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Roles of Abscisic Acid in Fruit Ripening

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Abstract

Abscisic acid (ABA) is a plant growth regulator, and it plays a variety of important roles throughout a plant's life cycle. These roles include seed development and dormancy, plant response to environmental stresses, and fruit ripening. ABA concentration is very low in unripe fruit, but it increases as a fruit ripens, so it is therefore believed that ABA plays an important role in regulating the rate of fruit ripening. This article reviews the effect of ABA on ripening and quality of climacteric and non-climacteric fruits. The effects of ABA application on fruit ripening are subsequently discussed. Moreover, it is found that during fruit ripening, ABA also contributes to other functions, such as ethylene and respiratory metabolism, pigment and color changes, phenolic metabolism and nutritional contents, cell wall metabolism and fruit softening, and sugar and acid metabolism. These processes are all discussed as part of the relationship between ABA and fruit ripening, and the possibilities for its commercial application and use are highlighted.

Keywords: Abscisic acid, ethylene, fruit, quality, ripening

Introduction

Abscisic acid (ABA) plays important roles in many cellular processes including the adaptation of plants to various environmental stresses, seed development, dormancy, germination, and fruit growth and development [1-4]. The chemical structure, biosynthesis and catabolic pathway of ABA are shown in **Figure 1** [5]. Unlike other plant hormones, ABA is an interesting hormone because it regulates its concentration as it rises and falls dramatically in several kinds of tissues in response to environmental and developmental changes.

Fruit ripening is one developmental process which can be characterized into two groups. The first is climacteric; a ripening for these kinds of fruits is accompanied by a peak in respiration and an existing burst of ethylene. The second group is non-climacteric, where respiration does not show dramatic changes and ethylene production also remains at a very low level [6]. The effect of ethylene on the ripening mechanism in climacteric fruit has been well studied, but the mechanism involved in the ripening of non-climacteric fruits remains unclear. It might be regulated by a different mechanism. Not only ethylene, ABA also plays a crucial role in the regulation of fruit ripening since increasing ABA content during the ripening of climacteric and non-climacteric has been reported [7-10]. Moreover, ABA concentration is very low in unripe fruit, but it increases during fruit ripening. It is therefore believed that ABA plays an important role in regulating fruit ripening.

In climacteric fruit such as apples, the level of ABA increases from maturation to harvest, while in non-climacteric sweet cherries, the level of ABA increases before maturation and thereafter decreases until harvest [5,10]. The differing ABA levels suggest that the role of ABA may vary between fruits. To clarify the physiological role of ABA on regulating fruit ripening, it may be important to know how endogenous ABA and its related compounds change during fruit development and how exogenous ABA affects fruit ripening.

When exogenous synthetic ABA has been directly applied to the edible portion, is it safe for consumption needs to be considered. However, recent advances show that ABA not only exists in plants but is also present in a wide range of lower animals to higher mammals [11]. Similar to findings in plants, ABA regulates cell growth and development and immune systems mediate a signaling pathway in animal and human cells [11]. When functioning as a growth regulator, ABA does not have significant toxic side effects on animal cells [11]. In addition, research indicated that ABA is an endogenous immune regulator and has potential medicinal applications in several human diseases such as inflammation diseases and cancer [11]. As it has been demonstrated to possess medicinal potential on humans, therefore the application of ABA on fruit might be an effective tool for improving fruit quality and increasing health benefits. This review mainly discusses the roles of endogenously produced or exogenously applied ABA in fruit ripening and their effects on fruit quality.

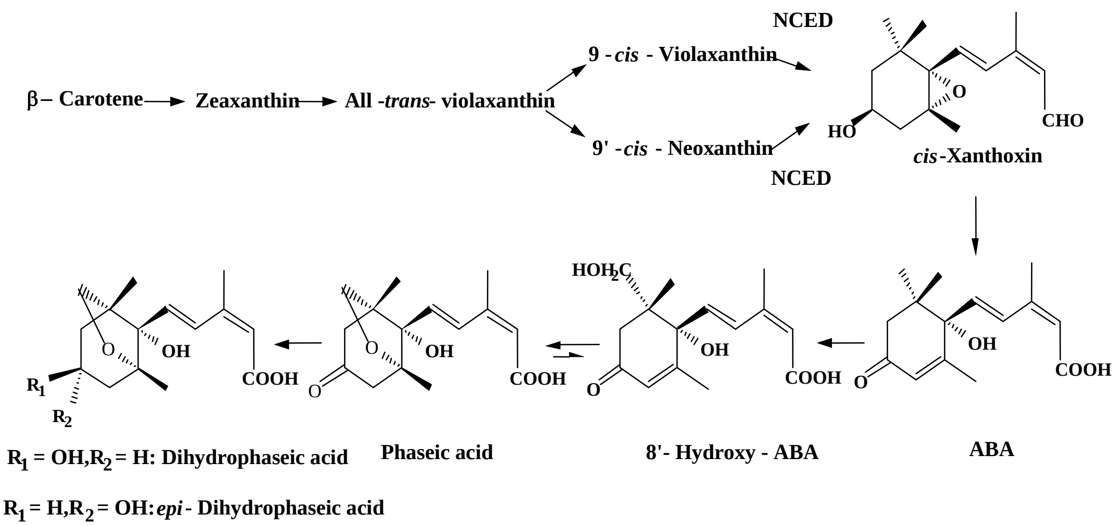

Figure 1 ABA biosynthesis and the major pathway for ABA catabolism in higher plants.
Source: Modified from [5].

