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ORIGINAL ARTICLE

ALDH2 genes are negatively correlated with natural deastringency in Chinese PCNA persimmon (*Diospyros kaki* Thunb.)

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Abstract Chinese pollination-constant and non-astringent persimmon (C-PCNA) has important application values in the genetic improvement of PCNA for its trait of natural deastringency controlled by a single dominant gene. However, the key genes and the regulatory networks are still not fully understood. The process of C-PCNA natural deastringency may be associated with the acetaldehyde-mediated coagulation of soluble tannins, but the functions of *ALDH2* genes related to the metabolism of acetaldehyde are not clear. In this work, three types of persimmon cultivars, 'Eshi 1' and 'Luotian Tianshi' (C-PCNA type), 'Youhou' (J-PCNA type), and 'Mopanshi' (non-PCNA type), were sampled. Two members of *ALDH2* family genes, *DkALDH2a* and *DkALDH2b*, were isolated from 'Eshi 1' persimmon fruit. Gene expression patterns indicated that they may be involved in "coagulation effect", which leads to natural deastringency in C-PCNA persimmon fruit. Transient expression in 'Eshi 1' leaves further demonstrated that their expression can reduce the consumption of soluble tannins and inhibit the astringency removal process. Therefore, *DkALDH2a* and *DkALDH2b* are negatively correlated with natural deastringency in C-PCNA persimmon.

Keywords Chinese PCNA · ALDH2 · Kaki-tannin (proanthocyanidin) · Natural deastringency

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Introduction

Based on whether the fruit astringency is lost or not naturally at maturity, persimmon (*D. kaki* Thunb.) is generally classified into pollination-constant and non-astringent (PCNA) and non-PCNA. The former is subdivided into Chinese PCNA (C-PCNA) and Japanese PCNA (J-PCNA) according to the differences in genetic characteristics of the PCNA trait (Akagi et al. 2011); the latter is further classified into pollination-variant and non-astringent (PVNA), pollination-variant and astringent (PVA), and pollination constant and astringent (PCA) (Sugiura and Tomana 1983; Yonemori et al. 2000). Natural astringency loss can occur in PCNA fruits without any special treatment at maturity, which is convenient for the fresh consumption, storage, and transportation of the fruits. PCNA persimmon is a key species in the world persimmon industry and an important target of persimmon genetic improvement. The natural deastringency trait of C-PCNA is controlled by a dominant single gene, which has great application values in PCNA genetic improvement. However, the key gene and its regulatory network are still not fully understood.

The mechanism of natural deastringency is different between C-PCNA and J-PCNA fruit. Deastringency in J-PCNA fruit is realized by the cessation of tannin cell enlargement and tannin biosynthesis in the early stages of fruit development; with the development of fruit, the proportion of tannin cells and soluble tannin content decrease for deastringency at about 8 weeks after full bloom (WAB), which is called the

"dilution effect" (Yonemori et al. 1983; Yonemori and Matsushima 1985). In contrast, deastringency in C-PCNA fruit is not completed until maturity, and its soluble tannin content decreases with the increase of insoluble tannins. These facts indicate that the natural deastringency of C-PCNA fruit is related to both the "dilution effect" and its typical "coagulation effect," which means that with the fruit ripening, part of soluble tannins are transformed to be insoluble, and acetaldehyde probably plays a key role in the transformation (Su et al. 2014; Zhang et al. 2013). In our previous study, persimmon transcriptome database was obtained from the "Luotian Tianshi" fruit treated with 5% ethanol (Luo et al. 2014), and obvious changes were found in the expression patterns of some key genes coding the acetaldehyde metabolism. The genes related to acetaldehyde metabolism, including *DkADH1*, *DkPDC1*, and *DkPDC2*, were cloned and further analyzed (Mo et al. 2016). Alcohol dehydrogenase (ADH) family gene *DkADH1* was significantly down-regulated, and *DkADH3* and pyruvate decarboxylase (PDC) family gene *DkPDC* were up-regulated remarkably. Besides, the expression of aldehyde dehydrogenase (ALDH) family gene *DkALDH2* was clearly down-regulated. These results indicate that the genes related to acetaldehyde metabolism are probably involved in the natural deastringency of C-PCNA fruit. In transient over-expression with persimmon leaves, the over-expression of *DkADH1* and *DkPDC2* could significantly reduce the soluble tannin content, implying that both genes are involved in the C-PCNA natural deastringency via "coagulation effect" mediated by acetaldehyde. The key enzymes of acetaldehyde metabolism in plants include PDC, ADH, and ALDH. In addition, ALDH2 plays a key role in the downstream of the metabolic pathway (Tsuji et al. 2003). Analysis of the transcriptome data suggested that *DkALDH2* genes may also participate in "coagulation effect" (Luo et al. 2014), but their roles in natural deastringency of C-PCNA type persimmon are still not clear. Here, two cultivars of C-PCNA type persimmon, 'Eshi 1' and 'Luotian Tianshi,' were sampled, and 'Youhou' (J-PCNA type) as well as 'Mopanshi' (non-PCNA type) were used as the control. Cloning, expression pattern analysis, and function verification were conducted for *DkALDH2* genes based on the information of sequence fragments. The relationship between the genes involved in acetaldehyde metabolism and natural deastringency of C-PCNA persimmon fruit was further investigated. In most previous studies, 'Luotian Tianshi' was used as the typical representative of C-PCNA and many relevant studies have been carried out. Therefore, it was taken as the main experiment materials of gene expression patterns compared with 'Eshi 1'.

