Transcriptome Changes Associated with Boron Applications in Fruits of Watercore-susceptible Pear Cultivar

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Research Article

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Abstract

Watercore is a common physiological disorder in pear and is closely related to excessive accumulation of sorbitol and sucrose. Our previous research found the watercore incidence of 'Akibae' (*Pyrus pyrifolia* cv. Akibae) fruit significantly decreased after boron application (BA). Moreover, foliar spray of boric acid also significantly improved fruit quality. To uncover the mechanisms underlying pear fruit response to BA, a comprehensive transcriptome analysis was performed in this study. Transcriptome results revealed a total of 3146 up-regulated and 1145 down-regulated differently expressed genes (DEGs) between control and treated fruits of 'Akibae' pear, respectively. BA significantly induced expression of sorbitol metabolism and sucrose metabolism genes. Besides, BA also increased the expression of starch degradation, fatty acid synthesis, IAA (indole-3-acetic acid) degradation, GA (gibberellin acid) synthesis and inhibit the expression of ethylene synthesis genes. Overall, these findings suggested that BA alleviated 'Akibae' watercore occurrence and improve fruit quality by regulating the decrease of sorbitol and sucrose, increased of fatty acid and a balance of plant hormone. Our results provided further information for understanding the molecular mechanism of the effect of boron application on pear fruit.

Introduction

Watercore is a physiological disorder which occurs frequently in the Rosaceae fruit and often occurs in the ripening stage of fruit ¹. But at present, the watercore in pear has not been paid enough attention, which often causes great economic losses. A wide range of environmental and physiological factors have been implicated in the incidence of watercore. In general, the mineral nutrition unbalance contributing to watercore incidence. A number of physiological disorders associated with low fruit calcium content can develop in the orchard or become evident after a period of storage in both apple and pear ². Besides, boron appear to have a fundamental role not only in fruit quality but also in physiological disorders, affecting basic structural features and physiological processes in plant ³. 'Akibae' pear is a self-compatible cultivar and resistant to black spot disease, however, highly susceptible to watercore ⁴. Our previous study found boron application can reduce the occurrence of 'Akibae' pear ⁵. However, there are few studies on the relationship between boron and watercore at molecular level.

Boron (B) plays important roles in numerous metabolic and physiological processes of higher plants, including sugar transport, cell wall synthesis and lignification, cell wall structure maintenance, carbohydrate metabolism, RNA metabolism, respiration, plant hormone metabolism, phenol metabolism, and membrane transport ⁶. Boron applications is one of the important measures to improve fruit quality in plant production. For instance, boron application resulted in an increase in flower percentage, final fruit set, firmness of the fruit, TSS content, fruit juice ascorbic acid and fruit yield in various economic crops ⁷⁻¹². However, the transcriptional changes of fruit quality as influenced by boron applications are still rarely reported.

Hereby, the purpose of current research was to provide important information for further functional studies of novel boron induced gene expression of 'Akibae' related to inhibit watercore occurrence and quality metabolism using RNA-Seq technology.

Materials And Methods

Plant materials and treatments

The 'Akiba' (*Pyrus pyrifolia* cv. Akibae) which grafted onto *Pyrus calleryana* in the pear orchards of the Yangzhou University, Jiangsu Province, China, were used in the present study. Pear trees were planted in double-layer trellis training and pruning and the fruit had high consistency. The 80 days after flowering (DAF) fruits were treated with the concentration of 0.1% boron acid. The control fruits were treated with purified water. Five uniform fruits of each treatment were randomly collected from the outer crown of the tree after ten days treatment and named BA (Boron Application) and CK (Control), respectively. The samples were treated with liquid nitrogen immediately and were stored at -80°C .

