PCP
PLANT & CELL PHYSIOLOGY

Transcriptome Comparison of Global Distinctive Features Between Pollination and Parthenocarpic Fruit Set Reveals Transcriptional Phytohormone Cross-Talk in Cucumber (*Cucumis sativus* L.)

Ji Li[1,5], Zhe Wu[1,2,5], Li Cui[1,5], Tinglin Zhang[1], Qinwei Guo[1], Jian Xu[1], Li Jia[1], Qunfeng Lou[1], Sanwen Huang[3], Zhengguo Li[4] and Jinfeng Chen[1,*]

[1]State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China
[2]College of Horticulture, Shanxi Agricultural University, Shanxi 030801, China
[3]Key Laboratory of Horticultural Crops Genetic Improvement of Ministry of Agriculture, Sino-Dutch Joint Lab of Horticultural Genomics Technology, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China
[4]Genetic Engineering Research Center, School of Life Sciences, Chongqing University, Chongqing 400044, China
[5]These authors contributed equally to this work.
*Corresponding author: E-mail, jfchen@njau.edu.cn; Fax, +86-025-84396279.
(Received September 28, 2013; Accepted March 25, 2014)

Parthenocarpy is an important trait determining yield and quality of fruit crops. However, the understanding of the mechanisms underlying parthenocarpy induction is limited. Cucumber (*Cucumis sativus* L.) is abundant in parthenocarpic germplasm resources and is an excellent model organism for parthenocarpy studies. In this study, the transcriptome of cucumber fruits was studied using RNA sequencing (RNA-Seq). Differentially expressed genes (DEGs) of set fruits were compared against aborted fruits. Distinctive features of parthenocarpic and pollinated fruits were revealed by combining the analysis of the transcriptome together with cytomorphological and physiological analysis. Cell division and the transcription of cell division genes were found to be more active in parthenocarpic fruit. The study also indicated that parthenocarpic fruit set is a high sugar-consuming process which is achieved via enhanced carbohydrate degradation through transcription of genes that lead to the breakdown of carbohydrates. Furthermore, the evidence provided by this work supports a hypothesis that parthenocarpic fruit set is induced by mimicking the processes of pollination/fertilization at the transcriptional level, i.e. by performing the same transcriptional patterns of genes inducing pollination and gametophyte development as in pollinated fruit. Based on the RNA-Seq and ovary transient expression results, 14 genes were predicted as putative parthenocarpic genes. The transcription analysis of these candidate genes revealed auxin, cytokinin and gibberellin cross-talk at the transcriptional level during parthenocarpic fruit set.

Keywords: Cucumber (*Cucumis sativus* L.) • Fruit set • Parthenocarpy • Transcriptome.

Abbreviations: CPPU, *N*-(2-chloro-4-pyridyl)-*N*′-phenylurea, a diphenylurea-derived cytokinin; DEG, differentially expressed gene; dpa, days post-anthesis; ELISA, enzyme-linked immunosorbent assay; FDR, false discovery rate; GO, gene ontology; GUS, β-glucuronidase; PSG, parthenocarpic-specific expressed gene; qRT-PCR, quantitative real-time PCR; QTL, quantitative trait locus; TCA cycle, tricarboxylic acid cycle.

Introduction

In the majority of flowering plants, fruit set and development are triggered by pollen tube growth and developing seeds (Varga and Bruinsma 1986). However, pollinated/fertilized fruit set limits by narrow environmental constraints. In many species, extreme sensitivity of microsporogenesis and pollination to moderately low/high temperatures and inadequate humidity are the major negative factors of fruit initiation that greatly repress fruit production (Picken 1984). Parthenocarpic fruit set, which is independent of pollination/fertilization, was first observed and recorded by Sturtevant in the 1890s and is a desirable trait for fruit production under unfavorable pollination conditions.

Parthenocarpy was recognized to be closely related to plant hormones. Studies on several fruit crops revealed that the endogenous hormone levels of parthenocarpic cultivars were much higher than those of non-parthenocarpic cultivars (Gustafson 1939, Beyer and Quebedeaux 1974, Mapelli et al. 1978). On the contrary, endogenous hormones of the unpollinated ovary appear to be reduced prior to the onset of senescence (Pharis and King 1985, Gillaspy et al. 1993). Application of exogenous plant hormones such as auxin, gibberellin, cytokinin

Plant Cell Physiol. 55(7): 1325–1342 (2014) doi:10.1093/pcp/pcu051, available online at www.pcp.oxfordjournals.org

and brassinosteroid could induce parthenocarpy (Gillaspy et al. 1993, Vivian-Smith and Koltunow 1999, Serrani et al. 2007, Fu et al. 2008, de Jong et al. 2009a). Furthermore, artificially increasing the endogenous auxin levels of the ovary or endosperm by introducing the DefH9-iaaM auxin-synthesizing gene into cucumber (Yin et al. 2006), tomato (Rotino et al. 1997, Ficcadenti et al. 1999, Pandolfini et al. 2002, Carmi et al. 2003), eggplant (Acciarri et al. 2002), strawberry (Mezzetti et al. 2004) and grape (Costantini et al. 2007) could also stimulate parthenocarpy.

Genetic studies suggested that parthenocarpy is a quantitative trait that is controlled by multiple genes. To date, parthenocarpy quantitative trait loci (QTLs) have been mapped in several fruit plants. Five tomato parthenocarpy QTLs were mapped, *pat*, *pat4.1*, *pat4.2*, *pat5.1* and *pat9.1* (Beraldi et al. 2004, Gorguet et al. 2008). In eggplant, parthenocarpy QTLs 3.1 (*Cop3.1*) and *Cop8.1* were mapped in chromosomes 3 and 8 (Miyatake et al. 2012). QTL analyses of citrus revealed that *Fn2* or *Fn3* might be involved in parthenocarpy (García et al. 2000). Molecular biology studies, however, indicated that parthenocarpy could be controlled by a single gene such as that for a receptor or transcriptional factor of the phytohormone signaling pathways, e.g. in tomato, SlTIR1 (transport inhibitor response 1) acts as an auxin receptor and *SlTIR1*-overexpressing lines exhibited parthenocarpy (Ren et al. 2011). SlIAA9, a transcriptional regulator that mediates many aspects of plant responses to auxin from a distinct subfamily of Aux/IAA genes, was also reported to be able to induce a parthenocarpy phenotype (Wang et al. 2005). SlARF7 (Auxin Response Factor 7) was found to be expressed at a high level in unpollinated mature ovaries. Also, transgenic tomatoes formed parthenocarpic fruits induced by decreasing the *SlARF7* mRNA level (de Jong et al. 2009b). Arabidopsis mutations in ARF8 uncouple fruit initiation from fertilization, resulting in the formation of parthenocarpic fruit (Goetz et al. 2006). As regards the gibberellin signaling pathway, consistent with the SlDELLA loss of function, tomatoes display a gibberellin-constitutive response phenotype, including parthenocarpy (Martí et al. 2007, Carrera et al. 2012). It seems that parthenocarpy is a complex biological process, in terms of both physiology and genetics. Unraveling the molecular regulation of parthenocarpy will elucidate the mechanism of fruit development initiation and its link to plant signal transduction pathways. However, understanding of the mechanisms underlying parthenocarpy induction is limited.

Cucumber (*Cucumis sativus* L.) is originally from the Indian subcontinent but is now grown in most continents. Chinese cucumber production ranks first in the world. To date, the area of cucumber cultivation in China has reached 1.253 Mha (Hu et al. 2011, Deng et al. 2012). However, the low rate of fruit set has remained one of the major problems in cucumber production under the protected cultivation in China. Cucumber is rich in parthenocarpic germplasm resources (Yan et al. 2009). After the completion of its de novo genome sequencing (Huang et al. 2009), cucumber became a good model plant for parthenocarpy studies. Our previous study of cucumber parthenocarpic germplasms revealed that the cucumber parthenocarpy trait was controlled by two major additive-dominant-epistatic genes and additive-dominant polygenes, in either monoecious or gynoecious cucumbers (Yan et al. 2008, Yan et al. 2010). Ten parthenocarpic QTLs were detected in cucumber, defining four genomic regions (Sun et al. 2006). In this study, we have investigated the transcriptome of cucumber fruits and compared the global transcriptional events occurring in different cucumber fruits in order to distinguish the molecular mechanism of parthenocarpy from that of pollinated fruit set. Furthermore, the global study of parthenocarpy has also helped to discover valuable parthenocarpic genes for resolving the major problem of cucumber production in China.

Results

Morphological and cytological observation in different cucumber fruits

The elongation and radial expansion of cucumber fruit are stimulated by cell division and growth (Boonkorkaew et al. 2008, Fu et al. 2010). In this study, the morphological changes of cucumber ovaries which occur with bagging, pollination and synthetic cytokinin [*N*-(2-chloro-4-pyridyl)-*N*′-phenylurea (CPPU)] treatment were observed from 0 to 4 days post-anthesis (dpa). EC1 (a inbred line derived from 'Delta star') was used as a parthenocarpic cucumber cultivar, and 8419s-1 (a inbred line derived from 'Thaminbeit alpha') was used as a non-parthenocarpic cultivar. The elongation of pollinated and parthenocarpic (EC1 and CPPU-treated 8419s-1) ovaries started after treatment, while senescence also occurred in the bagging-treated (unpollinated) 8419s-1 ovaries (**Fig. 1**). Cytological observation showed that the growth of cucumber fruit was initiated with an increase of cell numbers in the pericarp and placenta tissues by which the cavities between the inner epidermises were rapidly occupied by newly divided cells (**Fig. 2**). Meanwhile, the cell division of aborted ovaries was interrupted. The size of the epidermis and vascular bundle tissues decreased, which may have been caused by cell atrophy or apoptosis (**Fig. 2**). Ovules also stopped developing in the aborted ovaries at 2 dpa. Interestingly, although not pollinated, ovules of parthenocarpic ovaries kept on developing and formed integuments and embryo sac structures that were similar to those of the pollinated ovules (**Fig. 2**).

