Comparative analysis of transcriptome profiling to identify genes involved in bulged surface of pear fruits (*Pyrus bretschneideri* Rehd. cv. Yuluxiangli)

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Research article

Keywords: Pear (*Pyrus*); bulged surface; paclobutrazol; auxin; DEGs

Posted Date: May 1st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-22697/v1>

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Abstract

Background: Pear (*Pyrus* spp.) belongs to the genus *Pyrus*, in the family Rosaceae. Some varieties of pear fruits exhibit bulged surface, which seriously affect the quality and commodity value. In this study, we performed anatomical, physiological and transcriptomic analysis, to explore the mechanism of paclobutrazol (PBZ) on the bulged surface of pear fruits.

Results: The vascular bundles of flesh were more evenly distributed and the cells arranged more compactly with smaller size. However, the auxin (IAA) content of flesh decreased significantly when treated with PBZ. Further, the GO and KEGG analysis of differentially expressed genes (DEGs) showed that auxin, phenylpropanoid metabolic pathways and transcriptional factor genes were significantly enriched on the relieved bulged surface of pear fruits. The promoter region analysis of selected DEGs showed that some genes contained auxin responded cis-elements.

Conclusion: We conclude that PBZ might play a negative role in the cell division, cell elongation and vascular bundle development on bulged surface of pear fruits through the involvement of auxin related genes. This study will provide theoretical basis for the regulation of bulged surface of pear fruits by growth retardant agent.

Background

The pear tree is a specie of genus *Pyrus* in the family Rosaceae, bearing the pomaceous fruit of the same name, which is the third largest temperate fruit tree and widely cultivated in the world [1, 2]. However, some high-quality cultivated species, such as 'Korla' fragrant pear (*Pyrus sinkiangensis* Yu) and its bred variety 'Yuluxiangli' (*Pyrus bretschneideri* Rehd.), have the phenomenon of bulged surface fruits, which results in low-quality of fresh pear fruits with poor appearance and affects their value of commodity [3].

Plant growth retardants, negatively regulate plant growth and development by inhibiting hormones and help in partitioning of assimilates effectively [4–6]. Spraying growth retardants, such as paclobutrazol (PBZ), a member of the triazole family, at full bloom stage could significantly relieve bulged surface of pear fruits and improve its appearance [3]. It was reported that PBZ inhibited IAA and other hormones effects to delay growth vigor [7]. Therefore, the unbalanced distribution of endogenous hormones, might lead to uneven growth of various parts of the fruits, which might be one of the key factors leading to bulged surface of fruits. Previously, It was showed that the vascular bundles at the calyx end of young fruit were significantly retarded in pear by growth retardant PBZ [8, 9]. Vascular tissues were responsible for the transport of water, nutrients and photosynthetic products during plant growth and development [10–12]. Thus, we speculated that the growth and development of vascular bundles might directly affect the bulged surface of pear fruits. Furthermore, PBZ caused palisade and spongy leaves with more compact cell arrangement and smaller cell size [13, 14].

IAA plays an important role in vascular development through the auxin carrier, such as pin-formed 1 (PIN1), auxin efflux carrier, which affects the development of vascular bundles [15–17]. Some previous

studies showed that auxin influx carrier LAX2 was involved in vascular bundle development [18, 19]. Does PBZ affect the growth and development of vascular bundles by negatively regulating auxin and retarding strong growth vigor, thus relieving the bulged surface in pear fruits?

Lignin is distributed in cell walls of lignogenic vascular machinery, rigidifying them and rendering them impermeable to water. Thus, lignin plays important roles in increasing the strength of the cell wall and enhancing the ability of water transport in plants [20]. In lignin biosynthesis process, some genes such as *F5H* (*ferulic acid-5-hydroxylase*), *4CL* (*4-coumaric acid CoA ligase*) and *COMT* (*caffeic acid/5-hydroxyferulic acid-O-methyltransferase*) genes could also affect the growth and development of vascular tissue [21–23]. In addition, bHLH transcription factor, such as target of *monoperos 5* (*tmo5*) interacted directly with ARF (auxin responsive gene), and playing a significant role in the procambium tissue, where the vascular bundles split and bHLH interacted directly with another bHLH transcriptional factor LHW to act on ARF5/MP [24, 25]. Some other transcriptional factors, such as MYB, were also found to be involved in the accumulation of lignin, during vascular bundle growth and development [26–28]. Are these genes related to vascular development involved in pear-bulged surface and regulated by PBZ?

