Comparative transcriptome analyses of fruit development among pears, peaches, and strawberries provide new insights into single sigmoid patterns

Mao-Song Pei, Su-Hao Cao, Lei Wu, Guo-Ming Wang, Zhi-Hua Xie, Chao Gu* and Shao-Ling Zhang*

Abstract

Background: Pear fruit exhibit a single sigmoid pattern during development, while peach and strawberry fruits exhibit a double sigmoid pattern. However, little is known about the differences between these two patterns.

Results: In this study, fruit weights were measured and paraffin sections were made from fruitlet to maturated pear, peach, and strawberry samples. Results revealed that both single and double sigmoid patterns resulted from cell expansion, but not cell division. Comparative transcriptome analyses were conducted among pear, peach, and strawberry fruits at five fruit enlargement stages. Comparing the genes involved in these intervals among peaches and strawberries, 836 genes were found to be associated with all three fruit enlargement stages in pears (Model I). Of these genes, 25 were located within the quantitative trait locus (QTL) regions related to fruit weight and 90 were involved in cell development. Moreover, 649 genes were associated with the middle enlargement stage, but not early or late enlargement in pears (Model II). Additionally, 22 genes were located within the QTL regions related to fruit weight and 63 were involved in cell development. Lastly, dual-luciferase assays revealed that the screened bHLH transcription factors induced the expression of cell expansion-related genes, suggesting that the two models explain the single sigmoid pattern.

Conclusions: Single sigmoid patterns are coordinately mediated by Models I and II, thus, a potential gene regulation network for the single sigmoid pattern was proposed. These results enhance our understanding of the molecular regulation of fruit size in Rosaceae.

Keywords: Pear, Peach, Strawberry, Single sigmoid pattern, QTL, Gene regulation network

Background

Within the Rosaceae, different fleshy fruit types showed two different fruit growth patterns. Pome fruits such as apple and pear exhibit a single sigmoid pattern in which fruits undergo extensive cell division during the first few weeks immediately following fertilization, after which almost all growth is due to cell enlargement [1–4]. Stone fruits such as late-maturing peach exhibit a double sigmoid pattern in which two rapid-growth stages are separated by a slow-growth stage [2, 5, 6]. Interestingly, strawberry fruits exhibit either a single or double sigmoid pattern in different varieties [7–12]. These results indicate that fruit growth patterns are determined by other factors than by the type of fruit.

The distinction between single and double sigmoid patterns is whether a slow-growth period occurs during fruit enlargement. A previous study reported that cooperation between the velocity and duration of fruit swelling...
determines fruit size [13], which is determined by both cell number and size [14–16]. Currently, little is known about the genes that control fruit size, with the exception of genes involved in the cell cycle, cell wall metabolism, cytochrome, and ubiquitin [13, 17, 18]. Specifically, cyclin, RNA polymerase II transcription, and mitogen-activated protein kinase kinase kinase are involved in the cell cycle [19–21], xyloglucan galactosyltransferase, glycosyltransferase, cellulose synthase, β-galactosidase, and microtubule-associated proteins are associated with cell wall metabolism [22–25], and transcription factors, including basic region/leucine zipper motif (bZIP) [26], NAM/ATAF1/2/CUC2 (NAC) [27], v-myb avian myeloblastosis viral oncogene homolog (MYB) [28], basic/helix-loop-helix (bHLH) [29], and WRKY [30], are components of the fruit size regulation network.

A combination of transcriptome sequencing and QTL is an effective method for screening candidate genes of specific traits and has been used in plants, including peaches, pears, and tomatoes [27, 28, 31, 32]. Currently, fruit size is known to be anchored by 28 QTLs distributed on 11 chromosomes in tomatoes [33, 34]. In pome, fruit weight, height, and width were individually anchored by 14, 3, and 4 QTLs, respectively, on 5 chromosomes and 9 scaffolds in pears [35–37], as well as anchored by 10, 7, and 10 QTLs, respectively, on 7 chromosomes in apples [38]. In drupe, fruit weight, height and width were individually anchored by 7, 6, and 12 QTLs, respectively, on all 8 chromosomes in peaches [39–41], and anchored by 6, 2, and 2 QTLs, respectively, on 4 chromosomes in sweet cherries [42, 43]. Moreover, fruit weight was anchored by 3 QTLs on Chr2 in strawberries [44, 45]. These reported QTL regions provide a good reference for screening candidate genes of agronomic traits.

Single and double sigmoid patterns have been reported in the last century in Rosaceae fruit species [2], but the difference between these patterns has not been explored at the molecular level until now. In Rosaceae, pear is a pome fruit and was selected for studies of single sigmoid pattern, while peach is a drupe fruit that presents a double sigmoid pattern and was selected as a control. Moreover, strawberry is an aggregate fruit that presents a double sigmoid pattern and was selected as a control. The fruits in these three species were selected and performed for transcriptome sequencing to explore the difference between single and double patterns. First, developing and matured fruits were collected for measuring fruit weight and calculating cell number to clarify the periods of rapid enlargement and intervals in peaches and strawberries. Based on the completed genome projects of pears, peaches, and strawberries [46–48], transcriptome sequencing was conducted to screen for differentially expressed genes (DEGs) related to fruit enlargement in the three Rosaceae species. Validation of the candidate genes was conducted based on reported QTLs [35, 39, 44], functional annotation was also performed. Moreover, a dual-luciferase assay was conducted between the screened transcription factors and cell expansion-related genes. Finally, molecular regulation of the single sigmoid pattern was discussed. This is the first study to report on the difference between single and double sigmoid patterns at the molecular level. These results will enhance our understanding of the molecular regulation of fruit size in Rosaceae.

**Results**

**Investigation of growth curves in pear, peach, and strawberry fruits**

To investigate the growth curves of pear, peach, and strawberry fruits, the fruit weight of each species was measured from fruitlet to maturation. Results revealed a single sigmoid pattern growth curve in pear fruits (Fig. 1a; Figure S1), but a double sigmoid pattern in peach and strawberry fruits (Fig. 1a). Further analysis of paraffin section showed that the cell number of per fruit in three fruit species mainly increased before 42, 21, and 19 days after full blooming (DAFB), respectively, and then maintained stable until to fruit ripening (Fig. 1b). In contrast, the cell size changed little before 42, 21, and 19 DAFB, respectively, and then the cell expansion was happened (Fig. 2). These results revealed that pear, peach, and strawberry fruits underwent extensive cell division before 42, 21, and 19 DAFB, respectively, and then underwent cell enlargement. In addition, both the single and double sigmoid curve occurred after these days, we concluded that both single and double sigmoid patterns were a result of cell expansion, not cell division [1–4].