Transcriptome analysis of 2 dpa cucumber fruits

Based on the morphological and cytological observations, we found that the cucumber ovaries showed critical developmental changes at 2 dpa. Therefore, the 2 dpa cucumber fruits including pollinated fruits, natural parthenocarpic fruits, artificially induced parthenocarpic fruits and aborted fruits were harvested for study of the transcriptome. The transcriptome data were generated through Illumina II HiSeq^{TM} 2000 sequencing. In total, 30 million reads, each 50 nucleotides long, were generated, with approximately 7.4 million reads from each

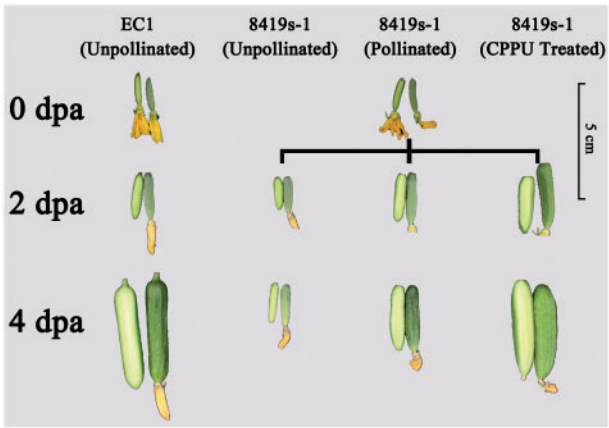

Fig. 1 Morphological changes of cucumber fruit during the early developmental stage. EC1 (an inbred line derived from Delta star) is a parthenocarpic cucumber cultivar. 8419s-1 (an inbred line derived from Thamin beit alpha) is a non-parthenocarpic cucumber cultivar. Morphological observation of the cucumber fruit during the early developmental stage (0–4 dpa, days post-anthesis) showed that the ovaries of 8419s-1, including pollinated and synthetic cytokinin CPPU-treated ovaries, and those of EC1 initiated longitudinal and radial growth at 2 dpa. At the same time, the bagging-treated 8419s-1 ovaries (unpollination treated) presented the etiolated and atrophied phenotypes.

sample. Statistical analysis was conducted to summarize the number of clean reads that were aligned to the reference genes, which provided the general information for the project (**Table 1**).

Genes with differential expression in setting fruits were filtered by the transcriptome of the aborted fruit. In multiple tests, the false discovery rate (FDR) method was used to determine the threshold of the *P*-value, which corresponded to differential gene expression. An FDR of $\leq$0.001 and an absolute value of $|\log_2 \text{ratio} \geq 1|$ were used as the threshold to judge the significance of the differential gene expression. The number of differentially expressed genes (DEGs) was counted (**Fig. 3A**). To validate the RNA-Seq results, expression levels of a selected set of up-/down-regulated and unaffected genes (**Supplementary Table S1**) was analyzed by quantitative real-time PCR (qRT-PCR). Close correlations ($R = 0.9150$, 0.9247 and 0.9031) were observed between relative expression levels measured with RNA-Seq and qRT-PCR (**Fig. 3B–D**).

The profiles of the DEGs were clustered based on the Euclid distance clustering method. Although the three DEG groups had similar expression patterns in the heat map, the transcription profiling of parthenocarpic fruits (EC1 and CPPU-treated 8419s-1) was clustered into one group (**Supplementary Fig. S1**). Venn analysis was used to screen the parthenocarpy-specific expressed genes (PSGs) (**Fig. 4**). The FDR of $\leq$0.001 and the absolute value of $|\log2\text{ratio} \geq 1.5|$ were used as the threshold for generating the DEGs. A total of 4,360 DEGs were common in the EC1 DEG and CPPU-treated 8419s-1 DEG groups. Of those, 3,021 DEGs were common with the pollinated 8419s-1 DEG group and were considered to be constitutively expressed during fruit development. The remaining 1,339 DEGs were defined as PSGs and included 759 up-regulated and 580 down-regulated genes. The gene ontology (GO) enrichment analysis provided all the GO terms that were significantly enriched in the DEGs as compared with the genome background. Finally, the DEGs corresponding to biological function were filtered. The GO analysis demonstrated that the PSGs of parthenocarpic fruits were mainly distributed in the 'primary metabolic process' (GO:0044238, GO-biological process), 'protein binding' (GO:0005515, GO-molecular function), and 'chromosome' (GO:0005694, GO-cellular component) (**Supplementary Fig. S2**). Herein, 'gene function' refers to the Arabidopsis database (http://www.arabidopsis.org/index.jsp) by the homology-based gene function prediction method.

Cell cycle, division and growth genes showed similar expression patterns in parthenocarpic and pollinated fruits

At the early stage of cucumber fruit development, the inner epidermis cavities were rapidly occupied by the newly divided cells after fruit set (**Fig. 2**). In the unpollinated ovaries, the inner epidermis cavities were expanded and vascular bundle cavities were formed (**Fig. 2**). Cytological observation also showed that there are more cells between the vascular bundle and epidermis of the parthenocarpic ovaries as compared with the pollinated ovaries (**Fig. 2**), suggesting that cell division was more active in the parthenocarpic fruits.

The RNA-Seq results showed that the genes in the GO categories of 'cell cycle', 'cell division' and 'cell growth' showed similar transcript abundance in the parthenocarpic and pollinated cucumber fruits (**Supplementary Table S2**). The expression data annotated by the GO category 'cell cycle' (GO:0007049) consisted of a group of 31 genes. Fifteen cyclins were identified, 14 of which showed an increased expression, the exception being *SDS* (SOLO DANCERS, Csa5M023910.1) which showed a decreased expression during fruit set. The GO category 'cell division' (GO:0051301) consisted of a group of 13 genes. Most of these genes had an increased expression in the expanding ovaries of both parthenocarpic and pollinated cucumber fruits, except for the *SNAP* (soluble *N*-ethylmaleimide-sensitive factor adaptor protein, Csa1M012100.1) gene, which was down-regulated in the CPPU-induced parthenocarpic fruits. The GO category 'cell growth' (GO:0016049) consisted of a group of 17 genes, most of which showed increased expression. Interestingly, there were more cell cycle-, division- and growth-related DEGs in the parthenocarpic fruits, which could explain why the growth rate of parthenocarpic fruits was higher than that of pollinated fruits (**Fig. 2**).

Response of chromosome organization- and DNA repair-related genes to parthenocarpic fruit set

Cell division consists of four distinct phases: G_1, G_2, S (DNA synthesis) and M (cell mitosis) phases. Many studies have confirmed that cells possess regulatory checkpoints that control

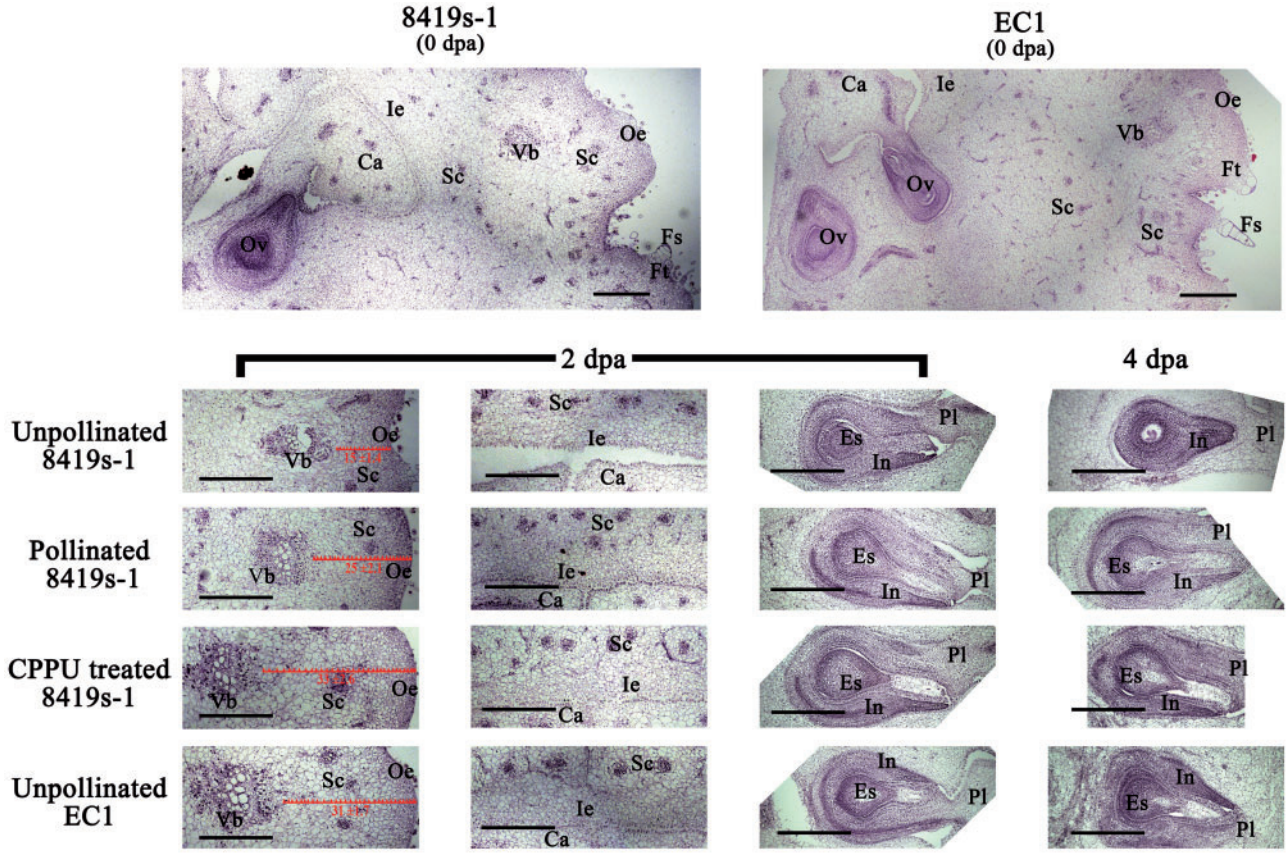

Fig. 2 Cytological observation of the cucumber ovaries during the early developmental stage. The histological sections (described in **Fig. 1**) were stained with HE. The aborted ovaries, due to no pollination treatment, showed atrophy or apoptosis of the cells that formed larger cavities between the inner epidermises. The values and arrow-rules in red color indicate the numbers of the cell layers from the outer epidermis to the vascular bundle. Each value represents the mean ± SE of three replicates, and for each replicate the numbers of the cell layers were measured 10 times. Ie, inner epidermis; Oe, outer epidermis; Vb, vascular bundle; Ca, carpel; Ov, ovule; Sc, secretory cavity; Ft, fruit tumor; Fs, fruit spines; In, intergument; Es, embryo sac; Pl, placenta. Scale bar = 0.5 mm.

