Comparative transcriptome profiling and morphology provide insights into endocarp cleaving of apricot cultivar (*Prunus armeniaca* L.)

Xiao Zhang1,2†, Lijie Zhang3†, Qipeng Zhang1,2, Jiayu Xu1, Weisheng Liu2* and Wenxuan Dong1*

Abstract

**Background:** A complete and hardened endocarp is a typical trait of drupe fruits. However, the ‘Liehe’ (LE) apricot cultivar has a thin, soft, cleavable endocarp that represents 60.39% and 63.76% of the thickness and lignin content, respectively, of the ‘Jinxihong’ (JG) apricot (with normal hardened-endocarp). To understand the molecular mechanisms behind the LE apricot phenotype, comparative transcriptomes of *Prunus armeniaca* L. were sequenced using Illumina HiSeq™ 2500.

**Results:** In this study, we identified 63,170 unigenes including 15,469 genes >1000 bp and 25,356 genes with Gene Function annotation. Pathway enrichment and expression patterns were used to characterize differentially expressed genes. The DEGs encoding key enzymes involved in phenylpropanoid biosynthesis were significantly down-regulated in LE apricot. For example, CAD gene expression levels, encoding cinnamyl alcohol dehydrogenase, were only 1.3%, 0.7%, 0.2% and 2.7% in LE apricot compared with JG cultivar at 15, 21, 30, 49 days after full bloom (DAFB). Furthermore, transcription factors regulating secondary wall and lignin biosynthesis were identified. Especially for *SECONDARY WALL THICKENING PROMOTING FACTOR 1* (*NST 1*), its expression levels in LE apricot were merely 2.8% and 9.3% compared with JG cultivar at 15 and 21 DAFB, respectively.

**Conclusions:** Our comparative transcriptome analysis was used to understand the molecular mechanisms underlie the endocarp-cleaving phenotype in LE apricot. This new apricot genomic resource and the candidate genes provide a useful reference for further investigating the lignification during development of apricot endocarp. Transcription factors such as *NST1* may regulate genes involved in phenylpropanoid pathway and affect development and lignification of the endocarp.

**Keywords:** Apricot candidate genes, Comparative transcriptomes, Endocarp cleaving, Transcription factors

**Background**

*Prunus* (*P. armeniaca* L.) is a typical drupe of the family Rosaceae with eight pairs of chromosomes (*2n* = 16) [1]. Cultivated apricots are widely cultivated around the world (Asia 59.9%, Europe 21.6%, Oceania 0.4%, Africa 15.8%, and Americas 2.3%. FAO, 2013–2014) and apricot production has a relatively high economic value. Apricot fruit has rich nutritional value, including dietary fiber, organic acids, vitamin C, carotene, and trace elements [2]. Furthermore, the kernel is a natural plant protein resource, which used as medicine and food [3].

The pericarp develops from the ovary, and the innermost layer is the endocarp. The hardened endocarp has a vital role in seed protection and dispersal in some important economic fruits, such as peach, apricot, plum, almond, cherry, mango, olive, and coffee [4]. In plant evolution, the function of the heavily lignified endocarp is to ensure secure environment for seed development [5, 6]. Endocarp hardening is a significant trait of mature
Drupe fruits. It is caused by the secondary wall formation and lignin deposition [6]. Biochemical analysis has found that the endocarps of olive and peach contained much more lignin than poplar stem [7], suggesting that a relatively extreme degree of secondary wall formation occurs in fruit endocarp tissues. Lignin is an aromatic polymer that is widely found in the secondary walls of plants, as well as most enzymes and regulatory steps in the lignin biosynthetic pathway (phenylpropanoid pathway) have been identified [8]. Endocarp lignification in Arabidopsis has been adequately studied in relation to dehiscence, and even the transcriptional regulatory network has also been examined [9]. For drupe fruits, Ryugo [10] observed the regulation of lignin biosynthesis and accumulation in peach stones in the early 1960s. Lignification in peach endocarp is a highly coordinated process, which has been shown by subsequent developmental studies [11]. Recently, a transcriptional network dominated by NAC and MYB genes was observed in a well-conserved regulatory pathway, which causes Arabidopsis dehiscence or peach endocarp formation [4], and plays an essential role in the secondary wall formation and lignification via stimulating the pathway. Furthermore, several MADs-box genes involved in the formation of fruit endocarp, including SHP1, SHP2, STK, and FUL were identified [12]. These TFs co-function with IND, ALC [13], and RPL [14] to stimulates endocarp differentiation.

However, there is wide variation in the phenotypes of Prunus endocarps, such as thickness, hardness, and brittleness of almond endocarps. The endocarp of “split pit” peach does not seal along the suture, leaving the seed severely exposed to pests and disease [15]. Callahan [16] found a natural “stoneless” plum in a wild-type P. domestica, which had imperfectly developed endocarp resulting in a partially naked seed. China has a great wealth of germplasm genetic resources of apricot that have important breeding values [17]. ‘Liehe’ (LE) apricot is an extremely rare cultivar that originated in Linyuan City of Liaoning Province and was introduced into National Germplasm Repository for Plums and Apricots (Xiongyue, Liaoning, China) in the 1983 [18]. The endocarp of LE apricot is thin, soft, and cleavable, and some seed partially exposed (Fig. 1). Both flesh and kernel have a tasty flavor and aroma.

