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Advances in metabolism and genetic control of astringency in persimmon (*Diospyros kaki* Thunb.) fruit: A review

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ABSTRACT

Persimmon fruits accumulate proanthocyanidins during development. Proanthocyanidins form insoluble complexes with saliva proteins, which promote a sensation of dryness in the mouth called astringency. Persimmon cultivars are classified according to pollination and the presence of astringency at harvest. The different characteristics between persimmon cultivars lead to different metabolic pathways for the loss of astringency. The aim of this review is to gather information about the advances in the literature on the metabolism of astringency in persimmon fruit. Persimmon proanthocyanidins are mostly catechins or epicatechins, biosynthesized from substrates of the shikimic acid pathway, via biosynthetic pathways of phenylpropanoids and flavonoids, from gallic acid units, or from precursors of the anthocyanin pathway. Two enzymes have been identified as key enzymes to this pathway: leucoanthocyanidin reductase and anthocyanidin reductase. Non-astringent cultivars accumulate proanthocyanidins during fruit development, however, the expression of genes that encode essential enzymes of the biosynthetic pathway is reduced in the early stages, interrupting the accumulation of proanthocyanidins. Acetaldehyde is the responsible compound for the polymerization of proanthocyanidins and the consequent reduction of astringency. In certain non-astringent cultivars, proanthocyanidins are polymerized by acetaldehyde in the final stages of fruit development and transported to the vacuole, blocking the biosynthetic pathway. Two transport proteins, GST and MATE, are supposed to be essential in this process. In other non-astringent cultivars, the loss of astringency can happen due to the dilution of proanthocyanidins as a result of the increase in fruit volume. The removal of astringency in astringent cultivars occurs through the application of postharvest treatments that induce the synthesis of acetaldehyde by the fruit. Exposure to ethylene, high CO_2 concentration or ethanol are the most used technologies for this purpose. Despite the advances made in the last decade, the astringency metabolism is complex and involves several metabolic pathways that still need to be elucidated for a better understanding of the effects of astringency removal in persimmon fruit.

1. Introduction

The development of persimmon fruit (*Diospyros kaki* Thunb.) is marked by the accumulation of proanthocyanidins, also known as condensed tannins (Min et al., 2014) or soluble tannins, which make the flesh astringent (Taira, 1996). Astringency is perceived in the mouth as an unpleasant sensation of dryness, due to the formation of insoluble complexes between oral proteins and proanthocyanidins released from the vacuole of specialized cells in the food matrix during the chewing process (Dinnella et al., 2009). In some cultivars, the astringency is lost during fruit development, while in others, the fruit remains astringent even when ripe. Depending on pollination and flesh color, persimmon cultivars can be divided into two groups: (1) Pollination Constant (PC), when pollination does not change the color of the flesh; and (2) Pollination Variant (PV), when flesh color darkens due to the presence of seeds, or in the absence of these, it remains light (Hume, 1914). Depending on the presence or absence of astringency at the time of harvest, cultivars can also be divided into non-astringents (NA) or astringents (A). Based on these classifications, persimmon cultivars can be classified into four groups: (1) Pollination Constant Non-Astringent (PCNA); (2) Pollination Variant Non-Astringent (PVNA); (3) Pollination Variant Astringent (PVA); and (4) Pollination Constant Astringent

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(PCA) (Ito, 1971; Kitagawa and Glucina, 1984; Itoo, 1986).

Astringency natural loss occurs in PCNA and PVNA types of fruits through acetaldehyde-mediated coagulation of soluble tannins (Kitagawa and Glucina, 1984; Xu et al., 2017). Differently, PCA and PVA fruits must undergo artificial removal of astringency before consumption, also called deastringency, which is carried out by the induction of tannins polymerization, also mediated by acetaldehyde (Matsuo and Itoo, 1982). Several studies address the artificial astringency removal process in persimmons (Antoniolli et al., 2000; Ben-Arie and Sonego, 1993; Ben-Arie and Guelfat-Reich, 1976; Del Bubba et al., 2009; Kato, 1990; Novillo et al., 2015; Salvador et al., 2007), and deastringency-related genes and transcription factors differently regulated in the astringency removal process, whether natural or artificially induced, were recently identified (Fang et al., 2016; Jin et al., 2018; Wang et al., 2017a; Xu et al., 2017; Zhu et al., 2018a). Edagi and Kluge (2009a, 2009b) reviewed the artificial removal of astringency in persimmons regarding its biochemical, physiological, and technological aspects, while Akagi et al. (2011) reviewed the proanthocyanidins biosynthesis in persimmon fruit. Some more recent publications were also found, such as those published by Wu et al. (2022) and Dong et al. (2022). However, Wu et al. (2022) provide a comprehensive synthesis on astringency while Dong et al. (2022) discusses the current status of persimmon in China, addressing phytotechnical aspects, such as rootstocks and breeding programs. Despite such publications, there is still a lack of recent studies broadly addressing the biochemical and genetic control mechanism of astringency in persimmon fruit. Based on these considerations, this review aims to gather information available in the literature on the biochemical metabolism and genetic control related to astringency of persimmons, from the process of formation and accumulation of proanthocyanidins in the fruit flesh to the loss of astringency, whether natural or artificial.

