyzed by the cytochrome P450 (CYP) monooxygenase to transform into 8'-hydroxy-ABA which is unstable and subsequently rearranges to phaseic acid (PA), and then is converted to dihydro phaseic acid (DPA) or possible epi-dihydrophaseic acid (epi-DPA) (Krochko et al., 1998; Jacobsen et al., 2002; Nambara & Marion-poll, 2005). Phaseic acid has an extremely low biological activity, and DPA has no physiological activity (Sharkey & Raschke, 1980; Uknes & Ho, 1984). Active ABA and sugar or amino acids can combine to form no activity bound ABA, which are mainly ABA-glucosylester (ABA-GE) and ABA glucoside, catalyzed by ABA glucosyltransferase (ABA-GTase), and they are the transportation form of ABA in screens or conduits. Free state ABA contents are lower under normal than stressful environmental conditions. Under stress conditions, a lot of bound ABA will change to free state ABA, and after stress is relieved, it will be recovered to bound ABA again and stored in plant tissues. In these processes, there is a key enzyme protein that is β -glucosidase 1 (AtBG1) which catalyzes the release of bound form ABA-GE back into active free ABA in order to rapidly regulate ABA levels (Lee et al., 2006). However, several studies have also found that under drought stress ABA levels increased rapidly through the biosynthesis of a great amount of ABA, rather than the hydrolysis of bound ABA (Zeevaart, 1980; Bano et al., 1994; Seo & Koshiba, 2002). These findings suggest several complex signal transduction networks and regulation mechanisms for ABA accumulation and catabolism.

Over the past three decades, genetic engineering, molecular genetics, biochemical and pharmacological studies have identified ABA biosynthetic and metabolic pathways, and numerous genes and enzymes involved in these processes. In addition, we also have to focus on current research progress linking ABA's regulation roles in fruit growth and development, quality formation, coloring and softening, ripening and senescence.

The regulation roles of ABA in fruit growth and development, quality formation, ripening and senescence

The plant hormone ABA not only regulates seed dormancy, bud activation, plant growth, stomatal closure, and responses to environmental stresses, but also regulates fruit ripening and senescence. In recent years, ABA is believed to play a key role in the growth and development, ripening and senescence of fruit. In the maturation process according to whether there is an increase of respiration and ETH production, usually the fruits are divided in two types: climacteric and non-climacteric (Giovannoni, 2001). For a long time, ETH has been considered the critical hormone for fruit ripening and senescence. Numerous studies have reported the role of ETH in the climacteric fruit ripening process (Trainotti et al., 2005). It has been demonstrated that in climacteric fruit, ETH is one of the key signals associated with the maturation-inducing specific gene expression, and the increasing ETH production resulted in the beginning of the fruit ripening process (Zaharah et al., 2011). Recently, people began to emphasize that the regulatory role of another ripening and senescence hormone (i.e., ABA) is more important in the fruit ripening process. Not only in climacteric fruit but also in the process of ripening and senescence of non-climacteric fruit, ABA plays an important role. Studies have shown that exogenous ABA treatment can contribute to the ripening process of fruit via increasing the ETH production and respiration rate, and accelerating the ripening and senescence of fruit. Thus, ABA may also regulate the ripening and senescence of non-climacteric and climacteric fruit. It is considered that ABA plays a more important role than that of ETH in fruit maturation and senescence (Zhang et al., 2009a).

Immature fruits have low levels of endogenous ABA; exogenous ABA treatment can accelerate fruit ripening and pulp softening, and a large number of ABA accumulation and expression of genes which are related to ABA biosynthesis during the fruit ripening and senescence processes. The above results suggest that ABA plays an important role in the processes of fruit ripening and senescence. Research on climacteric fruits [e.g., peach (Zhang et al., 2009b; Soto et al., 2013), avocado (Chernys & Zeevaart, 2000), tomato (Zhu et al., 2003; Galpaz et al., 2008; Sheng et al., 2008; Zhang et al., 2009a; Sun et al., 2011; Sun et al., 2012; Wang et al., 2013; Freitas et al., 2014), banana (Jiang et al., 2000; Lohani et al., 2004), apple (Zhang et al., 2001; Giulia et al., 2013) and mango (Kondo et al., 2004; Zaharah et al., 2012; Zaharah et al., 2013)], non-climacteric fruits [e.g., grape (Ban et al., 2003; Jeong et al., 2004; Deytieux et al., 2005; Cantín et al., 2007; Deytieux-Belleau et al., 2007; Lacampagne et al., 2010), citrus (Rodrigo et al., 2003; Rodrigo et al., 2006; Rodrigo et al., 2013; Shalom et al., 2014), cucumber (Wang et al., 2012; Wang et al., 2013), litchi (Liu et al., 2013), blueberry (Zifkin et al., 2012), bilberry (Karpinen et al., 2013), sweet cherry

(Luo et al., 2013) and strawberry (Chai et al., 2011; Jia et al., 2011; Li et al., 2011; Symons et al., 2012; Daminato et al., 2013; Jia et al., 2013)], and on melon (Sun et al., 2013; that has features of both climacteric and non-climacteric fruits) supports the important regulation role of ABA during fruit ripening.