Materials and methods

Plant materials

Three types of persimmon cultivars grown in the Persimmon Repository of Huazhong Agricultural University (HZAU), Wuhan, China, were sampled, including 'Eshi 1' and 'Luotian Tianshi' (C-PCNA type), 'Youhou' (J-PCNA type), and 'Mopanshi' (non-PCNA type). Fruits were sampled at 2.5, 5, 10, 15, 20, and 25 weeks after full bloom (WAB) (Fig. S1). The flesh from the equatorial region of fruits was detached and immediately frozen in liquid nitrogen, and then stored at − 80 °C for later use. Samples of flowers, stems, and leaves were obtained at full blossom, and calyx, peel, core, and seeds were collected at 20 WAB. All treatments were performed with three biological replicates (10 fruits for each replicate).

Analysis of tannin content

Folin-Ciocalteu method was used to determine the soluable/insoluble tannin contents at different stages of fruit development (Oshida et al. 1996). Three biological replicates were performed per sampling point.

Extraction of RNA and synthesis of cDNA

Total RNA was extracted by using RNAplant Plus (TIANGEN) kit according to the manufacturer's instructions. RNA purity and integrity were detected by 1% gel electrophoresis, and the absorbance value at 260 nm was measured by Nano Drop 2000 and used for quantitative analysis. cDNA was synthesized using PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa) according to the manufacturer's instructions.

qRT-PCR expression analysis

The primers used for quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis are shown in Table S1, and the *actin* gene (GenBank Accession No. AB219402) was used as the reference gene. qRT-PCR was conducted on LightCycler® 480 II Real-Time PCR equipment (Roche, Germany) using SYBR® Green Realtime PCR Master Mix fluorochrome (TOYOBO, Japan). The reaction system was operated according to the manuals. The qRT-PCR was programmed at 95 °C for 30 s, followed by 45 cycles of 95 °C for 5 s, 58 °C for 10 s, and 72 °C for 15 s, then 95 °C for 60 s, and 40 °C for 30 s. The qRT-PCR was performed for three technical replicates per sample, and the data represent the means ± SD ($n = 3$).

Isolation and sequence analysis of *DkALDH2a* and *DkALDH2b* genes

The full-length cDNA fragments of *DkALDH2a* and *DkALDH2b* genes were amplified with PCR from the 'Eshi 1' persimmon fruit. Primers in Table S2 were designed based on the fragments in the transcriptome data (Chen et al. 2017). PCR was conducted using SMARTer® RACE 5′/3′ Kit (Clontech, USA). The reaction system was operated according to the manuals. PCR products were separated on an agarose gel via electrophoresis and visualized using EB dye. Bands were excised from gels to isolate the products, which were cloned into a pEASY™-Blunt Cloning Vector (TransGen Biotech, Beijing, China) to obtain pEASY-DkALDH2a and pEASY-DkALDH2b constructs for sequencing and analyzing. The gene sequences were confirmed with BLAST in GenBank. Softberry tool was used to predict the protein sequences coded by *DkALDH2a* and *DkALDH2b* (http://linux1.softberry.com/). The ALDH2 protein multiple sequence alignment was performed through DNAMAN. The phylogenetic tree was constructed on the MEGA6 with neighbor-joining method. In addition, the cDNA fragments of the two genes were also amplified from the 'Luotian Tianshi' persimmon fruit in the same way, but a small part of the 5′ end was missed in full-length *ALDH2a*. The results of protein sequence alignment are presented in Fig. S2 and Fig. S3.

In vivo transient transformation of *DkALDH2a* and *DkALDH2b* in persimmon leaves

To determine the roles of *DkALDH2a* and *DkALDH2b* genes in the deastringency process of persimmon, the transient over-expression and silencing by *Agrobacterium tumefaciens*-mediated transformation were conducted in 'Eshi 1' leaves. A gateway technique was applied to construct binary vectors for gene expression. The full-length coding regions of *DkALDH2a* and *DkALDH2b* genes were amplified with the primers in Table S3. Target genes were cloned into the over-expression vector pK2GW7 to form the fusion constructs pK2GW7-Pro35S: *DkALDH2a* and pK2GW7-Pro35S: *DkALDH2b*. pK2GW7-Pro35S: *GFP* was used as the control. Then, the fragments with length of 303 and 226 bp, respectively, at the 5′ end of *DkALDH2a* and *DkALDH2b* genes were amplified with the primers in Table S4. The amplified products were inserted into the silencing vector pH7GWIWG2 to form the fusion constructs pH7GWIWG2-Pro35S: *DkALDH2a* and pH7GWIWG2-Pro35S: *DkALDH2b*. pH7GWIWG2-Pro35S: *GFP* acted as the control. The verified fusion and control plasmids were introduced into the competent *Agrobacterium tumefaciens* strain GV3101. The procedure was conducted in accordance with the description by Ratanasut et al. (2015). Transient expression (Agrobacterium culture, infiltration buffer) was carried out via the protocol described by Mo et al. (2015). The leaves of 'Eshi 1' approximately 4 cm in length were selected for injection. Ten days later, 100 mg tissue from each infiltrated leaf was excised for qRT-PCR and tannin content analysis, with a total of 10 leaves for each replicate. The statistical significance of differences was calculated using Student's *t* test ($p < 0.05$), which was performed with SPSS17.0.