**Transcriptome sequencing and differential expression analyses**

To uncover the difference between single and double sigmoid patterns, the fruit at fruitlet, first enlargement, interval, second enlargement, and maturation (Table S1) in peach and strawberry were selected for transcriptome sequencing. These five stage samples were designated as PP1 to PP5 in peaches (Prunus persica) and FA1 to FA5 in strawberries (Fragaria × ananassa) (Table S1), respectively. In pears (Pyrus bretschneideri), the fruitlet (28 DAFB), early enlargement (98 DAFB), middle enlargement (126 DAFB), late enlargement (140 DAFB), and maturation (168 DAFB) stage fruits were selected and designated as PB1 to PB5, respectively. A total of 1.41, 1.56, and 1.44 Gb raw reads were generated from pear, peach, and strawberry fruits, respectively. After removing low-quality reads, an average of 91.47, 100.01, and 92.3 Mb clean reads were used for mapping to the reference genome in pear, peach, and strawberry fruits, respectively. The average percentages of total mapped reads were 73.99, 88.64, and 72.27% in pear, peach, and strawberry fruits, respectively. A total of 31,910 genes were
detected in pear fruits. Of these genes, 25,633 were commonly expressed in all tested fruits, while 1303, 231, 162, 214, and 254 were specifically expressed from PB1 to PB5, respectively (Figure S2). A total of 27,747 genes were detected in peach fruits. Of these genes, 22,480 were commonly expressed in all tested fruits, while 652, 189, 149, 113, and 291 were specifically expressed from PP1 to PP5, respectively (Figure S2). A total of 35,502 genes were detected in strawberry fruits. Of these genes, 27,023 were commonly expressed in all tested fruits, while 1156, 303, 265, 702, and 325 were specifically expressed from FA1 to FA5, respectively (Figure S2).

To isolate the genes associated with fruit enlargement, a differential expression analysis was conducted to compare fruits at the rapid enlargement and fruitlet or maturation stages. In pears, 4096 (PB2-DEG), 2478 (PB3-DEG), and 3831 (PB4-DEG) genes were differentially expressed in PB2, PB3, and PB4 compared to PB1 and PB5 (Fig. 3). In peaches, 5656 (PP2-DEG) and 4536 (PP4-DEG) genes were differentially expressed in PP2 and PP4 compared to PP1 and PP5 (Fig. 3). In strawberries, 3210 (FA2-DEG) and 2238 (FA4-DEG) genes were differentially expressed in FA2 and FA4 compared to FA1 and FA5 (Fig. 3). These isolated genes were possibly associated with fruit enlargement in corresponding species. Moreover, 3799 (PP3-DEG) genes were differentially expressed between PP3 and PP2/PP4 in peaches, while 2863 (FA3-DEG) genes were differentially expressed between FA3 and FA2/FA4 (Fig. 3). The differential expression of these genes may be involved in the intervals of fruit enlargement.

Overlap of DEGs with QTL regions related to fruit size
Fruit size, which includes fruit weight, height, width, and depth, has been widely studied in pear, peach, and strawberry fruits. In pears, fruit size was anchored by 21 QTL regions covering 1279 genes [35–37], of which, 123, 72, and 117 genes were detected in PB2-, PB3-, and PB4-DEGs, respectively (Table S2). In peaches, fruit size was anchored by 36 QTL regions covering 7100 genes [39–41], of which, 1198 and 1001 genes were detected in PP2- and PP4-DEGs, respectively (Table S2). In strawberries, fruit size was anchored by three QTL regions covering 192 genes within a 150 kb range [44, 45], of which, four genes were detected in FA2-DEG (Table S2). In strawberries, fruit size was anchored by three QTL regions covering 192 genes within a 150 kb range [44, 45], of which, four genes were detected in FA2-DEG (Table S2). These results revealed that the DEGs overlapped with QTLs related to fruit size, indicating that screening for fruit enlargement candidate genes is reliable based on the transcriptome analyses of these three Rosaceae species.

Conception of fruit enlargement in Rosaceae
Pear, peach, and strawberry fruits are Rosaceae species that have relatively close genetic backgrounds. However,
Pear fruits exhibit a single sigmoid pattern, while peach and strawberry fruits exhibit a double sigmoid pattern (Fig. 1a). These growth patterns are a result of the differences between the middle enlargement stage in pears (PB3) and interval in peaches and strawberries (PP3/FA3). In peaches, two fruit enlargement stages were mediated by PP2- and PP4-DEGs, while the interval was controlled by genes exhibiting the opposite trend in PP3-DEG compared to PP2- and PP4-DEGs. This conclusion was also observed in strawberry fruits. Therefore, it was speculated that the genes involved in the interval stages must possess correlated expression profiles with the fruit growth patterns of peaches and strawberries. In pears, the genes involved in the middle enlargement stage correlated with the expression profiles of fruit growth patterns of peaches and strawberries. In pears, 592 genes were upregulated and 335 were downregulated, which overlapped among PB2-, PB3-, and PB4-DEGs (Fig. 4a, b), correlating with the single sigmoid pattern in pears. In the second model (Model II), the intervals in peach and strawberry fruits were independent of other fruit enlargement stages, resulting in the single sigmoid pattern, which was mediated by the genes involved in the middle enlargement stage, but not the early or late enlargement stages in pears.

**Identification of cell development genes in Model I**

To examine the possibility of Model I, the genes that present correlated expression profiles with the two fruit growth patterns were identified in the three Rosaceae species. In peaches, 90 genes were upregulated in PP2- and PP4-DEGs, but downregulated in PP3-DEG; this gene set was designated as PPP. Meanwhile, 162 genes were downregulated in PP2- and PP4-DEGs, but upregulated in PP3-DEG; this gene set was designated as PPN. In strawberries, 43 genes were upregulated in FA2- and FA4-DEGs, but downregulated in FA3-DEG; this gene set was designated as FAP. Moreover, 26 genes were downregulated in FA2- and FA4-DEGs, but upregulated in FA3-DEG; this gene set was designated as FAN. In pears, 592 genes were upregulated and 335 were downregulated, which overlapped among PB2-, PB3-, and PB4-DEGs (Fig. 4a, b), correlating with the single sigmoid pattern in pears. The up- and
downregulated gene sets were designated as PBPC and PBNC, respectively. Among these 927 genes, 155 were involved in cell development, including cell wall biogenesis, cell growth, and cell division (Figure S3A). However, the comparative analysis of the genes in PBPC, PPP, and FAP detected no orthologous genes among the three Rosaceae species, nor did the comparative analysis of the genes in PBNC, PPN, and FAN. These results were inconsistent with Model I, therefore, the genes associated with all three fruit enlargement stages in pears were different from the genes involved in the intervals in peaches and strawberries.

