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RESEARCH ARTICLE

Identification and expression patterns of alcohol dehydrogenase genes involving in ester volatile biosynthesis in pear fruit

QIN Gai-hua[1, 2*], QI Xiao-xiao[2, 3*], QI Yong-jie[1, 2], GAO Zheng-hui[1, 2], YI Xing-kai[1, 2], PAN Hai-fa[1, 2], XU Yi-liu[1, 2]

[1] *Horticultural Research Institute, Anhui Academy of Agricultural Sciences, Hefei 230031, P.R.China*
[2] *Anhui Key Laboratory of Genetic Improvement and Ecophysiology of Horticultural Crop, Hefei 230031, P.R.China*
[3] *College of Agriculture, Medicine and Health, Anhui Radio and Television University, Hefei 230022, P.R.China*

Abstract

Alcohol dehydrogenase (ADH) catalyzes the interconversion of aldehydes and their corresponding alcohols, and is a key enzyme in volatile ester biosynthesis. However, little is known regarding ADH and ADH encoding genes (*ADH*s) in pear. We identified 8 *ADHs* in the pear's genome (*PbrADHs*) by multiple sequences alignment. The *PbrADH*s were highly homologous in their coding regions, while were diversiform in structure. 9 introns were predicted in $PbrADH_3$–$PbrADH_8$, while 8 introns, generated through exon fusion and intron loss, were predicted in $PbrADH_1$ and $PbrADH_2$. To study the genetic regulation underlying aroma biogenesis in pear fruit, we determined the *PbrADH* transcripts, ADH activities and volatile contents of fruits during ripening stage for Nanguoli and Dangshansuli, two cultivars having different aroma characteristics. ADH activity was strongly associated with the transcription of ADH_6 in the two cultivars during fruit ripening stage. The higher ester content paralleling to a higher ADH activity was detected in Nanguoli than in Dangshansuli, so it is induced that the lower ester content in Dangshansuli fruit may be the result of weak ADH activity. The present study revealed that total ADH activity and volatile ester production correlated with increased $PbrADH_6$ transcript levels. $PbrADH_6$ may contribute to ADH activity catalyzing aldehyde reduction and ester formation in pear fruit.

Keywords: alcohol dehydrogenase, ester volatile, gene expression, pear

1. Introduction

Aroma, an important trait for fruit and its products, is composed of many volatiles (Hadi *et al*. 2013; Xu *et al*. 2015). Ester endowing 'fruity' aroma is important volatile for fruits. According to previous studies, the dynamics of the production of aroma volatiles in fruit is affected by a range of enzymes such as lipoxygenase (LOX), hydroperoxide lyase (HPL) and alcohol acetyltransferases (AAT). LOX pathway activity is responsible for many of the volatile induced by cellular disruption, which are overwhelmingly represented by C-6 aldehydes (Dixon and Hewett 2000; Fellman *et al*. 2000). Alcohol dehydrogenase (ADH, EC1.1.1.1) is an important enzyme catalyzes the interconversion of aldehydes and their corresponding alcohols (Dixon and Hewett 2000). Many studies have focused on the last step of ester formation, especially on AAT (Cumplido-laso *et al*. 2012; Freilich

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QIN Gai-hua, E-mail: 59672211@qq.com; Correspondence XU Yi-liu, Tel: +86-551-62160121, Fax: +86-551-65160937, E-mail: yiliuxu@163.com
* These authors contributed equally to this study.

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et al. 2015; Yang *et al*. 2016). While the studies on ester production in pear fruit supplemented with their precursors suggests that the critical step for ester formation may be regulated by alcohol dehydrogenase (ADH) or its upstream enzymes, which catalyze alcohol formation (Qin *et al*. 2014).