Results

Changes of tannin content during fruit development

An obvious decreasing trend in soluble tannin content was detected with the fruit development in C-PCNA, non-PCNA, and J-PCNA persimmons (Fig. 1a). The soluble tannin content of 'Luotian Tianshi' (C-PCNA) reduced to 0.6% at 25 WAB, which was close to the accomplishment of deastringency. The soluble tannin content of 'Youhou' (J-PCNA) decreased markedly at early fruit developmental stage. It fell to below 0.1% at 15 WAB and stayed at a very low level thereafter, suggesting complete deastringency. This result was consistent with findings in previous studies (Ikegami et al. 2005; Akagi et al. 2009). The soluble tannin content of 'Mopanshi' (non-PCNA), which was still above 1.0% at 25 WAB and brought a very strong astringent taste, was higher than that of 'Luotian Tianshi' and 'Youhou'. Although the insoluble tannin contents in different types of persimmon showed a generally decreasing trend, the insoluble tannin content of 'Luotian Tianshi' presented an increase before decreasing from 10 to 25 WAB, which was different from the case of 'Youhou' and 'Mopanshi' (Fig. 1b).

Cloning and sequence analysis of *DkALDH2a* and *DkALDH2b* genes

Full-length cDNAs of *DkALDH2a* and *DkALDH2b* genes were isolated from C-PCNA persimmon fruit by RACE and RNA-Seq data. They were 1984 and 1933 bp in length and encoded 542 and 540 amino acids, respectively. The amino acid sequences of DkALDH2a and DkALDH2b were imported in NCBI for BLASTp comparison. Their sequences were found to be 79% similar and were highly consistent with ALDH2 protein sequences in many plants. For example, the sequences of DkALDH2a and DkALDH2b were 86 and 79% similar to apple MdALDH2B4 (XP_008374451.1), 86 and 78% similar to grape VvALDH2B4 (XP_002283132.1), 79 and 76% similar to *Arabidopsis* AtALDH2B4 (NP_190383.1), and 79 and 78% similar to *Arabidopsis* AtALDH2B7 (NP_564204.1), respectively. The results of multiple sequence alignment showed that DkALDH2a and

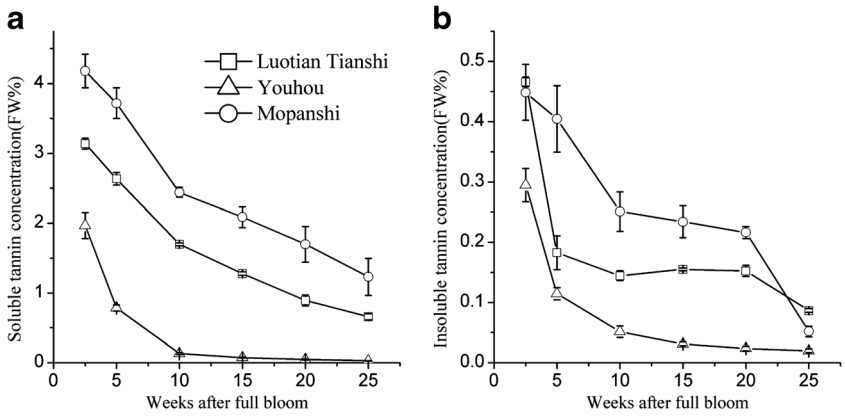

Fig. 1 Variations of tannin contents in three types of persimmon fruits at different developmental stages. **a** Soluble tannin content analysis. **b** Insoluble tannin content analysis. Errors bars indicate SEs from three biological replicates ($n = 3$)

DkALDH2b are highly conserved with ALDH2 sequence, which is related to acetaldehyde metabolism in many plants, and possess ALDH_F2BC, ALDH-SF superfamily, and PLNO2466 conserved motifs (Fig. 2). Besides, DkALDH2a and DkALDH2b showed close genetic relationships with the protein related to pyruvate dehydrogenase (PDH) bypass pathway and ALDH2 protein associated with stress, respectively (Fig. 3). The results showed that DkALDH2a and