2. Astringency, biosynthesis and tannin accumulation in the fruit flesh

Persimmons are characterized by astringency in the flesh (Taira, 1996), due to the accumulation of high levels of soluble condensed tannins (Akagi et al., 2009; Luo et al., 2014) in the tannin cell vacuoles (Ikegami et al., 2005; Kitagawa and Glucina, 1984; Tessmer et al., 2016). Tannins are flavonoid-like phenolic compounds (Dixon et al., 2005), formed in secondary metabolism (Kays, 1991). The presence of tannins is perceived as astringency when they are found in their condensed form. Condensed tannins are molecules of high molecular weight (Ikegami et al., 2005), also known as proanthocyanidins (Akagi et al., 2009), which receive this nomenclature because the extension units of condensed tannins are converted to anthocyanidins in acid hydrolysis, conferring the fruit pigmentation (Porter, 1989).

2.1. Chemical structure

Proanthocyanidins (Fig. 1A) are mostly polyphenols originated from flavan-3-ols building blocks (catechins or epicatechins) (Fig. 1C–F) with flavan-3,4-diol units (leucoanthocyanidins) (Dixon and Paiva, 1995; Dixon et al., 2005; Xie and Dixon, 2005) (Fig. 1B). Four distinct types of flavan-3-ol are known due to the existence of asymmetric atoms in the C2 and C3 positions: (1) (2R, 3R)-cis-flavan-3-ols or (-)-epicatechin (Fig. 1C); (2) (2S, 3S)-cis-flavan-3-ols or (+)-epicatechin (Fig. 1D); (3) (2S, 3R)-trans-flavan-3-ols or (-)-catechin (Fig. 1E), and (4) (2R, 3S)-trans-flavan-3-ols or (+)-catechin (Fig. 1F) (Qian et al., 2015). The structure of proanthocyanidins can vary depending on the origin of the flavan-3-ol units (hydroxylation pattern and stereochemistry), as well as other factors such as degree of polymerization of the molecule, stereochemistry of the linkage to the lower unit, and presence or absence of modifications (Dixon et al., 2005).

2.2. Biosynthesis

Understanding the biosynthetic pathway of proanthocyanidins is a difficult task due to multiple potential stereochemistries, difficulty in finding substrates for laboratory studies, and instability and easy oxidation of the compounds (Dixon et al., 2005). Greater advances have been achieved from genetic and molecular studies (Akagi et al., 2011;