Application of ABA

ABA exogenously applied to intact plants, certain organs, and tissue show various biological activities which are closely related to the physiological roles of endogenous ABA. The application of ABA on fruit ripening differs depending on several factors such as the species of fruit, the degree of fruit maturation, the concentration of applied ABA, and the method of application (**Table 1**). Different cultivars show different responses to ABA application as has been reported in apples and grapes [12,13]. The stage of maturation is an important factor that responds to ABA treatment; for example, ABA plays a different role at different stages of tomato fruit ripening by regulating the ethylene biosynthesis system [7]. For the method of application, exogenous ABA has been applied to fruits by several methods such as injection, infiltration, dipping, and spraying [13-17].

When applying ABA by injection, the ABA solution in the syringe is directly applied to the stylar scar along the fruit axis. This method has been used to study the role of ABA in ‘Granny Smith’ apples, in citrus, and in tomatoes [15,18,7]. The advantages of the injection method are that a definite amount of ABA can be applied to the pulp tissue, even in fruit with a tough skin. Moreover, the isomerization of *cis*- to *trans*- ABA and ABA breakdown from sunlight can be avoided. On the other hand, a needle wound reaction is of concern when using the injection method.

The application of ABA by vacuum infiltration and dipping has been used to observe the role of ABA in mature and unripe green bananas [16,17]. The acceleration of banana ripening by ABA infiltration was greater at 10^{-3} M than at 10^{-4} M [16].

For experiments employing an ABA solution treatment, the solution should be prepared at the desired concentration and include a surfactant such as 0.05 % of tween 20. The sample is then immersed in the solution for 1-3 min, air-dried and stored according to the optimal storage temperature of the sample. For pre-harvest spraying, the ABA solution is sprayed directly on to the fruit surface at the desired volume; this method has been used to apply ABA solutions mostly at the beginning of the ripening stage. For example, an ABA solution at 0.38 - 1.14×10^{-6} M has been sprayed on to grape berries during the onset of fruit ripening [13,20]. In climacteric fruits such as mangos and peaches, the ABA solution has been sprayed directly to the fruits at the beginning of maturation [21,22].

Although ABA application has been successful for improving the quality of several fruits, it seems that current commercial applications for ABA are minimal or non-existent. Rapid metabolism and photo destruction might be why there is limited use of the native compound [23]. In general, the plant hormones that are used as commercial products are synthesized analogues with high biological activity and stability, both in the plant and in the environment. This should be similarly considered when synthesizing an ABA analogue in order to increase its commercial application.

Table 1 Different application methods and concentrations of ABA applied in several fruits.

Fruit	Method of application	Degree of fruit maturity at the time of application	ABA	Reference
Apple	injection	pre- and climacteric rise	10^{-3} M	[14,15]
Banana	vacuum infiltration	mature green	10^{-3} - 10^{-5} M	[16]
Banana	dipping	beginning of maturation	10^{-4} M	[17]
Citrus	injection	pre-and post-harvest	0.2, 0.6, or 1.2×10^{-6} M	[18]
Grape	pre-harvest spraying	during and after veraison	0.38×10^{-3} M	[13]
Grape	pre-harvest spraying	during veraison	0.57 or 1.14×10^{-6} M	[20]
Mango	pre-harvest treatment	beginning of maturation	10^{-6} M	[21]
Peach	pre-harvest spraying	beginning of maturation	1.89×10^{-3} M	[22]
Strawberry	dipping	maturation	10^{-4} or 10^{-6} M	[19]
Sweet Cherry	pre-harvest spraying	before coloring stage	3.78×10^{-3} M	[9]
Tomato	injection	mature green	10^{-4} M	[7]

Involvement of ABA in fruit ripening

Fruit ripening is a complex process, which sees dramatic changes in color, texture, flavor, and aroma of a fruit. Due to the economic importance of fruit crops, their ripening processes have been studied extensively. The study gives particular focus to the regulating factors during fruit ripening.

Generally, the ripening of climacteric fruit is regulated by ethylene. However, ABA displays a similar change as well as ethylene during fruit maturation. The increase of ABA levels during fruit ripening has been observed in both kinds of fruits. Generalizations regarding the involvement of ABA in fruit ripening and its relative effect on fruit quality are shown in **Table 2**. The involvement of ABA in ethylene and respiratory metabolism, pigment and color changes, phenolic metabolism, and nutritional contents, cell wall metabolism and fruit softening, and sugar and acid metabolism are all discussed.

Ethylene metabolism

The ethylene biosynthesis pathway is well elucidated in higher plants [6]. Ethylene is formed from methionine via *S*-adenosyl-L-methionine (AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC) by two catalyzing enzymes that are ACC synthase (ACS) and ACC oxidase (ACO). The relationship between ethylene and ABA during ripening and senescence has been investigated in several fruits [7,8,15-16].