Abscisic acid regulation roles on climacteric fruit growth, development and ripening. In the climacteric fruit ripening process, molecular mechanisms of physiological effects and receptor recognition of ETH have been gradually explained clearly (Adams-Phillips et al., 2004). However, there are fewer investigations on the trigger signal and upstream regulation for the climacteric fruit ripening process. It is not clear whether ABA is the upstream regulatory signal of ETH biosynthesis and its possible regulation mechanism. In the ripening process on climacteric fruits, such as peach, tomato and banana, ABA reached the peak of biosynthesis earlier than ETH; this suggests that ABA might be the trigger and regulation signal of ETH biosynthesis.

Abscisic acid-deficient tomato mutant only showed 25% of wild-type tomato ABA levels, and both total and mean fresh fruit weights were reduced; However, carotenoid contents were 30% higher on ABA-deficient than on wild-type tomato fruits (Galpaz et al., 2008). Moreover, suppression of NCED biosynthesis-related gene expression in tomato fruit can reduce the ABA levels, and extend the shelf life of fresh fruits (Sun et al., 2012). NCED biosynthesis-related gene over-expressing tomato mutants showed that they could promote ABA accumulation, strengthen drought tolerance, and reduce the level of transcription (Thompson et al., 2007). In addition, NCED-suppressed fresh fruits of tomato mutants were firmer, and these fruits have a longer shelf life than wild-type tomatoes (Sun et al., 2012). These phenomena indicated that ABA is essential and significant for fruit growth, development and ripening.

The application of exogenous ABA and high endogenous ABA contents have also been reported to (1) induce increases in the production of ETH, and (2) promote the ripening process of fruits. Zhang et al. (2009a) reported that exogenous ABA treatment could up-regulate the expression of ETH-production related genes, such as *Le-ACO1* and *Le-ACS2*, which encode 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) and 1-aminocyclopropane-1-carboxylic acid synthase (ACS), and could accelerate tomato fruit ripening. On the other hand, the application of Fluridone or NDGA (which can block ABA biosynthesis), could inhibit the transcriptional level and expression production of *Le-ACO1* and *Le-ACS2* genes, and in turn constrains tomato fruit ripening (Zhang et al., 2009a; Zaharah et al., 2013).

Abscisic acid may play a key physiological role in fruit cell wall catabolism which relates to fruit ripening and softening at a molecular level. Sun et al. (2012) reported that ABA ac-

cumulation and *SINCE1* transcriptional levels in the transgenic fruit were down-regulated to about 20-50% of the levels on control fruits. This significant decline in NCED levels and activity directly caused the down-regulation in the transcriptional levels of genes encoding for cell wall metabolic enzymes. This led to a self-evident extension of the shelf life to about 15-29 d in contrast to the shelf life of the control group which was only a week; there was also an increase of 30-45% of fruit flesh firmness at the mature stage (Sun et al., 2012). Later, further research (Soto et al., 2013) found that ABA could modulate fruit ripening, softening and senescence interacting not only with ETH and cell wall, but with auxin-related genes. These genes were up- or down-regulated to either speed up or slow down, respectively, the fruit ripening process.

Some earlier reports indicated that endogenous ABA levels would increase in the skin and pulp of mango fruit at the harvesting stage (Kondo et al., 2004). A previous study showed that the accumulation of endogenous ABA levels during the mango fruit ripening process might initiate ETH production (Zaharah et al., 2012). Later reports (Zaharah et al., 2013) indicated that the application of ABA could accelerate mango fruit ETH production by improving the activities of ETH biosynthesis-related enzymes. However, these phenomena were significantly delayed and/or suppressed in the NDGA-treated group.

These results suggest that ABA is involved in regulating climacteric fruit development and ripening and its effects are, at least in part, regulated by changes in ETH biosynthesis and production. However, the specific detailed mechanism by which ABA regulates ETH biosynthesis and climacteric fruit ripening has not been examined methodically and studied comprehensively.