Table 1 Statistics of RNA-Seq alignment[a]

Map to gene[b]	EC1 unpollinated		8419s-1 unpollinated		8419s-1 pollinated		8419s-1 CPPU treated	
	No. of reads	Percentage	No. of reads	Percentage	No. of reads	Percentage	No. of reads	Percentage
Total reads	7,211,591	100.00%	7,463,026	100.00%	7,415,306	100.00%	7,125,780	100.00%
Total base pairs	353,367,959	100.00%	365,688,274	100.00%	363,349,994	100.00%	349,163,220	100.00%
Total mapped reads	4,936,153	68.45%	4,882,848	65.43%	5,191,826	70.01%	5,133,019	72.03%
Perfect match	3,825,754	53.05%	3,794,044	50.84%	4,455,266	60.08%	4,404,917	61.82%
≤2 bp mismatch	1,110,399	15.40%	1,088,804	14.59%	736,560	9.93%	728,102	10.22%
Unique match	4,215,362	58.45%	4,113,464	55.12%	4,430,622	59.75%	4,433,721	62.22%
Multiposition match	720,791	9.99%	769,384	10.31%	761,204	10.27%	699,298	9.81%
Total Unmapped Reads	2,275,438	31.55%	2,580,178	34.57%	2,223,480	29.99%	1,992,761	27.97%

[a] The gross statistics of the three replicates of RNA-Seq alignments.
[b] The reference gene database is from the open website: http://cmb.bnu.edu.cn/Cucumis_sativus_v20/ (Huang et al. 2009).

cell cycle progression in response to chromosome replication and DNA damage (Hartwell et al. 1989, Murray et al. 1992, Murray et al. 1995). Blocking replication or repair of DNA would inhibit the onset of mitosis (Weinert et al. 1994, Paulovich et al. 1995). Seventeen genes in the enriched GO category of 'chromosome organization' (GO:0051276) showed an increased expression in cucumber parthenocarpic ovaries. Histones are involved in the packaging of nucleosomes which is

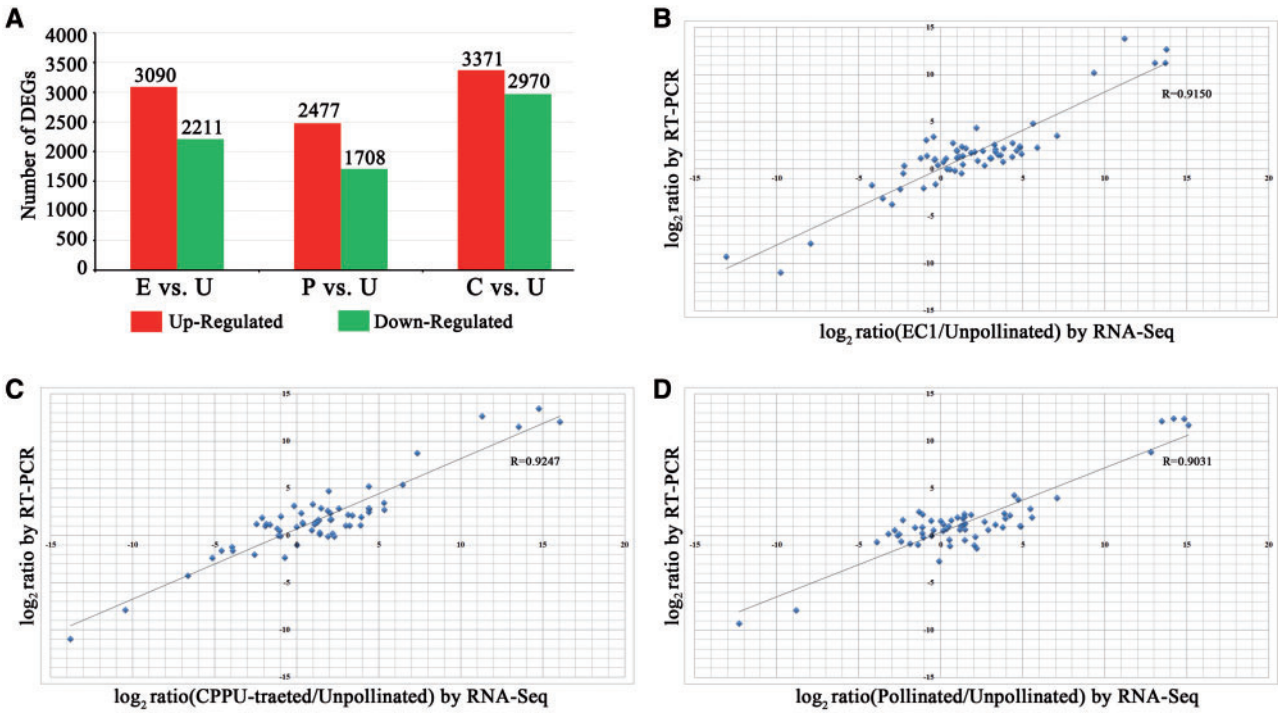

Fig. 3 Statistical analysis of the differentially expressed genes (DEGs) and correlation between RNA-Seq and qRT-PCR. The transcriptome of the four kinds of cucumber fruits, i.e. EC1 (E), pollinated 8419s-1 (P), CPPU-treated 8419s-1 (C) and unpollinated (bagging-treated) 8419s-1 fruits (U) were separately investigated by RNA-Seq. The transcriptome of the unpollinated 8419s-1 cucumber fruits (U) was considered as the control for filtering the DEGs from the other three transcrptomes (E, P and C). (A) The red columns indicate the up-regulated DEGs and the green columns indicate the down-regulated DEGs (FDR of $\leq$0.001 and an absolute value of $\log_2$ratio $\geq$1). (B) Close correlations (R = 0.9150, 0.9247 and 0.9031) were observed between relative expression levels measured with RNA-seq and qRT-PCR, validating the RNA-Seq methodology described here for quantitative analysis of the cucumber transcriptome.

a critical procedure for chromosome replication (Edenberg et al. 1975, Han et al. 1987). Most of the 'chromosome organization'-related PSGs were histone-encoding genes, including two histone methyltransferase genes (Csa1M275930.1 and Csa3M881620.1) and one histone kinase gene (Csa3M895060.1) (**Supplementary Table S3**). DNA repair is a process by which cells identify and correct the damage to DNA and thus ensure the integrity of the genome during chromosome replication. A total of 11 'DNA repair'-(GO:0006281) related genes were recognized in setting fruit, 10 of which were significantly increased only in the parthenocarpic ovaries (**Supplementary Table S3**).

Response of pollination- and gametophyte development-related genes to parthenocarpic fruit set

The differentiation and development of the ovules in the flowering plants are usually pollination dependent. However, it is interesting to find that the development of ovules in pollinated and parthenocarpic cucumber was the same from 0 to 2 dpa (**Fig. 2**). Furthermore, the genes annotated by the GO category of 'pollination' (GO:0009856) showed a greater transcript abundance in the parthenocarpic ovaries as compared with the pollinated ovaries (**Supplementary Table S3**). We speculated that the non-pollinated/fertilized parthenocarpic ovaries simulated the process of pollination by expressing pollination-related genes, therefore stimulating fruit initiation. The genes involved in 'gametophyte development' in the GO category (GO:0048229) also exhibited an enhanced transcriptional activity in the parthenocarpic ovaries, further supporting that parthenocarpy may be a process that mimicks pollination fruit set (**Supplementary Table S3**). *EDA3* and *EDA9* (Embryo sac Development Arrest 3 and 9; Csa4M285690.1 and Csa3M199630.1) were up-regulated. Disruption of these genes could arrest the nuclear division of megagametogenesis at the two-nuclear stage (Pagnussat et al. 2005). *SYN3* (Sister Chromatid Cohesion 1 PROTEIN 3, Csa4M153290.1) and *CTF7* (a DNA-directed DNA polymerase gene, Chromosome Transmission Fidelity 7, Csa3M781550.1) were also specifically expressed in the parthenocarpic ovaries; these genes plays important roles in chromosomal synthesis and arrangement (Schubert et al. 2009, Bolaños-Villegas et al. 2013).

Comparison of the carbohydrate-related responses between parthenocarpic and pollinated cucumber fruits

Based on the KEGG database, the pathway enrichment analysis identified significantly enriched metabolic pathways or signal

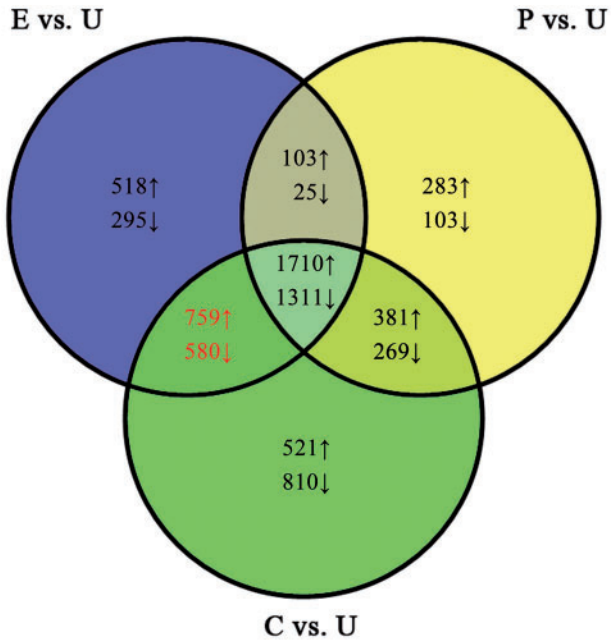

Fig. 4 Venn diagrams of DEGs. The DEG sets (E vs. U, P vs. U and C vs. U) described in **Fig. 3** were analyzed by using the Venn method. The numbers marked in the diagram indicate the number of common genes significantly up- (upward arrows) and down-regulated (downward arrows) among the three DEG sets ($\log_2$-fold change $\geq$1.5 and FDR-corrected *P*-value $\leq$0.001). The red numbers show the DEGs that are particularly expressed in parthenocarpic fruits.

transduction pathways in DEGs as compared with the whole-genome background. It revealed that the expression of glycometabolism-related genes was widely affected in the parthenocarpic fruits.

Response of glycometabolism-related genes. The expression of polysaccharide (starch and sucrose) and monosaccharide (fructose and mannose) synthesis-associated genes dramatically changed in the parthenocarpic cucumber ovaries. The genes encoding carbohydrate biosynthesis enzymes showed decreased transcript abundance, while carbohydrate degradation genes showed increased transcript abundance in the parthenocarpic ovaries (**Supplementary Table S4**). For example, the glycoside hydrolase-encoding genes including Csa5M 160230.1, Csa6M000120.1 and Csa6M421040.1 which assist in the hydrolysis of glycosidic bonds in complex sugars (Xu et al. 2004), as well as the amylase-encoding genes including Csa7M213190.1 and Csa7M213180.1 which catalyze the hydrolysis of starch into sugars (Silverman 2002) showed an increased expression in the parthenocarpic ovaries. The carbohydrate biosynthesis genes, such as glycosyltransferase which establishes natural glycosidic linkages, including the biosynthesis of disaccharides, oligosaccharides and polysaccharides (Ünligil et al. 2000), including Csa5M322500.2 and Csa6M 520240.1, were down-regulated during parthenocarpic fruit set (**Supplementary Table S4**). However, the transcription of these genes was unaffected in the pollinated ovaries (**Supplementary Table S4**). Measurement of the total sugars showed that the sugar content of the parthenocarpic ovaries was less than that of the pollinated ovaries, and this phenomenon existed not only in the ovaries of EC1 and CPPU-treated 8419s-1 but also in other parthenocapic cucumber cultivars (**Fig. 5A**).

Response of TCA cycle-associated genes. We speculated that the small sugars which are produced from starch or sucrose could be further degraded through the tricarboxylic acic cycle (TCA cycle) to generate energy by oxidization of the acetate derived from carbohydrates, fats and proteins into carbon dioxide. KEGG analysis revealed that the transcript abundance of TCA cycle-associated genes was increased in the parthenocarpic ovaries, suggesting that energy metabolism was more active during parthenocarpic fruit set. The qRT-PCR results supported that the expression of the TCA cycle-related genes was also increased in other parthenocarpy cultivars (**Fig. 5B**), e.g. E1 component subunit-encoding genes (Csa1M435750.1 and Csa6M095870.1) and dihydrolipoyllysine-residue acetyltransferase-encoding genes (Csa5M608580.1 and Csa7M 284430.1).