This study investigated the mechanisms of endocarp development and phenotype formation, using Illumina sequencing and expression pattern analysis of candidate genes in apricot fruits during the stages of endocarp development and lignification.

Results

Differences in endocarp development in LE and JG apricot

The growth of LE apricot fruit was compared with JG apricot. The horizontal and vertical diameter of the two cultivars increased continuously during fruit development. Growth patterns were similar, with formal double-sigmoid growth curves (Fig. 2). The equation of two apricot cultivars and their first derivatives were also similar (Additional file 1: Table S1). In addition, the transcript level of ACO1 and PEPCK divided the development of two apricots into same four stages (Additional file 2: Figure S1). Based on these patterns and fit equations, apricot fruit growth was assessed at four growth stages: S1, first exponential growth stage (before 30 DAFB); S2, slow-growth stage (30–49 DAFB); S3, second exponential growth culminating in fruit ripening stage (49–83 DAFB); S4, fruit ripening stage (after 83 DAFB). The changes in the growth pattern during fruit development in LE and JG apricot were almost the same.

Fig. 1 Morphology and structure of mature fruit and endocarp of LE and JG apricot in this study. Left: LE apricot with thin, soft and cleavable endocarp; Right: JG apricot with thick, hard and complete endocarp. The scale in this figure was 5 mm.
endocarp of LE apricot started to cleave, and these areas increased along with the progression in fruit development, which occurred in virtually each replicate sample of LE apricot. Endocarp thickness was significantly different after 30 DAFB subsequently (Fig. 3e, f), when cleaving areas of endocarp became more obvious in LE apricot (Fig. 3b, c).

The lignin deposition process was discernible from fruit transverse sections (at 24 DAFB) showing color reaction between the phloroglucinol and lignin (Fig. 3b). The lignin deposition process began at the tip of the endocarp and gradually completed over 25 days (24–49 DAFB). Interestingly, LE cultivar exhibited incomplete areas of endocarp that had little or no lignification (Fig. 3a, b). The thickness and lignin content were significantly lower in LE endocarp, which estimated by 60.4% and 63.3%, respectively; out of that in JG endocarp (Fig. 3d, e). Thus the differences in endocarp development and lignification were significant.

Illumina sequencing and assembly

The LE endocarp cleaving occurred at 15 DAFB and rapidly increased at 21 DAFB (Fig. 3a). Thus, RNA from these two fruits stages of LE and JG apricot was used for RNA-seq, with two replicates per fruit, which generated 40,145,230,606 raw reads. After removing low-quality reads and trimming adapter sequences, 159,378,508 remained (Table 1). The assembly data were confirmed by an N50 value (1689 bp) and average length (868.72 bp). The number of transcripts (length ≥ 200 bp) was 152,146, constituting 99.99% of the total, with average lengths of 1579.75 bp (Table 1). Transcripts assembled 63,170 genes from LE and JG apricot. Among these genes, 15,469 had lengths of ≥ 1000 bp, constituting 24.49% (15,469/63,170) of the total (Table 2). Length distribution of all genes is shown in Table2.

Functional annotation and identification of unigenes

Based on the sequence similarity, 25,356 genes were matched to the Japanese apricot and peach genome databases (Additional file 3: Table S2). All of these genes were aligned using BLASTx (E values ≤ 10^-5) searches against the NR, Swiss-Port, GO, COG, and KOG protein databases, and KEGG pathway databases. A total of 25,356 (40.14%) genes had more than one match, and 39.78% were annotated to the NR database (Table 3). Among the annotated genes in the NR database, 74.08% had an E-value ≤ 1.0 E^-5 and showed very strong homology to the gene sequence in the database. The remaining 50.38% of genes had an E-value ranging from 1.0 E^-6 to 1.0 E^-60 (Additional file 4: Figure S2). We further analyzed the BLAST results in the NR database and investigated the best-hit species distribution, and the top two matched plant species were P. mume (63.67%) and P. persica (26.23%) (Additional file 4: Figure S2).

The functions of predicted genes were classified by GO analysis. A total of 16,506 genes annotated in the GO database were categorized into 57 functional groups,
belonging to three main GO ontologies: biological processes, cellular components, and molecular functions (Additional file 5: Table S3; Additional file 6: Figure S3). 'metabolic process' (8408 genes, 50.93%), were dominant among the functional groups. In addition, assembled genes were searched against the COG database to estimate the gene function (Fig. 4). In general, 7724 putative proteins were clustered into 25 functional categories. Among these categories, 'general function prediction only' (2188, 28.33%) accounted for the largest amount, followed by 'replication, recombination and repair' (14.29%) and 'transcription' (13.85%).

In addition, 4.41% of assembled genes were assigned to secondary metabolites biosynthesis, transport, and catabolism, reflecting the large amount of secondary metabolites that were present in the apricot. The 'nuclear structure' (0.01%), 'cell motility' (0.17%), and 'chromatin structure and dynamics' (0.93%) accounted for the least amounts.