Increased ABA concentration precedes the climacteric increase in ethylene production [5,7]. In peaches, the ABA concentration rapidly reached a peak and then declined again before the harvest stage, which coincided with the peak of ethylene production [8]. Over the entire period of grapes development, which includes ripening and senescence, ethylene remained at low levels, but the ABA gradually increased and reached high levels at the beginning of ripening [8].

Application of ABA can accelerate banana ripening by regulating ethylene evolution and respiration, and subsequently induces color change and fruit softening [16]. However, ABA induced banana ripening was not observed in a fruit treated with an ethylene inhibitor, 1-methylcyclopropene (1-MCP). This was especially true when ABA was applied after exposure to 1-MCP [16]. Thus, ABA promoted ripening in intact bananas and was at least partially mediated by ethylene. Exposure of ABA treated banana fruit to ethylene at 0.1×10^{-6} M for 24 h resulted in an increase of ethylene production and respiration, and was clearly associated with skin color change and fruit softening [16]. The data suggests that ABA facilitates the initiation and progress of the sequence of ethylene-mediated ripening events, possibly by enhancing sensitivity to ethylene.

The application of exogenous ABA or ACC hastens fruit ripening by accelerating ethylene production and respiration [7]. Out of the interaction between ABA and ethylene during the maturation of 'Granny Smith' apples, it was hypothesized that there was a synthesis of *de novo* ACO protein, causing an increase in endogenous ethylene levels, which subsequently enhanced ACS expression, either directly or through a modification of ABA levels [15,24]. In 'Tsugaru' apples, the ABA application increased ACC concentration and ACO activity, and it increased the transcription levels of *MdACS1* and *MsACO1* in both the pre-climacteric and the climacteric stage [unpublished data].

In order to understand the role of ABA in fruit ripening, *NCED* genes that encode 9-*cis*-epoxycarotenoid dioxygenase (NCED), a key enzyme in ABA biosynthesis have been cloned in several fruits such as tomatoes, peaches and grapes [7]. For tomatoes, it was shown that the expression of *LeNCED1* occurred before the ethylene biosynthesis genes *LeACS* and *LeACO*, which encode ACS and ACO, respectively. It suggests that ABA triggers ethylene biosynthesis by regulating the expression of ACS and ACO genes during the fruit ripening process. The expression of *PpNCED1* and the *VvNCED* in peaches and grapes were observed only at the beginning of ripening, when ABA accumulation was high [8]. Therefore, the expression of those genes is initiated by ABA biosynthesis at the onset of fruit ripening. The *NCED* genes were expressed at the beginning of maturation, and then ABA began to accumulate. After this, the expression of the *NCED* genes was not detected, indicating that because of abundant expressions of these genes at the beginning of maturation, ABA could rapidly accumulate and continuously remain at a high level to complete physiological and biochemical reactions during fruit ripening [8].

Inhibitors of ABA biosynthesis have been used in attempts to study the function of ABA in plants. Several compounds such as fluridone, norflurazon, and nordihydroguaiaretic acid (NDGA) are widely used as ABA inhibitors [25,26]. They suppress ABA biosynthesis by inhibiting the biosynthesis of carotenoids. As carotenoids are precursors for ABA, inhibitors that affect carotenoid biosynthesis will also inhibit the biosynthesis of ABA. Reducing ABA content by fluridone or NDGA application effectively postponed the maturity and softening of peaches and grapes [8]. The effect of ABA and NDGA application on the ethylene production at different stages of ripening has been studied in tomatoes [7]. At the mature green stage, exogenous ABA treatment increased ABA content and promoted ethylene biosynthesis and ripening while they were inhibited when treated with NDGA. However, at the breaker stage (45 days after anthesis), NDGA treatment could not block ABA accumulation and ethylene synthesis [7].

Table 2 The effect of ABA in the fruit ripening processes.

Attribute or process affected	Mechanism of action, enzyme activity or associated gene expression	Increased (+) or decreased (-)
Ethylene metabolism	Ethylene perception	+
	Ethylene production	+
	ACC synthase (ACS) expression and activity	+
	ACC oxidase (ACO) expression and activity	+
Respiratory metabolism	Respiration rate	+
Rate of ripening	Hasten rate of ripening	+
Pigments	Anthocyanins accumulation	+
Phenolic metabolism	Phenolic content	+
	Phenylalanine ammonia lyase (PAL)	+
	Chalcone synthase (CHS)	+
	Chalcone isomerase (CHI)	+
	Dihydroflavonol 4-reductase (DFR)	+
	UDP-glucose-flavonoid: 3-*O*-glucosyltransferase (UFGT)	+
	Proanthocyanidins (condensed tannins)	-
	Leucoanthocyanidin reductase (LAR)	-
	Anthocyanidin reductase (ANR)	-
Nutritional	Antioxidant activity	+
	Anthocyanins content	+
Cell wall metabolism and fruit softening	Softening	+
	Pectin methyl esterase (PME)	+
	Pectate lyase (PL)	+
	Cellulase	+
Sugar and acid metabolism	Total sugar content	+
	Glucose and fructose	+
	Total soluble solid (TSS)	+
	Titrable acidity (TA)	-
	TSS:TA	+
	Amylase	+
	Invertase	+
	Sorbitol oxidase (SOX)	+

Pigment and color changes

Fruit color is an important deciding factor for consumers. Several reports showed that changes in ABA content are closely correlated with color changes and pigment content in fruit [12-13,19-20]. The skin color of red and black grapes is evaluated mainly by anthocyanin and flavonoid content [27]. Grape anthocyanin biosynthesis is regulated by the UDP-glucose: flavonoid 3-*O*-glucosyltransferase (UFGT) [28,29]. Anthocyanin accumulation in grapes begins at veraison, the onset of maturation. This accumulation appears to be regulated, at least in part by ABA [30].