Although the genes in PBPC were different from the genes in PPP and FAP, it was not clear whether all the genes in PBPC were associated with fruit enlargement as the orthologous genes in peach and strawberry fruits may be included in PPN/FAN. A similar inference was made for the genes in PBNC. To verify these speculations, a comparative analysis was conducted on the genes in PBPC, PPN, and FAN. Results revealed that 44 genes in PBPC were orthologous to the genes in PPN or FAN, suggesting that these genes were not associated with the single sigmoid pattern; the remaining 548 genes in pears may be involved in fruit enlargement (Fig. 4a). In these 836 DEGs in pears, 90 were involved in cell development, including the cell cycle and cell wall metabolism (Table S3). Notably, of these genes, 25 were located within the fruit weight QTL regions (Table 1). Clearly, several of these genes were specifically associated with fruit enlargement in pears and the difference between the single and double sigmoid patterns may result from these genes.

Identification of cell enlargement genes in Model II
To examine the possibility of Model II, the genes that mediate the middle enlargement stage, but not early or late enlargement, were isolated in pears. As a result, 512 upregulated and 204 downregulated genes were detected in PB3-DEG; these gene sets were designated as PBPO and PBNO, respectively (Fig. 4c, d). Among these 706 genes, 22 were involved in cell wall biogenesis, ubiquitin, and auxin (Figure S3B). However, comparative analysis of the genes in PBPO, PPP, and FAP detected no orthologous genes among the three Rosaceae species, nor did the comparative analysis of the genes in PBNO, PPN, and FAN. These results were inconsistent with Model II, therefore, the genes involved in the middle enlargement stage in pears were different from the genes involved in the intervals of peaches and strawberries.

Although the genes in PBPO were different from the genes in PPP and FAP, it was not clear whether all the
genes in PBPO were associated with fruit enlargement as the orthologous genes in peach and strawberry fruits may be included in PPN/FAN. A similar inference was made for the genes in PBNO. To verify these speculations, a comparative analysis was conducted among the genes in PBPO, PPN, and FAN. Results revealed that 47 genes in PBPO were orthologous to the genes in PPN or FAN, suggesting that these genes were not associated with the single sigmoid pattern. The remaining 465 genes in pears may be involved in fruit enlargement (Fig. 4c). Similarly, 20 genes in PBNO were orthologous to the genes in PPP or FAP, suggesting that these genes were not associated with the single sigmoid pattern. The remaining 184 genes in pears may be involved in fruit enlargement (Fig. 4d). Of these 649 DEGs in pears, 63 were involved in cell development, including cell wall biogenesis, cell growth, and cell metabolism (Table S4). Notably, of these genes, 22 were located within the fruit weight QTL regions (Table 1). Clearly, several of these genes were specifically associated with fruit enlargement.
Table 1: The identified genes from Model I and Model II overlapped with fruit size QTL markers

| Gene ID          | Annotation                                      |
|------------------|-------------------------------------------------|
| Pbr035270.1      | IRK-interacting protein-like (LOC103934871)     |
| Pbr038258.1      | uncharacterized LOC103933313 (LOC103933313)     |
| Pbr012946.1      | transcription repressor OFP12-like (LOC103960152) |
| Pbr009000.1      | probable transmembrane GTPase FZO-like (LOC103957273) |
| Pbr020465.2      | alphaalpha-trehalose-phosphate synthase         |
| Pbr033649.1      | kinesin-3-like (LOC103948751)                   |
| Pbr08977.1       | chloroplast stem-loop binding protein of 41 kDa a (LOC103957266) |
| Pbr08967.1       | chloroplast stem-loop binding protein of 41 kDa a (LOC103957266) |
| Pbr017191.1      | zinc finger CCCH domain-containing protein 53-like (LOC103963791) |
| Pbr039708.1      | ubiquitin thioesterase OTU1-like (LOC103951354)  |
| Pbr022797.1      | BTB/POZ domain-containing protein At1g03010 (LOC103967390) |
| Pbr024215.1      | rab3 GTPase-activating protein non-catalytic subunit (LOC103926877) |
| Pbr030227.1      | E3 SUMO-protein ligase MMS21-like (LOC103931527)  |
| Pbr038269.1      | D-amino-acid transaminase, chloroplastic-like (LOC103932682) |
| Pbr038230.1      | uncharacterized LOC103932372 (LOC103932372)     |
| Pbr008947.1      | protein MARD1-like (LOC103957220)               |
| Pbr022822.1      | ATP-dependent zinc metalloprotease FTSH 2 (LOC103967414) |
| Pbr033637.2      | aminopeptidase M1-like (LOC103948738)           |
| Pbr039758.1      | GDP-L-galactose phosphorylase 2 (LOC103951401)   |
| Pbr025844.1      | uncharacterized LOC103927937 (LOC103927937)     |
| Pbr024234.1      | chitin-inducible gibberellin-responsive protein 1-like (LOC103926896) |
| Pbr022819.1      | zuotin-like (LOC103967411)                      |
| Pbr036709.1      | uncharacterized LOC103949957 (LOC103949957)     |
| Pbr017194.1      | uncharacterized LOC103963818 (LOC103963818)     |
| Pbr040573.1      | chitin-inducible gibberellin-responsive protein 1-like (LOC103932823) |
| Pbr012884.1      | FBD-associated F-box protein At4g10400-like (LOC103960072) |
| Pbr012619.1      | nudix hydrolase 18, mitochondrial-like (LOC103959904) |
| Pbr003047.2      | uncharacterized LOC103940404 (LOC103940404)     |
| Pbr020827.1      | U-box domain-containing protein 13-like (LOC103944939) |
| Pbr036583.1      | uncharacterized LOC10395645 (LOC103956455)      |
| Pbr020491.1      | LRR-receptor-like serine/threonine-protein kinase EFR (LOC103965424) |
| Pbr035276.1      | F-box/LRR-repeat protein At4g14103-like (LOC103926900) |
| Pbr008975.1      | uncharacterized LOC103957269 (LOC103957269)     |
| Pbr022826.1      | nuclear poly(A) polymerase 1-like (LOC103967418) |
| Pbr017829.1      | LRR receptor-like serine/threonine-protein kinase At3g47570 |
| Pbr012938.1      | 50S ribosomal protein L24-like (LOC103960110)    |
| Pbr035475.1      | stress enhanced protein 2 (LOC103935051)        |
| Pbr014977.1      | uncharacterized LOC103961245 (LOC103961245)     |
| Pbr017817.2      | tRNA-specific 2-thiouridylase MnmA (LOC103963898) |
| Pbr012885.1      | ---NA---                                        |
| Pbr020496.1      | LRR receptor-like serine/threonine-protein kinase At3g47570 (LOC103965422) |
| Pbr009970.1      | C2 domain-containing protein At1g53590-like (LOC103931542) |
| Pbr009987.1      | probable solanesyl-diphosphate synthase 3, chloroplastic (LOC103931560) |
| Pbr039779.1      | 65-kDa microtubule-associated protein 1-like (LOC103951420) |
| Pbr038272.1      | L-type lectin-domain containing receptor kinase IX.1-like (LOC103933343) |
| Pbr041406.1      | scarecrow-like protein 21 (LOC103938496)         |
| Pbr036594.1      | protein DETOXIFICATION 42-like (LOC103935654)    |

Pei et al. BMC Plant Biology (2020) 20:108
in pears. Thus, the difference between the single and double sigmoid patterns may be a result of Model II. Collectively, the genes detected in Models I and II in pear fruits may regulate the single sigmoid pattern.