We used the KEGG pathway database to search the functional networks of biological interactions. In total, 4830 genes were identified in the KEGG database and were assigned to 118 KEGG pathways (Additional file 7: Table S4). The majority of genes was classified into pathways for 'carbohydrate metabolism' (905 genes),
Table 1 Summary of RNA-seq and de novo assembly of *P. armeniaca* L. unigenes

| Sequence                  | Number     |
|---------------------------|------------|
| Total nucleotides         | 40,145,230,606 |
| Numbers of clean reads    | 159,378,508 |
| Numbers of 200–300 bp contigs | 17,108,433 (99.71%)a |
| Mean length of contigs (bp) | 39.07 |
| N50 length of contigs (bp) | 42 |
| Numbers of ≥200 bp transcripts | 152,146 (99.99%)b |
| Mean length of transcripts (bp) | 1579.75 |
| N50 length of transcripts (bp) | 2598 |
| Numbers of unigenes       | 63,170 |
| Mean length of unigenes (bp) | 868.72 |
| N50 length of unigenes (bp) | 1689 |

The expression patterns of the genes among LE1, LE2, JG1, and JG2 were calculated using the FPKM method. A total of 5385 DEGs were identified by comparing the four libraries in paired comparisons, as illustrated in Fig. 5. The most prominent library was LE1_vs_JG1. In each library, LE1_vs_LE2, LE1_vs_JG1, JG1_vs_JG2, and LE2_vs_JG2 had 2763, 2887, 1085, and 886 DEGs respectively. Four libraries had 17 common DEGs and 1301 DEGs in LE1_vs_LE2, as well as 1314 DEGs in LE1_vs_JG1, 480 DEGs in JG1_vs_JG2, and 220 DEGs in LE2_vs_JG2. These results indicated that early fruit development of apricot is a highly active process, and key genes that are related to endocarp development were significantly expressed.

Table 2 Length distribution of *P. armeniaca* L. unigenes

| All combination unigenes length (bp) | Total number | Percentage (%) |
|--------------------------------------|--------------|----------------|
| 200–300                              | 19,728       | 31.23          |
| 300–500                              | 15,585       | 24.67          |
| 500–1000                             | 12,388       | 19.61          |
| 1000–2000                            | 8153         | 12.91          |
| 2000+                                | 7316         | 11.58          |

Endocarp hardening occurs via secondary cell wall formation and lignification. In the phenylpropanoid pathway, p-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol are the end products that form the different types of lignin monomers [8]. From the KEGG enrichment analysis, phenylalanine metabolism, phenylpropanoid biosynthesis, and hormone signal transduction were the foremost pathways and contained the most number of DEGs in LE1_vs_JG1 (Additional file 7: Table S4; Additional file 8: Figure S4). Thirty-four DEGs associated with the phenylalanine pathway were differentially expressed.

The expression level of genes which involved in phenylpropanoid pathway was down-regulated in LE compared with JG cultivar, in both the replicates and development stages (Table 4, Figs. 6, 8). These included genes encoding shikimate O-hydroxycinnamoyltransferase (HCT, unigene c42130.c0 and c26167.c0) [2.3.1.133], caffeic acid O-methyltransferase (COMT, unigene c43821.c0) [EC 2.1.1.6]. Among the seven annotated Peroxidase [EC 1.11.1.7] genes, two were down-regulated (unigene c10367.c0 and c36804.c0) in LE relative JG cultivar. Cinnamyl alcohol dehydrogenase (CAD, unigene c10104.c0) [EC 1.1.1.195] in particular, were involved in lignin biosynthesis and catalyzed the final step specific to the production of lignin monomers [19]. The expression level of CAD was always down-regulated in LE relative to JG cultivar during S1 stage. The fold change data of each selected candidate gene in the phenylpropanoid pathway are shown in Fig. 6, and detailed information is presented in Table 4.

Several TFs were identified that mediated the endocarp development, including SHP and STK. ALC and IND promoted endocarp differentiation and negative regulation was achieved by FUL and RPL. Meanwhile, NST1, NST3, and several MYB-box genes are associated with secondary wall formation and lignin biosynthesis. By the RNA-seq, the majority of these TFs and genes were identified and showed in Fig. 8. The expression of SHP, FUL, and MYB32 were up-regulated in LE relative to JG cultivar. However, STK, MYB46–1, MYB46–2, and NST1 were down-regulated significantly in LE compared with JG cultivar (Table 5, Fig. 8).

We used common expression patterns to further analyze the DEGs between LE and JG apricot at 15 and 21 DAFB. Based on this method, 5383 DEGs were placed into seven clusters (Fig. 7; Additional file 9: Table S5). Most of the candidate DEGs was categorized in either Cluster 1 (408 genes) or Cluster 6 (311 genes). Compared with JG apricot, the expression of DEGs of LE apricot was up-regulated in Cluster 1, and some DEGs were present as CHS1, F3H, CSLG3, CSLA9, KATAM, ARR5, and ARR16 (Additional file 9: Table S5). Conversely, the expression of DEGs such as DFR and XTH2 in LE apricot was down-regulated in Cluster 6.