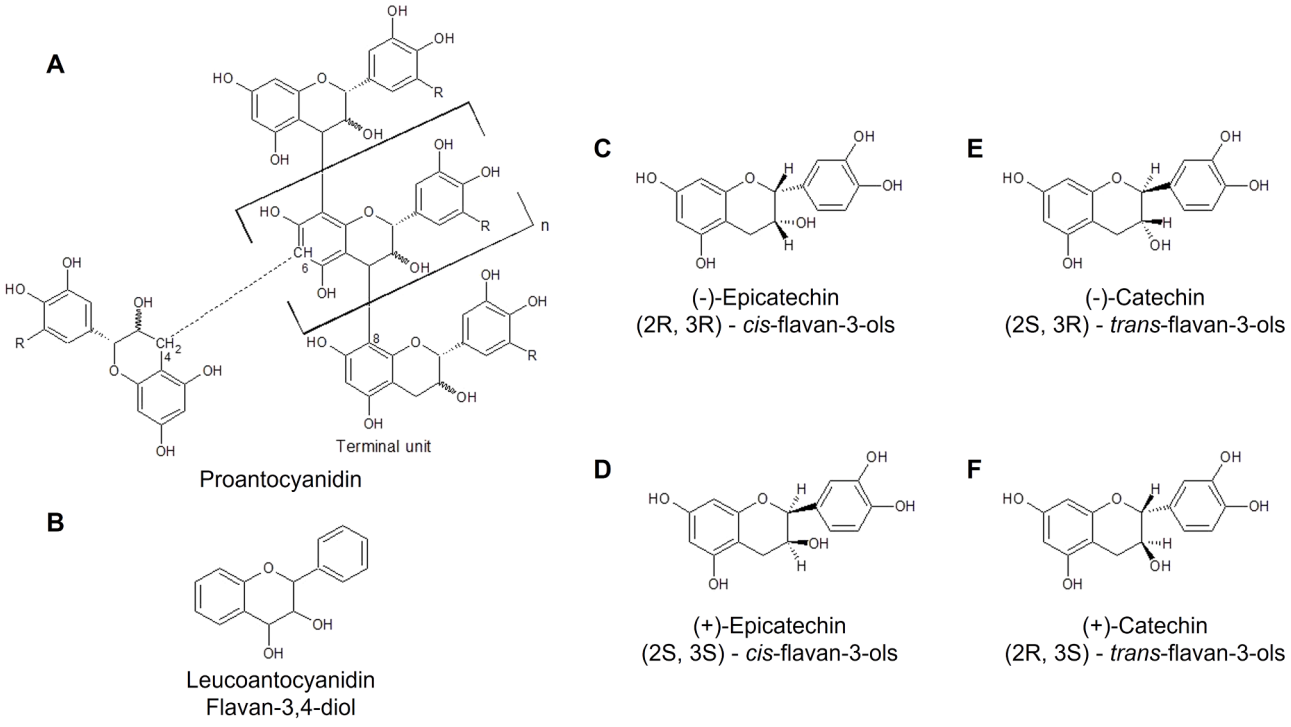

Fig. 1. Structure of proanthocyanidin (A), leucoanthocyanidin (B), (-)-epicatechin (C), (+)-epicatechin (D), (-)-catechin (E) and (+)-catechin (F). R from the proanthocyanidin molecule (A) = H or OH.

Dixon et al., 2005; Jin et al., 2018; Wang et al., 2017a; Xu et al., 2017; Zhu et al., 2018a), which unveiled the genetic basis of proanthocyanidin synthesis in astringent and non-astringent persimmon cultivars.

The catechin and epicatechin units that give rise to proanthocyanidins are formed in the secondary metabolism from metabolites of the shikimic acid pathway (Dixon et al., 2005; Kays, 1991) and from the biosynthetic pathways of phenylpropanoids and flavonoids (Akagi et al., 2011; Xie et al., 2003) (Fig. 2).

Among the possible biosynthesis pathways, proanthocyanidins may originate directly from gallic acid units (Fig. 2) that are transported to the vacuole of specialized tannin cells. Proanthocyanidins synthesis can also originate from phenylalanine, a product of the shikimic acid pathway, which normally results in the formation of anthocyanins through phenylalanine ammonia lyase (PAL). However, the anthocyanin formation pathway is diverted by the activity of leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR), which promote the biosynthesis of the proanthocyanidin starter units, catechin and epicatechin, respectively (Fig. 2).

In vitro assays revealed that at an early point of the pathway, the biosynthesis of catechin and epicatechin also depends on the action of a dihydroflavonol reductase (DFR) enzyme, which leads to the formation of leucodelphinidin and leucocyanidin (Dixon et al., 2005). Leucodelphinidin and leucocyanidin are formed from dihydromyricetin and dihydroquercetin, respectively (Fig. 2). LAR activity generates gallocatechin (2,3-trans-flavan-3-ols) from leucodelphinidin, or catechin (2,3-trans-flavan-3-ols) from leucocyanidin. For this, leucodelphinidin and leucocyanidin are primarily converted to delphinidin and cyanidin, by the action of the enzyme anthocyanidin synthase (ANS). Then ANR catalizes the formation of epigallocatechin (2,3-trans-flavan-3-ols), from delphinidin, and epicatechin (2,3-cis-flavan-3-ols), from cyanidin (Fig. 2). Afterwards, the flavan-3-ols units are transported to the vacuole and used for the synthesis of proanthocyanidins.

Two specific genes of proanthocyanidin biosynthesis, *DkLAR* and *DkANR* have been identified and isolated in persimmon (Akagi et al., 2009; Ikegami et al., 2005; Ikegami et al., 2007). Previously, Xie et al. (2003) and Tanner et al. (2003) described LAR as one of the key enzymes in the proanthocyanidin formation pathway. Bogs et al. (2006) demonstrated that the expression of *LAR* and *ANR* is essential for proanthocyanidin synthesis and determines the accumulation and composition of proanthocyanidins in grape berries. Akagi et al. (2009) demonstrated that LAR and ANR are critical enzymes in the biosynthesis of proanthocyanidins in persimmons. The *ANR* gene is one of the key regulators of the content and composition of proanthocyanidins in non-astringent persimmon fruit and in fruits submitted to the astringency removal process. Using suppression subtractive hybridization to isolate genes that are differentially expressed in astringent fruit and in fruit treated with ethanol for astringency removal, it was observed that the *ANR* gene is down-regulated, supporting the presence of epicatechins in astringent persimmons (Ikegami et al., 2007). Additionally, the reduction in the proanthocyanidin content in PCNA-type persimmon was found to be more related to the downregulation of *ANR* (Akagi et al., 2009), which drives the carbon flow to the branch of epicatechins synthesis. The existence of (epi)gallocatechin, epigallocatechin-3-O-gallate and epicatechin-3-O-gallate in proanthocyanidins of astringent persimmon was also confirmed previously (Gu et al., 2008). During fruit development, persimmon proanthocyanidins consist of molecules originated from the condensation of epigallocatechin and epigallocatechin-3-gallate units, catechin, gallocatechin, epicatechin and 2,3-cis epicatechin-3-O-gallate (Akagi et al., 2009).