‘Yuluxiangli’ is a Chinese cultivated pear variety, famous for its high production and good quality fruits. However, producing bulged surface fruits is one of the major problems, facing this variety. Recently, researches were performed to investigate the cause of its bulge surface fruits and find out the prevention measures [3, 29]. However, the molecular mechanism of pear fruits bulged surface is still unclear. For this reason, in our study, the plant of full blooming flower of ‘Yuluxiangli’ was treated with the growth retarding agent PBZ, to explore the causing factors of the bulged surface of fruits, from structural, physiological and transcriptomic data. This study will provide theoretical as well as molecular basis for the regulation of bulged surface of pear fruits by growth retardant agent.

Results

The morphological and anatomical features of bulged surface of pear fruits

PBZ plant growth retardant was sprayed at the full blooming stage of ‘Yuluxiangli’, then fruit samples were collected after 85 days of plants full blooming stage. The surface of fruits in the control group were significantly bulged (Fig. 1a and 1b, CK; Additional file Fig. S1), whereas the fruits with smooth surface and diagonally round shape were treated with PBZ (PK)(Fig. 1a and 1b). Further, 10.8 and 6 number of vascular bundles were observed and sprayed with ddH₂O (CK) and PBZ (PK), respectively (Fig. 1c). Moreover, the result showed that the cross section of fruits vascular bundles were dense and irregular in shape of the control group, when sprayed with water; whereas the vascular bundles were distributed uniformly and developed gradually and its shape was round, when treated by growth retardant agent PBZ (Fig. 1d).

The content of IAA in relieved bulge surface of pear fruits

The IAA content of pear fruits was 24.86 ng/g in control group. But when treated with PBZ it was 18.27 ng/g (Fig. 2). The IAA content was higher in CK group than that of treated group (PK).

The paraffin section and its characteristic of bulged surface of fruits

The result of paraffin wax sections showed that the cells were sparse and irregular in control group of fruits, however the result of the cells of fruits were round and smaller in size, with more compaction observed when treated by PBZ (Fig. 3a). Furthermore, the number of cells was 9.27 and 20.53/100 $\mu\text{m} \times 100 \mu\text{m}$ in CK and PK groups, respectively (Fig. 3d); the number of cell layers was 9.67 and 15.40/400 $\mu\text{m} \times 400 \mu\text{m}$ in CK and PK groups, respectively (Fig. 3c). And the length of fruit cell was 70.64 μm and 12.27 μm in CK and PK groups, respectively (Fig. 3b). The paraffin wax section showed that the length of fruit cells in control group was significantly longer than that of treated by PBZ. Further, the elongated and morphological irregular cell sizes were observed in control group. And the number of cells and cell layers were significantly reduced in control group.

Identification genes involved in bulged surface of pear fruits

Sequencing of samples was the same as 2.1. A total of 442 differentially expressed genes (DEGs) were identified in this study, of which 249 were up-regulated and 193 were down-regulated in response to growth retardant agent PBZ (Additional file Fig. S2 and Additional file Table S1).

All DEGs were annotated by blasting their sequences against eight publically available databases such as COG, GO, KEGG, KOG, NR, Pfam, Swiss-prot and eggNOG. Significant annotation matches were found for 440 DEGs (99.5%) in all databases (Additional file Table S2 and Additional file Table S3).

In order to explore the relationship of expressed genes, involved in the function of regulating bulged surface of fruits, we determined the important biological functions of 442 DEGs by GO and KEGG analysis. The selected up- and down-regulated DEGs were assigned to two main GO categories: molecular function and biological process, significantly enriched in phenylpropanic metabolic pathway, which were related to the lignin biosynthesis of vascular bundle. The selected DEGs of the molecular function were further distributed into four different groups, namely, trans-cinnamate 4-monooxygenase activity (GO:0016710, *Pbr013141*), electronic carrier activity (GO:0009055, *Pbr040547* and *Pbr013141*), iron ion binding (GO:0005506, *Pbr040547*, *Pbr013141*), DNA binding (GO:0003677, *Pbr035111*, *Pbr013141*), sequence specific DNA-binding transcription factor activity (GO:0003700, *Pbr007589*); whereas the selected DEGs of the biological process were attributed into two groups, which were related to the metabolic process (GO:0008152, *Pbr012851*) and the oxidation reduction process (GO:0055114, *Pbr024635*, *Pbr013141* and *Pbr040547*) (Fig. 4a and Additional file Table S4).