Expression profiles of fruit enlargement candidate genes
To confirm the transcriptome assay, 12 candidate genes associated with cell wall, cell cycle, ubiquitination, phytohormones, cytochrome, ankyrin, transcription factor, and leucine-rich repeats (LRR) from Models I and II were selected for quantitative real-time polymerase chain reaction (qRT-PCR) analysis of pear, peach, and strawberry fruits. Results revealed that these genes had similar expression profiles as the transcriptome assay results, indicating that these findings were reliable (Figure S4). Moreover, to test whether the candidate genes exhibited similar expression profiles in different pear cultivars, the ‘Housui’, ‘Cuiguan’, and ‘Xueqing’ cultivars were investigated at the same five fruit enlargement stages and analyzed by qRT-PCR (Figure S1). Results revealed that the expression profiles of the 12 aforementioned genes in the three cultivars were almost identical to the ‘Dangshansuli’ cultivar (Fig. 5), suggesting

![Fig. 5 Expression profiles of the candidate genes in the ‘Housui’, ‘Cuiguan’, and ‘Xueqing’ pear cultivars. SEs were calculated from 3 biological replicates. Different lowercase letters indicate significant differences (p < 0.05); * and ** indicate significant correlations (p < 0.05 and p < 0.01, respectively)](image-url)
that these candidate genes identified from ‘Dangshansuli’ were also involved in the middle enlargement stages of other pear cultivars. Therefore, these candidate genes may be associated with the single sigmoid pattern in pear fruits.

**Regulation of bHLHs in cell expansion-related genes**

In a previous study, bHLH was defined as the upstream factor regulating cell expansion-related genes in peaches [13]. In this study, four bHLH genes, bHLH3 (Pbr009044.1), bHLH30 (Pbr016145.1), bHLH106 (Pbr013890.1), and bHLH144 (Pbr039557.1), were detected in the genes associated with the single sigmoid pattern, as well as cell expansion-related genes, including cellulose synthase (CES), glycosyltransferase (GT), microtubule-associated protein (MAP), UDP-glycosyltransferase (UGT), COP9 signalosome complex subunit (CSN), exocyst complex component (EXOC), expansin (EXP), xyloglucan galactosyltransferase (XG GalT), and xyloglucan glycosyltransferase (XG GT). To investigate the role of the four bHLH transcription factors (TFs) on the expression of cell expansion-related genes, full-length sequences of the four bHLH genes were amplified and inserted into pSAK277 as effectors. Additionally, ~2000 bp sequences in the promoter of cell expansion-related genes were inserted into pGreen 0800-LUC as reporters (Fig. 6a). The dual luciferase assay results revealed that bHLH3, bHLH30, and bHLH106 enhanced the activities of firefly luciferase (LUC), which were driven by the promoters of 11 of the 14 selected genes, while bHLH144 enhanced the activities of LUC and was driven by the promoters of 10 of the 14 selected genes (Fig. 6b). Notably, LUC was driven by the promoters of cellulose synthase, glycosyltransferase-1, glycosyltransferase-2, UDP-glycosyltransferase-2, expansin, xyloglucan galactosyltransferase-1, and xyloglucan glycosyltransferase, and exhibited higher activities in tobacco leaves overexpressing any of the bHLH genes compared to tobacco leaves transformed with an empty vector. These results suggested that the four bHLH TFs interacted with most promoters of cell expansion-related genes and drove their expression.

**Discussion**

**Potential gene regulation network for the pear single sigmoid pattern**

Single and double sigmoid patterns have been reported over the last century in Rosaceae fruit species [2], however, the difference between these patterns had not been explored at the molecular level until now. In this study, based on the investigated physiological phenotypes, two models of fruit enlargement in pears were proposed. By comparative transcriptome analyses of the selected fruits in three Rosaceae fruit species, several genes associated with the cell cycle [19–21], polysaccharides and cell development [22–24, 49–56], were detected in Models I and II (Tables S3 and S4). Therefore, models I and II play important roles in generating the single sigmoid pattern.

In addition to the above genes, several transcription factors were also detected in Models I and II (Table 1; Tables S3 and S4), including zinc finger proteins (ZFPs), which control cell size during plant organogenesis [57], and bHLHs, which regulate cell extension by transducing auxin signaling [27]. bZIP mediates cell expansion by affecting the biosynthesis and signal transduction of gibberellins and auxins [24, 58], while ankyrin repeat proteins enhance auxin biosynthesis via bZIP [59]. WRKY and NAC are involved in cell development [30, 60, 61], as well as auxin biosynthesis [62, 63], while proliferating cell factor (TCP) and MYB are only associated with auxin biosynthesis [64, 65]. Clearly, auxin signal transduction may be involved in the middle enlargement stage of pear fruits.

---

**Fig. 6** Detection of transcriptional activation of the 4 bHLHs in 14 candidate genes associated with the pear single sigmoid pattern. a Constructed effectors and reporters. b Dual-luciferase assay of effectors on reporters. Cell development-associated genes include CES (Pbr038537.1), GT (Pbr016727.1, Pbr023514.1, and Pbr023516.2), MAP (Pbr011537.1 and Pbr039779.1), UGT (Pbr018679.1 and Pbr021540.1), CSN (Pbr025986.3), EXOC (Pbr029906.1), EXP (Pbr013129.1), XG GalT (Pbr036086.1 and Pbr004891.1), and XG UGT (Pbr005326.1). * and ** indicate significant correlations (p < 0.05 and p < 0.01, respectively).
In this study, auxin-induced factors, including auxin response factor, auxin-induced proteins, and auxin-responsive proteins, were detected in Models I and II, which are associated with cell expansion [66, 67]. Moreover, the detected cytochrome p450 proteins stimulate plant organ growth by increasing cell size [17]. The transcriptional activation of the four bHLHs in 14 candidate genes associated with the pear single sigmoid pattern of the two models was also confirmed by the dual-luciferase assay. These results revealed that the promoters of the 14 genes were activated by bHLHs (Fig. 6). Based on these results, a potential gene regulation network was proposed for the pear single sigmoid pattern. This network indicates that phytohormone auxin was regulated by MYB, WRKY, TCP, NAC, and ankyrin-mediated bZIP, which thereby triggered the expression of bHLH and auxin-induced factors. Simultaneously, bZIP triggered gibberellin signal transduction to activate gibberellin-responsive proteins. Auxin-induced factors along with bHLH, ZFP, cytochrome P450, and gibberellin-responsive proteins promoted the expression of genes related to cell expansion, which resulted in the middle enlargement of pear fruits.