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There was a linear correlation \((R = 0.9188, P \leq 0.0001)\) between RNA-seq data and qPCR in our study (Additional file 10: Figure S5). TFs (STK, SHP, FUL, NST1, MYB46, and MYB32) and key candidate genes could regulate fruit endocarp growth, development, and lignification (Fig. 8). These genes involved in the biosynthesis of plant hormones (GA3ox1, ARR5, and ARR16), phenylpropanoid pathway (4CL, HTC, COMT, and CAD), flavonoid biosynthesis (F3H and DFR), and cellulose-related pathway (CSLG3, CSLA9, and KATAM). STK, NST1, GA3ox1, HTC, COMT and CAD were down-regulated and MYB32 was up-regulated in LE apricot, compared with JG, in RNA-seq data and gene expression, respectively. Furthermore, Pearson's correlation analysis indicated that there was a significant association between CAD expression and endocarp thickness in LE apricot at the 0.05 level. HTC expression and lignin content also showed the same result (Additional file 11: Table S6). The special endocarp development and lignification in LE were caused by the effects of several TFs and genes involved in phenylpropanoid pathway.

**Discussion**

The hardened endocarp has a vital role in seed protection and dispersal in some important economic fruits, such as peach, apricot, plum, almond, cherry, mango, olive, and coffee [4]. Endocarp hardening is a significant trait of fruit matures of any types of drupes, which caused by the secondary wall formation and lignin deposition [6]. Phenylpropanoid biosynthesis played a crucial role in endocarp lignification in both LE and JG apricot. Sequence analysis of transcriptome revealed a series of differentially expressed genes involved in the phenylpropanoid pathway, such as 4CL, HTC, COMT and CAD. Knockout of 4CL in Arabidopsis had no significant effect on either lignin content or monomeric composition [20]. However, RNAi silencing of HCL in

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**Table 3** Summary of assembled *P. armeniaca* L. unigenes

| Database type      | Number of unigenes length ≥ 300 bp | Number of unigenes length ≥ 1000 bp | The total number of annotated unigenes | Percentage (%)a |
|--------------------|------------------------------------|-------------------------------------|----------------------------------------|---------------|
| COG_Annotation     | 1405                               | 5713                                | 7724                                   | 12.23         |
| GO_Annotation      | 3081                               | 7856                                | 12,347                                  | 19.55         |
| KEGG_Annotation    | 1087                               | 3214                                | 4830                                    | 7.65          |
| KOG_Annotation     | 3104                               | 8290                                | 12,735                                  | 20.16         |
| Pfam_Annotation    | 3656                               | 11,592                              | 16,506                                  | 26.13         |
| Swiss–prot_Annotation | 4498                             | 10,587                              | 16,897                                  | 26.75         |
| nr_Annotationb     | 7813                               | 13,685                              | 25,126                                  | 39.78         |
| All_Annotation     | 7899                               | 13,706                              | 25,356                                  | 40.14         |

aPercentage means the proportion of 63,170 unigenes
bnr_Annotation means NCBI non-redundant sequence database
Arabidopsis and Radiata pine reduced lignin content and changed the monomeric composition [21, 22]. In COMT antisense Leucaena leucocephala, the lignin content was reduced to 72% by decreasing 60% of OMT activity [23]. CAD1 made a significant contribution to the synthesis of coniferyl alcohol, and down-regulated CAD1 in wild-type tobacco has a moderate impact on G unit content of the non-condensed lignin fraction [24]. CAD activities were drastically reduced in null mutants of Arabidopsis (AtCAD-D and AtCAD-C), and affected sinapyl alcohol dehydrogenase activity in these mutants. AtCAD-D had a significant influence on lignin content and proportion of conventional S lignin [25]. In LE cultivar, expression of 4CL were up-regulated, yet expressions of HCL and COMT were down-regulated, which relative to JG cultivar (Figs. 6, 8). In particular, CAD expressed down-regulated in LE apricot compared with JG, in both RNA-seq data and relative gene expression.