Response of amino sugar and nucleotide sugar metabolism. Sugars could provide derivatives such as amino sugars and nucleotide sugars that are consumed in numerous biochemical reactions. In cucumber parthenocarpic ovaries, transcription of the amino sugar and nucleotide sugar metabolic enzyme-encoding genes was increased (**Supplementary Table S4**), e.g. ADP-glucose pyrophosphorylase (Csa7M 030510.1) and galacturonosyltransferase (Csa1M179740.1) genes. An acidic endochitinase-encoding gene (Csa5M 139760.1) which has an important function in early somatic embryo development (De Jong et al. 1992) was down-regulated in the parthenocarpic ovaries.

Glycosylation reactions. Nucleotide sugars act as glycosyl donors in glycosylation reactions. The increasing transcription of sugar and nucleotide sugar metabolic genes implied that glycosylation was enhanced in parthenocarpic fruits. Indeed, the qRT-PCR analysis showed that the expression of *N*-glycan biosynthesis-related genes was frequently higher in the pathenocarpic ovaries than in the pollinated ovaries (**Fig. 5A**; **Supplementary Table S5**).

Expression of phytohormone-related genes during cucumber fruit set

High concentrations of auxin, cytokinin and gibberellin are required for fruit set (Gillaspy et al. 1993, Vivian-Smith and Koltunow 1999, Serrani et al. 2007, de Jong et al. 2009a). The endogenous hormones of cucumber ovaries were analyzed during fruit set by enzyme-linked immunosorbent assay (ELISA) (**Fig. 6**). The concentration of auxin, cytokinin and

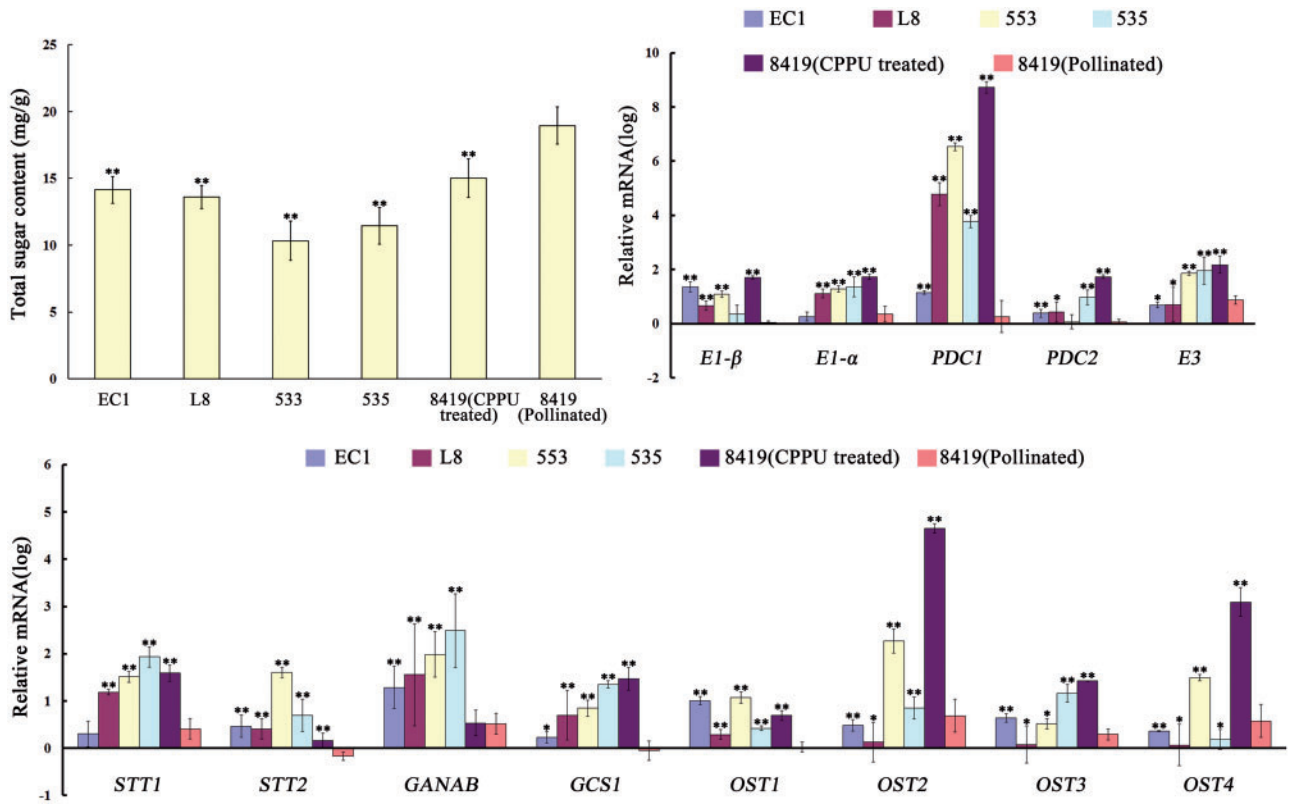

Fig. 5 The total sugar measurement and gene expression analysis of the TCA cycle and *N*-glycan biosynthesis of the related genes in the cucumber fruits of different cultivars. (A) Measurement of the total sugar content of the parthenocarpic and pollinated cucumber fruits. The sugar levels were generally low in the parthenocarpic cucumber fruits as compared with those of the pollinated fruits. Each value represents the mean ± SE of three replicates. (B) The qRT-PCR analysis of the TCA cycle-related genes in the parthenocarpic and pollinated cucumber fruits. (C) The qRT-PCR analysis of the *N*-glycan biosynthesis-related genes in the parthenocarpic and pollinated cucumber fruits. E1-β, pyruvate dehydrogenase E1 component subunit beta; PDC1 and 2, dihydrolipoyllysine-residue acetyltransferase components of the pyruvate dehydrogenase complex; E1-α, pyruvate dehydrogenase E1 component subunit alpha; E3, dihydrolipoyl dehydrogenase; STT1 and 2, ataurosporine and temperature sensitive; OST1-4, lipid-to-protein transfer of oligosaccharides; GANAB, glucosidase II α-subunit; GCS1, glucosidase. The genes are annotated in **Supplementary Table S6**. The marks indicate that the total sugar levels (A) or mRNA levels (B and C) were significantly different compared with the pollinated 8419s-1 (*t*-test) with statistical significance at $^{*}P < 0.05$; $^{**}P < 0.01$. Each value represents the mean ± SE of three replicates.

gibberellin showed a general upward trend but fluctuated during pollination and parthenocarpic fruit set. The elevation of auxin, cytokinin and gibberellin usually starts at 2 dpa. However, in the ovaries of 8419s-1 with CPPU treatment, the increase in the above endogenous hormones was delayed until 4 dpa. However, the CPPU-treated 8419s-1 showed a similar shape and sizeas the pollinated 8419s-1 (**Fig. 1**). In general, a burst of auxin post-anthesis is a clear sign of successful pollination/fertilization (Gillaspy et al. 1993, Vivian-Smith and Koltunow 1999). However, an abnormal transient decrease in auxin was observed in pollinated fruits of 8419s-1 and EC1. Conversely, a burst of auxin occurred in unpollinated 8419s-1 and EC1 from 0 to 2 dpa. This indicated that the peak's shift of auxin was closely related to pollination/non-pollination treatment. Interestingly, phytohormone measurement showed that, whether pollinated or not, the concentration and rate of change of endogenous hormone in EC1 was stable and relatively lower than those the 8419s-1 fruits (**Fig. 6**; **Supplementary Table S6**). ABA increased in unpollinated 8419s-1 (aborted ovaries) at 2 dpa, then decreased quickly from 2 to 4 dpa and increased again from 4 to 6 dpa, The dynamic changes in ABA in aborted fruits confirmed that ABA plays important roles in the process of fruit senescence and abscission (Kano et al. 1981, Archbold et al. 1984, Jia et al. 2013). However, ABA also changed during fruit set, and an opposite trend was seen in set fruit in comparison with aborted fruits (**Fig. 6**), suggesting that in addition to auxin, cytokinin and gibberellin, ABA maybe also plays an important role in regulation of fruit set.

Based on the RNA-Seq results, DEGs were filtered from the transcriptome of the pollinated and parthenocarpic cucumber fruits and compared against the transcriptome of the aborted fruits (**Fig. 4**). About 8.7% of the DEGs were phytohormone related, including 285 phytohormone biosynthesis genes and