**Positive selection was observed in pear and peach fruit enlargement genes**

Plant domestication is a long-term, people-based plant interaction that favors the alleles of genes that control certain traits of interest in increased frequency [68]. In fleshy fruits, fruit enlargement is a typical phenotype observed during fruit development and maturation. In a previous study, tomato fruits exhibited a single sigmoid pattern during fruit growth [2], and fruit mass was domesticated from the small fruits of their ancestors to the big fruits of modern tomatoes [55, 69]. The similar domestication of fruit size was detected in pears [70], peaches [71, 72], apples [73] and sweet cherries [43].

In this study, the dynamic weight of developing and mature pear, peach, and strawberry fruits was measured. Results confirmed that pear fruits exhibited a single sigmoid pattern during fruit growth, while peach and strawberry fruits exhibited a double sigmoid pattern. However, it is unclear whether the fruit growth patterns of these three Rosaceae species undergo positive selection. Previously, the selective sweep regions of fruit size were isolated in peach and pear fruits [70, 72, 74]. In peaches, 707 and 507 genes correlated with the first and second enlargement stages, respectively, and were located within the selective sweep regions. Moreover, 395 genes correlated with the first and second enlargement stages [71, 72]. These results indicate that the first and second enlargement stages were domesticated during the evolution of these species (Table S5). Similarly, in pears, 66, 32, and 74 genes correlated with the early, middle, and late enlargement stages, respectively, and were located within the selective sweep regions. Moreover, 21 genes correlated with the three fruit enlargement stages (Table S5). These results suggest that the three fruit enlargement stages were domesticated during

![Fig. 7 Potential gene regulation network of the pear single sigmoid pattern showing TCP, NAC, MYB, WRKY, and ankyrin-mediated bZIP-regulated auxin expression. bZIP triggered gibberellin signal transduction to activate gibberellin-responsive proteins. Auxin-induced factors along with bHLH, ZFP, cytochrome P450, and gibberellin-responsive proteins promoted the expression of genes related to cell expansion, which resulted in the middle enlargement of pear fruits](image-url)
the evolution of these species. Collectively, these findings indicate that each fruit enlargement stage was domesticated in pear and peach fruits.

In previous studies, the double sigmoid pattern of peach fruits was explained by two hypotheses: the competition for assimilates between the seed and pericarp, and the hormonal control of pericarp growth by the seed [6]. In this study, these hypotheses may co-exist in the three fleshy fruits. First, strawberry fruit development is dependent on seed fertilization-induced hormones [75], indicating that hormones are necessary for fruit enlargement. Theoretically, hormone-controlled fruit enlargement should not be interrupted. However, when strawberry fruits were bleaker in color (Figure S5), both hormones and assimilates were used for anthocyanin biosynthesis or fruit enlargement, leading to slow fruit growth. Similarly, when peach fruits exhibited stone hardening (Figure S5), both hormones and assimilates were provided for endocarp lignification or fruit enlargement, inducing slow fruit growth. In contrast, due to the lack of dramatic changes observed during pear fruit development (Fig. 1a), both hormones and assimilates were used for fruit enlargement, which resulted in the single sigmoid pattern.

Conclusions
In this study, pear fruits exhibited a single sigmoid pattern, while peach and strawberry fruits exhibited a double sigmoid pattern. These patterns resulted from cell expansion, not cell division. By comparative transcriptome analysis among pear, peach, and strawberry fruits, 836 genes were found to be associated with all three fruit enlargement stages in pear fruits (Model I), while 649 genes were associated with middle enlargement stage, not early or late enlargement (Model II). Therefore, the pear single sigmoid pattern appeared to be coordinately mediated by these proposed models. Interestingly, most genes in these models were annotated and correlated with cell development. Moreover, 47 genes were located within the QTL regions related to fruit weight. Based on previous reports and the dual-luciferase assay, a potential gene network of the single sigmoid pattern was drafted in this study (Fig. 7).

Methods
Plant materials
The ‘Dangshansuli’ (P. bretschneideri Rehd.), ‘Cuiguan’ (P. pyrifolia Nakai), ‘Xueqing’ (P. bretschneideri Rehd.), and ‘Housui’ (P. serotina Rhed.) pear cultivars were maintained at the Jiangpu orchard of Nanjing Agricultural University, Nanjing, China. Pear fruits were collected every 14 days from 14 DAFB to maturation. The ‘Hongyan’ strawberry cultivar (F. × ananassa) was grown in a greenhouse at Zhen Jiang Agricultural Academy, China. A total of 300 flowers were selected and marked at the initial flowering stage; the fruits derived from these flowers were collected every 4 days from three DAFB to maturation. Fruit weight was measured using 15 fruits from each period for all fruit species.

‘Dangshansuli’, ‘Liangyuan’, and ‘Hongyan’ fruits were cut into pieces and fixed in a fixative solution (90 mL 50% ethanol, 5 mL formol, 5 mL glacial acetic acid) for > 24 h. After dehydration by gradient alcohol application and embedding into paraffin, samples were sliced using an RM2016 LEICA slicer (Leica, Wetzlar, Germany). Pictures of the paraffin sections were visualized using a Nikon ECLIPSE E100 system (10 × 10) (Nikon, Tokyo, Japan). Cell numbers were recorded using imageJ 1.47v (National Institute of Health, Bethesda, USA).

Moreover, to analyze the expression profiles of whole predicted genes during fruit swelling in pear, peach, and strawberry fruits, each collection had a minimum of three independent replicates. The collected pear and peach fruits were snap frozen in liquid nitrogen and ground into a fine powder. Similarly, after removing the seeds from the surface of strawberry fruits, the remaining tissues were mashed in liquid nitrogen and stored at −80 °C for further analysis.

Library preparation and sequencing
Total RNA was extracted using an RNAprep Pure Plant kit (Tiangen Biotech, Beijing, China). RNA degradation and contamination were monitored on 1% agarose gel. RNA purity was checked using a NanoPhotometer spectrophotometer (Implen, CA, USA). RNA concentrations were measured using a Qubit® RNA Assay kit in a Qubit® 2.0 fluorometer (Life Technologies, CA, USA). RNA integrity number (RIN) of pear, peach, and strawberry fruits was > 8.8, which met the requirement for building libraries. After qualitative and quantitative detections of total RNA, ribosomal RNA (rRNA) was depleted using an Epicentre Ribo-zero™ rRNA Removal kit (Epicentre, WI, USA). rRNA-deleted RNA fragmentation was used to synthesize first and second cDNA strands with a random hexamer primer in NEBNext First Strand Synthesis Reaction buffer (5×). Then, the NEBNext adaptor was added to adenylated DNA fragments followed by purification and screening of cDNA fragments, which were 150–200 bp in length. A trueSeq PP Cluster kit v3-cBot-HS (Illumina, CA, USA) was used to perform clustering. Then, the libraries for ‘Dangshansuli’, ‘Liangyuan’, and ‘Hongyan’ were sequenced on an Illumina HiSeq 4000 platform (Illumina, CA, USA) with three replicates.
Quality control and transcriptome assembly

