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Research article

Molecular identification and characterization of the pyruvate decarboxylase gene family associated with latex regeneration and stress response in rubber tree

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ABSTRACT

In plants, ethanolic fermentation occurs not only under anaerobic conditions but also under aerobic conditions, and involves carbohydrate and energy metabolism. Pyruvate decarboxylase (PDC) is the first and the key enzyme of ethanolic fermentation, which branches off the main glycolytic pathway at pyruvate. Here, four *PDC* genes were isolated and identified in a rubber tree, and the protein sequences they encode are very similar. The expression patterns of *HbPDC4* correlated well with tapping-simulated rubber productivity in virgin rubber trees, indicating it plays an important role in regulating glyco-metabolism during latex regeneration. *HbPDC1*, *HbPDC2* and *HbPDC3* had striking expressional responses in leaves and bark to drought, low temperature and high temperature stresses, indicating that the *HbPDC* genes are involve in self-protection and defense in response to various abiotic and biotic stresses during rubber tree growth and development. To understand ethanolic fermentation in rubber trees, it will be necessary to perform an in-depth study of the regulatory pathways controlling the *HbPDC*s in the future.

1. Introduction

Ethanolic fermentation is an ancient and traditional metabolic pathway in all species. In plants, the pathway has been studied during flooding in the past few decades and was found to take part in ATP production under anaerobic conditions (Kato-Noguchi and Morokuma, 2007; Peschke and Sachs, 1993; Sachs et al., 1996; Vartapetian and Jackson, 1997). In addition to the flooding tolerance, the pathway has recently been found to have new functions, such as anther development and response to disease and stress, under aerobic conditions (Kato-Noguchi and Yasuda, 2007; TadegeDupuis and Kuhlemeier, 1999). During the fermentation period, pyruvate decarboxylase (PDC) is the key enzyme that irreversibly converts pyruvate into acetaldehyde and CO_2. Depending on the environment, two different metabolic pathways produce acetaldehyde, which is very toxic to plants (Chen and Han, 2011; Tadege et al., 1999). Under anaerobic conditions, the acetaldehyde is subsequently converted to ethanol, regenerating NAD^+ for the continuation of glycolysis to supply ATP. In the other pathway, acetaldehyde is converted to acetyl-CoA under aerobic conditions, supporting energy production, and lipid and amino acid biosynthesis.

In plants, PDC drives the fermentation pathway involved in energy and material metabolism, responding to development, and biotic and abiotic stresses, such as flowering, anoxia, cold, salt, wounding and pathogen infection (Tadege et al., 1999). PDC is encoded by a multigene family, and there are at least four *PDC* genes in both of the model plants *Arabidopsis* and rice (Hossain et al., 1996; Kursteiner et al., 2003; Rivoal et al., 1997). Among the *PDC* gene family of *Arabidopsis*, both *PDC1* and *PDC2* have been significantly induced to express during hypoxia and anoxia, and have been demonstrated to play an important role in the tolerance to submergence by mutant and transgenic experiments (Mithran et al., 2014). In rice, the expression of *PDC1*, *PDC2* and *PDC4* is also strongly up-regulated during flooding, which improves the tolerance under long-term anoxia (Hossain et al., 1996; Rivoal et al.,

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1997). Additionally, the rice *PDC3* gene specifically expresses in pollen and plays a role in aerobic alcoholic fermentation in mature pollen (Chen and Han, 2011; Li et al., 2004). In petunia, *PDC2* is highly and exclusively expressed in anthers and pollen, and an analysis of the *pdc2* mutant phenotype indicates the participation of *PDC2* in pollen tube elongation (Gass et al., 2005). Overexpression of *PDC1* in *Arabidopsis* enhances the low-temperature sweetening tolerance in the transgenic potato (Pinhero et al., 2011). Overall, the *PDC* gene family regulates sugar metabolic processes in response to environmental stress and development in plants.

Natural rubber, *cis*-polyisoprene, is an essential industrial substance and strategic material, and is mainly acquired from *Hevea brasiliensis*, the Pará rubber tree. Rubber biosynthesis occurs in the cytoplasm (latex) of laticifers, which are a strong sink and highly specialized cells in the phloem (Lewinsohn, 1991; Metcalfe, 1967). In latex, sugar metabolism is necessary to provide the carbon and energy demands for rubber biosynthesis. Intermediate products of glycolysis, acetyl-CoA or glyceraldehyde-3-phosphate and pyruvate, are considered as precursors of natural rubber. Physiology and biochemistry, as well as molecular biology, experiments demonstrate that the supply and utilization of sucrose is a major factor for latex regeneration affecting the rubber yield (Bouteau et al., 1991, 1992; BouteauDellis and Rona, 1999; Jacob et al., 1988; Silpi et al., 2006, 2007; Tang et al., 2010; Tupy, 1973). In addition, the rubber yield is also affected by environmental factors, such as cold, wind and disease, and especially wounding during tapping (Gébelin et al., 2013; Li et al., 2010; Sookmark et al., 2002; Venkatachalam et al., 2009). In other plants, PDC not only adjusts the concentration of pyruvate and acetyl-CoA but also responds to biotic and abiotic stresses. However, there were few references regarding PDC in rubber trees.

In this study, four *PDC* genes (*HbPDC*s) were isolated and identified from a rubber tree for the first time. The expression patterns of the *HbPDC*s were analyzed under different experimental conditions. The results demonstrate that *HbPDC4* plays an important role in the fermentation of laticifers, and the other *HbPDC*s might be involved in self-protection and defense in response to various abiotic and biotic stresses.