Other important enzymes in proanthocyanidin biosynthesis that act upstream of LAR and ANR, are flavonoid-3′-hydroxylase (F3′H) and flavonoid-3′5′-hydroxylase (F3′5′H). F3′H catalyzes the formation of eriodictyol from naringenin as well as the formation of dihydroquercetin from dihydrokaempferol. F3’5’H shares the same substrates, naringenin and dihydrokaempferol for the formation of pentahydroxyflavonone and dihydromyricetin, respectively (Fig. 2). In grapevines, these

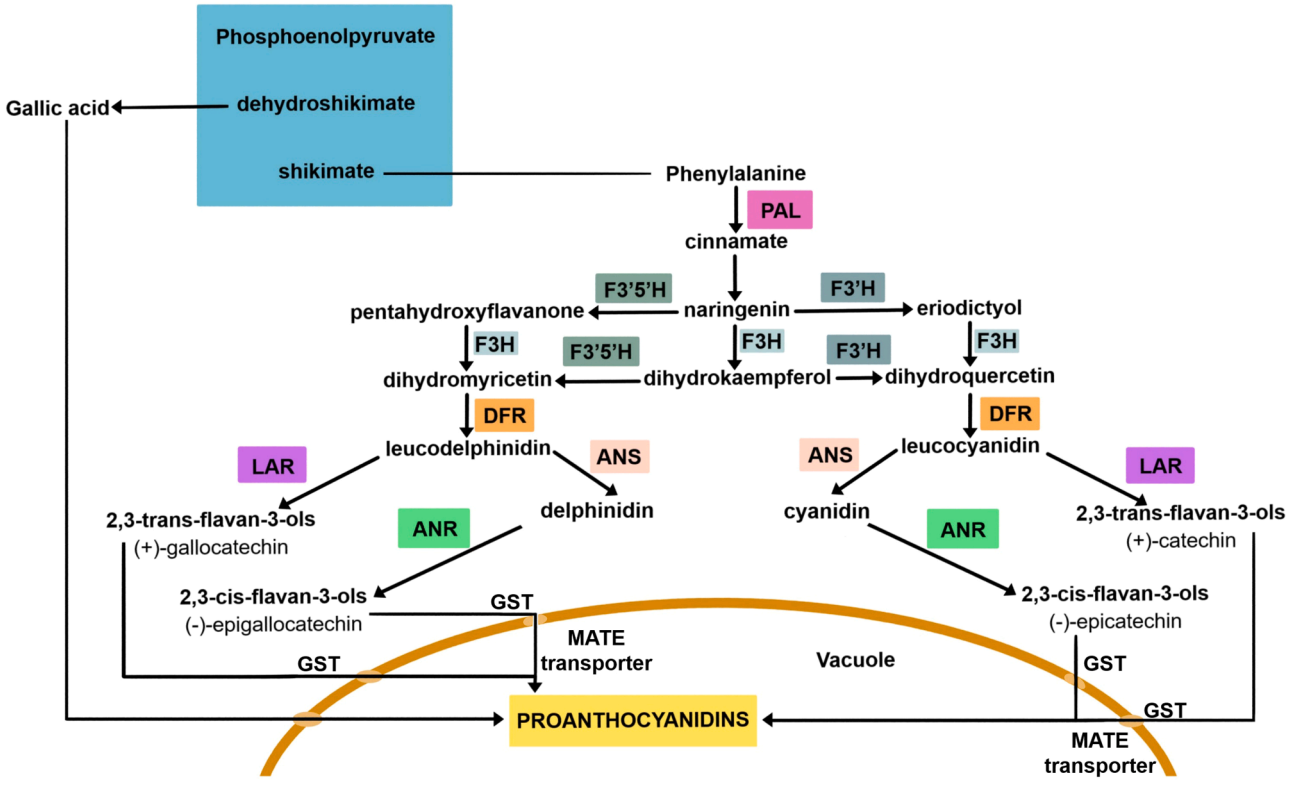

Fig. 2. Proanthocyanidin formation pathways. PAL: phenylalanine ammonia lyase; F3H: flavonol-3-hydroxylase; F3’5’H: flavonoid-3’5’-hydroxylase; F3’H: flavonoid-3’-hydroxylase; DFR: dihydroflavonol 4-reductase; LAR: leucoanthocyanidin reductase; ANR: anthocyanidin reductase; GST: glutathione S-transferase; MATE: multidrug transporter and toxic compound extrusion transporter.

enzymes hydroxylate the B-ring of flavonoids in a cytochrome b5-dependent manner (Bogs et al., 2006). In non-astringent persimmon (PCNA), *F3′5′H* is remarkably downregulated during the period of reduced proanthocyanidin biosynthesis, while *F3′H* expression remained continuous (Akagi et al., 2009). The results found by Akagi et al. (2009) suggest that F3′5′H may also be involved in reducing astringency in PCNA cultivars.