To further study the biological pathways of DEGs triggered by growth retardant agent PBZ, they were annotated by blast analysis against the KEGG database. PBZ treatment relieved the bulged surface of pear fruits, through highly complex biological processes. However, the three important pathways namely, plant hormones (Ko04075), phenylpropane (Ko00940) and phenylalanine pathway (Ko00360) were significantly involved in the vascular bundle growth and development (Additional file Table S5). KEGG metabolic pathway analysis showed that six DEGs were involved in hormone signaling pathway. For example *auxin efflux carrier* genes (*PIN*, k13947, *Pbr028379*, *Pbr038852*), *auxin responsive* genes (*GH3*, k14487, *Pbr030587*, *Pbr030571* and *Pbr021158*) and *auxin influx carrier* genes (*LAX*, ko04075, *Pbr009498*). However, only *GH3* and *PIN* gene expression were up-regulated, but the other auxin-related genes were down-regulated in the fruit in response to PBZ. Seven DEGs related to phenylpropanic biosynthesis were down-regulated in pear flesh treated by PBZ reagent, including *ferulic acid-5-hydroxylase* (*F5H*, k09755, *Pbr040547*) [EC:1.14.-], *caffeic acid 3-O-methyltransferase* (*COMT*, k13066, *Pbr013510*) [EC:2.1.1.68], *peroxidase* (*PRX*, k04430, *Pbr031894*) [EC:1.11.1.7], *caffeoyl shikimate esterase* (*CAE*, k18368, *Pbr002415*) [EC:3.1.1.-], *4-coumaric acid coenzyme ligase* (*4CL*, k01904, *Pbr024635*, *Pbr012851*) [EC:6.2.1.12] and *trans-cinnamic acid 4-monooxygenase* (*CYP73A2*, k00487, *Pbr013141*) [EC:1.14.13.11]. And four genes, which were also involved in the phenylpropane pathway, participated in flavonoid synthesis (Ko00941, *Pbr013141*), stilbenid, dialheptanoid and ginger biosynthesis (Ko00945, *Pbr013141*), ubiquinone and other terpenoid quinone biosynthesis (Ko00130, *Pbr012851*, *Pbr013141* and *Pbr024635*) and phenylalanine metabolism (Ko00360, *Pbr012851*, *Pbr013141* and *Pbr024635*) (Fig. 4b, Table 1 and Additional file Table 5). Meanwhile, the expression of cellulose synthesis DEGs (*CesA*, k10999, *Pbr038537*, *Pbr034219*) [EC:2.4.1.12] were down-regulated in response to PBZ (Fig. 4c and Table 1). Further we found some transcriptional factor genes from 442 DEGs, such as, *bHLH130* (*Pbr032736*), *bHLH93* (*Pbr001646*), *MYBS3* (*Pbr015587*), *NAC002* (*Pbr020642*) and *DOF4.6* (*Pbr035111*), which were down-regulated in response to PBZ (Fig. 4c and Additional file Table S6).

The selected expression profile of DEGs, which might be involved in bulged surface of pear fruits, were analyzed and the candidate DEGs with similar expression patterns were clustered together based on their up- and down-regulated nature (Fig. 4c and Additional file Table S6).

Table 1
Cis-acting elements identified from the promoter regions of candidate genes.

Gene name	Cis-element	Sequence	Species	Function
<i>Pbr001646</i>	AuxRR-core	GGTCCAT	Nicotiana tabacum	cis-acting regulatory element involved in auxin responsiveness
<i>Pbr013141</i>	AuxRR-core	GGTCCAT	Nicotiana tabacum	cis-acting regulatory element involved in auxin responsiveness
<i>Pbr013510</i>	AuxRE	TGTCTCAATAAG	Glycine max	part of an auxin-responsive element
<i>Pbr024913</i>	AuxRR-core	GGTCCAT	Nicotiana tabacum	cis-acting regulatory element involved in auxin responsiveness
<i>Pbr030571</i>	AuxRR-core	GGTCCAT	Nicotiana tabacum	cis-acting regulatory element involved in auxin responsiveness
<i>Pbr034219</i>	AuxRR-core	GGTCCAT	Nicotiana tabacum	cis-acting regulatory element involved in auxin responsiveness
Supplementary Materials				
Supplementary information accompanies this paper at https://				