It has been reported that the application of ABA to 'Cabernet Sauvignon' (*Vitis vinifera* L.) berries stimulated the accumulation of mRNA coding for several enzymes involved in anthocyanin biosynthesis, including UFGT [31]. By HPLC analysis for anthocyanins concentration in 'Noble' grapes, ABA treatment increased the concentration of individual anthocyanins (i.e. delphinidin, cyanidin, petunidin, peonidin and malvidin 3,5-diglucoside) throughout the ripening period. Among the anthocyanins, cyanidin 3,5-diglucoside had the highest increase by ABA application [13].

The improvement of grapes skin color has also been reported in others grape cultivars, such as 'Flame Seedless' [32] and 'Redglobe' grapes [33]. Cantin *et al*. [20] found that treatment with ABA at 1.14×10^{-6} M advanced the harvest date of 'Crimson Seedless' by 10 - 30 days compared to grapes treated with ethephon, or non-treated grapes. The application of ABA advanced the harvest date because it rapidly improved grape color. Treatment with ABA did not reduce grape quality in any way, and after cold storage, the coloration of ABA-treated grapes was superior to that of grapes treated with ethephon or those that were not treated. Thus, treatment with ABA is an effective alternative to treatment with ethephon for improving the color of 'Crimson Seedless' grapes. Moreover, there is also the added benefit of improving the visual appearance of stored grapes and reducing the rate of rachis browning during postharvest storage [20].

Endogenous ABA plays a role in the color development of strawberries during ripening by the up-regulation of ethylene production [19]. ABA treatment accelerated fruit color in harvested strawberries cv. Everest by increasing anthocyanin, phenolic contents and PAL activity during storage [19]. Moreover, ABA also induces anthocyanin accumulation in grapes (*Vitis* spp.) and in rambutans (*Nepelium lappaceum* L.) [34,35].

The involvement of ABA in fruit color development has also been observed in peaches and apples [8,12,36]. ABA contents in the peel and pulp of 'Jonagold' apples continuously increased in parallel with anthocyanin contents from pre-harvest through postharvest, which suggested that endogenous ABA may regulate anthocyanin biosynthesis at the preharvest stage and was involved in the senescence process, which was indicated by the increase of membrane permeability at the postharvest period [36]. In early-harvest cultivars, 'Tsugaru' apples showed that increasing ABA content coincided with anthocyanin content [12]. In peaches, ABA peaked when the color turned white and thereafter, the ABA decreased [8]. Therefore, it is suggested that ABA may play an important role in the coloring of both climacteric and non-climacteric fruits.

Phenolic metabolism

Phenolic compounds are secondary metabolites produced by plants as a defense mechanism against various biotic and abiotic stresses. They play an important role in the color and sensory characteristics of fruits and vegetables. The protective effects of these compounds against various chronic diseases such as cancer and cardiovascular diseases are well recognized [37]. The phenolic compounds in plants are regulated by genetics, cultural practices, and environmental factors such as temperature, light, soil, rainfall, fertilizers, and plant growth regulators [13].

Various agronomic strategies such as the alteration of environmental conditions and revised cultivation practices have been used to manage the phenolic content in crops [38]. The pre-harvest application of ABA is one strategy that can be used to increase phenolic compounds in several grape cultivars [13,20]. ABA treatment stimulates anthocyanins and total phenolic concentration as well as enhancing the antioxidant capacities in 'Muscadine' grapes [13,20]. The concentration of ellagic acid and flavonols (myricetin, quercetin and kaempferol) in 'Noble' grapes are increased by ABA treatment, but they are not found in 'Alachua' grapes [13]. This suggests that the effect of ABA on phenolic content may vary depending upon the cultivar and other environmental factors. However, ABA application of certain fruit species at critical periods of their developmental stages has a potential to enhance the key photochemicals that attract health conscious consumers and also increase the marketability of fruit.

Proanthocyanidins or condensed tannins are important polyphenolic compounds for grape and wine quality. In addition to their natural protective properties in fruit, they also have a benefit for human health [38,39]. They contribute to taste by conferring bitterness, astringency, and color stability [40,41]. To date, two enzymes for proanthocyanidin biosynthesis, anthocyanin reductase (ANR), and leucoanthocyanin reductase (LAR) have been characterized [42-44]. These two enzymes are responsible for the production of (-) epicatechin and (+) catechin, respectively. In a study about the involvement of ABA for controlling tannins biosynthesis in grape skin, it was found that ABA influenced tannin content and was indeed involved in tannin biosynthesis by decreasing LAR and ANR activities in green grapes during veraison without modifying their composition [45].

Cell wall metabolism and fruit softening

Fruit ripening is characterized by many physiological and biochemical changes. Progression in ripening and degradation of cell wall components results in fruit softening. These changes can affect various cell wall hydrolases. The increase of cell wall hydrolases activities may be regulated by ripening-related hormones. Ethylene has been reported to contribute to the increase activities of cell wall-modifying enzymes such as polygalacturonase and pectin esterase [46].