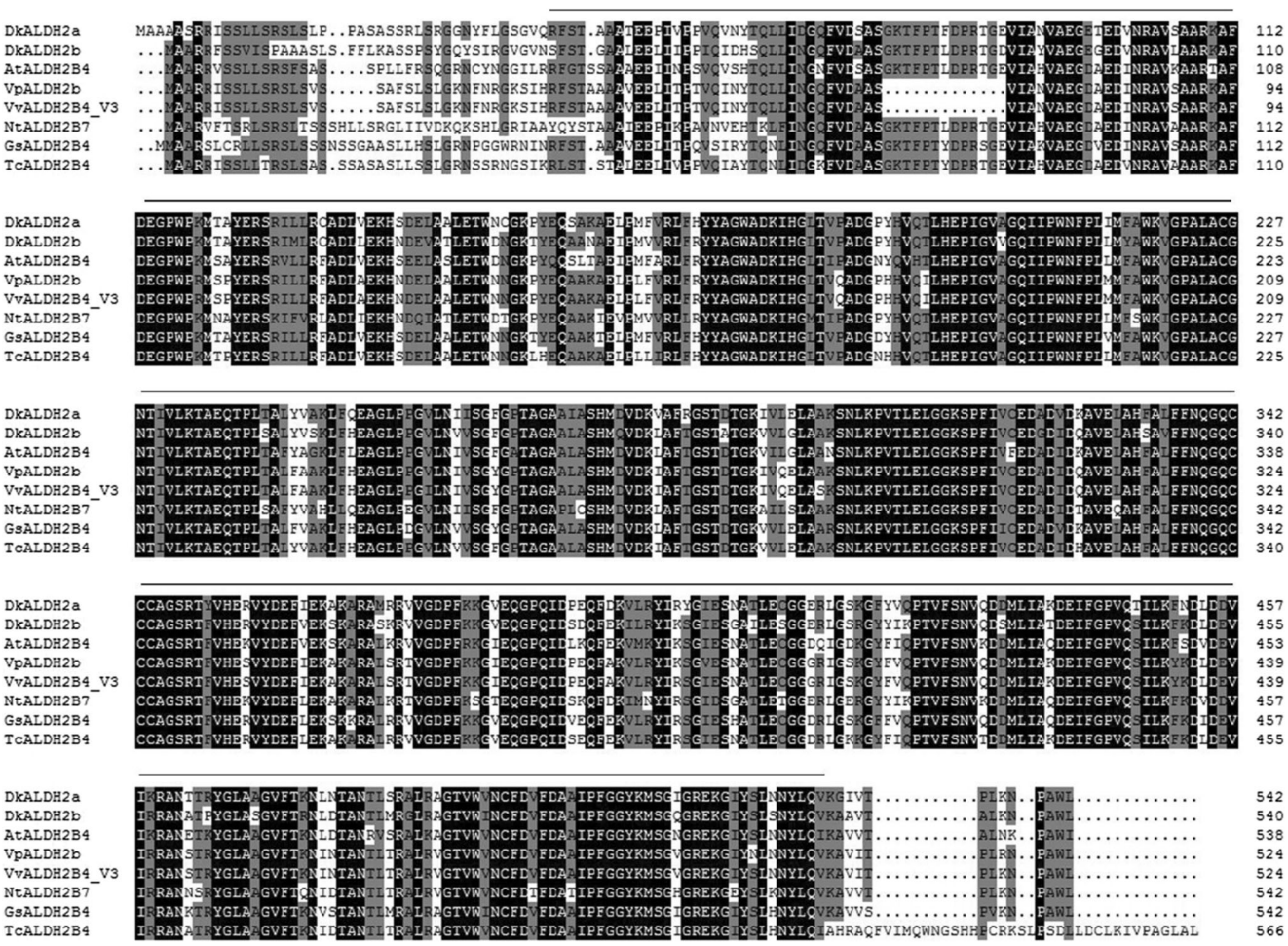

Fig. 2 Multiple alignment of DkALDH2 and ALDH2 proteins in plants. The PLN02466 domain which means an ALDH2 family member is marked with black lines

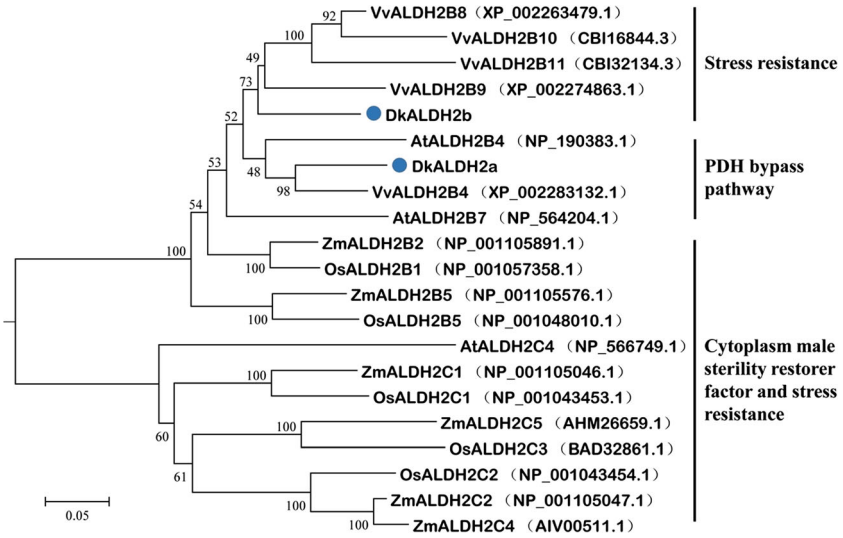

Fig. 3 Phylogenetic tree of ALDH2 proteins in plants. The deduced amino acid sequences of ALDH2s were obtained from NCBI. The accession numbers are indicated. Persimmon ALDH2 proteins are shown in bold. The phylogenetic tree was constructed using MEGA6

DkALDH2b are probably related to C-PCNA natural deastringency via participating in acetaldyhyde metabolism.