Predication of cis-regulatory elements and their distribution in candidate genes caused the bulged surface of pear fruits

To better understand transcriptional regulation of the candidate genes (Additional file Table S6), the cis-acting elements located in the 1.5-kb regions upstream of their start codons (ATG) were predicted by searching against the PlantCare online tool. The results showed that, the six genes (*Pbr001646*, *Pbr013141*, *Pbr013510*, *Pbr024913*, *Pbr030571* and *Pbr034219*) contained auxin responsive cis-regulatory elements AuxRR-core (GGTCCAT) or AuxRE (TGTCTCAATAAG) (Table 1). This indicated that auxin might involve in regulating bulged surface of pear fruits.

Validation of DEGs by real-time qRT-PCR analysis

To validate the RNA-seq data, we randomly selected nine DEGs namely; *PIN*, *LAX1*, *CYP73A2*, *COMT*, *CesA*, *bHLH*, *MYB*, *GH3.1* and *GH3.6* for qRT-PCR analysis in pear fruits in response to PBZ treatment. Transcriptional levels of these six genes by qRT-PCR showed that (*LAX1*, *CYP73A2*, *COMT*, *CesA*, *bHLH*, *MYB*), involved in plant hormone transporting, phenylpropionic acid pathway, cellulose pathway and plant transcription, were significantly down-regulated in response to PBZ treatment, however only *GH3* and *PIN* genes were up-regulated compared with the control group (Fig. 5). Similar patterns of genes expression were observed between the qRT-PCR and RNA-seq-generated data.

Discussion

It had been reported that the bulged surface of fruits could be significantly improved through cultivation measures and exogenous spraying of growth retardant agent [3]. However, the research on mechanism is still unknown. In this study, we explored the mechanism of growth retardant agent (PBZ) relieving bulged surface by means of histomorphology, exogenous hormones and transcriptome analysis.

PBZ retarded the growth and development of cell and vascular bundles

Schematic analysis revealed that the surface of pear fruit and their vascular bundles were unevenly distributed in the bulge part of the fruits in the control group (CK) . But it was evenly distributed in the transverse direction in fruits treated with growth retardant PBZ (PK)(Fig. 1, Additional file Fig. S1). It was previously reported that the developed vascular bundles were densely distributed at the calyx end of the bulged surface of pear fruits. However, these vascular bundles were developed slowly and distributed loosely at the calyx end of fruits in PBZ treated group [4,5]. And phloem cells appeared to become much smaller and tighter treated by PBZ than that of CK group [6]. These findings suggest that PBZ might affect the growth and development of the vascular bundles in the fruits, thus affecting the bulge shape on the surface fruits.

The cells were well division and development in CK group. And the length of cell became long in CK group (Fig. 3a). Furthermore, the control group showed that the cell arrangement was relatively wobbly, the cell sizes and layers were significantly increased in the course of making paraffin wax section. Whereas the cells in the transverse section near the fruit pericarps, treated with growth retardant PBZ, were found compact, small and arranged; and cell layer increased in PK group (Fig. 3b-3d). Researchers showed that palisade and spongy cells became smaller when sprayed with PBZ [6]. Meanwhile, the ratio of palisade to sponge layer thickness was increased[7,31], but cell longitudinal growth was inhibited in PBZ group [30-32]. Our data, together with previously reported studies, suggested that the growth retardant agent (PBZ) could affect cell size, cell layer arrangement and inhibit cell longitudinal growth.

PBZ reduced the content of IAA bulged surface of fruits

Auxin is one of the important endogenous hormones, which plays an essential role in many processes of plant growth and development, such as promoting cell division and elongation [33-35] and vascular tissue formation and differentiation [36-38]. Our observation of auxin results indicated that IAA content was dropped in fruits treated by PBZ compared with CK group (Fig. 2). Our results were consistent with previous studies showing that IAA content was reduced in plant tissue treated by PBZ [11-13]. In addition, the result further showed that bulged surface fruits caused by strong growing vigor contained with high IAA and was relieved by the way of balancing phytohormone, especially IAA, among tissues.

PBZ degraded the expression of auxin and lignin related genes

GO and KEGG analysis revealed that auxin transport-related genes *LAX* were down-regulated and auxin responded *GH3* genes and *auxin efflux carrier (PIN)* genes were up-regulated in pear fruits treated by PBZ. In addition, we found that lignin synthesis genes *CYP73A2*, *COMT* and *cellulose synthesis* genes involved in construction of vascular bundle were found down-regulated in the pear fruits treated by PBZ.