### Table 4 DEGs between LE and JG apricot that involved in phenylpropanoid pathway

| Unigene ID | Fold change (log2 JG/LE) | Annotation |
|------------|--------------------------|------------|
| c36405.c0  | 3.72                     | Phenylalanine ammonia–lyase 1 [P. mume] |
| c39178.c0  | 1.47                     | 4–coumarate–CoA ligase [A. thaliana] |
| c13354.c0  | 2.45                     | Cytochrome P450 98A2 [P. mume] |
| c14455.c0  | 2.63                     | Cytochrome P450 98A2 [P. mume] |
| c48482.c0  | 1.72                     | Caffeoyl-CoA reductase 1 [A. thaliana] |
| c27758.c0  | 1.24                     | Caffeoyl-CoA reductase 1–like [P. mume] |
| c15115.c0  | 7.29                     | Cinnamoyl-CoA reductase 1 [A. thaliana] |
| c43821.c0  | –0.21                    | Caffeic acid 3-O-methyltransferase [P. mume] |
| c42130.c0  | –2.77                    | Shikimate O-hydroxycinnamoyltransferase [A. thaliana] |
| c26167.c0  | –3.74                    | Shikimate O-hydroxycinnamoyltransferase [A. thaliana] |
| c24524.c0  | 2.73                     | Caffeoyl-CoA O-methyltransferase 1 [A. thaliana] |
| c10104.c0  | –4.56                    | Cinnamyl alcohol dehydrogenase [A. thaliana] |
| c9752.c0   | –5.52                    | Cinnamyl alcohol dehydrogenase [A. thaliana] |
| c10367.c0  | –3.93                    | Peroxidase 72 [A. thaliana] |
| c32572.c0  | 3.59                     | Peroxidase 29 [A. thaliana] |
| c34865.c0  | 1.77                     | Peroxidase 12 [A. thaliana] |
| c36804.c0  | –1.39                    | Peroxidase 42 [A. thaliana] |
| c41877.c0  | 2.55                     | Peroxidase 4 [V. vinifera] |
| c35483.c0  | 2.36                     | Peroxidase 17 [A. thaliana] |
| c35595.c0  | 1.63                     | Peroxidase 51 [A. thaliana] |
| c45746.c0  | –1.64                    | Aspartate aminotransferase [D. carota] |
| c41467.c2  | 1.24                     | aminotransferase TAT2 [A. thaliana] |
| c10544.c0  | 2.28                     | Cytochrome P450 98A3 [A. thaliana] |
These results indicated that thickness and incomplete endocarp are unlikely to result from mutation of one specific phenylpropanoid pathway gene. In fact, expression levels of CAD and HCT had significant correlation with endocarp thickness and lignin content in LE apricot (Additional file 11: Table S6). These genes or TFs may be responsible for the defects in endocarp development and lignification in LE apricot.

SHP, STK and NST1 were specifically expressed in endocarp of peach. In exocarp and mesocarp, the negative regulator FUL exhibited a high expression level. However, the expression of IND and ALC was insignificant [4]. In Arabidopsis stk shp1 shp2 triple mutants, the integuments were changed into carpel-like structures leading to complete sterility [26]. Over-expression of FUL caused no lignin deposition in valve tissues in Arabidopsis [27]. Furthermore, in tomato, over-expression of FUL2 lead to a thinner pericarp, and reduced stem cell layer [28]. In a split pit resistant variety of peach, SHP expression was low, however in the sensitive variety, FUL expression was significantly elevated [15]. Our analysis found that STK, SHP, and FUL were discovered in DEGs, but IND and ALC were not. Expression of SHP and FUL had significant different between LE and JG at 9 DAFB, while STK remained down-regulated during the S1 stage significantly. RNA-seq data and qPCR analysis reflected that SHP, STK, and FUL were highly expressed and essential for endocarp development (Table 5; Fig. 8). IND

Table 5 DEGs between LE and JG apricot that involved in secondary wall biosynthesis

| Unigene ID    | Fold change (log₂ JG/LE) | Annotation                                                                 |
|---------------|--------------------------|----------------------------------------------------------------------------|
| c33854.c0     | −0.30                    | Agamous–like MADS–box protein AGL11 [A. thaliana]                         |
| c37786.c0     | 0.49                     | Agamous–like MADS–box protein AGL1 [A. thaliana]                          |
| c31419.c1     | 0.37                     | Agamous–like MADS–box protein AGL8 [A. thaliana]                         |
| c32638.c0     | 1.30                     | BEL1–like homeodomain protein 9 [A. thaliana]                             |
| c10986.c0     | −1.93                    | NAC domain–containing protein 43 [A. thaliana]                            |
| c23553.c0     | −1.69                    | NAC domain–containing protein 7 [A. thaliana]                             |
| c45336.c0     | −3.56                    | Gibberellin 3–beta–dioxygenase 1 [P. sativum]                             |
| c34462.c0     | −0.03                    | Transcription factor MYB46 [A. thaliana]                                  |
| c36170.c1     | −0.55                    | Transcription factor MYB46 [A. thaliana]                                  |
| c37609.c0     | 1.98                     | Transcription factor MYB32 [P. mume]                                      |
directly activates GA3ox1, which is an indispensable enzyme catalyzing the last step of GAs biosynthesis in the separation layer of Arabidopsis. IND induces GAs accumulation to degrade DELLA protein, resulting in release ALC [29]. In atga3ox1 atga3ox2 double mutants of Arabidopsis, synthesis of cellulose, hemicelluloses, and lignin were suppressed obviously [30]. NST1 and NST3 (SND1) have been proven as master switches that regulate the secondary wall biosynthesis and lignification in Arabidopsis [31], Medicago [32], and Poplar [33]. In the transcriptional network, the downstream transcription factor MYBs is activated by NST1 and SND1, and multiple genes are involved in secondary wall biosynthesis [34]. In nst1 mutants, valve margins were obvious in the absence of the secondary wall, meanwhile in nst1 nst3 double mutants, only vascular vessels conserved secondary wall formation [35]. The SND1, and VND1–5, VND6–7 were not detected as DEGs in this study. The expression levels of VND4 were always lower in LE relative to JG cultivar at 15 and 21 days after full bloom (Additional file 3: Table S2). NST1, the domain of that regulates biosynthesis of secondary wall, lignin and xylanase always had low expression levels in LE fruit (Table 5; Fig. 8). This might