It was reported that the MBW complex (MYB-bHLH-WD40), composed by the transcription factors DkMYB2 and DkMYB4, as well as DkMYC1 (basic helix-loop-helix (bHLH) transcription factor) and DkWDR1 (WD40-repeat gene), contribute to the process of proanthocyanidin biosynthesis in persimmon (Naval et al., 2016). The protein complex acts on the regulation of the BANYLUS gene (BAN), involved in the activation of ANR in Arabidopsis, through the conversion of anthocyanidins in the initial unit 2,3-transflavan-3-ols (Xie et al., 2003; Baudry et al., 2004).

The biosynthesis of proanthocyanidins in persimmon is complex and still unclear. Although many gaps have been filled in the last few years, much remains to be unreaveed in this secondary metabolism pathway. Zheng et al. (2021) generated a full-length reference transcriptome of the hexaploid persimmon, which may help future discoveries about the genetic control network of proanthocyanidins in persimmon. The authors also identified a SBP protein, a bZIP transcriptor factor and a MYB protein as novel candidates to regulate proanthocyanidin biosynthesis in Chinese persimmon.

3. Natural loss of astringency in persimmons

Non-astringent cultivars (PCNA and PVNA) lose their astringency until harvest time due to specific metabolic factors. This type of cultivar was found to have originated from a spontaneous phenotypic mutation (Ikegami et al., 2005; Yonemori et al., 2000a, 200b). Non-astringent fruits accumulate large amounts of proanthocyanidins during development (Chen et al., 2017), however, tannin accumulation is ceased in the early stages of fruit development, when the expression of flavonoid biosynthesis genes, such as *PAL, F3H, F3′H, F3′5′H,* and *DFR* is down-regulated (Ikegami et al., 2005), and the process of natural loss of astringency begins.

PCNA persimmon cultivars are commonly classified into two groups, according to their genotypic differences: the Chinese PCNA (CPCNA) and the Japanese PCNA (JPCNA) (Chen et al., 2017). Both genotypes occur as a result of spontaneous mutations, and have distinct genetic control (Zheng et al., 2021). The genetic traits of astringency loss in JPCNA are controlled by a single locus *AST* (*ASTRINGENCY*) recessive allele, (Kanzaki et al., 2001; Yonemori et al., 2000a), while the genetic trait of CPCNA persimmon is controlled by the *CPCNA* (*CHINESE PCNA*) locus, dominant allele (Ikegami et al., 2006). There are differences regarding the natural astringency loss pathways of different types of non-astringent cultivars, mainly between JPCNA and CPCNA (Mo et al., 2016). PVNA persimmons are not classified into groups.

3.1. Natural loss of astringency in CPCNA persimmons

Acetaldehyde is responsible for the polymerization and insolubilization of proanthocyanidins during the natural astringency loss process, as well as in the induced deastringency (Ito, 1971; Matsuo and Itoo, 1982; Mo et al., 2016; Novillo et al., 2015; Pesis and Ben-Arie, 1984; Taira et al., 1996; Wang et al., 2017b). After insolubilization, proanthocyanidins do not bind to saliva proteins, making astringency unnoticeable. The mechanism of astringency loss in CPCNA persimmons is likely induced by the polymerization of soluble to insoluble proanthocyanidins during the later stages of fruit development. The genes *ADH* and *PDC*, encoding the enzymes alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC), respectively, are key regulators of this process in CPCNA persimmons (Mo et al., 2016).

Alcohol dehydrogenase and pyruvate decarboxylase are the main enzymes that catalyze acetaldehyde production (Strommer, 2011). The analysis of 454 transcriptome sequences of CPCNA persimmons treated with ethanol showed that *ADH* and *PDC* play an important role in the polymerization process of proanthocyanidins during the loss of astringency promoted by ethanol treatment (Luo et al., 2014). However, the authors did not determine whether *ADH* and *PDC* act in the uninduced coagulation of tannins.

The synthesis of acetaldehyde in persimmon fruit can occur in two pathways (Fig. 3). In the first pathway, PDC produces acetaldehyde from the pyruvate formed in glycolysis, with the release of CO_2. In the other pathway, ADH catalyzes the conversion of ethanol into acetaldehyde, regenerating NAD^+ for glycolysis (Ben-Arie and Sonego, 1993; Ito, 1971; Taira, 1996; Yamada et al., 2002). The reaction catalyzed by ADH is reversible, and the biosynthesized acetaldehyde can also be converted to ethanol, with the expense of NAD^+.