Meanwhile, transcriptional factor genes such as *bHLH* and *MYB* were down-regulated in PBZ treated pear fruits (Fig. 5). Real Time qRT-PCR analysis of these differentially expressed genes were consistent with that of RNA-seq data, which confirmed the reliability of the experimental data. Previous reports showed that auxin affected vascular bundle formation through auxin efflux carrier PIN [16, 39,40]. *Auxin efflux carrier* genes were highly expressed in mutant vascular bundles [15,40,41]. Similar studies were reported that auxin influx carrier LAX supported vascular bundle development [18,42]. In addition, the active state of IAA was inactivated by *GH3* genes [26,43]. Further our results proved that the PBZ treatment might change the auxin pathway genes activity to reduce IAA content, and could affect the distribution of transverse vascular bundles, cell division and cell elongation on the surface fruits, thus regulating the bulged surface in pear fruits.

In addition, it had been reported that, phytohormones, lignin synthesis related genes such as phenylpropane metabolic pathway genes *CYP73A2* and *COMT* [44,21,22], transcriptional factor genes *bHLH* and *MYB*, as well as cellulose synthetase genes were involved in vascular tissue, growth, development and metabolism of its secondary cell wall. On the other hand, *bHLH* genes played an important role in early vascular bundle development [45,46]. MYBs, directly act on VND6 and VND7, which were involved in the formation of vessel and xylem of secondary cell walls [47-49,27,50]. Furthermore, overexpression of *MYB52* and *MYB54* up-regulates *cellulose synthase 8 (CesA8)* activity [51,52] and *4-coumarate-CoA ligase (4CL)* [53,54] in cellulose and lignin synthesis. Some of the above differentially expressed genes showed that there were six genes which responds to auxin. These results indicated that PBZ treatment might affect vascular bundle development by changing the genes in response to auxin (Fig. 2 and Additional file Table S5).

In a word, this research presented a comprehensive overview of PBZ, which might regulate genes function involved in auxin metabolic pathway. Finally vascular development and cell division were affected by auxin. Thus it was concluded from our data that vascular bundles distribution and cells division might affect the bulged surface of pear fruits. This study will provide theoretical basis for the regulation of bulged surface of pear fruits by growth retardant agent (Fig. 6).

Conclusions

PBZ regulated the growth and development of vascular bundles related genes and cell division by negatively regulating auxin signal pathway and retarding strong cell growth vigor, which affected the cell

division, cell elongation and vascular bundle development on bulged surface of pear fruits, thus relieving the bulged surface in pear fruits.

Methods

Source of plants

A Chinese pear fruits variety 'Yuluxiangli' was used from Pomology Institute, Shanxi Academy of Agricultural Sciences, Taigu of China. Paclobutrazol (PBZ) plant growth retardant was sprayed at the full blooming stage of 'Yuluxiangli' with a concentration of 1500 times dilution ratio (PK: treated group), and ddH₂O was sprayed on control group (CK: control group). Random block design (RBD) was used, each treatment was repeated and applied three times. At full blooming stage, after 85 days of plants full blooming the treated young fruits were collected and immediately snap-frozen in liquid nitrogen and stored at -80 °C until RNA and DNA extraction. The fruits were immediately treated for hormone detection and tissue morphology observation. Pear genome sequences were downloaded from [website\(http://gigadb.org\)](http://gigadb.org)

Auxin hormone determination

A total of 0.15 g flesh of young fruit was accurately weighed and were used to determine the content of IAA using a method developed by Trione [55].

Staining Method

After fruits coloring from the fruit stalk, each fruit was crosscut from the middle to its core with a sharp blade using a method presented by Ma [56].

Paraffin wax section

Each fruit was cut perpendicularly from the pericarp to the core of the fruits with a sharp blade. Further, excised three long fruit tissues with a cross-section size of 3 mm × 3 mm. Immediately were fixed for no more than 24 h with Carnot fixation solution of FAA (5% formaldehyde: 5% acetic acid: 50% ethanol). Subsequently dehydrated by washing with a series of ethanol, cleaned by dipping in dimethylbenzene and packed in paraffin. Finally portioning was performed by microtome. Portioned samples were stained with safranin and fast green. Olympus biological microscope (IX81, Tokyo, Japan) was used for sample observation. The length of cells were calculated according to the scale of the light microscope. However, the number of cells and its layers were calculated at the same time. Paraffin sections were prepared a method developed by Yang [57] and Chen [3].