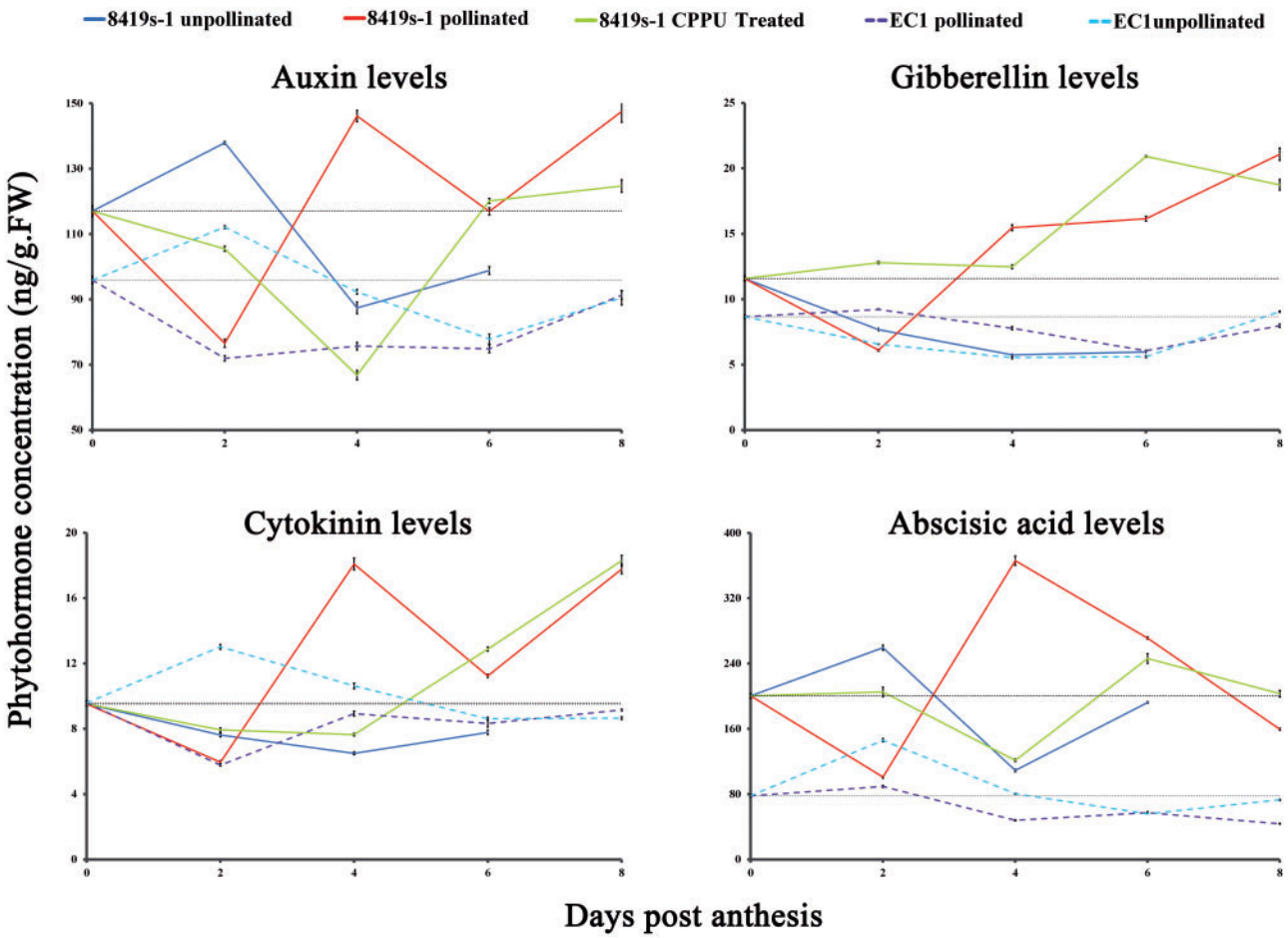

Fig. 6 Phytohormone measurement in cucumber fruits. The concentrations of the plant hormones auxin (IAA), cytokinin (ZR), gibberellin (GA_3) and ABA were analyzed by ELISA. Average phytohormone concentrations (ng g FW) were measured at 0, 2, 4, 6 and 8 dpa in the ovaries of unpollinated, pollinated, CPPU-treated 8419s-1 and unpollinated and pollinated EC1. The results are presented as the mean ± SE of three biological replicates and three technical replicates. The dotted lines are lines to show the up or down trend of the hormone levels (gray line, assigned a value for the initial hormone concentration of 0 dpa 8419s-1; light gray line, assigned a value for the initial hormone concentration of 0 dpa EC1).

384 phytohormone signal transduction genes. Interestingly, either up-regulation or down-regulation of auxin, cytokinin and gibberellin biosynthesis DEGs occurred during cucumber fruit set (**Fig. 7A**). This suggested that the trend in the dynamic level of the hormones may be caused by the changing ratios of up-/down-regulated hormone synthesis genes. The statistics of auxin, cytokinin and gibberellin signal transduction-related DEGs showed that the numbers and category of hormone-related DEGs were similar in parthenocarpic and pollinated fruits (**Fig. 7B**), which perhaps resolved the question of why EC1 can stimulate fruit set under low endogenous hormone levels (**Fig. 6**). Usually auxin, cytokinin and gibberellin are recognized as the major regulators of fruit set. Recent studies showed that brassinosteroids could act as positive regulation factors during cucumber fruit set (Fu et al. 2008). The RNA-Seq results showed that the highest proportion of the hormone-related DEGs were brassinosteroid genes (>30%; **Fig. 7B**), suggesting that brassinosteroids may play the most important role during cucumber fruit set.

Preliminary prediction and verification of parthenocarpic genes through ovary transient expression experiments

Many genes related to phytohormone biosynthesis and signal transduction have been proved to to regulate parthenocarpic fruit set (Wang et al. 2005, Martí et al. 2007, Ren et al. 2011, Carrera et al. 2012). In this study, 14 hormone-related PSGs were predicted as putative parthenocarpic regulatory genes which showed highly differential expression in parthenocarpic fruits ($|\log_2 \text{ratio} \geq 2|$) and were distributed throughout the hormone signaling cascades (**Supplementary Table S7**).

In order to verify the parthenocarpic function of the candidate genes, an ovary transient expression system was built.

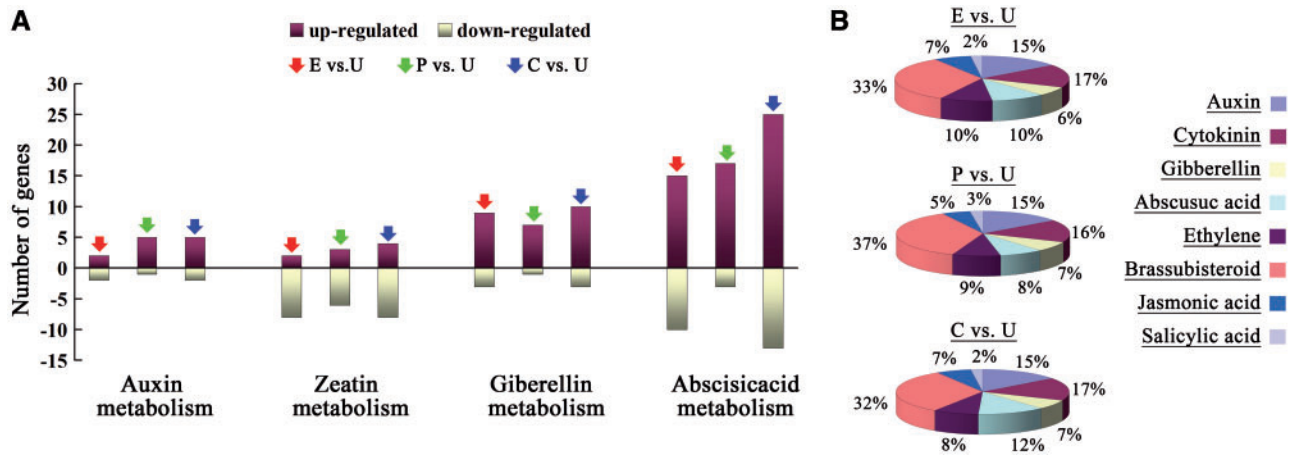

Fig. 7 Statistical analysis of the phytohormone-related DEGs. (A) The columns indicate the numbers of the phytohormone synthesis-related DEGs involved in the auxin, cytokinin, gibberellin and ABA metabolism processes. (B) The portions of the pie chart show the percentages of total DEGs (including the up- and down-regulated DEGs) separately involved in eight phytohormone signal transduction pathways.

According to the RNA-Seq results, the sense and antisense transcripts of the 14 genes were cloned for construction of the overexpression or underexpression vectors and then transformed into C58 *Agrobacterium*. The transformed *Agrobacterium* were suspended in the infection fluid and injected into −2 dpa ovaries of 8419s-1 then incubated in the dark by bagging treatment for 2 d. The morphological changes of the treated ovaries were observed 10 days after injection (equal to 8 dpa). β-Glucuronidase (GUS) staining was used to identify whether the ovaries were positively treated (**Supplementary Fig. S3**). Morphological observation showed that all the candidate genes were capable of inducing ovary expansion (**Table 2**; **Supplementary Fig. S3**). Conversely, reversing the up-/down-regulation of the candidate genes according to the RNA-Seq results would induce opposite effects. For example, RNA-Seq showed that *CsTIR1a* and *CsTIR1b* (transport inhibitor response 1 genes; Csa7M393970.1 and Csa3M597350.1) were down-regulated during cucumber parthenocarpic fruit set, while transient overexpression of *CsTIR1a/b* in the EC1 ovaries (parthenocarpic cultivar) could induce fruit abortion (**Table 2**, right column).

The treated ovaries exhibited three types of growth: ovary swelling, stationary development and ovary abortion. For every individual transient expression experiment, only about 5–50% of the positively treated ovaries expanded but over 40–85% of the ovaries were in a frozen state (**Table 2**). Therefore, we speculated that according to the function, parthenocarpic genes can be divided into two groups: fruit abortion inhibitors and fruit swelling promoters. A part of the positively treated ovaries (5–10%) underwent fruit abortion. We speculated that the reason for this is that the *Agrobacterium*-treated ovaries are kinds of chimeras, including transformed and untransformed tissue; if the amount of transformed tissue is small, the effect on the transiently expressed genes should be weak. The exact function of the candidate genes should be identified by genetic transformation. So far, 13 tomato transgenic lines (T_3) of three genes (Csa7M393970.1, Csa3M597350.1 and Csa5M166390.1) have been obtained and showed the parthenocarpic phenotype (**Supplementary Fig. S4**). Also putative cucumber transformants (T_0 generation) are ready for growth in a greenhouse (data not show).

Transcriptional characteristics of the phytohormone-related genes were analyzed in expanding ovaries after transient expression

The expanding ovaries induced by the transient expression experiments were harvested for gene expression analysis. Transcription of 19 genes related to auxin, cytokinin and gibberellin biosynthesis and signaling transduction were analyzed in the expanding ovaries (**Supplementary Table S8**). The 8 dpa unpollinated 8419s-1 aborted fruits were used as the negative control (U). The 8 dpa EC1 (E) and CPPU-treated 8419s-1 (C) parthenocarpic fruits were considered as the positive control (**Fig. 8**). The qRT-PCR results were arrayed according to the hormonal signaling cascades. The referent genes and ovary samples were arranged in columns and rows. Red color clumps indicated the |log| expression in the parthenocarpic ovaries (**Fig. 8**). Differential expression is indicated by the intensity of the red color.

Phytohormone signaling was transduced and enlarged by multilevel components of signaling cascades (Abel 1996, Hirt 1997, Sun 2000, Hwang and Sheen 2001). Transcription profiling of the expanding ovaries revealed that transcriptional regulation occurred among the components. In the *CsPNO*- (Csa2M379350.1 pyridine nucleotide-disulfide oxidoreductase, an auxin synthesis gene) induced expanding ovaries, most of the downstream genes of *CsPNO* along the auxin signaling cascade have changed their expression (**Fig. 8**; the top part of the first column). However, no feedback transcriptional regulation was observed. In the *CsAUX1*- (Csa5M201310.1, auxin receptor

Table 2 Statistics of transient expression experiments

Genes	ID	Constructs	8419s-1			EC1	
			Fruit abortion[a]	Frozen state[b]	Fruit expansion[c]	Fruit abortion	Fruit expansion
Pyridine nucleotide-disulfide oxidoreductase,	Csa2M379350.1	Overexpression	3/30[d]	16/30	11/30	–	–
Cytokinin trans-hydroxylase, CYP735A	Csa5M166390.1	Overexpression	2/32	13/32	17/32	–	–
Ent-kaurene oxidase, KO	Csa3M015360.1	Overexpression	4/29	11/29	14/29	–	–
Auxin transporter-like protein, AUX1	Csa5M201310.1	Underexpression	6/20	11/20	3/20	–	–
	Csa6M011040.1	Overexpression	2/18	14/18	2/18	–	–
Auxin-responsive protein, AUX/IAA	Csa6M497220.1	Overexpression	5/21	14/21	2/21	–	–
	Csa1M397130.2	Overexpression	3/21	17/21	1/21	–	–
Auxin-induced protein, SAUR	Csa3M866530.1	Overexpression	4/19	11/19	4/19	–	–
	Csa6M137590.1	Overexpression	2/21	18/21	1/21	–	–
Glucan endo-1,3-beta-glucosidase-like protein, CRE1	Csa4M280410.1	Overexpression	4/24	18/24	2/24	–	–
	Csa6M420530.1	Overexpression	3/25	17/25	5/25	–	–
Histidine-containing phosphotransfer protein, AHP	Csa6M067360.1	Underexpression	4/23	17/23	2/23	–	–
Transport inhibitor response 1, TIR1	Csa7M393970.1	Overexpression	19/19	0/19	0/19	4/8	4/8
		Underexpression	11/38	17/38	10/38	0/6	6/6
	Csa3M597350.1	Overexpression	19/19	0/19	0/19	7/10	3/10
		Underexpression	8/29	13/29	8/29	0/10	10/10
Agrobacterium control[e]			30/30	0/30	0/30	0/10	10/10
Infection solution control[f]			30/30	0/30	0/30	0/10	10/10

The expansion rate, described in the Material and Methods, was used to classify treated ovaries.
Ovary transient expression for each putative parthenocarpic gene has three replicates at a different time or place.
[a] Aborted fruit (≤0% expansion rate with yellow or shrinkage symptoms, S12A).
[b] Frozen state fruit (expansion rate between 0% and 10%).
[c] Expanding fruit (with ≥10% expansion rate).
[d] The data presented in the table, e.g. '3/30', mean that 30 ovaries were positively treated, of which three ovaries were aborted ovaries.
[e] The 8419s-1 and EC1 ovaries injected with the C58 *Agrobacterium* containing an empty vector were considered as negative controls.
[f] The 8419s-1 and EC1 ovaries injected with the infection solution were also considered as negative controls.