Chen et al. (2021) demonstrated that DkMYB14 is an important transcription factor in the process of loss of astringency in CPCNA type, acting in a bifunctional way. According to these authors, DkMYB14 acts as a repressor of proanthocyanidin biosynthesis, preventing the formation of new molecules, and also acts as an activator, promoting acetaldehyde biosynthesis and, in turn, the insolubilization of proanthocyanidins. As a repressor, DkMYB14 inhibits the expression of the *DkF3′5′H* and *DkANR* genes, and as an activator, it upregulates the expression of *DkADH1* and *DkPDC,* promoting the formation of acetaldehyde. Additionally, the laccase gene, *DkLAC2*, has been considered as a candidate in the regulation of the proanthocyanidin biosynthesis pathway in CPCNA persimmons (Zaman et al., 2022). It was shown that *DkLAC2* was downregulated by DkmiR397, a microRNA in 'Eshi 1′ persimmon (Zaman et al., 2022) that may be a regulator of proanthocyanidin polymerization in CPCNA persimmons.

Mueller et al. (2000) mention that AN9 is a GST (Glutathione S-transferase) transporter protein found in *Petunia hybrida* and essential for the transport of anthocyanins from the synthesis site in the cytoplasm to the vacuole, where they are stored. The *Bz2* gene was identified in maize and encodes a type III GST during the last step of the anthocyanin synthesis pathway, when the pigment is transported to the vacuole as glutathionated cyanidin 3-glucoside (Alfenito et al., 2007). In *Arabidopsis*, a transporter from the multidrug and toxic compound extrusion transporter (MATE) family acts as a flavonoid/H^+ antiporter in vacuolar membranes of cells where proanthocyanidin is synthesized (Marinova et al., 2007). Yang et al. (2016) characterized the *MATE1* gene, which also encodes a protein of the MATE family in CPCNA persimmons, and described its role as a proanthocyanidin transporter in the vacuolar membrane (Fig. 2). Chen et al. (2017) suggested that the biosynthesis of proanthocyanidins in CPCNA persimmons occurs in the endoplasmic reticulum (cytosolic part) and that the transport of tannin cells to the vacuole occurs in the final stages of fruit development through GST and MATE transporters (Fig. 2). In the vacuole, proanthocyanidins insolubilization is mediated by acetaldehyde, resulting in the loss of astringency in fruit. Some cultivars showing CPCNA phenotype are 'Eshi 1′, 'Luotian-Tianshi' and 'Tian bao gai' (Ikegami et al., 2011; Sato and Yamada, 2016).

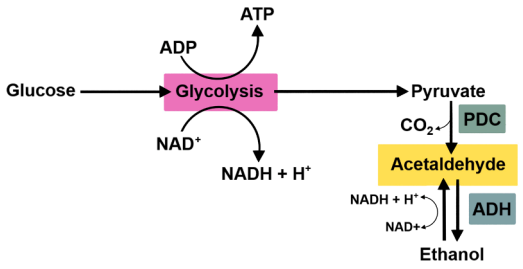

Fig. 3. Acetaldehyde formation pathways through ethanol and pyruvate. PDC: pyruvate decarboxylase; ADH: alcohol dehydrogenase.

3.2. Natural loss of astringency in JPCNA persimmons

The loss of astringency in JPCNA persimmons is mainly due to the dilution of proanthocyanidins during fruit development, where the increase in fruit volume leads to the dilution of proanthocyanidins (Yonemori et al., 2003). Tannin cell development ceases in the early stages of fruit growth. Thus, the volume of tannin cells is smaller in relation to the fruit size (Yonemori and Matsushima, 1985), which reduces proanthocyanidin concentration in the flesh. The tannins present in this type of fruit do not readily coagulate when exposed to acetaldehyde (Yonemori et al., 2003). The genetic trait of JPCNA is controlled by the recessive allele, *ast*, in a single *AST* (*ASTRINGENCY*) locus (Akagi et al., 2011; Nishiyama et al., 2018a; Yonemori et al., 2000a). Nishiyama et al. (2018a, 2018b) delimited the AST locus region in hexaploid persimmon using the shuttle mapping approach, and observed that AST may be a transcriptional regulator involved in the proanthocyanidin biosynthetic pathway. It was found that the expression of genes encoding proanthocyanidin biosynthesis enzymes is downregulated during the development of JPCNA type persimmons. (Akagi et al., 2009).