However, ABA may also directly affect the activity of fruit-softening enzymes; a study of ‘Zihua’ mango found that exogenous application of ABA at 19×10^{-6} M increased polygalacturonase activity and consequently promoted fruit softening [47].

Likewise, ABA has also been reported to induce the maturation of ‘Nam Dokmai’ and ‘Nang Klangwan’ mangoes [48]. ABA content in the skin and pulp of mangoes increased toward harvest [48]. This suggests that ABA may induce the maturation of mangoes. In bananas, ABA stimulates the activities of pectin methyl esterase (PME), cellulose, and it was most evident for pectate lyase (PL). ABA can also enhance softening with or without ethylene [17].

In addition, the increase in ABA coincides with a decrease in fruit firmness. In sweet cherries, ethylene concentration was low and had no direct effect in fruit ripening [49]. In contrast, ABA concentrations in the pulp of sweet cherries were low at the early stage of fruit development and reached a maximum before maturation, and finally declined during maturation. The significant increases in ABA after 29 days after full bloom coincided with softening, suggesting that ABA may play a role in inducing fruit maturation in sweet cherries [10]. In peaches, firmness decreased rapidly as ABA content decreased, but ethylene content increased [8]. In grapes, fruit firmness decreased with the decrease of ABA content, but there was no peak in the content of ethylene [20]. In addition, the application of ABA by injection into citrus fruit drops showed that the number of drops increased in proportion to the increase in ABA concentration [18]. ABA may induce cellulase and polygalacturonase activities, which are related to the abscission of citrus fruit.

Sugar and acid metabolism

It has been reported that accumulation of sugar content in fruit can be stimulated by plant hormone application, including by ABA [50]. Endogenous ABA is correlated to sucrose uptake, which may function to enhance sugar accumulation. In non-climacteric fruit such as strawberries, ABA content gradually accumulates with sugar accumulation, and acidity decreases during the late stage of fruit development [19]. ABA is associated with fruit maturation as well as sugar accumulation in sweet cherries [51]. Peaches begin to ripen at about 10 days after the stone-hardening stage and coincidentally with an increasing in endogenous ABA. Thereafter, ABA decreases and coincides with an increase in ethylene content and with the sugar-acid ratio [8]. Kobashi *et al.* [22] reported that spraying of ABA at 1.89×10^{-3} M at the onset of fruit ripening on ‘Hakuho’ peaches increases sugar concentration by temporarily increasing ABA content. The activities of sugar metabolizing enzymes have also been determined.

Regarding the activity of a predominant enzyme for sorbitol translocation, sorbitol oxidase (SOX) it is stimulated by ABA application in peaches. However, ABA application does not affect the activities of sucrose synthase, sucrose phosphate synthase and acid invertase [22].

ABA has a role in increasing the sugar content of citrus fruits since the glucose and fructose concentrations were increased by ABA injection without affecting the organic acid content [18]. The possible reasons are that ABA may release sugar from stored carbohydrates. It may also cause the import of sugar from leaves or other fruit parts via phloem transport. In order to understand the effect of ABA on sugar metabolism, further research is needed to investigate changes in carbohydrate content in the different parts of fruits, peels, and in phloem transport.

Sugar levels and ABA are linked to chilling injury effects in fruit. Mature green tomatoes stored at 2 ^{o}C for 12 days result in a 2 to 3 fold increase in free ABA [52]. Sucrose levels also increase at chilled temperatures in several tomato cultivars. Reducing sugar content in the peel of "Marsh" grapefruit (*Citrus paradise*) is highest when seasonal resistance due to chilling injury is the highest [53]. Moreover, water stress also induced an increase in ABA content in the fruit, which resulted in accelerated sugar accumulation in peaches by activating the sorbitol metabolism [54]. Recent reports provide strong evidence that the interaction between ABA and sugar may be a core mechanism in the regulation of the ripening of non-climacteric fruit [55].

Conclusions

There is much evidence that shows the role of ABA in fruit ripening and its involvement with fruit quality. Endogenous signals and environmental factors might affect ethylene biosynthesis primarily through ABA biosynthesis. In climacteric fruit, ABA might be involved in fruit ripening, at least through ethylene biosynthesis, by regulating the activity of ACO and ACS. The possible mechanism of ABA on regulating ripening of non-climacteric fruit may be through an ethylene independent gene expression by an unknown mechanism. Another hypothesis is that ABA plays a directly role through ABA receptors and several transcription factors from signaling pathways in order to regulate changes during ripening without mediating ethylene dependence or independent genes. However, the mechanism of how this happens is still unclear and further clarification is needed in this regard. The general scheme that shows this relationship between ABA and fruit ripening is shown in **Figure 2**.

ABA improves fruit color and nutrition levels by increasing anthocyanin and phenolic concentration, and therefore enhances antioxidant activity. The increase of cell wall hydrolases activities and fruit softening may also be regulated by ABA. Moreover, ABA is associated with fruit maturation and sugar accumulation. In addition, ABA not only regulates plant growth and development and stress responses but also functions as a medicine in animals and humans, therefore further research on ABA application needs to be extended to determine its potential benefits to human health.