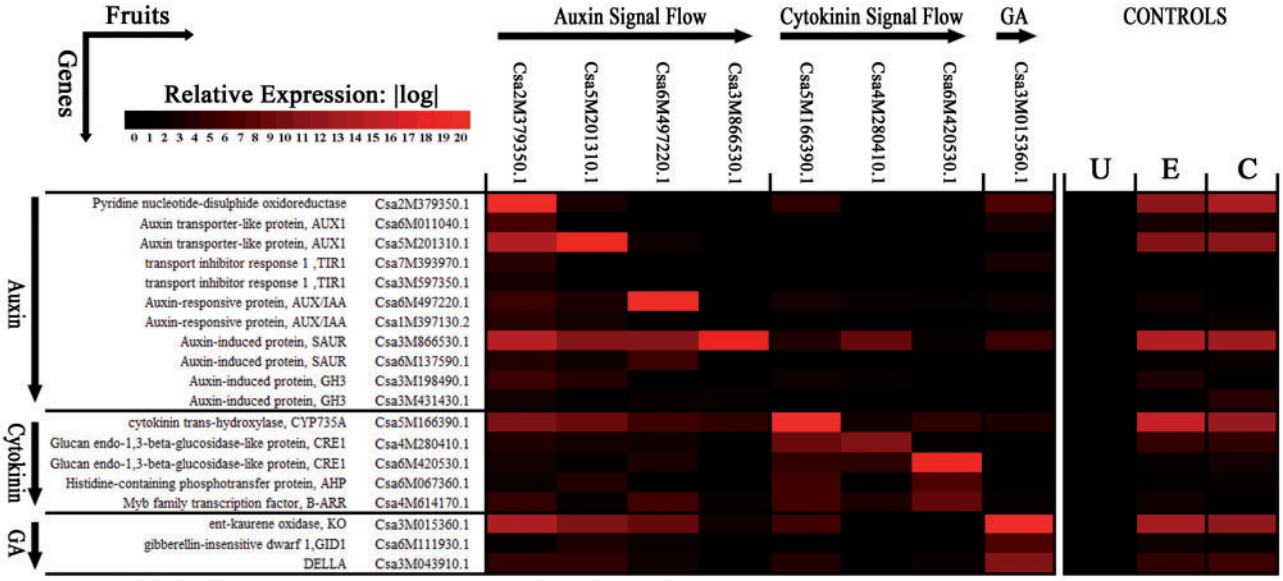

Fig. 8 The matrix graph of the relative expression of the putative parthenocarpic genes in the expanding fruits induced by transient expression. The absolute values of the relative expression (|log|) of the predicted parthenocarpic genes are indicated by the depth of color. The abscissa of the matrix graph shows the different types of expanding fruits, which were induced by transient expression of the predicted parthenocarpic genes (described in **Table 2**). The 8 dpa fruits of EC1 (E) and CPPU-treated 8419s-1 (C) were considered as positive controls, and the unpollinated 8419s-1 fruit (U) as negative controls. The vertical ordinate of the matrix graph shows the name of the investigated genes. Both the fruits and the genes were arrayed according to the hormonal signal transduction flows, which were classified into three groups, auxin, cytokinin and gibberellin related.

gene) induced expanding ovaries, downstream genes of *CsAUX1* showed differential expression, but the expression of *CsPNO*, the upstream gene of *CsAUX1*, was unaffected (**Fig. 8**; the top part of the second column). The same transcriptional events were also observed in the expanding ovaries induced by cytokinin- or gibberellin-related genes (**Fig. 8**; the lower part of the columns).

The involvement of various plant growth regulators indicated an interconnected complex hormone network in parthenocarpic fruit set (Gillaspy et al. 1993, Vivian-Smith and Koltunow, 1999; Serrani et al. 2007, Fu et al. 2008, de Jong et al. 2009a). The expression analysis of the phytohormone-related genes in the expanding ovaries revealed hormone cross-talk that occurred at the transcriptional level, e.g. in *CsPNO*- (Csa2M379350.1) induced expanding ovaries, the differential expression of cytokinin synthesis signal transduction genes (cytokinin trans-hydroxylase, *CsCYP735A*; glucanendo-1, 3-beta-glucosidase-like protein, *CsCRE1*) and the gibberellin synthesis gene (ent-kaurene oxidase, *CsKO*) was induced (**Fig. 8**, first column). Furthermore, the transcriptional interactions of hormone-related genes performed hierarchical cross-talk. For example, in the *CsCYP735A*-induced expanding ovaries, the expression of cytokinin- and gibberellin-related genes, such as *CsCRE1* and *CsKO*, was changed (**Fig. 8**, first column), but in the *CsKO*-induced expanding ovaries, despite the expression of downstream genes such as *CsGID1* (gibberellin-insensitive dwarf 1) being changed, the expression of the auxin- and cytokinin-related genes was less affected (**Fig. 8**, fifth and eighth column).

Discussion

In the present study, global transcriptional events of cucumber fruit set were investigated by RNA-Seq. The results were jointly analyzed with cytomorphological observations and physiological analysis to distinguish globally parthenocarpy from pollinated fruit set.

Distinctive sugar responses of the cucumber parthenocarpy

Sugar is the main component of soluble solids in fruits that are directly related to fruit sweetness and flavor. Many people feel that seedless fruit has lower sweetness than seeded fruit, such as seedless muskmelon and loquat (Chen et al. 2006; X.X. Li et al. 2011). Our findings that the total sugar content of parthenocarpic cucumber fruits was significantly lower than that of the pollinated fruits supported this concept to some extent (**Fig. 5A**). The carbohydrate economy of the developing fruit is determined by whole-plant source–sink relationships. However, the fate of the imported photoassimilate partitioned to the fruit sink is controlled by the carbohydrate metabolism of the fruit tissue. We speculated that the reduction of the sugar contents in the parthenocarpic cucumber fruits was caused by up-/down- regulation of carbohydrate metabolism genes. The RNA-Seq results indicated that expression of the glycometabolism-related genes was more active in the parthenocarpic ovaries. Furthermore, the carbohydrate biosynthesis genes showed decreased transcript abundance, while expression of carbohydrate degradation genes was up-regulated in the parthenocarpic ovaries (**Supplementary Table S4**).

Smaller sugar molecules are further degraded through the TCA cycle to generate energy. The RNA-Seq and qRT-PCR results revealed that the expression of the TCA cycle-related genes was generally up-regulated in the parthenocarpic fruits (**Fig. 5B**; **Supplementary Table S4**). However, transcription of these TCA cycle-related genes was unaffected in the pollinated fruits and even some related genes were greatly down-regulated, e.g. Csa7M284430.1, a pyruvate dehydrogenase complex unit-coding gene (**Supplementary Table S4**). Sugars, representing the most important organic resources, are known to be indispensable for cell division (Wobus and Weber 1999). Cytological observations revealed that the parthenocarpic fruits had more intense cell division activities as compared with the pollinated cucumber fruits (**Fig. 2**). The synthesis of DNA, RNA and proteins was highly active during parthenocarpic fruit set, reflected indirectly by the RNA-Seq result (**Supplementary Table S5**, **S6**, **S9**). This implied that the parthenocarpic fruit set is a high energy-consuming process. Furthermore, in order to maintain highly active synthesis processes, a high consumption of sugars in the parthenocarpic fruits supplied not only the energy but also the synthetic compounds, e.g. glycosyl units for ribonucleic and amino acid synthesis or protein glycosylation. The RNA-Seq result showed that the transcription of amino sugar, nucleotide sugar and glycoprotein metabolic enzymes was up-regulated in the parthenocarpic fruits (**Supplementary Table S4**). The qRT-PCR analysis also supported that the transcript abundance of the glycan biosynthesis genes in the parthenocarpic fruits was much higher than that of the pollinated fruits (**Fig. 5C**).

Sugars are not only important energy sources and structural components but also have important signaling functions throughout all the stages of the plant's life cycle (Steeves and Sussex 1989, Brusslan and Tobin 1992, Graham et al. 1992, Bernier et al. 1993, Sheen 1994, Dangl et al. 1995). Sugars are indispensable for cell division (Wobus and Weber 1999). Studies on the effects of sugar on gene expression indicated that a potential sugar signal for cell cycle regulation is not generated by uptake, but may result from a metabolic reaction, e.g. by hexokinase, which mirrors the endogenous hexose concentration (Sheen et al. 1999), and the length of the S and the G_2 phase could be modified by changing the endogenous monosaccharide levels (Hartig and Beck 2005). Hartig and Beck (2006) also indicated that the cell cycle of root and shoot meristems could be derived and modulated by the cross-talk of sugar and phytohormone signals. We considered that the enhancement of cell division in the parthenocarpic fruits might be regulated by a biochemical framework consisting of the mitogenic hormonal and sugar signals.