2. Methods

2.1. Plant materials

Reyan7-33-97 (CATAS7-33-97 or RY3-33-97) rubber trees (*H. brasiliensis*) selected for this study were cultivated at the experimental plantation of the Rubber Research Institute of the Chinese Academy of Tropical Agricultural Sciences (CATAS, Danzhou, Hainan, China). These trees were regularly tapped for latex collection in a half spiral pattern, every three days (S/2, d/3), without ET stimulation. To study the tissue-specific expression of the *HbPDC*s, all tissues were collected for RNA extraction from 10-year-old mature trees of Reyan7-33-97 that had been tapped for the last 2 years. The same type of tree was also used to examine the effects of various hormones on *HbPDC*. To analyze the effect of tapping and wounding on the *HbPDC*s expression levels, 8-year-old mature virgin (never tapped) trees were selected.

2.2. Isolation of HbPDC genes

To isolated and identify the *HbPDC* sequences, the *PDC* genes of *Arabidopsis* and *Oryza sativa* were used to search the transcriptome database of *H. brasiliensis* (Rahman et al., 2013). According to the sequences of the resulting contigs, multiple pairs of primers were designed and used to amplify the cDNA and genomic DNA of *PDC* genes from *H. brasiliensis* (Table 1). The PCR products were cloned into the pMD18-T cloning vector (TaKaRa Biotechnology, Dalian, China), and then transformed into *Escherichia coli* DH5α cells. The obtained sequences were submitted to the NCBI database for BLAST searching, and other bioinformatics analyses. Exon/intron structures of the *PDC* genes were analyzed by comparing the cDNA sequences and their genomic DNA sequences using the web server GSDS (http://gsds.cbi.pku.edu.cn/).

2.3. Construction of a phylogenetic tree

A phylogenetic tree of PDC was obtained by analyzing the deduced amino acid sequence from *H. brasiliensis* (KJ 599632, *HbPDC1*; KJ 599633, *HbPDC2*; KJ 599634, *HbPDC3*; and KJ 599635, *HbPDC4*), *Glycine* (XP003529168.1, XP00355267.1, XP003522062.1, XP003516954.1, XP003542972.1), *Medicago* (XP003623318.1, XP00003604737.1, XP003593734.1), Populus (XP002322997.1, XP002305755.2, XP002317431.1, XP002308230), *Ricinus* (XP002530500.1, XP002522545.1), *Arabidopsis* (Q9FFT4.1, O82647.1, Q9M040.1, Q9M039.1), *Sorghum* (XP002439941.1, XP002465414.1), *Zea* (NP001105422.1, NP001105052.1), *Oryza* (A2Y5L9.1, A2YQ76.2, NP001049811.1), *Brachypodium* (XP003558149.1) and *Setaria* (XP004984863.1), using the Neighbor-Joining method in MEG 5.05 software. A bootstrap analysis was performed using 1000 replicates.

2.4. Hormones, tapping and wounding treatments

To determine the expression patterns of *HbPDC*s in response in

Table 1
Primer sequences used in this paper.

Primer code	Primer sequence	Use
HbPDC1-F	5′-CAG CCT CCA AAA CCA CAA AAC AGA GTC TAA T-3′	PCR amplification primers for the *HbPDC*s
HbPDC1-R	5′-ACA TCA TAC AAT GGT TAC AAT CAC ATG GCT CG-3′	PCR amplification primers for the *HbPDC*s
HbPDC2-F	5′-CCT TTG CAT TCT GCG AAA ACC CAT TTG TTG-3′	PCR amplification primers for the *HbPDC*s
HbPDC2-R	5′-ACA TCA TAC AAT GGT TAC AAT CAC ATG GCT CG-3′	PCR amplification primers for the *HbPDC*s
HbPDC3-F	5′-CAA AAC AGA AAA AAA TTC TCC TTC TCA TGG ACA CC-3′	PCR amplification primers for the *HbPDC*s
HbPDC3-R	5′-GAT CAC TCA CAG GAG TGG GTC TCA TTC AC-3′	PCR amplification primers for the *HbPDC*s
HbPDC4-F	5′-TCA AAA CAG TAA CCA AAA CAG AAA AAA ATT CTC CTT CT-3′	PCR amplification primers for the *HbPDC*s
HbPDC4-R	5′-GCG CCA TTA ACA TTA GAA ATG AAA CTT CAA AGA TTG TC-3′	PCR amplification primers for the *HbPDC*s
HbPDC1-Q-F	5′-TGC TTA TAG TGA GAA TTT GCC CG-3′	Real-time PCR primers for the *HbPDC*s
HbPDC1-Q-R	5′-GCA GTG TCA ATC TGT TCG TGC-3′	Real-time PCR primers for the *HbPDC*s
HbPDC2-Q-F	5′-TGC GAA TCA AAT GGG CTC TG-3′	Real-time PCR primers for the *HbPDC*s
HbPDC2-Q-R	5′-GTT ACA GCA GCC GAT CAC G-3′	Real-time PCR primers for the *HbPDC*s
HbPDC3-Q-F	5′-GAC CAC CAA ATC CTC AGT AGA ATT-3′	Real-time PCR primers for the *HbPDC*s
HbPDC3-Q-R	5′-GAG TCC GGA GAA CAT CAA ACT G-3′	Real-time PCR primers for the *HbPDC*s
HbPDC4-Q-F	5′-CCA AGG TCC AAT GTG AGG AG-3′	Real-time PCR primers for the *HbPDC*s
HbPDC4-Q-R	5′-ATC TTG TCC TGA AAG AAC AAA GGA-3′	Real-time PCR primers for the *HbPDC*s
YLS8-Q-F	5′-CCT CGT CGT CAT CCG ATT C-3	Real-time PCR primers for the housekeeping gene
YLS8-Q-R	5′-CAG GCA CCT CAG TGA TGT C-3′	Real-time PCR primers for the housekeeping gene