Expression patterns of *DkALDH2a* and *DkALDH2b* genes during fruit development

To better understand the correlations between the expression of *DkALDH2a* and *DkALDH2b* genes and the changes of tannin content, the two genes were further analyzed using qRT-PCR at different developmental stages of C-PCNA persimmon fruit (Fig. 4). J-PCNA and non-PCNA cultivars were used as the control. In 'Luotian Tianshi' and 'Mopanshi,' the expression level of *DkALDH2b* was higher than that of *DkALDH2a*. The expression of *DkALDH2a* in 'Luotian Tianshi' first decreased before rising during 2.5–10 WAB, and then decreased gradually with fruit ripening; in 'Mopanshi,' it displayed a continuous decreasing trend during 2.5–20 WAB with a slight increase during 20–25 WAB; in 'Youhou', it reached the peak at 2.5 WAB and then decreased gradually, showing a consistent trend with the variation of soluble tannin contents. The expression of *DkALDH2b* in 'Luotian Tianshi' was transiently increased before decreasing slowly during 2.5–20 WAB, and then increased gradually to the highest level with fruit maturity; in 'Mopanshi', it increased gradually with fruit development, particularly after 15 WAB; in 'Youhou', it stayed at a relatively low level with modest variation during fruit developmental stages (Fig. 4). In C-PCNA persimmon, *DkALDH2a* expression seemed to be negatively correlated with soluble tannin content during the late fruit developmental stages, and it was the opposite case for *DkALDH2b*.

Expression of *DkALDH2a* and *DkALDH2b* genes in different tissues and organs in C-PCNA persimmon

The expression of *DkALDH2a* and *DkALDH2b* in different tissues and organs of 'Luotian Tianshi' was analyzed via qRT-PCR. The expression of *DkALDH2a* was relatively

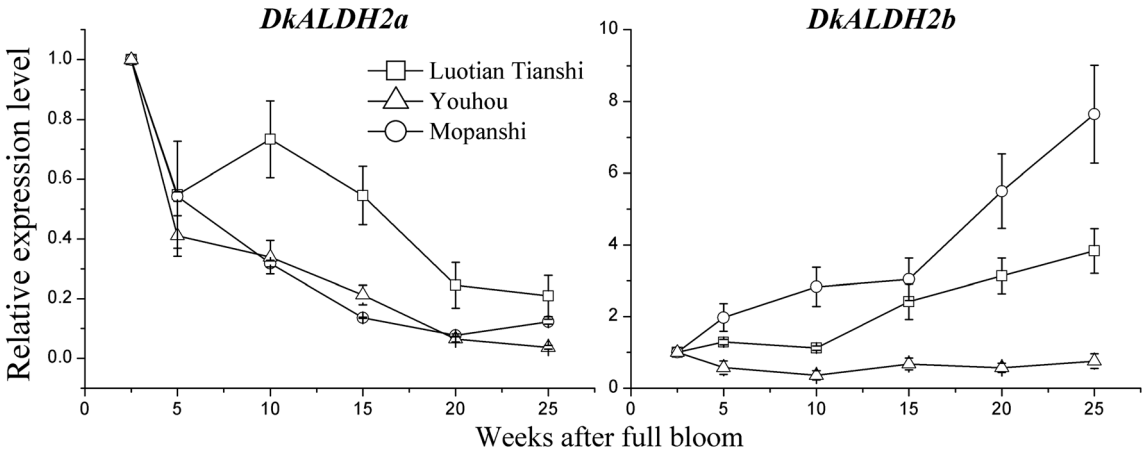

Fig. 4 qRT-PCR analysis of the expression of *DkALDH2* genes. Flesh from three types of persimmon fruits from 2.5 to 25 weeks after full bloom was utilized for the expression analysis. Errors bars indicate ±SE (n = 3)

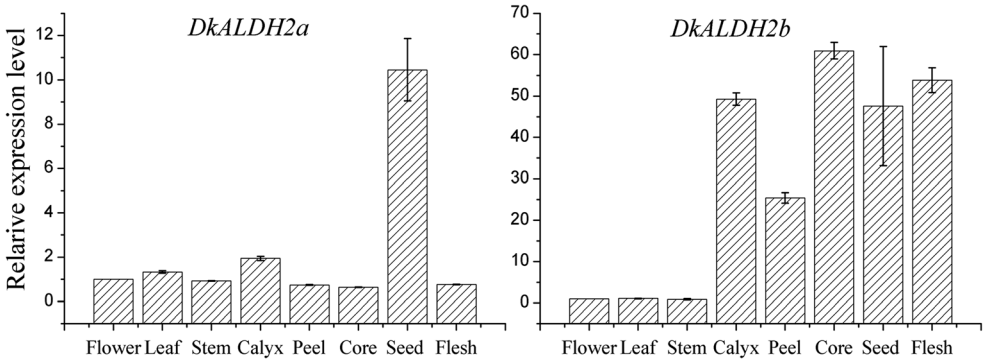

Fig. 5 Expression pattern of *DkALDH2* genes in vegetative tissues and reproductive organs of 'Luotian Tianshi' by qRT-PCR. Flowers, leaves, and stems were sampled at full bloom period, and other tissues (calyx, peel, core, seeds, and flesh) were sampled at 20 weeks after full bloom. Error bars indicate SEs from three biological replicates ($n = 3$)