Some of the transcription factors involved in the synthesis of proanthocyanidins in persimmons are members of the MYB family (Akagi et al., 2009). It was demonstrated that the expression of Myb4 is reduced only at the end of the JPCNA persimmon fruit development (Akagi et al., 2011), supporting the hypothesis that reduces proanthocyanidin production in the flesh with the advancement of development, in addition to the dilution of proanthocyanidins. Some examples of JPCNA cultivars are 'Youhou', 'Gosho', 'Fuyu', 'Yubeni', 'Suruga', 'Taishuu', 'Izu', 'Shinshuu', 'Soshu', 'Kanshu', 'Kishu', 'Kurokuma', 'Yamatogosho' and 'Hanagosho' (Sato and Yamada, 2016).

3.3. Natural loss of astringency in PVNA persimmons

The seeds of PVNA cultivars produce a sufficient amount of volatile compounds, including acetaldehyde and ethanol, capable of polymerizing proanthocyanidins in the flesh. In addition, PVNA persimmons are more permeable to volatile compounds (Akagi et al., 2011). The *PDC2* gene, which encodes a pyruvate decarboxylase in *D. kaki*, seems to control the production of ethanol in the seeds (Itai et al., 2007). Some examples of cultivars with PVNA phenotype are 'Huashi 1', 'Zenjimaru', 'Mizushima', 'Shogatsu' and 'Amahyakune' (Ito, 1971; Sato and Yamada, 2016).

4. Deastringency in astringent cultivars

Astringent cultivars are characterized by the accumulation of marked amounts of soluble tannins in the flesh (Chen et al., 2017; Ito, 1971; Luo et al., 2014; Taira, 1996), becoming edible after postharvest treatments that remove the astringency (Matsuo and Itoo, 1982; Yamada et al., 2002). Such cultivars may belong to either the PCA or PVA groups. For astringency not to be perceived, proanthocyanidin levels should be less than 0.1% fresh mass (Taira, 1996). Some technologies were developed for astringency removal of persimmon fruit (Ikegami et al., 2005). These techniques include exposing the fruits to (1) ethanol (Antoniolli et al., 2000; Matsuo et al., 1976; Novillo et al., 2015); (2) atmospheres rich in CO_2 (concentrations usually above 60%, depending on the cultivar) in order to induce hypoxia (Kato, 1990; Matsuo et al., 1976; Novillo et al., 2014); or (3) ethylene applications (Itamura et al., 1997; Taira, 1996).

4.1. Mechanism of ethanol and carbon dioxide astringency removal

During glycolysis, the six-carbon glucose (C6H12O6) is broken down to the three-carbon pyruvate (C3H6O3). Under normal oxygen conditions in the mitochondria, pyruvate is oxidized to carbon dioxide in the citric acid cycle (Voet et al., 2014). Under anaerobic conditions, pyruvate is eventually metabolized in alcoholic and lactic fermentation reactions, during anaerobic metabolism in the cytosol (Nelson and Cox, 2014).

The exposure of astringent persimmons to high concentrations of CO_2 promotes hypoxia that activates the enzymes pyruvate decarboxylase and alcohol dehydrogenase in the alcoholic fermentation pathway (Fig. 3), with production of acetaldehyde and ethanol (Chen et al., 2017; Nelson and Cox, 2014; Taira, 1996). After the biosynthesis of pyruvate, in the fermentative pathway, the regeneration of NAD^+ from NADH is necessary for the continuity of glycolysis (Voet et al., 2014) and for the reduction of acetaldehyde concentration, which is toxic for the cell (Hribal et al., 2000). Alcohol dehydrogenase then rapidly metabolizes acetaldehyde to ethanol, regenerating NAD^+, in a reversible reaction.

With the application of ethanol vapor to persimmon fruit, the activity of alcohol dehydrogenase takes place in the reverse reaction (Nelson and Cox, 2014; Oshida et al., 1996) (Fig. 3), catalyzing the formation of acetaldehyde from ethanol, without the activity of pyruvate decarboxylase. Acetaldehyde later reacts with the proanthocyanidins in the flesh and polymerizes these compounds making them insoluble.

The induction of hypoxia is another strategy used for astringency removal in persimmon fruit. Ethylene response factors (ERFs) are transcription factors belonging to a family that is responsible for signaling the ethylene response pathway (Müller and Munné-Bosch, 2015) and have been reported as major regulators of the hypoxia-induced stress response (Licausi et al., 2010; Papdi et al., 2015). In *Arabidopsis*, the role of four ERFs has been previously described in the hypoxia response mechanism, *HRE1, HRE2, RAP2.2* and *RAP2.12* (Licausi et al., 2010; Papdi et al., 2015). The action of ERFs in response to low O_2 concentration occurs, in part, through the transcription of genes encoding the enzymes pyruvate decarboxylase and alcohol dehydrogenase (Yang et al., 2011).