RNA extraction and sequencing

RNA of each sample was isolated using the Trizol Kit (Promega, USA) following the manufacturer's instructions. To gain insight into the transcriptomic dynamics of boron application fruit, RNA-seq analysis was performed using pulp tissues in three biological replicates. For each sample, 1 μg of RNA was used to generate RNA-seq library with NEB Next® Ultra™ RNA Library Prep Kit for Illumina (NEB, Ipswich, USA) and library quality was assessed on the Agilent Bioanalyzer 2100 system. The libraries were sequenced on the Illumina Novaseq platform by Novogene (Beijing, China). Raw data (raw reads) of fastq format were first processed and clean reads were obtained by removing reads containing adapter and poly-N and those of low quality. Meanwhile, Q20, Q30 and GC content of the clean data were also calculated. The high quality clean data was mapped to the reference genome of *Pyrus bretschneideri* Rehd¹³ using Hisat2. Gene expression levels were estimated with Fragments Per Kilobase of transcript sequence per Millions (FPKM). Differential expression analysis of two groups was performed using the DESeq2 R package, and *P*-values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. The differentially expressed genes (DEGs) were screened according to the following criteria: $|\log_2^{\text{foldchange}}| \geq 1$ and corrected $P < 0.05$. The selected DEGs were also annotated with the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

Results

RNA sequencing and analysis of differentially expressed genes

The transcriptome changes of BA pulp were investigated through RNA-Seq analysis. More than 40 million reads were generated per sample. Of these reads, the Q30 percentage (sequencing error rate < 1%) was over 93%, and GC content was approximately 47% for the libraries. Among all the libraries, 64.85–66.32% of unique reads were mapped to the *Pyrus bretschneideri* Rehd (Table 1).

Table 1				
Statistics of sequencing data of the six libraries.				
Library	Clean reads	Q30 ratio (%)	GC content (%)	Mapping ratio (%)
BA1	42034166	93.59	46.93	27270180(64.88%)
BA2	44906822	93.64	46.91	29120166(64.85%)
BA3	41265050	93.64	47.02	27011504(65.46%)
CK1	45391358	93.73	47.11	30102908(66.32%)
CK2	43646738	93.86	46.82	28547478(65.41%)
CK3	42463764	93.6	46.67	27619994(65.04%)

A total of 3146 genes and 1145 genes in BA pulp were significantly up-regulated and down-regulated, respectively (Fig. 1A). All the DEGs were subjected to GO and KEGG analysis. In the GO database, thylakoid, thylakoid part, photosynthetic membrane, photosystem, et al were the most enriched in the ‘cellular component’ category; iron ion binding, DNA binding transcription factor activity, et al were the most enriched in the ‘molecular function’ category cellular component (Fig. 1B). Pathways showing significant change (Q value ≤ 0.05) in BA pulp were identified using the KEGG database. The DEGs were mainly involved in the pathways of plant hormone signal transduction, starch and sucrose metabolism, photosynthesis, phenylpropanoid biosynthesis, flavonoid biosynthesis, carotenoid biosynthesis, brassinosteroid biosynthesis and et al (Fig. 1 C).

Changes of sugar metabolism-related gene expression in BA fruit

Sorbitol metabolism related gene analysis showed that a sorbitol synthesis gene, S6PDH (LOC103966946), was significantly down-regulated. The expression levels of three sorbitol dehydrogenase genes (LOC103960512, LOC103930819 and LOC103930934), seven sorbitol transporter genes (LOC103936915, LOC103964420, LOC103948508, LOC103929477, LOC103936910, LOC103936324 and LOC103964421) and a starch degradation gene (LOC103934256) were significantly increased in BA fruit. Four sucrose-phosphate synthase genes (LOC103946723, LOC103937917, LOC103952486 and LOC103956574) and two sucrose synthase genes (LOC103927263 and LOC103946870) were significantly down-regulated and up-regulated after the treatment of boric acid, respectively (Table 2).