The ADH is a Zn-binding enzyme that reversibly converts aldehydes to their corresponding alcohols. A low ADH activity level in fruit can lead to a reduced and altered flavor (Speirs *et al*. 2002). Due to the role of ADH in alcohol generation, several ADH enzymes and their corresponding genes have been studied in fruit, such as tomato (Moummou *et al*. 2012), grape (Tesnière and Verriès 2000), apple (Defilippi *et al*. 2005) and mango (Singh *et al*. 2010). They are members of the well-studied medium-length dehydrogenase/reductase protein superfamily that is comprised of ~370 residues (Strommer 2011) related to volatile ester biosynthesis processes. Some members of the more distantly related plant short-chain dehydrogenase/reductase superfamily also function as ADHs, having ~250 amino acids and having the characteristic catalytic domain and Zn co-factors (Tonfack *et al*. 2011). *ADH* genes expressed in a developmentally regulated manner, particularly during fruit ripening stage, were also detected (Manríquez *et al*. 2006). In addition to tomato (Moummou *et al*. 2012), detailed studies focusing on the role of *ADH* genes in relation to ripening and aroma synthesis have been studied in grapes, apricot and melon (Tesnière and Verriès 2000; Defilippi *et al*. 2009; Gonzalez-Aguero 2009). However, the role of ADH in ester aroma biosynthesis and *PbrADHs* expression pattern in pear fruit during ripening stage has not been reported, to our knowledge.

Pear (*Pyrus* L.) is an important fruit crop that is cultivated worldwide. Dangshansuli (*Pyrus bretschneideri* Rehd.) is an important pear cultivar widely planted in worldwide, and Nanguoli (*Pyrus ussuriensis* Maxim.) is an excellent pear cultivar mainly planted in the northeast of China. The two cultivars grow well in the Liaoning Province of China and have similar bloom and maturity dates; however, the composition and concentration of their aroma volatiles are distinctly different. *P. bretschneideri* Rehd. Dangshansuli fruit contains many straight-chain aldehydes and alcohols, which gives the fruit a 'green' note. Volatile ester is an important volatile of the ripened *P. ussuriensis* Nanguoli fruit and they produce a 'fruity' note (Qin *et al*. 2014). The majority of plant volatiles, on quantitative and qualitative bases, originate from saturated and unsaturated fatty acids (Boom *et al*. 2004). ADH, a key enzyme in the fatty acid metabolism pathway may play important roles in volatile ester biosynthesis. In this study, the role of the ADH in ester biosynthesis of pear fruit and candidate *ADH* genes participating in the ester biosynthesis of pear fruit were studied.

2. Materials and methods

2.1. Plant materials

In this study, Dangshansuli and Nanguoli fruits were harvested from an orchard of the Liaoning Institute of Pomology, Xiongyue, China (39°55′N, 121°56′E). All of the management procedures, such as fertilization and irrigation, were the same for the two cultivars in the orchard. In total, fifteen fruits having a uniform size and lacking visible defects or decay were acquired at 115, 125 and 135 days after full bloom (DAFB) and labeled as S1, S2 and S3, respectively. Another fifteen fruits were harvested at 135 DAFB and exposed to the air for 5 d at 25°C to allow for full ripening, and labeled as S4. A portion of each fruit, without skin and core, was diced in liquid nitrogen and kept frozen in bags at –80°C until analyzed.

2.2. Identification of pear ADH genes

The keyword 'alcohol dehydrogenase' was used to search the pear (*Pyrus* L.) genome (http://peargenome.njau.edu.cn). In addition, to further confirm the identification of the selected genes, the putative protein sequences deduced from the ADH-like gene sequences were compared to ADH proteins in other species using a BLASTP algorithm.

2.3. Sequence analysis

Predictions of the ADH protein characteristics, including amino acid number, calculated molecular weight and isoelectric point, were performed using the SIB Bioinformatics Resource Portal (http://expasy.org/). The presence of functional domains was confirmed by the Pfam database (PfamWeb: http://pfam. xfam.org/). Multiple alignments were carried out with other known plant ADH proteins using the DNAMAN5.0 Program. To place the *ADH* gene on a phylogenetic tree, a multiple alignment of the pear *ADH* gene with those of other species was performed using the MEGA5.0 (http://megasoftware.net) Program based on the neighbor-joining method (minimum evolution criterion and bootstrap values performed on 1 000 replicates). The positions of the introns and exons in the *ADH* genes were determined using Spidey (http://www.ncbi.nlm.nih.gov/spidey/).

2.4. RNA isolation and cDNA synthesis

Total RNA was extracted using the cetyl trimethyl ammonium bromide method from fruit tissues (Zhang *et al*. 2012). The RNA was then treated with DNaseI according to the manufacturer's instructions (TaKaRa Bio Inc., Shiga, Japan) to