Quality control of raw data (raw reads) in fastq format was conducted according to previously described methods [76]. After removing unqualified raw reads, clean reads were mapped to the reference genomes using Bowtie2 v2.2.8 and HISAT2 v2.0.4 [77]. The reference genomes of pear, peach, and strawberry fruits consisted of the Pyrus genome v1.1 (http://peargenome.njau.edu.cn), Prunus persica v2.0.a1 (https://www.ncbi.nlm.nih.gov) and Fragaria vesca v2.0.a1 (http://ftp.bioinfo.wsu.edu), respectively. The clean reads mapped to the reference genomes and assembled using StringTie v1.3.1 wsu.edu), respectively. The clean reads mapped to the reference genomes and assembled using StringTie v1.3.1 following a reference-based approach [78]. StringTie was used for its novel network flow algorithm and optional de novo assembly step for assembling and quantifying full-length transcripts that represent multiple splice variants for each gene locus.

Differential expression analysis

Gene expression levels were calculated based on the fragments per kilobase of exon per million fragments mapped (FPKM) reads using Cuffdiff v2.1.1 [79]. After filtering unreliable data in python scripts (https://github.com/Peims/Batch-screening-unqualified-data). A differential expression analysis was conducted using limma packages (https://bioconductor.org/packages/limma/). The criterion for distinguishing significant gene differences was \( p < 0.05 \) [80, 81].

Gene function annotation

Gene Ontology (GO) enrichment analysis of the DEGs was implemented using PlantRegMap (http://plantregmap.cbi.pku.edu.cn/). The screening criteria of significantly enriched terms was \( p < 0.05 \). KOBAS v3.0 (http://kobas.cbi.pku.edu.cn/) software was used to calculate the enrichment of KEGG pathway DEGs. Gene functional annotations were conducted using local BLAST 2.2.29+ against the NCBI NR database.

Chromosome location of DEGs and orthologous analysis

The location of DEGs was retrieved based on mRNA information. DEGs were located within the reported QTL regions were statistically analyzed in python script (https://github.com/Peims/Calculate-the-distance-between-the-gene-and-the-marker). An orthologous genes analysis was conducted using OrthoFinder v2.3.3 [82].

qRT-PCR

Total RNA was extracted using a Plant Total RNA Isolation kit plus (Gore Gene, Chengdu, China). The first strand of cDNA was synthesized using FastKing qDNA Dispelling RT SuperMix kits (Tiangen Biotech, Beijing, China), which was subsequently used as a template for qRT-PCR. qRT-PCR was performed on a Lightcycler 480 SYBR Green I Master mix (Roche, Basel, Sweden). The reaction mixture was conducted as previously reported [74]. The gene-specific primers used in this study are provided (Table S6). All PCR experiments were performed using three independent biological samples, which included three technical replicates. The pear SNF, peach TEF, and strawberry DBP genes were used as normalizers. An independent sample t-test was used to analyze the qRT-PCR results between each sample. A correlation analysis between transcriptome and qRT-PCR results was conducted in python script (https://github.com/Peims/calculate-the-pearsoner).

Vector constructs and dual luciferase assays

To detect the transcriptional activation of four bHLHs on 14 candidate genes associated with the single sigmoid pattern of Models I and II, full-length sequences of the four bHLHs were amplified using specific primers (Table S7). Phanta* Max Super-Fidelity DNA polymerase (P505, Vazyme Biotech Co., Ltd., China) was introduced into the pSAK277 vector to construct the effectors (Fig. 6a). The ~2000 bp promoter sequence of the 14 genes was inserted into the pGreen 0800-LUC vector to construct reporters. Then, the correctly recombinant plasmids were transformed into the Agrobacterium strain GV3101, using pSoup for pGreen 0800-LUC vector transformation. The GV3101 strain contained an effector and reporter was co-infiltrated into tobacco leaves [83]. The empty pSAK277 transient expression vector was used as the negative control. For each promoter-TF comparison, at least six infiltrated leaves using the same Agrobacterium culture were used in the dual-luciferase assay. LUC and Remilia luciferase (REN) activity were assayed using a Dual-Luciferase® Reporter Assay system (Promega, WI, USA) following the manufacturer’s instructions. Relative LUC activities were calculated as the ratio of LUC/REN for each comparison and normalized to the control in each experiment. Statistical analyses were conducted using SPSS v17.0 software (SPSS Inc., CHI, USA). Student’s t-tests were performed to determine significant differences (\( p < 0.05 \)).

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12870-020-2317-6.

Additional file 1: Figure S1. Measurements of fruit weight in Cvs. ‘Housui’, ‘Cuiguan’ and ‘Kueqing’.

Additional file 2: Figure S2. Commonly and specially expressed genes in five period of three fruit species.

Additional file 3: Figure S3. GO enrichment of candidate genes identified in Model I and Model II.

Additional file 4: Figure S4. Expression profiles of the differential expressed genes related to fruit enlargements in pears, peaches and strawberries, respectively.
Additional file 5: Figure S5. The pictures of strawberries and peaches in the intervals.

Additional file 6: Table S1. Stages of peaches and strawberries selected for transcriptome sequencing.

Additional file 7: Table S2. Overlap of differential expressed genes with QTL regions related to fruit size.

Additional file 8: Table S3. Candidate genes involved into fruit enlargement in Model I.

Additional file 9: Table S4. Candidate genes involved into fruit enlargement in Model II.

Additional file 10: Table S5. The gene involved into fruit enlargements undergo positive selection in both pears and peaches.

Additional file 11: Table S6. Primers for real-time quantitative PCR.

Additional file 12: Table S7. Primers for dual luciferase assay.

Abbreviations
DAFB: Days after full blooming; DEG: Differentially expressed gene; FA: Strawberry; FAN: Genes downregulated in FA2- and FA4-DEGs, but upregulated in FA3-DEG in strawberries; FAP: Genes upregulated in FA2- and FA4-DEGs, but downregulated in FA3-DEG in strawberries; PB: Pear; PBPC (PBNC): Overlapping downregulated and upregulated genes among PB2-, PB3-, and PB4-DEGs in pears; PP: Peach; PPN: Genes downregulated in PP2- and PP4-DEGs, but upregulated in PP3-DEG in peaches; PPP: Genes upregulated in PP2- and PP4-DEGs, but downregulated in PP3-DEG in peaches; qRT-PCR: Quantitative real-time polymerase chain reaction; QTL: Quantitative trait locus

Acknowledgments
We thank Zhen Jiang Agricultural Academy (Hangzhou, China) for providing the experimental materials. We thank the LabPub language editing company for their excellent language editing work for this manuscript.