higher in the seeds, but was very low in the peel, core, and flesh compared with that of *DkALDH2b*. However, except for in the flowers, leaves, and stems, the expression of *DkALDH2b* was significantly higher than that of *DkALDH2a* in other tissues and organs (Fig. 5). Therefore, the expression of *DkALDH2b* was more active than that of *DkALDH2a* in the critical period of fruit natural deastringency, and *DkALDH2a* was specifically expressed in seeds. Seed is an important organ for the production of acetaldehyde in persimmon fruit (Sugiura and Tomana 1983; Sugiura et al. 1979). Our previous study has shown the continuous accumulation of acetaldehyde in the seeds of C-PCNA persimmon fruit during its natural deastringency process (Mo et al. 2016), which is consistent with the decreasing trend of soluble tannin content observed in this study. It could be indicated that the acetaldehyde produced by the seed contributes to the coagulation effect, resulting in the decrease of soluble tannin content.

Expression of genes related to acetaldehyde metabolism during fruit development

To confirm the relationship between the genes related to acetaldehyde metabolism and natural deastringency in C-PCNA persimmon, expression of the six previously amplified genes was further analyzed at different fruit developmental stages (Fig. 6). J-PCNA and non-PCNA cultivars were used as the control. The *DkADH* genes (mainly *DkADH2*) were highly expressed at the maturity stage (20–25 WAB) of 'Luotian Tianshi', and in 'Youhou', they were highly expressed at the

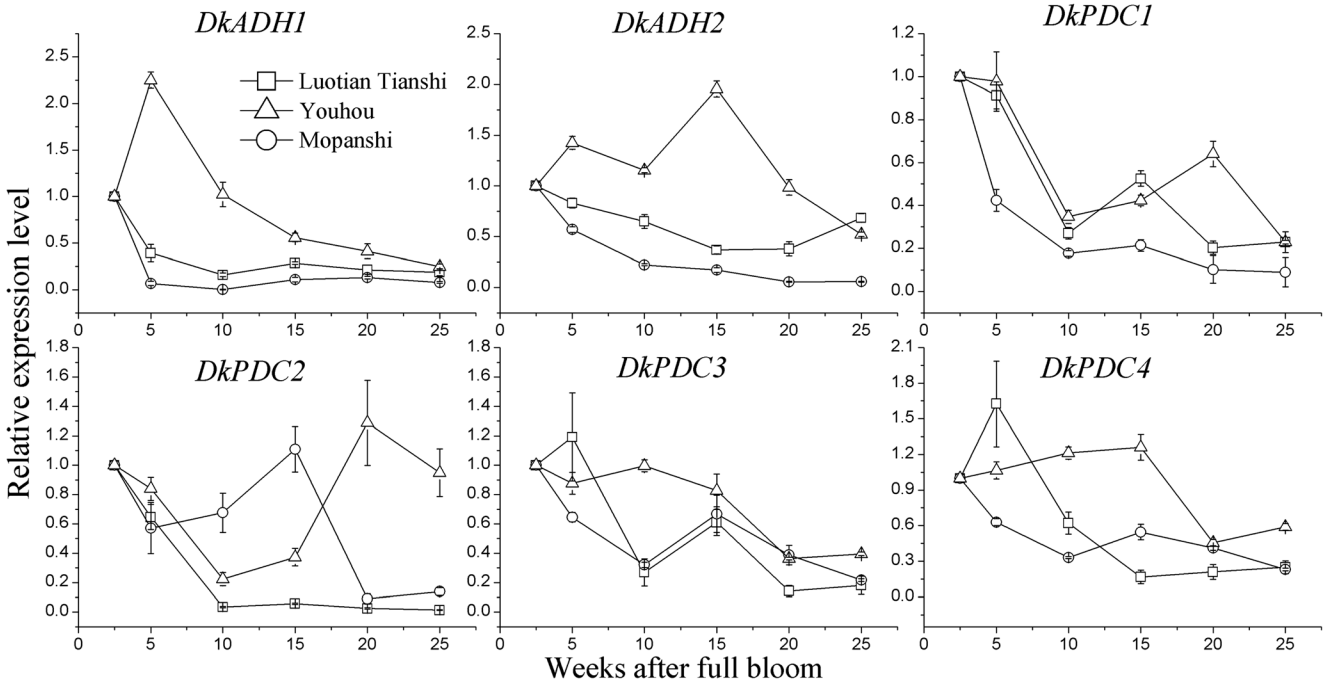

Fig. 6 qRT-PCR analysis of *DkADH* and *DkPDC* genes. Flesh from three types of persimmon fruits from 2.5 to 25 weeks after full bloom was utilized for the expression analysis. Errors bars indicate ±SE ($n = 3$)