Table 2		
List of DEGs relate to sorbitol, sucrose and starch metabolism		
ID	Gene description	log ₂ ^{Fold Change}
Sorbitol metabolism		
LOC103966946	D-sorbitol-6-phosphate dehydrogenase, S6PDH	-1.59
LOC103960512	Sorbitol dehydrogenase, SDH	1.34
LOC103930819	Sorbitol dehydrogenase, SDH	1.46
LOC103930934	Sorbitol dehydrogenase, SDH	8.02
LOC103936915	Sorbitol transporter, SOT	4.67
LOC103964420	Sorbitol transporter, SOT	4.28
LOC103948508	Sorbitol transporter, SOT	5.99
LOC103929477	Sorbitol transporter, SOT	8.85
LOC103936910	Sorbitol transporter, SOT	6.96
LOC103936324	Sorbitol transporter, SOT	5.73
LOC103964421	Sorbitol transporter, SOT	4.65
Sucrose metabolism		
LOC103946723	Sucrose-phosphate synthase, SPS	-2.29
LOC103937917	Sucrose-phosphate synthase, SPS	-2.55
LOC103952486	Sucrose-phosphate synthase, SPS	-3.80
LOC103956574	Sucrose-phosphate synthase, SPS	-2.54
LOC103927263	Sucrose synthase, SS	1.25
LOC103946870	Sucrose synthase, SS	3.26
Starch degradation		
LOC103934256	β-amylase	3.08

Changes of fatty acid synthesis-related gene expression in BA fruit

Six genes involved in fatty acid biosynthesis were significantly regulated induced by BA. These genes included two malonyl CoA-acyl carrier protein transacylase genes (LOC103945733 and LOC103935263), a 3-oxoacyl-[acyl-carrier-protein] synthase gene (LOC103951262), a 3-hydroxyacyl-[acyl-carrier-protein] dehydratase gene (LOC103967963) and three enoyl-[acyl-carrier-protein] reductase genes (LOC103964860, LOC103959004 and LOC103940555) (Table 3).

Table 3		
List of DEGs relate to fatty acid biosynthesis		
ID	Gene description	log ₂ Fold Change
Fatty acid biosynthesis		
LOC103945733	Malonyl CoA-acyl carrier protein transacylase	1.50
LOC103935263	Malonyl CoA-acyl carrier protein transacylase	1.35
LOC103951262	3-oxoacyl-[acyl-carrier-protein] synthase	1.23
LOC103967963	3-hydroxyacyl-[acyl-carrier-protein] dehydratase	1.40
LOC103964860	Enoyl-[acyl-carrier-protein] reductase	1.83
LOC103959004	Enoyl-[acyl-carrier-protein] reductase	2.43
LOC103940555	Enoyl-[acyl-carrier-protein] reductase	1.61

Changes of plant hormone metabolism-related gene expression in BA fruit

Analysis of DEGs found that two indole-3-acetaldehyde oxidase genes (LOC103958137 and LOC103929079) and six Peroxidase genes (LOC103948174, LOC103951047, LOC103934752, LOC103964015, LOC103958862 and LOC103953577) which involved in IAA synthesis and degradation, respectively, were significantly down-regulated and up-regulated in BA fruit. Moreover, the expression levels of two GA synthesis genes (LOC108865311, LOC103948777 and LOC103939254) were significantly increased in BA fruit. Four key genes of ethylene synthesis (LOC103948231, LOC103943975 and LOC103939367) were significantly decreased in BA fruit (Table 4).

Table 4		
List of DEGs relate to plant hormone metabolism		
ID	Gene description	$\log_2$ Fold Change
IAA degradation		
LOC103958137	indole-3-acetaldehyde oxidase	-4.93
LOC103929079	indole-3-acetaldehyde oxidase	-4.50
LOC103948174	Peroxidase, POD	1.60
LOC103951047	Peroxidase, POD	6.54
LOC103934752	Peroxidase, POD	2.43
LOC103964015	Peroxidase, POD	3.11
LOC103958862	Peroxidase, POD	1.80
LOC103953577	Peroxidase, POD	6.48
GA synthesis		
LOC108865311	ent-kaurene oxidase, KO	3.90
LOC103948777	ent-kaurenoic acid oxidase, KAO	1.81
LOC103939254	ent-kaurenoic acid oxidase, KAO	3.83
Ethylene synthesis		
LOC103948231	1-aminocyclopropane-1-carboxylate oxidase, ACO	-7.01
LOC103943975	1-aminocyclopropane-1-carboxylate oxidase, ACO	-9.88
LOC103939367	1-aminocyclopropane-1-carboxylate oxidase, ACO	-1.74
LOC103956316	1-aminocyclopropane-1-carboxylate synthase, ACS	-2.39