Hierarchical hormonal interactions occurred during cucumber parthenocarpic fruit set at the transcriptional level

The phytohormone cross-talk is well known to have synergistic or antagonistic effects and can take place at different regulatory levels (Coenen and Lomax 1997). Auxin, cytokinin and gibberellin were recognized as the major regulators for inducing parthenocarpy (Gillaspy et al. 1993, Vivian-Smith and Koltunow 1999, Serrani et al. 2007, Fu et al. 2008, de Jong et al. 2009a). In this study, the transcriptional profiling of hormone-related genes in natural and induced parthenocapic fruit revealed hierarchical hormone cross-talk at the transcriptional level (**Fig. 8**). It is widely known that polar auxin transport and the establishment of an auxin gradient are important determinants of plant growth and morphological patterning (Feraru et al. 2008). Based on our studies, auxin was considered as the primary regulatory hormone for cucumber parthenocarpic fruit set, because the transcription of cytokinin- and gibberellin- related genes can be regulated by the auxin-related genes (**Supplementary Table S9**). Cytokinin has been implicated in a broad range of developmental processes including germination, root and shoot meristem function, and leaf senescence (Miller et al. 1955, To et al. 2008). The classical cross-talk of auxin and cytokinin is the opposite interaction during lateral root initiation. During root development, auxin promotes lateral root initiation while cytokinin opposes this response (Blilou et al. 2005, Laplaze et al. 2007). However, both auxin and cytokinin have positive effects on fruit set and development.We also speculated that cytokinin was the secondary regulator for cucumber fruit set. The qRT-PCR results showed that cytokinin-related genes have no effect on the transcription of auxin-related genes but could regulate the expression of gibberellin-related genes during parthenocarpic fruit set (**Fig. 8**). Gibberellin was considered as the third-line regulator. Previous studies indicated that auxin is able to regulate the biosynthesis of gibberellin (Davies et al. 1975, Yang et al. 1996). Our study also supported that gibberellin synthesis genes can be regulated by auxin-related genes. A study has shown that gibberellin can repress the effects of cytokinin on the morphology of the leaf and flower through SPINDLY in Arabidopsis (Greenboim-Wainberg et al. 2005). However, our findings showed the opposite result in that gibberellin had no effect on cytokinin while cytokinin could regulate the expression of gibberellin-related genes. Interestingly, application of synthesized cytokinin as well as gibberellins had a better efficiency for the induction of parthenocarpy than exogenous auxin (data not show). We speculated that auxin may be just a trigger of fruit set that promoted the biosynthesis of cytokinin and gibberellins which were downstream regulators but had a greater impact on parthenocarpy induction.

Previous studies showed that brassinosteroids could act as positive regulatory factors during cucumber fruit set (Fu et al. 2008), and there was a significant overlap between the auxin- and brassinosteroid-responsive gene sets (Goda et al. 2004). Generally, common target genes that were repressed or induced by auxin were also regulated by brassinosteroids (Nemhauser et al. 2004). The RNA-Seq results showed that the highest proportion of the hormone-related DEGs were brassinosteroid related (**Fig. 7B**), suggesting that brassinosteroids perhaps play the most important role during cucumber fruit set.

A working hypothesis of cucumber parthenocarpic fruit set

Developing seeds are usually considered to be essential determinants of fruit growth (Nitsch 1950, Archbold and Dennis 1985) by synthesizing high levels of plant growth hormones (Eeuwens and Schwabe 1975, Sponsel 1983, Talon et al. 1990, García-Martínez et al. 1991a, García-Martínez et al. 1991b, Ben-Cheikh et al. 1997, Rodrigo et al. 1997). Increases in endogenous auxins and gibberellins were observed in the fruit of plants exhibiting naturally occurring parthenocarpy (George et al. 1984, Talon et al. 1990, Talon et al. 1992). Exogenous application of plant growth hormones could induce artificial parthenocarpy (Gillaspy et al. 1993, Vivian-Smith and Koltunow 1999, Serrani et al. 2007, Fu et al. 2008, de Jong et al. 2009a). It has been assumed that exogenous plant growth hormones could substitute for hormones synthesized by the developing seeds. We proposed a hypothesis whereby parthenocarpy induced fruit set by simulating the processes of pollination and seed formation. The evidence provided by the present work supported this viewpoint to some extent.

Cytological observation showed that the embryo sac and integuments of cucumber ovules formed at 2 dpa which were signs of a mature embryo (**Fig. 2**). This phenomenon was similar to that of orchids, the ovaries of which contained immature ovules prior to pollination (Wirth and Withner 1959, Withner et al. 1974). Moreover, our previous study on pollen of cucumber found that pollen remained at the bicellular stage in the mature anthers then restarted delayed development after pollination (Cao et al. 2004). We speculated that in cucumber, ovule differentiation and pollen maturation were triggered by pollination then promoted fruit set. Interestingly, the development of ovules in parthenocarpic cucumber was similar to that of pollinated ovules from 0 to 2 dpa (**Fig. 2**). RNA-Seq results also indicated that the expression of the pollination- and gametophyte development-related genes was increased in the parthenocarpic ovaries (**Supplementary Table S3**). We were surprised that the pollen allergens generally carried by the pollen were present in unpollinated cucumber ovaries, e.g. the transcription of a calcium-binding pollen allergen gene (Csa5M561760.1), annotated by the GO category of 'cell growth' (GO:0016049), was found that was greatly enhanced in the parthenocarpic fruits. The pollen allergens were recognized to have an expansin activity, like that of the *Zea mays* pollen allergens, which are small extracellular proteins that promoted the turgor-driven extension of plant cell walls (Cosgrove et al. 1998, Pezzotti et al. 2002, Yennawar et al. 2006).

Overall, a working hypothesis of the cucumber parthenocarpic fruit set was proposed whereby cucumber mimicked the process of pollination by up-regulating the expression of pollination- and gametophyte development-related genes, thereby inducing parthenocarpy (Fig. 9). Moreover, the simulation of seed formation in order to increase the biosynthesis of the plant growth hormones might not be necessary for parthenocarpic fruit set, since in this study we found that EC1, a cucumber parthenocarpic variety, can promote fruit set under relatively low hormone levels whether pollinated or unpollinated (Fig. 6). Cytological studies have been conducted on double fertilization and early embryogenesis after pollination with irradiated pollen and this phenomenon was defined as stenospermocarpy (Vassileva-Dryanovska 1966a, Vassileva-Dryanovska 1996b, Vassileva-Dryanovska, 1996c, Nishawama and Uematsu 1967). In cucumber, fruit set can also be induced by pollinating with irradiated pollen. However, many other researchers have defined this phenomenon as 'induced parthenocarpy' (Pandey and Phung 1982, Denissen et al. 1987, James et al. 1985, Sniezko and Visser 1987, Zhang and Lespinasse 1991). In any case, we speculated that either in stenospermocarpic or in parthenocarpic fruit set, the same mechanism (simulating pollination/fertilization) was performed to promote fruit set.

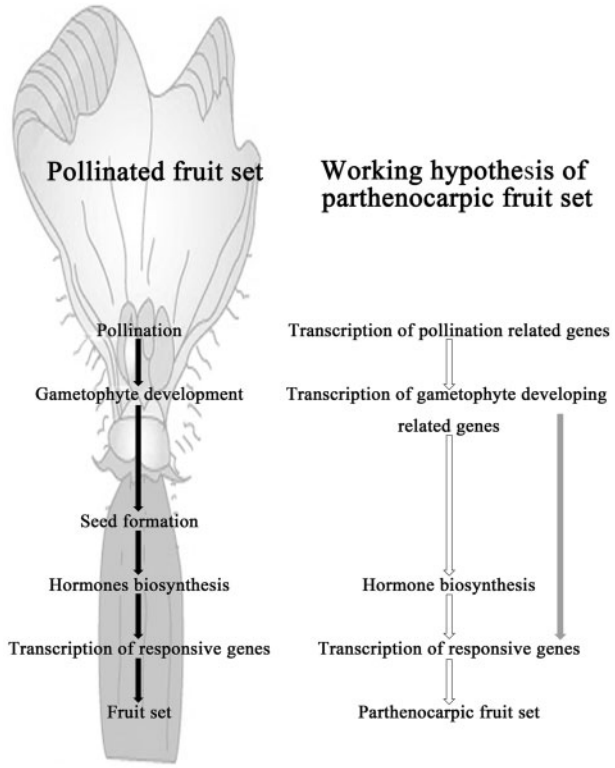

Fig. 9 A working hypothesis of cucumber parthenocarpic fruit set. The hypothesis was proposed that cucumber mimicked the process of ovary pollination by up-regulating the expression of the genes involved in pollination and gametophyte development, thereby inducing the expression of the genes responsible for hormone synthesis and the downstream responsive genes, which could finally induce parthenocarpic fruit set. Stimulation of seed formation in order to increase the biosynthesis of the plant growth hormones might not be necessary for parthenocarpic fruit set (gray arrow), since we found that EC1, a cucumber parthenocarpic variety, maintained a relatively low level of the hormones no matter whether pollinated or not pollinated, but still promoted fruit set.

Materials and Methods

Plant materials

In this study, EC1 (an inbred line derived from Delta star), L8 (an F_1 cultivar), S12533 (an F_1 cultivar) and S12535 (an F_1 cultivar) (parthenocarpic rate $\geq$95%) were used as the parthenocarpic cucumber lines, and 8419s-1 (an inbred line derived from Thamin beit alpha) as the non-parthenocarpic line (parthenocarpic rate $<$5%; parthenocarpy occurred in the end stage of the cucumber life cycle at the $\geq$20th node of the stems). All the cucumbers were cultivated and stored in our lab. The plants were grown in a greenhouse with a 12 h photoperiod, a mean daily air temperature of 29/17°C (day/night), a relative humidity of 85% and a photosynthetic photo flux density of 800 μmol $m^{-2} s^{-1}$ at Nanjing Agricultural University. The female flowers of the above cultivars, at the 12–15th node of the main stem, were treated prior to bagging in order to prevent pollen contamination on the day before anthesis. This was followed by three kinds of treatments, bagging treatment, pollination treatment (hand pollination, Kim et al. 1992) and synthetic cytikinin CPPU treatment (100 mg l^{-1}, 20 μl per female flower by spraying) on the day of anthesis. The ovaries of the treated female flowers were harvested at 2 dpa. A part of the materials was immediately fixed in formalin–acetic acid–alcohol (FAA) solution (Phillips and Hayman 1970) at room temperature for paraffin sectioning and hematoxylin and eosin (HE) staining (Kierna 2008). The remained materials were stored at −80°C for RNA extraction.

RNA isolation and transcriptome analysis

As described above, four kinds of ovary samples were collected for RNA-Seq analysis, i.e. bagging-treated EC1 and 8419s-1, pollination-treated 8419s-1, and CPPU-treated 8419s-1. For each sample, 20 individual ovaries were ground into powder and mixed in liquid nitrogen (three replicates). Total RNAs were isolated using Trizol (Invitrogen) according to the manufacturer's instructions. The isolated RNA samples were sent to the Beijing Genomics Institute for RNA-Seq analysis via Illumina HiSeq™ 2000. The reads with $>$10% unknown bases and those of low quality were removed from the raw sequencing data by using the software Illumina GA Pipeline (v1.6). The filtered reads were mapped to the reference sequences using SOAPaligner/soap2 (Li et al. 2009). The reference genome and gene database were from the open website: http://cmb.bnu.edu.cn/Cucumis_sativus_v20/ (Huang et al. 2009). Mismatches of no more than two bases were allowed in the alignment. The gene expression level was calculated by RPKM (reads per kilobase per million; Mortazavi et al. 2008). A strict

algorithm was utilized to identify the differentially expressed genes between different transcriptomes (Audic and Claverie 1997). The RNA-Seq data of bagging-treated ovaries of 8419s-1 were used as the control. An FDR of $\leq$0.001 and an absolute value of $\log_2$ratio $\geq$1 were used as the threshold for the significance of differential gene expression. More criteria could be used with higher fold changes (e.g. fold $\geq$1.5) to identify the DEGs. Genes were annotated by reference to the cucumber database (http://www.icugi.org/cgi-bin/ICuGI/genome/index.cgi?organism=cucumber) using three methods: cDNA-EST (expressed sequence tag), homology based and ab initio (Huang et al. 2009, Z. Li et al. 2011). Gene function throughout the manuscript was referred to the Arabidopsis database (http://www.arabidopsis.org/index.jsp) by the homology-based gene function prediction method. A close correlation was also observed between relative expression levels measured with RNA-Seq and qRT-PCR, validating the RNA-Seq methodology described above for quantitative analysis of the cucumber transcriptome. GO enrichment analysis provided all the GO terms that were significantly enriched in the DEGs as compared with those of the genome background. The DEGs corresponding to biological functions were filtered.