RNA extraction and RNA-seq data analysis

A total of 0.1 g flesh of fruits were placed in a precooled mortar with liquid nitrogen. The improved CTAB method was used to extract RNA from the flesh fruits following the manufacturer's instructions. Extracted RNA was dissolved in nuclease-free water and their quality and integrity were measured by

NanoPhotometer® spectrophotometer (IMPLEN, CA, USA) and 1% agarose gel electrophoresis, respectively. The RNA concentration was estimated using a Qubit® RNA Assay Kit in Qubit®2.0 Fluorometer (Life Technologies, CA, USA). Following RNA isolation and quality assessment, cDNA libraries were constructed and remaining nucleic acids was used for transcript analysis. An equal amount (500 ng) of total RNA was pooled from the biological replicates from each sample for each cDNA library construction. The libraries were sequenced using an Illumina HiSeq4000 platform. The above protocol steps developed by Zhang were followed[23]. The sequencing of raw data was deposited in RNA-seq data, GO and KEGG analysis were performed using BMKCloud (www.biocloud.net) online tool. Genes with an adjusted p-value < 0.05 ($|\log_2(\text{fold change})| \geq 1$) found by DESeq were considered as DEGs. Gene expression pattern analysis were carried out by BMKCloud (<https://www.biocloud.net/>) online tool.

Quantitative RT-PCR analysis

A total of 500 ng amount of RNA was used to synthesize first-strand cDNA, using TURScript 1st-Strand cDNA SYNTHESIS Kit (Aidlab Biotechnologies Co., Ltd, China) as templates, following the manufacturer's protocol. The diluted cDNA was used as a template and Real Time PCR Detection System was carried out by Analytikjena-qTOWER2.2 system (Germany). The detailed procedure and primers were listed in Additional file files (Additional file Table S7). The Tublin gene (Tubulin-F: TGGGCTTTGCTCCTCTTAC; Tubulin-R: CCTTCGTGCTCATCTTACC) was used as a reference primer [58]. Three biological replicates were carried out for each sample. The qRT-PCR data were analyzed by the $2^{-\Delta\Delta C_t}$ quantitative method to determine the differences. The expression data of these genes were transformed in $\log_2$ (Change Fold) values and used for heat map generation.

Cis-element analysis in promoter regions

To perform cis-element analysis, 1-1.5 kb of genomic DNA sequences upstream of each genes initiation codon (ATG) were downloaded, and then were detected by PlantCARE database (<http://www.dna.affrc.go.jp/PLACE/>) [60].

Statistics of analysis

The number of vascular bundles at different treatments were calculated at the size of 10 mm × 10 mm and cell layer was at the width of 400 μm × 400 μm, respectively. The cell numbers were calculated at the size of 100 μm × 100 μm at 15 visual fields. Data of Analysis of variance (ANOVA) was performed and multiple comparisons of the means were conducted by the student test at a P value < 0.05.

Abbreviations

IAA: Auxin; GH3 Gretchen hagen3; KEGG: Kyoto Encyclopedia of Genes and Genomes; PBZ: Paclobutrazol; PK: Paclobutrazol treatment; CK: ddH₂O treatment; DEGs: Differentially expressed genes; PIN: pin-formed 1; AUX: auxin influx carrier; COMT: Caffeic acid 3-O-methyltransferase; 4CL: 4-coumarate-CoA ligase 1; CYP73A2: Cytochrome P450 73A2; CSE: Caffeoyl shikimate esterase; NAC: domain-containing protein 2; DOF4.6: Dof zinc finger protein; CesA: Cellulose synthase A

Declarations

Ethics approval and consent to participate

This article did not include any experiments with human participants or animals performed by all the authors.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the above research are available from the Additional file or corresponding author. The pear genome datasets used during the current study are available in the website (<http://gigadb.org>). The RNA-seq data were deposited in NCBI bioproject (<https://www.ncbi.nlm.nih.gov/bioproject/>) under accession number PRJNA627328 and in BMKCloud platform (<https://international.biocloud.net/zh/mydata/private/05889c21-f545-4119-ab38-b87c64ea925c>).