**Fig. 7** Common expression analysis based on DEGs expression patterns. Left: Numbers under the x-axis indicated different sample, numbers under the y-axis indicated the log₂(fpkms + 1). Right: Red color represents higher log₂(fpkms + 1) data of genes; Green color represents lower log₂(fpkms + 1) data of genes. Black color represents log₂(fpkms + 1) = 0. LE1–1 and LE1–2 indicate two biological replications of LE at 15 DAFB; LE2–1 and LE2–2 indicate two biological replications of LE at 21 DAFB; JG1–1 and JG1–2 indicate two biological replications of JG at 15 DAFB; JG2–1 and JG2–2 indicate biological replications of JG at 21 DAFB.
be one of the main cause of cleaving and thinning of endocarp in LE apricot. Hence, NST1 was regarded as an essential candidate gene in the development and phenylpropanoid biosynthesis in endocarp of LE apricot. MYB46 is also a decisive master switch and AtMYB46 was reported to be a direct target of ANAC012/SND1/NST3 [36], which adjusted secondary cell wall biosynthesis [37]. Dominant repression or over-expression of MYB46 has a considerable effect on secondary wall thickening of fibers and vessels and biosynthesis of lignin and cellulose [36]. We identified two differentially expressed MYB46 (Table 5). In addition, MYB46 could activate the expression of MYB4, MYB7, and MYB32 [38], and the MYB32 protein sequence was highly similar to that of MYB4 [39]. AtMYB4 regulates the expression of C4H, so that AtMYB32 could negatively regulate several genes implicated in phenylpropanoid biosynthesis [40]. Transactivation assays and transgenic studies also show that MYB32 appears to be a negative regulator of SND1 expression [32]. Interestingly, MYB32 was extraordinary up-regulated in LE, and expression of C4H down-regulated (Fig. 8) only in LE fruit. MYB32 of apricot might also play an important part in negative regulating the lignin biosynthesis in the secondary wall.

Common expression pattern analysis provided a new understanding of the expression and function of DEGs, and combines pathways with multiple candidate genes, which were related to the flavonoid pathway and cell function (Fig. 7; Additional file 9: Table S5). CHS1 and F3H expressed higher level in LE than JG, which may

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**Fig. 8** qPCR analyses of selected candidate genes involved in this study. Light–colored bars indicate LE apricot; dark–colored bars indicate JG apricot. Numbers under the x–axis indicate the days after full bloom. Label: “*” means the significant differences at $P < 0.05$ by DMRT.
cause by considerable down-expression of CAD. However, DFR had a down-regulated expression. Among 408 genes in Cluster 1, CSLG3, and related genes, CSLA9 [41] and KATAM had significant higher level expression in LE during stage S1 and S2 relative to JG cultivar. Furthermore, ARR5 and ARR16 regulators appeared to act as negative regulators of Cytokinin signaling [42], and showed significantly up-regulated expression in LE apricot.

Conclusions
Our results implied that cleaving of endocarp in LE apricot started at 15 DAFB, and this area increased during fruit development. The thickness and lignin content of the mature LE endocarp was only 60.39% and 63.25%, respectively, compared with JG endocarp (Fig. 3). RNA-Seq to sequencing and de novo assembly of the fruit transcriptomes of two cultivars of P. armeniaca (L.) showed discrepancies in development and lignification of the endocarp and explained the cleaving of endocarp in LE apricot. The DEGs and qPCR analysis data (Fig. 8) identified differentially expression genes involved in TFs (STK, SHP, FUL, NST1, MYB46, MYB32) and phenylpropanoid, flavonoid and hormone pathways (4CL, HCT, COMT, CAD, CHS1, F3H, DFR, GA3ox1, ARR5, and ARR16), consistent with endocarp phenotype and lignin content. Our results indicated that TFs especially NST1, may regulate genes of the phenylpropanoid pathway. Besides, low expression level of NST1 may inhibit the endocarp development and lignification of LE apricot.

Methods
Plant materials
‘Liehe’ (LE) apricot, synonym as ‘Luoren’ apricot, and ‘Jinxihong’ (JG) apricot are local cultivars originated respectively in Linyuan City and Jinx County of Liaoning Province and were collected into National Germplasm Repository (N40°10’ 1.18”, E122°09’ 39.41”) for Plums and Apricots at Xiongyue, Liaoing, China in 1983. LE and JG, with the accession number XC0347 and XC0015 respectively, compared with JG endocarp (Fig. 3). RNA-Seq to sequencing and de novo assembly of the fruit transcriptomes of two cultivars of P. armeniaca (L.) showed discrepancies in development and lignification of the endocarp and explained the cleaving of endocarp in LE apricot. The DEGs and qPCR analysis data (Fig. 8) identified differentially expression genes involved in TFs (STK, SHP, FUL, NST1, MYB46, MYB32) and phenylpropanoid, flavonoid and hormone pathways (4CL, HCT, COMT, CAD, CHS1, F3H, DFR, GA3ox1, ARR5, and ARR16), consistent with endocarp phenotype and lignin content. Our results indicated that TFs especially NST1, may regulate genes of the phenylpropanoid pathway. Besides, low expression level of NST1 may inhibit the endocarp development and lignification of LE apricot.