Abscisic acid regulation roles in non-climacteric fruit growth, development and ripening. Comparatively speaking, there are fewer studies about the triggering signal and signal regulation of the maturation process in non-climacteric fruits; a detailed explanation of the mechanism involved is also not very clear. Early research showed that the plant hormone ABA may breathe a non-climacteric fruit ripening in close contact. Most tests showed that elevated levels of endogenous ABA occur before the fruit get ripe fully. This seems to prove that increasing ABA levels induce the beginning of maturation rather than ripening and senescence lead to increased ABA levels. For non-climacteric fruit, the ripening signal may be not ETH, such as on grape and strawberry. Substantial evidences proved that ABA can induce growth, ripening and senescence on this fruit type. In non-climacteric fruit, levels of ETH are low and change smoothly, while ABA accumulates until reaching a peak. Therefore, ABA is considered an important factor to start the ripening process in non-climacteric fruit. There are a large number of studies which suggest that the role of ABA in fruit ripening and senescence is more im-

portant than that of ETH, and that ABA may trigger and start the maturation process (Cantín et al., 2007; Deytieu-Belleau et al., 2007; Lacampagne et al., 2010; Chai et al., 2011; Jia et al., 2011; Li et al., 2011; Symons et al., 2012; Daminato et al., 2013; Rodrigo et al., 2013; Shalom et al., 2014).

Abscisic acid is an important signal, regulatory molecule which can promote non-climacteric fruit (e.g., strawberry) growth, development, coloring and ripening. In RNA interference (RNAi) strawberry fruit, the expression of *FaNCED1* was down-regulated, resulting in a significant decrease in ABA levels and the formation of uncolored fruits; more importantly, the uncolored phenotype of the *FaNCED1*-downregulated RNAi fruits could be reverted by exogenous ABA (Jia et al., 2011). PYR/PYL/RCAR (Pyrabactin Resistance Proteins/PYR-Like Proteins/Regulatory Components of ABA Receptor) family proteins are considered to be the novel ABA receptors in *Arabidopsis* (Ma et al., 2009; Park et al., 2009). Subsequent studies revealed that when PYR/PYL/RCAR combines with ABA, they will interact with downstream effector proteins [type 2C protein phosphatase (PP2C)], and inhibit the PP2C activities, thereby affecting the life activities of higher plants (Fujii et al., 2009). Thereafter, fruit studies showed that the *Arabidopsis* ABA receptor gene *FaPYR1* acts as a positive regulator in the strawberry fruit ripening (Chai et al., 2011), while PP2C gene *ABI1* is a negative regulator of strawberry fruit ripening (Jia et al., 2013). These findings may suggest that ABA regulation roles and signaling transduction might be an extremely complex mechanism in non-climacteric fruit.

Additionally, the de-greening stage of the ABA-deficient orange mutant initiated later than in the wild-type, and at the same time it was synchronized with the accumulation of ABA during orange fruit ripening (Rodrigo et al., 2003). ABA was also involved in the regulation of ripening, and changes related with development and coloring in grape (Deytieu-Belleau et al., 2007; Wheeler et al., 2009). It has been demonstrated that the application of exogenous ABA could increase the anthocyanin levels in grape skins (Ban et al., 2003; Jeong et al., 2004), and could improve the pigment contents and quality of the grapes (Cantín et al., 2007; Peppi et al., 2006). Lacampagne et al. (2010) reported that ABA regulates the generation and activity of enzymes which are involved in tannin biosynthesis, and can increase the tannin levels of green grapes at veraison. These studies suggest that ABA plays a significant role in triggering the flavonoids and polyphenols biosynthetic pathways (Soto et al., 2013). Most studies making a thorough inquiry of the effects of exogenous ABA application have been implemented in grapes. Even more, Sandhu et al. (2011) hypothesized that exogenous ABA treatment affects the anthocyanin accumulation, antioxidant capacity, flavonol levels, and phenolic content and composition of red muscadine grapes. A grape proteomics study showed that ABA induces or promotes grape fruit ripening by affecting most proteins involved in the regulatory processes and networks at the ripening stage (Giribaldi et al., 2010).

Abscisic acid accumulation, not the increasing amount of ETH production, might play a significant role in the complex regulatory networks of fruit development, ripeness and senescence. Abscisic acid has previously been demonstrated to be an important signaling component regulating ripening initiation in grape fruit (Lund et al., 2008). In grape fruit the NCED gene fragments were highly expressed at the beginning of ripening when ABA contents were high, and thereafter ABA levels gradually increased from the beginning of ripening until reaching the highest level at 20 d before the harvesting stage (Zhang et al., 2009b). Further, the application of exogenous ABA or Fluridone (or NDGA) could promote or delay the endogenous ABA levels, and subsequently the softening and ripening of grape fruit (Zhang et al., 2009b).