Discussion

Many studies discovered that watercored had a consistently higher sorbitol content than healthy fruit in both apples and pears ². Moreover, Chun and our research also found the 'Akibae' is high-sucrose cultivar and the watercore fruit accumulated higher sucrose content ^{4,14}. Thus, the accumulation of sorbitol and sucrose has been speculated to be closely related to watercore. In pear production, it is commonly utilized from foliar applications of calcium at preharvest period ¹⁵. However, the relationship between calcium concentration and watercore occurrence is not always apparent and in some cases is contradicted ². Boron is imperative element for plant growth and it must be obtainable in adequate quantities for optimal

plant growth and productivity ¹⁶. In this study, the sorbitol metabolism-related and sucrose metabolism-related genes were significantly induced by BA which suggested that spraying boron during fruit development can reduce the occurrence of watercore by reducing sorbitol and sucrose accumulation. Moreover, boron has also been shown to form a stable complex with sorbitol and sucrose ^{17,18}. Although the function of boron in apple and pear remains unknown, it is becoming increasingly important to understand the significance of the formation of sorbitol-boron or sucrose-boron complexes in watercore development.

Pear starch in fruit started accumulating early and reached a maximum concentration during the middle phase of fruit development then decreased ¹⁹. In this study, BA significantly increased the expression of β -amylase and accelerated starch degradation which promoted the formation of fruit quality. In addition, fruit aromas consist of many aroma volatiles, and fatty acid metabolism is an important metabolic pathway involved in the biosynthesis of aroma volatiles in fruit ²⁰. Previous study found application of boron led to a better olive oil quality by improving fatty acid composition ²¹. In this study, BA also significantly induced the gene expression of fatty acid biosynthesis which indicated BA increase the formation of fruit aromas.

Boron affect the formation of fruit quality by regulating plant hormone metabolism. Hegazi found IAA and abscisic acid (ABA) decreased and GA increased in response to boron treatments which indicated a balance in endogenous hormones (IAA, GA, ABA) concentrations in olive tree has induced the maximum fruit set and yield ²². Islam found the Si + B treatment resulted in the lowest respiration and ethylene production ²³. In current study, BA significantly increased the expression of GA synthesis genes and inhibit the synthesis of ethylene and IAA which closely related to fruit size and delayed fruit softening.

Above all, we speculated a model which BA influence pear watercore occurrence and fruit quality (Fig. 6). The boron signal induced the decrease of sorbitol and sucrose synthesis gene expression or increase of gene expression of sorbitol transport, sorbitol degradation and sucrose degradation in pulp cells, resulting in the accumulation less sorbitol and sucrose in pulp and inhibited fruit watercored. Moreover, boron also affect the fruit aromas related genes and the balance of endogenous hormones which lead to the fruit internal and external quality. Hence, this study provided further information for understanding the molecular mechanisms of the influence of BA on pear.

Declarations

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Author contributions

XL, JW and C-L W conceived and designed the research. DH L, YS and JL analyzed the data and performed the bioinformatics analysis. XL wrote the manuscript.

Compliance with ethical standards

Conflict of interest

The authors assure that the manuscript has not been submitted for publication elsewhere. All the authors have contributed equally to this article, and all have agreed to submit it for publication in this journal. All the authors declare that there are no conflicts of interest to disclose.

Research involving human participants and/or animals

This research is about transcriptomic analysis in plant (pear). Human participants/animals were not involved in this study.

Informed consent

Not applicable in this study.

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Figures

Figure 1

Number of DEGs (A), GO classification (B) and KEGG enrichment (C) of DEGs.

Figure 2

A proposed model of BA influence on pear watercore occurrence and fruit quality. The red and green circle refers to promote and inhibit related gene expression or metabolic pathways, respectively.