Authors’ contributions
SLZ and CG conceived and designed the experiments. MSP carried out paraffin sections, data analysis, qRT-PCR validation, and writing of the original manuscript. SLZ and CG conducted instruction of data analysis and revision of the original manuscript. SHC, LW, GMW and ZHX performed sample collection and measurement of fruit weight. All authors have read and approved the final manuscript.

Funding
This work was funded by the National Key Research and Development Program of China (2018YFD1000107), Key Program of National Natural Science Foundation of China (31830081), the earmarked Fund for China Agriculture Research System (CARS-28) and Jiangsu Province Science and Technology Support Program (BE2018389). The funding bodies have no role in the study design, data analysis and interpretation, and manuscript writing, but just provide the financial supports.

Availability of data and materials
Illumina sequencing data from ‘pears, peaches and strawberries’ were deposited in NCBI SRA database under accession number SRP238133, bioProject accession: PRJNA596556. Data generated or analyzed during this study are included in this article and its supplementary information files.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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

Received: 29 November 2019 Accepted: 27 February 2020
Published online: 06 March 2020

References
1. Cuevas J, Salvador-Sola FJ, Gavilán J, Lorente N, Hueso JJ, González-Padierna CM. Loquat fruit sink strength and growth pattern. Sci Hortic (Amsterdam). 2003;98:131–7.
2. Dennis Jr, FG. Fruit development. In: Tesar MB, editor. Physiological Basis of Crop Growth and Development. Madison: American Society of Agronomy; 1988. p. 273.
3. Farinati S, Rasori A, Varotto S, Bonghi C. Rosaceae fruit development, ripening and post-harvest: an epigenetic perspective. Front Plant Sci. 2017;8:1247.
4. Opara LU. Fruit growth measurement and analysis. Hortic Rev. 2002;24:373–431.
5. Rasori A, Zillofott F, Botton A, Tadello A, Trainotti L, Bonghi C, Raminia A. Hormonal cross-talk between fruit and seed throughout development and maturation in peach. Acta Hortic. 2010;884:53–60.
6. Pavel EW, Delong TM. Relative growth rate and its relationship to compositional changes of nonstructural carbohydrates in the mesocarp of developing peach fruits. J Am Soc Hortic Sci. 1993;118:503–8.
7. Miura H, Imada S, Yabuuchi S. Double sigmoid growth curve of strawberry fruit. J Jpn Soc Hortic Sci. 1990;59:527–31.
8. Kassai T, Mosoni P, Patry R, Dénes F. Investigation of the dynamics of fruit growth in two strawberry varieties. Acta Hortic. 2002;567:377–9.
9. Ledesma NA, Nakata M, Sugiyama N. Effect of high temperature stress on the reproductive growth of strawberry cvs. ‘Nyoho’ and ‘Toyonokai’. Sci Hortic. 2008;116:186–93.
10. Vallarino JG, e Lima FD, Soria C, Tong H, Pott DM, Willnitzer L, Fernie AR, Nikoloski Z, Osorio S. Genetic diversity of strawberry germplasm using metabolomic biomarkers. Sci Rep. 2018;8:14386.
11. Kim SK, Baie RN, Hwang H, Kim MJ, Sung HR, Chun C. Comparison of bioactive compounds contents in different fruit tissues of June-bearing strawberry cultivars. J Am Soc Hortic Sci. 2010;128:946–53.
12. Lee CH, Min JH, Kim TI, Kim JG, Matsumoto K, Kim DY, Hwang YS. Comparison of wall polymers among three genetically closely related strawberry cultivars with different fruit firmness. Horti Environ Biotechnol. 2011;52:581–9.
13. Gu C, Zhou Y, Shu W, Cheng H, Wang L, Han Y, Zhang Y, Yu M, Joldersma D, Zhang S. RNA-Seq analysis unveils gene regulation of fruit size cooperatively determined by velocity and duration of fruit swelling in peach. Physiol Plant. 2018;164:320–36.
14. Mizukami Y. A matter of size: developmental control of organ size in plants. Curr Opin Plant Biol. 2001;4:333–9.
15. Sugimoto-Shirasu K, Roberts K. ‘Big it up’: endoreduplication and cell-size control in plants. Curr Opin Plant Biol. 2003;6:544–53.
16. Harada T, Kurashii W, Yanai M, Nakasa Y, Sato T. Involvement of cell proliferation and cell enlargement in increasing the fruit size of Malus species. Sci Hortic (Amsterdam). 2005;105:447–56.
17. Anastasiou E, Kunz S, Gerstung M, Mac Lean D, Timmer J, Fleck C, Lenhard M. Control of plant organ size by KLUH/CYP78A5-dependent intercellular signaling. Dev Cell. 2007;13:3843–56.
18. Osch S, Anastasiou E, Sharma VK, Lax T, Fletcher JC, Lenhard M. The E3 ubiquitin ligase BIG BROTHER controls Arabidopsis organ size in a dosage-dependent manner. Curr Biol. 2006;16:272–9.
19. Devitte W, Scofield S, Alcassas AA, Maughan SC, Menges M, Braun N, et al. Arabidopsis CYCD3 D-type cyclins link cell proliferation and endocycles and are rate-limiting for cytokinin responses. Proc Nat Acad Sci U S A. 2007;104:14337–42.
20. Ribeiro PA, Capella M, Chan RL. Functional characterization of the homeodomain leucine zipper I transcription factor ATHB13 reveals a crucial role in Arabidopsis development. J Exp Bot. 2015;66:5929–43.
21. Smelková V, Luptová I, Komis G, Samajová O, Ovečka M, Daskalović A, et al. Involvement of YODA and mitogen activated protein kinase 6 in Arabidopsis post-embryogenic root development through auxin up-regulation and cell division plane orientation. New Phytol. 2014;203:175–93.
22. Hu H, Zhang R, Tao Z, Li X, Li Y, Huang J, Li X, Han X, Feng S, Zhang G, Peng L. Cellulose synthase mutants distinctively affect cell growth and cell wall integrity for plant biomass production in Arabidopsis. Plant Cell Physiol. 2018;59:144–57.
23. Pesquet E, Korolev AV, Calder G, Lloyd CW. The microtubule-associated protein AtMAP70-5 regulates secondary wall patteming in Arabidopsis wood cells. Curr Biol. 2010;20:744–9.
24. Roach MJ, Mokshina NV, Badhan A, Snegireva AV, Hobson N, Deyholos MK, Gornikova TA. Development of cellulobisic secondary walls in flax fibers requires beta-galactosidase. Plant Physiol. 2011;156:1351–63.
25. Scheible WR, Pauli M. Gyscosyltransferases and cell wall biosynthesis: novel players and insights. Curr Opin Plant Biol. 2004;7:285–95.