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported in part by grants from the National Key R&D Program of China (2018YFD0201407) and Excellent PhD Reward Project Fund of Shanxi Province (SXYBKY201736). These fundings provided the financial support to the research projects, but did not contain project design, data collection, analysis, or preparation of the manuscript.

Author Contributions

LL planned and designed the research. BD collected the plant materials, performed the experiment, analyzed the data, interpreted results, and wrote the manuscript. CH and TC contributed to sample data collection. YS analyzed the part of data. RH and XF provided suggestions in drafting the manuscript and edited the manuscript. All authors have read and approved the final manuscript.

Acknowledgments: We would like to thank researcher Huangping Guo (Pomology Institute, Shanxi Academy of Agricultural Sciences, Taiyuan 030031, China) and Yuanyuan Chen (Institute of Farm Product Storage and Fresh-keeping, Shanxi Academy of Agriculture Sciences, Taiyuan 030031, China) for their materials; We thanked professor Yuanhui Han (College of Agriculture, Shanxi Agricultural University, Taigu 030801, Shanxi, China) for his kind suggestions to the manuscript. Finally, we thanked engineers from BMKCloud platform for their bioinformatic software.

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Supplementary Materials

Additional file 1: Fig. S1. Graph of bulged surface of pear fruits at different development stage. The bulged surface of pear fruits at the early, middle and late stage of fruits.

Additional file 2: Fig. S2. Differentially expressed vol diagram. The Y axis shows the log2 FPKM values, FPKM (Fragments Per Kilobase of exon model per Million mapped reads) values, X axis shows log2 fold change. Red, green and black indicate up, down and unchanged expression, respectively (Fold change ≥ 1 , p -value < 0.05)

Additional file 3: Table S1. Statistics of differentially expressed genes in response to PBZ.

Additional file 4: Table S2. The number of differential expression annotated genes in database.

Additional file 5: Table S3. The list of differentially expressed genes.

Additional file 6: Table S4. The list genes of GO enrichment.

Additional file 7: Table S5. The list of genes enriched on KEGG pathway

Additional file 8: Table S6. Identification genes involved in bulged surface of pear fruits

Additional file 9: Table S7 Primers used to perform qRT-PCR of selected candidate genes.

Additional file 10: Table S8. Quantitative RT-PCR method.

Figures

Figure 1

The bulged surface of pear fruits and schematic diagram of vascular bundles in CK and PK groups. (a) The bulged surface of pear fruits at the stage of young fruits. (b) The bulged surface of pear fruits at the stage of mature fruits. (c) Statistic number of vascular bundles. CK indicates control group treated by ddH₂O, PK indicates PBZ treatment. Scale bar = 10mm. (d) The cross section of pear fruits. Student t-test ($p < 0.05$) ($n = 15 \pm SE$).

Figure 2

The IAA content of measurement analysis in CK and PK groups. Error bars represent standard deviation of three biological replications, student t-test ($p < 0.05$).

Figure 3

Structure and statistic of pear fruits in CK and PK groups. (a) Structure of pear fruits. Scale bar = 100 μm . (b) The length of fruit cells. (c) The number of cell layers. Cell layers were at the size of 400 $\mu\text{m} \times 400 \mu\text{m}$. (d) The number of fruit cells. Cell numbers were at the size of 100 $\mu\text{m} \times 100 \mu\text{m}$. Letter indicated statistical difference between CK and PK by student t-test ($p < 0.05$) ($n = 15 \pm \text{SE}$).

Figure 4

DEGs of cluster analysis and enrichment on GO and KEGG. (a) Gene Ontology (GO) enrichment of DEGs. (b) KEGG enrichment of selected DEGs. X-axis represents the enrichment factor (rich factor) which is the ratio of the foreground value (the number of DEGs) and the background value (total gene amount). The Y-axis shows the pathway names. (c) Heatmap depicting relative expression profile.

Figure 5

qRT-PCR expression profile of candidate DEGs in RNA-seq data in CK and PK groups. The error bars with different letters (ab) denote significant differences in CK and PK groups and mean $\pm$ SD (standard deviation, $n=3$, $p<0.05$).

Figure 6

A hypothetical representation of regulatory network in relieved pear-bulged surface fruits treated by PBZ. Black dotted line with arrows denote indirectly positive regulation, whereas black solid line means directly positive regulation. black solid with blunt ends denotes negative regulation.

Supplementary Files

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