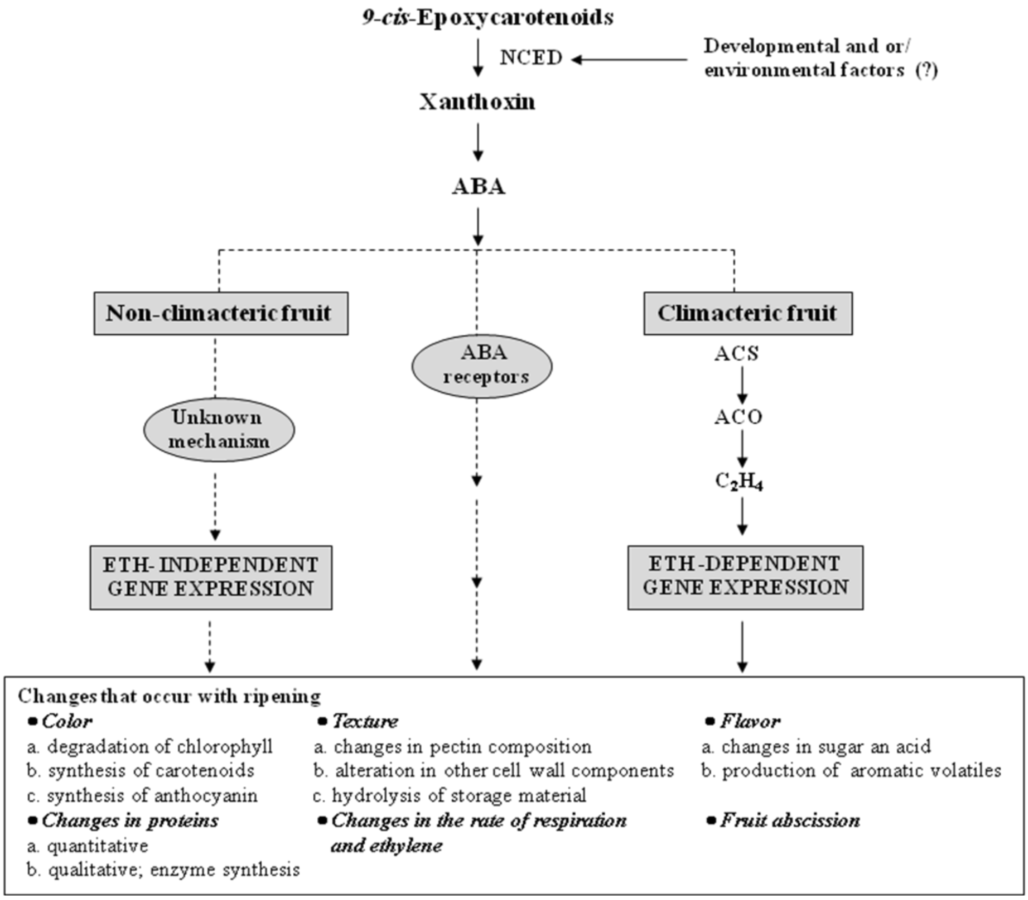

Figure 2 General scheme showing the relationship between ABA and fruit ripening of climacteric and non-climacteric fruit. ETH = ethylene. Solid lines indicate predicted interactions and dotted lines demonstrated interactions.

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References

[1] JAD Zeevaart and RA Creelman. Metabolism and physiology of abscisic acid. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 1988; **39**, 439-73.

[2] L Xiong and JK Zhu. Regulation of abscisic acid biosynthesis. *Plant Physiol.* 2003; **133**, 29-36.

[3] S Kondo, J Uthaibutra and H Gemma. Comparison of ACC, abscisic acid and anthocyanin content of some apple cultivars during fruit growth and maturation. *J. Jpn. Soc. Hort. Sci*. 1991; **60**, 505-11.

[4] S Kondo and A Tomiyama. Changes of free and conjugated ABA in the fruit of 'Satohnishiki' sweet cherry and the ABA metabolism after application of (s)-(+)-ABA. *J. Hort. Sci. Biotechol*. 1998; **73**, 467-72.

[5] S Setha, S Kondo, N Hirai and H Ohigashi. Xanthoxin, abscisic acid and its metabolite levels associated with apple fruit development. *Plant Sci*. 2004; **166**, 493-9.

[6] AB Bleecker and H Kende. Ethylene: A gaseous signal molecule in plants. *Annu. Rev. Cell. Dev. Biol*. 2000; **16**, 1-18.

[7] M Zhang, B Yuan and P Leng. The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. *J. Exp. Bot.* 2009; **60**, 1579-88.

[8] M Zhang, P Leng, G Zhang and X Li. Cloning and functional analysis of 9-*cis*-epoxycarotenoid dioxygenase (NCED) genes encoding a key enzyme during abscisic acid biosynthesis from peach and grape fruits. *J. Plant Physiol*. 2009; **166**, 1241-52.

[9] S Kondo and K Inoue. Abscisic acid (ABA) and 1-aminocyclopropane-1-carboxylic acid (ACC) content during growth of 'Satohnishiki' cherry fruit, and effect of ABA and ethephon application on fruit quality. *J. Hort. Sci*. 1997; **72**, 221-7.