Measurement of fruit growth and endocarp lignification
Fresh fruit diameter were measured (horizontal and vertical diameter) by digital Vernier caliper (0–150 mm ± 0.02 mm), and weighted using electronic scales (300 g/0.01 g). The fitting for each equation were used the Pearl-Reed logistic [45, 46] and normal logistic equation [47] as references. The first derivative of equation was calculated and drew by MATLAB 8.5 (Math Works, US). The expression levels of transcripts encoding ACO1 and PEPCK were analysis used the same method [48]. Flower buds, flowers and young fruits were observed using an Olympus SZX7 microscope to examine the cleaving of the endocarp. Developing endocarp areas were calculated using Olympus cellSens software. Fruit samples for lignin deposition observation were collected once every 3 days from 15 to 43 DAFB. Observation and lignin content tests were conducted using Alba’s method [49]. The measurements for each index were repeated three times in 10 samples. Means and analysis of variance (ANOVA) were separated using Duncan’s Multiple Range Test (DMRT) in SPSS 19.0 (IBM, US).

cDNA library preparation and Illumina sequencing
Total RNAs were extracted from fruit samples without kernels using Gambino’s method [50]. The RNA samples were examined with an Agilent 2100 Bioanalyzer (US). cDNA library preparation and sequencing of fruits at 15 DAFB (LE1, JG1) and 21 DAFB (LE2, JG2) with two replicates per each cultivar, were conducted by the Biomarker Technology Company (Beijing, China). The cDNA library used high-throughput sequencing (RNA-seq) with the Illumina HiSeq” 2500. Reads length of sequences was PE125.

Sequence assembly and functional annotation
A large number of raw reads was produced using Sequencing by Synthesis (SBS) from Illumina HiSeq” 2500. The Trinity method [51] was used for de novo assembly of Illumina reads of the two apricot cultivars. Clean reads were mapped to the genome of Prunus mume and Prunus persica using TopHat software [52]. Genes were first aligned using BLASTx (E value <10^-5) to the NCBI non-redundant protein databases (NR) [53]. The alignments from the NR database were used blast2GO (https://www.blast2go.com/) to get GO annotation [54]. The number of DEGs which matched to three categories was counted, and GO ontology figure was drawn by Graph-R Project. The statistical method of GO enrichment was “right sided Fisher exact test”. The term was Core ontology (go.obo, http://purl.obolibrary.org/obo/go.obo). The main parameter of BLASTx is “blastx -task blastx-fast -num_descriptions 100 -num_alignments 100 -evalue 1e-5”. This parameter was used to blast databases. The annotation of genes were performed using the method were as follows: Swiss-Port protein databases [55], COG [56], KOG [57] KEGG [58].
Predicted amino acid sequences were aligned by hidden Markov models (HMMER, E value <10^{-19}) [59] to the Protein family (Pfam) [60] to annotate the genes. Coding sequence (CDS) of genes were predicted by TransDecoder Software (http://transdecoder.github.io).

Differentially expression genes analysis
Gene expression levels were analyzed using fragments per kilobase of the transcript per million mapped reads (FPKM) method [61]. DESeq Software [13] was used to identify DEGs in pair-wise comparisons, and the results of all statistical tests were revised to account for multiple testing with the Benjamini–Hochberg false discovery rate (FDR <0.01). Sequences were determined to be significantly differentially expressed at a P value (<0.01), and Fold change (FC) >2. Common expression pattern analysis using BMKCloud (https://www.biocloud.net/) was applied twice to serial samples. Euclidean distance was used in the Distance method and K-means for hierarchical clustering. Hierarchical clustering was conducted using Spotfire DecisionSite 8.1 (Spotfire Inc., http://spotfire.tibco.com/).

Quantitative RT-PCR analysis
A total of 500 ng of RNAs was used to synthesize cDNA using PrimeScript™RT Kit (Cat. RR047A, TaKaRa, Japan). The cDNA was diluted five times, and then used as a template. The reaction solution contained SYBR® PremixExTaq™II (Tli RNaseH Plus) (Cat. RR820A, TaKaRa, Japan) and was conducted in an ABI 7500 Real Time PCR Detection System (Applied Biosystems, US). Quantitative primers for validation of DEGs were listed in Additional file 12: Table S7. The relative expression levels of the selected genes, normalized to peach ACT [62] and P. mume ACT7 (unigene, c48143.c0), were calculated using the 2^{-ΔCT} method. All reactions were performed with three biological replicates. Three technical replicates were in each biological replicate. The analysis of variance (ANOVA) was based on Duncan’s Multiple Range Test (DMRT) in SS 19.0 (IBM, US).