Min et al. (2014) treated persimmons with CO_2 to remove astringency and characterized twelve candidate ERF genes (*DkERF11-22*) regarding the response to high CO_2 concentration, from which *DkERF19* e *DkERF22* showed trans-activation effects on the promoters of two pyruvate decarboxylase genes (*DkPDC2* and *DkPDC3*). Treatment of persimmons with CO_2 was also related to the up-regulation of *DkADH1* (alcohol dehydrogenase regulator), *DkPDC1* and *DkPDC2*, promoted by *DkERF9* and *DkERF10* (Min et al., 2012). However, in addition to ERFs, other regulatory mechanisms of transcription factors involved in the response to persimmon deastringency remain unknown. Transcription factors from the MYB family (some involved in the regulation of proanthocyanidin synthesis) may also be involved in the response to stress caused by hypoxia acting earlier than ERFs, as supported by the observation that *DkMYB6* possibly shares the same target genes than ERFs, trans-activating *DkPDC2* and *DkPDC3*, but also trans-activating *DkERF9* and *DkERF19* (Fang et al., 2016), which suggests a link between MYBs and ERFs in a possible cascate of transcriptional regulation that has yet to be unraveled. Eight other transcription factors involved in the response to hypoxia induced by CO_2 destanization were characterized, including *bHLH1, MYB9/10/11, RH2-1, GT3-1, AN1-1, HSF1*, which belong to different families, such as *bHLH, Zinc finger, HSF* e *IAA* (Zhu et al., 2018b).

Astringency removal in persimmons with 95% CO_2 also revealed that acetaldehyde production is controlled by genes involved in carbohydrate metabolism, mainly *DkPFK*, encoding the phosphofructokinase enzyme that acts in the regulation of glycolysis. High CO_2 concentration led to anaerobic respiration, with consequent production of acetaldehyde, where *DkPFK* was shown to be a key gene regulating the variation of acetaldehyde production in different persimmon cultivars, supported by a positive correlation with the acetaldehyde content (Kou et al., 2021). In addition, these authors raise the discussion about the polymerization reaction between acetaldehyde and the proanthocyanidins may not be the only factor regulating the astringency sensation.

4.2. Mechanism of ethylene astringency removal

There is a lack of updated reports in the literature that fully elucidate the deastringency route through the application of ethylene. This is due, in part, to the fact that the use of atmospheres with high concentrations of CO_2 is considered worldwide as the main commercial tool for removing persimmons astringency. In Brazil, the fifth largest producer of persimmon fruit in the world (FAO, 2020), the use of ethylene is considered the main way to remove astringency (Prohort, 2016), mainly due to the national preference for 'Rama Forte' persimmons (PVA type) that have a succulent flesh, a condition provided by the removal of astringency with ethylene.

Ethylene is a well-known plant hormone responsible for signaling the main metabolic pathways associated with maturation and senescence. The astringency removal mechanism with ethylene application occurs with the induction of ripening in response to the gaseous hormone. Acetaldehyde can be produced in persimmon fruit during the ripening process (Pesis, 2005). Acetaldehyde accumulation is observed in 'Mopan' persimmons treated with ethylene, as well as the activity of the enzymes pyruvate decarboxylase and alcohol dehydrogenase (Min et al., 2012).

Many aspects of astringency removal associated with the regulation of ethylene-induced ripening remain unclear. It is known that ethylene induces the biosynthesis of volatile compounds responsible for the aroma of fruits, including acetaldehyde and ethanol (Edagi and Kluge, 2009a, 2009b). Interesting, ripening-related genes encoding alcohol dehydrogenase in bananas from the Cavendish subgroup 'Grand Nain' are also involved in the biosynthesis pathway of volatile compounds (Manrique-Trujillo et al., 2007). It was also shown that the action of acetaldehyde-producing enzymes (pyruvate decarboxylase and alcohol dehydrogenase) is controlled by ERFs and MYBs transcription factors (Fang et al., 2016; Min et al., 2012; Zhu et al., 2019), which reinforces the relationship between astringency removal and the ripening process, since ERFs such as *DkERF8/16/18* may act in the ripening of persimmon fruit, by promoting ethylene biosynthesis and changes in the cell wall associated with softening (He et al., 2020). From these, *DkERF8* and *16* are also responsive to hypoxia (Wang et al., 2017b), a condition associated with acetaldehyde production.

5. Final considerations

The metabolism and the genetic control involving astringency in persimmons is complex. Several metabolic pathways, enzymes and response factors are involved. Much progress has been made in the search for the elucidation of these pathways, to better understand the effects observed in persimmon fruit, however, some points, such as steps in the proanthocyanidin accumulation pathways, acetaldehyde formation and transcription factors involved in the astringency loss process still remain to be elucidated, and therefore need further studies.

Declaration of Competing Interest

None.

Data availability

No data was used for the research described in the article.

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