26. Fukazawa J, Sakai T, Ishida S, Yamaguchi I, Kamiya Y, Takahashi Y. Repression of shoot growth, a bZIP transcriptional activator, regulates cell elongation by controlling the level of gibberellins. Plant Cell. 2000;12:2901–15.
27. Zhou H, Lin-Wang K, Wang H, Gu C, Dare AP, Espley RV, He H, Allan AC, Han Y. Molecular genetics of blood-filled peach reveals activation of anthocyanin biosynthesis by NAC transcription factors. Plant J. 2015;82:105–21.
28. Yao G, Ming M, Allan AC, Gu C, Li L, Wu X, Wang R, Zhang Y, Qi K, Zhang S, Wu J. Map-based cloning of the pear gene MMY14 identifies an interaction with other transcription factors to coordinately regulate fruit anthocyanin biosynthesis. Plant J. 2017;92:437–51.
29. Yi K, Menand B, Bell E, Dolan L. A basic helix-loop-helix transcription factor gene, FRP1, regulates secondary cell wall development in apple (Pyrus spp.). J Exp Bot. 2014;65:5771–82.
30. Yang L, Zhao X, Yang F, Fan D, Jiang Y, Luo K, PrüWY919, a novel WRKY transcription factor, contributes to the regulation of pith secondary wall formation in Populus trichocarpa. Sci Rep. 2016;6:18643.
31. Gu C, Wang L, Wang W, Zhou H, Ma B, Zheng H, Fang T, Oogu C, Vilmolningkanta S, Han Y. Copy number variation of a conserved genomic cluster encoding endopolygalacturonase mediates flesh texture and stone adhesion in peach. J Exp Bot. 2016;67:1993–2005.
32. Illa-Berenguer E, Van Houten J, Huang Z, van der Knaap E. Rapid and accurate genetic linkage map construction and identification of fruit-related QTLs in Japanese pear (Pyrus pyrifolia N. F.) using SNP and SSR markers. J Exp Bot. 2014;65:17062.
33. Rajangam AS, Kumar M, Aspëborg H, Guerriero G, Arvestad L, Pansri P, et al. MAP 20, a microtubule-associated protein in the secondary cell walls of hybrid aspen, is a target of the cellulose synthesis inhibitor 2,6-dichlorobenzonitrile. Plant Physiol. 2008;148:1283–94.
34. Lin T, Zhu G, Zhang J, Xu Y, Yu Q, Zheng Z, et al. Genomic analyses provide insights into the history of tomato breeding. Nat Genet. 2014;46:220–6.
35. Cole RA, Synek L, Zaray V, Fowler JE, SEC5, a subunit of the putative Arabidopsis exocyst complex, facilitates pollen germination and competitive pollen tube growth. Plant Physiol. 2005;138:2005–18.
36. Schiess K, Kauiska S, Southam P, Bush M, Sablovsky R. JAGGED controls growth anisotropy and coordination between cell size and cell cycle during plant organogenesis. Curr Biol. 2012;22:1739–46.
37. Weiße C, Droge-Laser W. The Arabidopsis transcription factor bZIP11 activates auxin-mediated transcription by recruiting the histone acetyltransferase. Nat Commun. 2014;5:3883.
38. Böttner S, Iven T, Carjens CS, Droge-Laser W. Nuclear accumulation of the ankyrin repeat protein ANK1 enhances the auxin-mediated transcription accomplished by the bZIP transcription factors BZF1 and BZF2. Plant J. 2009;58:914–26.
39. Kwon Y, Kim JH, Jikumaru Y, Kamiya Y, Hong SW, Lee H. A novel Arabidopsis MYB-like transcription factor, MYB81, regulates hypocotyl elongation by enhancing auxin accumulation. J Exp Bot. 2013;64:3911–22.
40. Schuff-Mig, Spielman M, Tiwari S, Adams S, Fenby N, Scott RJ. The AUXIN RESPONSE FACTOR 2 gene of Arabidopsis links auxin signalling, cell division, and the size of seeds and other organs. Development. 2006;133:251–61.
41. Espartz AK, Lee SH, Wenger JP, Gonzalez N, Itoh H, Inzé D, Peer WA, Murphy AS, Overvoorde PJ, Gray WM. The SIAUR19 subfamily of SMALL AUXIN UP RNA genes promote cell expansion. Plant J. 2012;70:978–90.
68. Wang L, Stec A, Hey J, Lukens L, Doebley J. The limits of selection during maize domestication. Nature. 1999;398:236–9.
69. Zhu G, Wang S, Huang Z, Zhang S, Liao Q, Zhang C, et al. Rewiring of the fruit metabolome in tomato breeding. Cell. 2018;172:249–61.
70. Wu J, Wang Y, Xu J, Korban SS, Fei Z, Tao S, et al. Diversification and independent domestication of Asian and European pears. Genome Biol. 2018;19:77.
71. Cao K, Zheng Z, Wang L, Liu X, Zhu G, Fang W, et al. Comparative population genomics reveals the domestication history of the peach, Prunus persica, and human influences on perennial fruit crops. Genome Biol. 2014;15:415.
72. Yu Y, Fu J, Xu Y, Zhang J, Ren F, Zhao H, et al. Genome re-sequencing reveals the evolutionary history of peach fruit edibility. Nat Commun. 2018;9:5404.
73. Yao J, Xu J, Cornille A, Tomes S, Karunairetnam S, Luo Z, et al. A microRNA allele that emerged prior to apple domestication may underlie fruit size evolution. Plant J. 2015;84:417–27.
74. Cao K, Zhou Z, Wang Q, Guo J, Zhao P, Zhu G, Fang W, Chen C, Wang X, Wang X, Tian Z, Wang L. Genome-wide association study of 12 agronomic traits in peach. Nat Commun. 2016;7:13246.
75. Kang C, Darwish O, Geretz A, Shahan R, Alkharouf N, Liu Z. Genome-scale transcriptomic insights into early-stage fruit development in woodland strawberry Fragaria vesca. Plant Cell. 2013;25:1960–78.
76. Pei M, Gu C, Zhang S. Genome-wide identification and expression analysis of genes associated with peach (Prunus persica) fruit ripening. Sci Hort. (Amsterdam). 2019;246:317–27.
77. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9.
78. Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nat Protoc. 2016;11:1650–67.
79. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, Van Baren MJ, Salzberg SL, Wold BJ, Pachter L. Transcript assembly and quantification by RNA-seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010;28:511–5.
80. Law CW, Alhamdoosh M, Su S, Dong X, Tian L, Smyth GK, Ritchie ME. RNA-seq analysis is easy as 1–2–3 with limma, Genes and edgeR. F1000Res. 2016;5:1408.
81. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010;11:R106.
82. Emms DM, Kelly S. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol. 2015;16:157.
83. Hellens RP, Allan AC, Friel EN, Bolitho K, Grafton K, Templeton MD, Karunairetnam S, Gleave AP, Laing WA. Transient expression vectors for functional genomics, quantification of promoter activity and RNA silencing in plants. Plant Methods. 2005;1:13.

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