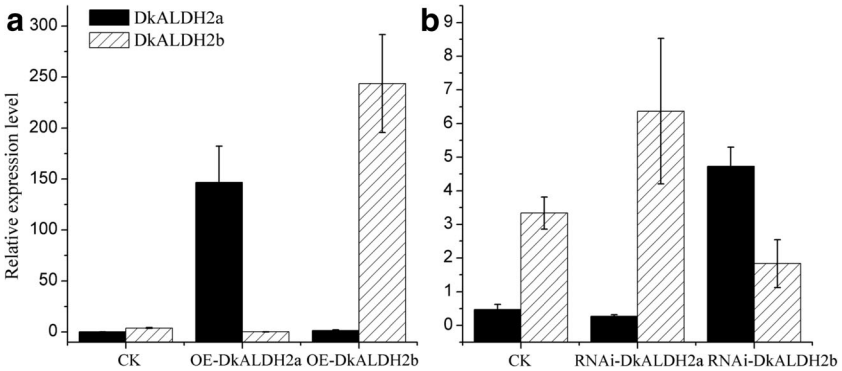

Fig. 7 Expression of *DkALDH2* genes after transient expression in 'Eshi 1' persimmon leaves. **a** Transient over-expression (OE). **b** Transient silencing (RNAi). Leaves infiltrated with *DkALDH2* genes were collected for qPCR analysis at 10 days after agroinfiltration. CK represents the empty vectors. Error bars indicate SEs from three biological replicates ($n = 3$)

earlier maturity stage (2.5–15 WAB), but their expression was low at the maturity stage (15–25 WAB). The expression of *DkPDC* genes in 'Luotian Tianshi' maturity stage (20–25 WAB) was between that of 'Mopashi' and 'Youhou' with a rising tendency, except for that of *DkPDC2*. The results indicated that *ADH* and *PDC*, two gene families related to acetaldehyde metabolism, to some extent, are involved in C-PCNA natural deastringency.

Transient expression of *DkALDH2a* and *DkALDH2b* in persimmon leaves in vivo

To confirm the putative function of *DkALDH2* genes in the coagulation of soluble tannins in vivo, transient over-expression and silencing of *DkALDH2a* and *DkALDH2b* were conducted by *Agrobacterium*-mediated infiltration in 'Eshi 1' leaves. The over-expression recombinant plasmids pK2GW7-DkALDH2a and pK2GW7-DkALDH2b were transformed and compared with the empty plasmid. The expression of both genes increased significantly in the leaves (Fig. 7a). Further analysis of tannin content showed that the contents of both soluble and insoluble tannins were markedly increased compared with the control (Fig. 8a). In contrast, when the silencing recombinant plasmids pH7GWIWG2-DkALDH2a and pH7GWIWG2-DkALDH2b were transformed and compared with the empty plasmid, the expression of the silenced genes was decreased, and at the same time, the expression of the other gene was increased (Fig. 7b). Tannin content showed no obvious changes with the silencing of *DkALDH2a*, but a marked decline with the silencing of *DkALDH2b* compared with the control (Fig. 8b). In addition, the ratio of soluble/insoluble tannins was practically unchanged and the two forms of tannins showed a consistent variation trend (Fig. 8). These results indicated that *DkALDH2a* and *DkALDH2b* participated in the coagulation of soluble tannins in vivo and they are negatively correlated with natural deastringency.

Discussion

C-PCNA possess great potential in the genetic improvement of PCNA, because its natural deastringency trait is controlled by a single dominant gene, which is different from the case of J-PCNA (Ikegami et al. 2004, 2006). In recent years, many studies focused on the identification of the key genes of deastringency in C-PCNA persimmon fruit (Wang et al. 2010; Hu et al. 2013; Su et al. 2014; Guan et al. 2016; Chen et al. 2017). The mechanisms of deastringency are

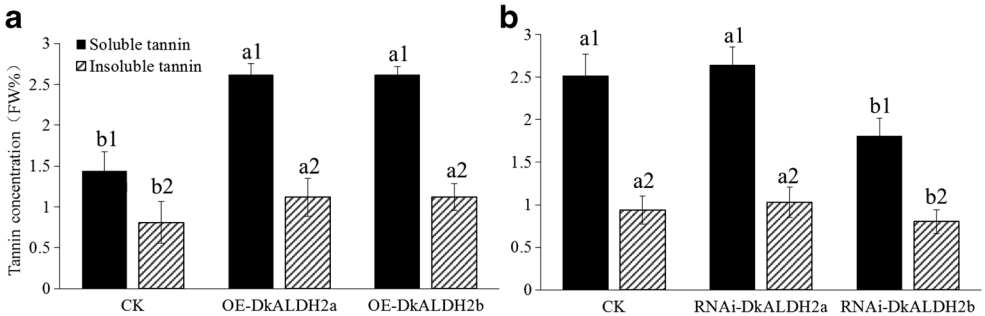

Fig. 8 Variations of tannin contents after transient expression of *DkALDH2* genes in 'Eshi 1' persimmon leaves. **a** Transient over-expression(OE). **b** Transient silencing (RNAi). Leaves infiltrated with *DkALDH2* genes were collected for soluble tannin content analysis at 10 days after agroinfiltration. CK represents the empty vectors. Numbers 1 and 2, respectively, represent the comparison of soluble and insoluble tannins. Error bars indicate SEs from 15 biological replicates ($^*p < 0.05$; $n = 15$)