Endogenous ABA levels may be regulated by its biosynthesis, catabolism and the transcriptional level and expression of related genes during fruit development and ripening. The application of ABA at the turning stage can promote cucumber fruit ripening, and ABA contents would reach a peak level in pulp before fruit get fully ripe (Wang et al., 2013). Abscisic acid and ETH may have a synergistic effect and interaction on the regulation of fruit ripening. Sun et al. (2013) reported that the ABA levels in young melon (*Cucumis melo* L. cv. Elizabeth) fruit were initially high and then gradually decreased; in addition, ETH production, 1-aminocyclopropane-1-carboxylic acid (ACC) contents, and ACC oxidase (ACO) activity were all at low levels during the early stages of fruit setting, while their peaks were observed 5 days after the ABA peak.

In higher plants, including horticultural products, the endogenous ABA levels were maintained in a dynamic balance of biosynthesis and catabolism before fruit was fully ripe. Exogenous ABA treatment promoted fruit softening, ripening and senescence by the upregulation and expression of ABA, ETH and cell wall enzyme synthesis genes. However, the application of ABA biosynthesis pathway inhibitors (i.e., NDGA and Fluridone) had a negative, even opposite effect on fruit development, ripening and senescence. These results may lay foundation for further revealing the molecular mechanism of ABA regulation in non-climacteric fruit development and ripening. The mechanism of ABA biosynthesis and catabolism, and the complete pathways in regulating fruit ripening should be studied extensively and examined in detail in further research. This will allow to understand more clearly and fully the ABA regulation roles in non-climacteric fruit growth, development, softening, coloring, ripening and senescence.

CONCLUSIONS AND PERSPECTIVES

As an important naturally occurring phytohormone, ABA plays a key role in the regulation of a wide range of critical physiological processes in higher plants. They include the induction of growth and development (particularly seed de-

velopment), fruit ripening and responses to environmental stresses. Abscisic acid can also control bud differentiation, regulate flowering, and has a great value in flowers. Additionally, ABA also has a critical role in the regulation or triggering of fruit functional active ingredients and natural antioxidants such as anthocyanin, flavonoid and polyphenol biosynthesis and accumulation.

Abscisic acid has a broad application prospect in agricultural production, and it might produce huge economic and social benefits. Meanwhile, ABA is obtained mainly by chemical synthesis to a high cost of production. Due to the expensive prices and differences in activity, ABA has not been widely used in agricultural production. Scientists from all over the world are looking for cheap, natural ABA production methods. Consequently, the regulation roles of ABA continue to be explored in terms of plant physiology, while people are trying to find ABA analogues or substitutes.

In recent years, the significant regulation roles of ABA in fruit growth and development, quality formation, coloring and softening, ripening and senescence and other aspects are full of opportunities and challenges. In-depth research and multifaceted tests on ABA, on the basis of biochemistry, molecular biology, plant physiology and genetic engineering theory and practice are needed to illustrate the mechanism of ABA biosynthesis, signal transduction, and receptor recognition. All this has an important practical significance for revealing the growth and development, quality formation, softening and coloring, and ripening and senescence of fruits.

Life is a painstaking, exquisite process, especially for postharvest fresh fruit products which still have life activities. They must endure the pressures of the environment or humans, such as dehydration, hypothermia and mechanical vibration. But the fruit will produce the stress hormone ABA and other plant hormones which will contribute to their adaptation to environmental stresses and enhancement of their own survival. Understanding the specific action mechanism of ABA will greatly help in maintaining the quality of fresh fruit at the molecular level. The application of ABA at the growth and morphological developmental stages of fruits can reasonably regulate their physiological activities and growth processes, enhancing the fruit's ability to adapt to the environment and adversities, reduce the use of pesticides and improve food quality and fruit nutritional value. Fresh produce, especially postharvest fruits and vegetable products, suppress the biosynthesis and metabolism of ABA during storage under fresh-keeping conditions. This can inhibit respiration reasonably, and contribute to form and maintain the good eating quality, and extend the shelf life and fresh quality of fruits and vegetables.

In summary, these pivotal regulation roles of ABA in fruit maturation indicate that ABA tends to participate and mediate a wide range of plant physiological activities and require consideration of multiple aspects in higher plants. We believe

that these findings can contribute to further developments and more in-depth inquiries in Agricultural and Horticultural Science.

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