Additional files

Additional file 1: Table S1. Growth curve equation and its first derivative of P. armeniaca L. (XLS 33 kb)

Additional file 2: Figure S1. Transcription levels of genes marking different phenological phases of apricots. (TIFF 484 kb)

Additional file 3: Table S2. Summary of DEGs and annotation. DEGs were generated for comparison between LE and JG apricot and JG was control sample. (XLS 2860 kb)

Additional file 4: Figure S2. E-value and NR distribution of assembled P. armeniaca L. unigenes. (TIF 891 kb)

Additional file 5: Table S3. Summary of GO enrichment analyses of assembled P. armeniaca L. unigenes. DEGs were generated for comparison between LE and JG apricot and JG was control sample. (XLS 46 kb)

Additional file 6: Figure S3. GO classification of assembled P. armeniaca L. unigenes and DEGs. The results were summarized in three main GO categories: cellular component, molecular function, and biological process. Metabolic process (50.93%), cellular process (42.41%), ‘single-organism process’ (36.39%), binding (37.30%), catalytic activity (40.42%), ‘cell part’ (31.31%), and ‘cell’ (31.17%) were dominant among the functional groups. DEGs were generated for comparison of LE and JG apricot and JG was control sample. The right y-axis indicated the number of assembled unigenes and DEGs. (TIFF 9700 kb)

Additional file 7: Table S4. KEGG pathway analysis of P. armeniaca L. assembled unigenes. (XLS 185 kb)

Additional file 8: Figure S4. KEGG enrichment analyses of DEGs between LE and JG apricot at 15 DAFB. Phenylalanine metabolism (Q value =0.032), Phenylalanine biosynthesis (Q value =0.055). Red color represents higher expression levels of genes in LE relative to JG apricot; Green color represents lower expression levels of genes in LE relative to JG apricot. (TIFF 3033 kb)

Additional file 9: Table S5. Annotation of DEGs between LE and JG apricot in Cluster1 and Cluster6. (XLS 264 kb)

Additional file 10: Figure S5. Correlation analysis of fold change data of RNA-seq with that from qPCR. 18 genes were selected for this analysis. (TIFF 183 kb)

Additional file 11: Table S6. Correlation between lignin related phenotypic measurements and DEGs expression data. (XLS 44 kb)

Additional file 12: Table S7. Primers used to perform qPCR of selected candidate genes. (XLS 35 kb)

Abbreviations
ACO1: 1-aminocyclopropane-1-carboxylic acid oxidase 1; ALC: Basic helix-loop-helix genes ALCATRAZ; ARR16: Two-component response regulator ARR16; ARRS: Two-component response regulator ARRS; CAD: Cinnamyl alcohol dehydrogenase; CDS: Coding sequence; CHS1: Chalcone synthase 1; COG: Clusters of orthologous groups databases; CSLA9: Glucosaminan 4-beta-mannosyltransferase; CSLG3: Cellulose synthase 3; DAFB: Days after full bloom; DEGs: Differentially expression genes; DFR: Dihydroflavonol-4-reductase; DMRT: Duncan’s Multiple Range Test; F3H: Flavanone 3-hydroxylase; FPKM: Fragments per kilobase of the transcript per million mapped reads; FUL: MADS-Box genes FRUITFUL; GA3ox1: Gibberellin 3-beta-dioxygenase 1; GO: Gene ontology databases; HCT: Shimikate O-hydroxycinnamoyltransferase; IND: Basic helix-loop-helix genes INDEHISCENT; KATAM: Xyloglucan galactosyltransferase KATAMARI1 homolog; KEGG: Kyoto encyclopedia of genes and genomes pathway databases; KOG: eukaryotic orthologous groups databases; NR: NCBI non-redundant protein databases; NST3: SECONDARY WALL THICKENING PROMOTING FACTOR; PEPCK: Phosphoenolpyruvate carboxkinase; qPCR: Quantitative real-time PCR; REPLUMLESS: NST1; RPL: BELL-like homeodomain gene; SHP: MADS-box genes SHATTERPROOF; STRK: MADS-box genes SEEDSTICK; TF: Transcription factor; VND1–7: Protein VASCULAR RELATED NAC-DOMAIN 1–7; XTH2: Xyloglucan endotransglucosylase/hydrolase 2

Acknowledgments
We thank Dr. Hamad, Dr. Zhang Qijing and Dr. Hou Yali for assisting with the experiments and commenting on the manuscript.

Funding
This work was supported by the Program of Conservation and Utilization of Crop Germplasm Resources-Apricot and Plum (2014–2016); National Natural Science Foundation of China (31401826); Hawthorn Program of National Crop Germplasm Resources Infrastructure (2014–2016).

Availability of data and materials
The data supporting the results presented in this article are included as additional files.

Authors’ contributions
Conceived and designed the experiments: ZX, LWS, and DWX. Performed the experiments: ZX, ZQP, and ZLJ. Analyzed the data: ZX, ZLJ, XJY and DWX.
Wrote the paper: ZX, LWS, and DWX. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

Research area
Fruit Germplasm Resources Evaluation and Utilization.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1College of Horticulture, Shenyang Agricultural University, Shenyang 110866, China. 2Liaoning Institute of Pomology, Yingkou 115009, China. 3College of Forestry, Shenyang Agricultural University, Shenyang 110866, China.

Received: 24 August 2016 Accepted: 30 March 2017

Published online: 11 April 2017

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