significantly different among C-PCNA, J-PCNA, and non-PCNA (Akagi et al. 2011; Yonemori et al. 2005). Our study displays that 'Luotian Tianshi' (C-PCNA type) almost accomplishes deastringency at 25 WAB, while 'Youhou' (J-PCNA type) completes its natural deastringency at early fruit developmental stage (before 10 WAB), which is sustained till fruit maturity. 'Mopanshi' (non-PCNA type) could not complete natural deastringency. Mo et al. (2016) showed that C-PCNA completes deastringency at 25 WAB. This finding is inconsistent with the results of this study, which may be due to a certain degree of climate change between years. Besides, our results show that the decrease of soluble tannin content is accompanied with the increase of insoluble tannin content in the later stage of fruit development of C-PCNA, which is consistent with the results of Mo et al. (2016) and Hu et al. (2013). These results together indicate that besides the same "dilution effect" as in J-PCNA, the natural deastringency of C-PCNA exhibits a unique "coagulation effect", namely the transformation of soluble tannins to insoluble tannins. Our results also show that as the key genes of acetaldehyde metabolism, *DkALDH2a* and *DkALDH2b* play vital roles in the natural deastringency of C-PCNA persimmon. Acetaldehyde is a key factor affecting the transformation of soluble tannins to insoluble tannins in persimmon fruit (Tanaka et al. 1994; Taira et al. 2001). In addition, numerous studies on the mechanism of artificial deastringency in non-PCNA persimmon fruit have also shown the important role of acetaldehyde (Matsuo and Ito 1982; Matsuo et al. 1991; Pesis et al. 1986, 1988; Taira et al. 1989, 2001; Yamada et al. 2002; Arnal and Río 2003; Min et al. 2012). Besides, Mo et al. (2016) found that *ADH* and *PDC* can lead to natural deastringency in C-PCNA by "coagulation effect", which is to some extent similar to the results in Fig. 6. In this study, *DkALDH2*, a key gene of downstream of acetaldehyde metabolism, showed different expression levels in the three types of persimmon during fruit development and was related to the variation of soluble tannin content (Figs. 1 and 4). In other words, the variation of *DkALDH2a* expression was consistent with that of soluble tannin content during fruit maturity stage, which was the opposite case for *DkALDH2b* (except for J-PCNA). In C-PCNA persimmon, *DkALDH2a* expression was significantly higher than that in J-PCNA and non-PCNA during fruit maturity stage (Fig. 4), which is consistent with the natural deastringency characteristics of C-PCNA. In addition, the soluble tannin content significantly increased after the transient over-expression of *DkALDH2a* and *DkALDH2b* (Fig. 8), confirming that both genes can suppress the coagulation effect to inhibit deastringency. Therefore, the two *ALDH2* genes play important roles in the natural deastringency process in C-PCNA. ALDH2 family belongs to the superfamily of ALDHs in plants and is closely related to acetaldehyde metabolism (Kirch et al. 2004). The family usually consists of several members with distinctive functions (Brocker et al. 2013; Zhang et al. 2012; Wei et al. 2009). In the present study, we also found that the two *ALDH2* genes affect natural deastringency in very different ways. As mentioned above, *DkALDH2a* expression was mainly detected in seeds and was low in C-PCNA during fruit developmental stages. Therefore, there was no significant change in soluble tannin content after transient silencing of the gene. In contrast, the expression of *DkALDH2b* was fairly high during fruit developmental stages and was detected in several organs of fruit, indicating that this gene has high expression in C-PCNA. Hence, there was a sharp decline in soluble tannin content after transient silencing of this gene. Our previous study showed that acetaldehyde content was continuously decreased during C-PCNA natural deastringency process, which is consistent with the increasing trend of *DkALDH2b* expression (Mo et al. 2016). These results demonstrate that *DkALDH2b* inhibits the coagulation effect to some extent, but the inhibition would not be strong enough to prevent the natural deastringency process in C-PCNA.

Conclusion

This study reveals that *DkALDH2a* and *DkALDH2b* negatively regulate the natural deastringency in C-PCNA. The former mainly acts on the seeds, and its expression gradually decreases during fruit development, resulting the generation of a large amount of acetaldehyde. The acetaldehyde is then transported from the seed to the pulp to promote the coagulation effect, which contributes to the deastringency. The latter is highly expressed in the pulp, which might be due to the accumulation of acetaldehyde, but the consumption of acetaldehyde is not sufficient to restrain deastringency of C-PCNA.

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Data archiving statement The cDNA sequences of *DkALDH2a* and *DkALDH2b* genes have been submitted to GenBank, and the accession numbers are MG189594 and MG189595, respectively.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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