[10] S Setha, S Kondo, N Hirai and H Ohigashi. Quantification of ABA and its metabolites in sweet cherries using deuterium-labeled internal standards. *Plant Growth Regul*. 2005; **45**, 183-8.

[11] HH Li, LR Hao, SS Wu, PC Guo, CJ Chen, LP Pan and H Ni. Occurrence, function and potential medicinal applications of the phytohormone abscisic acid in animals and humans. *Biochem Pharmacol*. 2011; **82**, 701-12.

[12] S Kondo, J Uthaibutra and H Gemma. Comparison of ACC, abscisic acid and anthocyanin content of some apple cultivars during fruit growth and maturation. *J. Jpn. Soc. Hort. Sci*. 1991; **60**, 505-11.

[13] AK Sandhu, DJ Gray, J Lu and L Gu. Effects of exogenous abscisic acid on antioxidant capacities, anthocyanins, and flavonol contents of muscadine grape (*Vitis rotundifolia*) skins. *Food Chem*. 2011; **121**, 982-8.

[14] M Vendrell and C Buesa. Relationship between abscisic acid content and ripening of apples. *Acta Hort*. 1989; **258**, 389-96.

[15] I Lara and M Vendrell. Development of ethylene-synthesizing capacity in preclimacteric apples; interaction between absicisic acid and ethylene. *J. Amer. Soc. Hort. Sci*. 2000; **125**, 505-12.

[16] Y Jiang, DC Joyce and AJ Macish. Effect of abscisic acid on banana fruit ripening in relation to the role of ethylene. *J. Plant Growth Regul*. 2000; **19**, 106-11.

[17] S Lohani, PK Trivedi and P Nath. Changes in activities of cell wall hydrolases during ethylene-induced ripening in banana: effect of 1-MCP, ABA and IAA. *Postharvest Biol. Tech*. 2004; **31**, 119-26.

[18] K Kojima, Y Yamada and M Yamamoto. Effect of abscisic acid contents of citrus fruit. *J. Japan Soc. Hort. Sci.* 1995; **64**, 17-21.

[19] Y Jiang and DC Joyce. ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. *Plant Growth Regul.* 2003; **39**, 171-4.

[20] CM Cantin, MW Fidelibus and CH Crisosto. Application of abscisic acid (ABA) at veraison advanced red color development and maintained postharvest quality of 'Crimson Seedless' grapes. *Posthavest Biol. Tech*. 2007; **46**, 237-41.

[21] VA Palejwala, B Amin, HR Parikh and VV Modi. Role of abscisic acid in the ripening of mango. *Indian J. Exp. Biol.* 1986; **24**, 722-5.

[22] K Kobashi, H Gemma and S Iwahori. Sugar accumulation in peach fruit as affected by abscisic acid (ABA) treatment in relation to some sugar metabolizing enzymes. *J. Jpn. Soc. Hort. Sci*. 1999; **68**, 465-70.

[23] BR Loveys. *How useful is a Knowledge of ABA Physiology for Crop Improvement*. *In*: Davies WJ and Jones HG (eds.). Abscisic Acid Physiology and Biochemistry. BIOS Scientific, UK, 1991, p. 245-60.

[24] I Lara and M Vendrell. Changes in abscisic acid levels, ethylene biosynthesis, and protein patterns during fruit maturation of 'Granny Smith' apples. *J. Amer. Soc. Hort. Sci.* 2000; **125**, 183-9.

[25] SY Han, N Kitahata, T Saito, M Kobayashi, K Shinozaki, S Yoshida and T Asamia. A new lead compound for abscisic acid biosynthesis inhibitors targeting 9-*cis*-epoxycarotenoid dioxygenase. *Bioorg. Med. Chem. Lett*. 2004; **14**, 3033-6.

[26] SY Han, N Kitahata, K Sekimata, T Saito, M Kobayashi, K Nakashima, K Yamaguchi-Shinozaki, K Shinozaki, S Yoshida and T Asami. A novel inhibitor of 9-*cis*-epoxycarotenoid dioxygenase in abscisic acid biosynthesis in higher plants. *Plant Physiol*. 2004; **135**, 1574-82.

[27] G Mazza. Anthocyanins in grapes and grape products. *Crit. Rev. Food Sci. Nutr*. 1995; **35**, 341-71.

[28] PK Boss, C Davies and S Robinson. Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L. cv. 'Shiraz' grape berries and the implications for pathway regulation. *Plant Physiol*. 1996; **111**, 1059-66.

[29] PK Boss, C Davies and S Robinson. Expression of anthocyanin biosynthesis pathway genes in red and white grapes. *Plant Mol. Biol*. 1996; **32**,565-9.

[30] T Ban, M Ishimaru, S Kobayashi, S Siosaki, NG Yamamoto and S Horiuchi. Abscisic acid and 2,4-dichlorophenoxyacetic acid affect the expression of anthocyanin biosynthetic pathway genes in 'Kyoho' grape berries. *J. Hort Science & Biotech.* 2003; **78**, 586-9.

[31] ST Jeong, N Goto-Yamamotob, S Kobayashi and M Esaka. Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. *Plant Sci*. 2004; **167**, 247-52.

[32] MC Peppi, MW Fidelibus and N Dokoozlian. Abscisic acid application timing and concentration affect firmness, pigmentation and color of 'Flame Seedless' grapes. *HortScience.* 2006; **41**, 1449-5.

[33] MC Peppi, MW Fidelibus and NK Dokoozlian. Application timing and concentration of abscisic acid affect the quality of 'Redglobe' grapes. *J. Hort. Sci. Biotech*. 2007; **82**, 304-10.

[34] S Kondo and M Kawai. Relationship between free and conjugated ABA levels in seeded and gibberellins-treated seedless, maturing 'Pione' grape berries. *J. Amer. Soc. Hort. Sci*. 1998; **12**, 3750-4.

[35] S Kondo, P Posuya, S Kanlayanarat and N Hirai. Abscisic acid metabolism during development and maturation of rambutan fruit. *J. Hort. Sci. Biotechnol*. 2001; **76**, 235-41.

[36] J Uthaibutra and H Gemma. Changes in absicisic acid content of peel and pulp of 'Jonagold' apples during pre- and post-harvest periods. *J. Japan. Soc. Hort. Sci.* 1991; **60**, 443-8.

[37] ICW Arts and PCH Hollman. Polyphenols and disease risk in epidemiologic studies1-4. *Am. J. Clin. Nutr*. 2005; **81**, 317S-325S.

[38] D Treutter. Managing phenol contents in crop plants by phytochemical farming and breeding-visions and constraints. *Int. J. Mol. Sci*. 2010; **11**, 807-57.

[39] PM Aron and JA Kennedy. Flavan-3-ols: nature, occurrence and biological activity. *Mol. Nutr. Food Res*. 2008; **52**, 79-104.

[40] F Brossaud and V Cheynier. Bitterness and astringency of grape and wine polyphenols. *Aust. J. Grape Wine Res*. 2001; **7**, 33-9.

[41] C Malien-Aubert, O Dangles and HJ Amiot. Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra- and intermolecular copigmentation. *J. Agric. Food Chem*. 2001; **49**, 170-6.

[42] DY Xie, SB Sharma, NL Paiva, D Ferreira and RA Dixon. Role of anthocyanidin reductase, encoded by BANYULS in plant flavonoid biosynthesis. *Science* 2003; **299**, 396-9.

[43] J Pfeiffer, C Kuhnel, J Brandt, D Duy, N Punyasiri, G Forkmann and TC Fischer. Biosynthesis of flavan-3-ols by leucoanthocyanidin 4-reductases and anthocyanidin reductases in leaves of grape (*Vitis vinifera* L.), apple (*Malus* × *domestica* Borkh.) and other crops. *Plant Physiol. Biochem.* 2006; **44**, 323-34.

[44] S Gagne, S Lacampagne, O Claisse and L Geny. Leucoanthocyanidin reductase and anthocyanidin reductase gene expression and activity in flowers, young grapes and skins of *Vitis vinifera* L. cv. Cabernet-sauvignon during development. *Plant Physiol. Biochem*. 2009; **47**, 282-90.

[45] S Lacampagne, S Gagné and L Gény. Involvement of abscisic acid in controlling the proanthocyanidin biosynthesis pathway in grape skin: New elements regarding the regulation of tannin composition and leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR) activities and expression. *J. Plant Growth Regul*. 2010; **29**, 81-90.

[46] Z Singh and SP Singh. *Mango*. *In*: Rees D and Orchard J (eds.). Crop Post-Harvest: Science and Technology: Perishables, Vol 3. Blackwell Publishing Ltd, Oxford, 2011, p. 108-42.

[47] YC Zhou, YL Tang, XJ Tan and JY Guo. Effects of exogenous ABA, GA_3 and cell-

wall-degrading enzyme activity, carotenoid content in ripening mango fruit. *Acta Phytophysiol Sin*. 1996; **22**, 421-6.

[48] S Kondo, K Sungcome, S Setha and N Hirai. ABA catabolism during development and storage in mangoes: influence of jasmonates. *J. Hortic. Sci. Biotechnol*. 2004; **76**, 891-6.

[49] C Hartmann. Biochemical changes in harvested cherries. *Postharvest Biol. Technol*. 1992; **258**, 89-96.

[50] J Daie. Carbohydrate partitioning and metabolism in crops. *Hort. Rev*. 1985; **7**, 69-108.

[51] S Kondo and H Gemma. Relationship between abscisic acid (ABA) content and maturation of the sweet cherry. *J. Japan Soc. Hort. Sci*. 1993; **62**, 63-8.

[52] PM Ludford and LL Hillman. Abscisic acid content in chilled tomato fruit. *HortScience* 1990; **25**, 1265-7.

[53] AC Purvis, K Kawada and W Grierson. Relationship between midseason resistance to chilling injury and reducing sugar level in grapefruit peel. *HortScience* 1979; **14**, 227-9.

[54] K Kobashi, H Gemma and S Iwahori. Abscisic acid content and sugar metabolism of peaches grow under water stress. *J. Amer. Soc. Hort. Sci*. 2000; **125**, 425-8.

[55] C Li, H Jia, Y Chai and Y Shen. Abscisic acid perception and signaling transduction in strawberry; A model for non-climacteric fruit ripening. *Plant Signaling & Behavior* 2011; **6**, 1950-3.