Phytohormone analysis

The auxin (IAA), cytokinin (zeatin riboside), gibberellin (GA_3) and ABA were extracted from ovaries of EC1 and 8419s-1 cucumbers. In total, 21 samples were investigated, comprising the 0, 2, 4, 6 and 8 dpa ovaries of the pollination-treated EC1 and 8419s-1, the bagging-treated EC1 and 8419s-1, and the CPPU-treated 8419s-1. Ten ovaries were mixed into a sample pool (three biological replicates). A 2 g aliquot of each samples was ground in an ice-cold mortar with 8 ml of 80% (v/v) methanol extraction medium containing 1 mM butylated hydroxytoluence as an antioxidant (Yang et al. 2001). The extracts were incubated at 4°C for 4 h then centrifuged at 3,500 r.p.m. for 8 min at 4°C. The supernatants were injected into Chromosep C18 columns (C18 Sep-Park Cartridge, Waters Corp.) to determine the binding hormones, and washed with 10 ml of 100% and 5 ml of 80% (v/v) methanol. The hormones were eluted with 10 ml of 100% (v/v) methanol and dried in N_2 gas. The residues were dissolved in 2 ml of phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween-20 and 0.1% (w/v) gelatin (pH 7.5).

Phytohormones were separately analyzed through ELISA using IAA, ZR, GA_3 and ABA ELISA Kits (Sangon Biotech Company) based on Weiler's method (Weiler et al. 1981). The results are presented as the mean ± SE of three biological replicates and three technical replicates.

Ovary transient expression

To perform a transient expression experiment, the coding sequences of the predicted parthenocarpic genes (**Supplementary Table S7**) were cloned from the cucumber fruit cDNA (2 dpa pollinated 8419s-1) and verified by sequencing and BLAST with the cucumber genome database (http://www.arabidopsis.org/index.jsp), then inserted into the binary vector pGreen0029 (kanamycin resistant) with the *Cauliflower mosaic virus* (CaMV) 35S promoter. The recombinant vectors were transformed into *Agrobacterium tumefaciens* (C58). The transformed *Agrobacterium* were cultured in LB liquid medium with 100 μg ml^{-1} kanamycin, 50 μg ml^{-1} rifampicin, and centrifuged with 20 μg ml^{-1} gentamicin and 10 μg ml^{-1} tetracycline at 28°C, 250 r.p.m. for 16 h in the dark until an OD_{600} of 1.0. A 2 ml aliquot of each transformed *Agrobacterium* culture was collected and resuspended in 1 ml liquid of Murashige and Skoog (MS) medium with 10 mmol l^{-1} MES pH 5.6, 20 g l^{-1} sucrose, 200 μmol l^{-1} acetosyringone as infection solution, and incubated at 22°C for 1 h in the dark (Spolaore et al. 2001).

The −2 dpa 8419s-1 and EC1 ovaries, with short closed petals (length 1.2–1.5 cm, diameter 3–5 mm), were used for the transient expression experiment (each time 15 ovaries were selected for each transformation, three replications). The ovaries were injected with 5 μl of the above liquid mixture by microinjector at the stigma site then isolated with bags. Both 8419s-1 and EC1 ovaries were injected with the C58 *Agrobacterium* containing an empty vector or pure infection solution which were considered as negative controls. All the ovaries were harvested and investigated 10 d after injection (equivalent to 8 dpa). GUS staining was used to identify whether the ovaries were positively treated. If the exogenous genes were successfully expressed in a transient treatment, the ovaries should be presented in blue color, because the recombinant vectors contained both expression cassettes of exogenous genes and GUS. The expansion rate '(diameter of treated ovary at 8 dpa – mean ovary diameter of 8419s or EC1 at 0 dpa)/mean ovary diameter of 8419 or EC1 at 0 dpa)' was used to classify expanded (with a $\geq$10% expansion rate), aborted ($\leq$0% expansion rate with yellow or shrinkage symptoms) and frozen state fruit (expansion rate between 0% and 10%). Ovary transient expression for each putative parthenocarpic gene has three replicates at a different time (spring and autumn of 2012) or place (the greenhouse of Jiangpu horticulture experimental station, Nanjing Agricultural University and the greenhouse of the Pailou breeding station, Nanjing Agricultural University).

Real-time PCR and total sugar measurement

RNA was isolated from the bagging-, pollination-, CPPU- and transient expression-treated ovaries and digested with DNase I (Fermentas) for 30 min at 25°C to remove DNA according to the manufacturer's instructions. cDNA was synthesized from 2 μg of total RNA by using a cDNA Synthesis Kit (Fermentas). The qRT-PCR was performed as described previously (Li et al. 2012) by using the SYBR Premix Ex Taq^{TM} Kit (TAKARA) following the description in the handbook of the Bio-Rad iQ1 real-time PCR system (Bio-Rad). The Ct value of each gene was investigated and normalized to the Ct value of *Cs-Actin*. To determine relative expression fold differences for each gene during different treatments, the formula $2^{-\Delta\Delta Ct}$ was calculated. The primers used for qRT-PCR are listed in

Supplementary Table S8. The qRT-PCR results were performed from three repeat reactions for each gene and sample.

Carbohydrate contents in cucumber fruits were measured by the anthrone colorimetric method following the procedure of Xue (1985). Briefly, 50 mg of dry plant material was extracted with 4 ml of 80% (v/v) ethanol at 80°C for 30 min and the extracts were centrifuged at 16,000×*g* for 10 min. The supernatants were combined, made up to 10 ml with 80% (v/v) ethanol and used for assaying total soluble sugars, sucrose, fructose and glucose. A 20 µl aliquot of the extraction was mixed with 80 µl of distilled water and 2.5 ml of anthrone reagent [150 mg of anthrone in 100 ml of 71.70% (v/v) H_2SO_4], and the resulting mixtures were incubated at 90°C for 15 min. After cooling, the absorbance was measured at 620 nm. Standard solutions of glucose were tested in the same way to obtain a calibration curve. The analysis was repeated three separate times. The statistical significance of the differences between the samples was investigated by the *t*-test at the 1% and 5% level.

Supplementary data

Supplementary data are available at PCP online.

Funding

This work was supported by the National Basic Research Program of China (973 program) [2012CB113904, 2009CB119001-01]; Special Fund for Agro-Scientific Research in the Public Interest [201403032]; Project supported by the National Natural Science Foundation of China [31071801, 31301781 and 31272174]; The National High Technology Research and Development Program of China (863 Program) [2012AA100202]; Research Fund for the Doctoral Program of Higher Education of China [20120097120037]; The Fundamental Research Funds for the Central Universities of China [KYZ201410]; Natural Science Foundation of Jiangsu Province [BK20130674]; Youth Science and Technology Innovation Fund program of Nanjing Agricultural University [KJ2012013] and Fundamental Research of Nanjing Agricultural University [Y0201100253].

Acknowledgments

We would like to thank Professor Yi Li (University of Connecticut, USA) and Professor Yuling Bai (Wageningen UR, The Netherlands) for critical comments on the manuscript.

Disclosures

The authors have no conflicts of interest to declare.

References

Abel, S. and Theologis, A. (1996) Early genes and auxin action. *Plant Physiol.* 111: 9–17.

Archbold, D.D. and Dennis, F.G. Jr (1984) Quantification of free ABA and free and conjugated IAA in strawberry achene and receptacle tissue during fruit development. *J. Amer. Soc. Hortic. Sci.* 109: 330–335.

Acciarri, N., Restaino, F., Vitelli, G., Perrone, D., Zottini, M., Pandolfini, T. et al. (2002) Genetically modified parthenocarpic eggplants: improved fruit productivity under both greenhouse and open field cultivation. *BMC Biotechnol.* 2: 4.

Audic, S. and Claverie, J.M. (1997) The significance of digital gene expression profiles. *Genome Res.* 7: 986–995.

Beyer, E.M. and Quebedeaux, B. (1974) Parthenocarpy in cucumber: mechanism of action of auxin transport inhibitors. *J. Amer. Soc. Hortic. Sci.* 99: 385–390.

Beraldi, D., Picarella, M.E., Soressi, G.P. and Mazzucato, A. (2004) Fine mapping of the parthenocarpic fruit (*pat*) mutation in tomato. *Theor. Appl. Genet.* 108: 209–216.

Ben-Cheikh, W., Perez-Botella, J., Tadeo, F.R., Talon, M. and Primo-Millo, E. (1997) Pollination increases gibberellin levels in developing ovaries of seeded varieties of citrus. *Plant physiol.* 114: 557–564.

Bernier, G., Havelange, A., Houssa, C., Petitjean, A. and Lejeune, P. (1993) Physiological signals that induce flowering. *Plant Cell* 5: 1147–1155.

Blilou, I. (2005) The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. *Nature* 433: 39–44.

Bolaños-Villegas, P., Yang, X., Wang, H.J., Juan, C.T., Chuang, M.H., Makaroff, C.A. et al. (2013) Arabidopsis CHROMOSOME TRANSMISSION FIDELITY 7 (AtCTF7/ECO1) is required for DNA repair, mitosis and meiosis. *Plant J.* 75: 927–940.

Boonkorkaew, P., Hikosaka, S. and Sugiyama, N. (2008) Effect of pollination on cell division, cell enlargement, and endogenous hormones in fruit development in a gynoecious cucumber. *Sci. Hortic.* 116: 1–7.

Brusslan, J.A. and Tobin, E.M. (1992) Light-independent developmental regulation of cab gene expression in *Arabidopsis thaliana* seedlings. *Proc. Natl Acad. Sci. USA* 89: 7791–7795.

Cao, Q.H., Chen, J.F., Qian, C.T. and Guo, J.Y. (2004) Cytological studies on meiosis and male gametophyte development in cucumber. *Acta Bot. Boreal Occident. Sin.* 24: 1721–1726.

Carmi, N., Salts, Y., Dedicova, B., Shabtai, S. and Barg, R. (2003) Induction of parthenocarpy in tomato via specific expression of the *rolB* gene in the ovary. *Planta* 217: 726–735.

Carrera, E., Ruiz-Rivero, O., Peres, L.E., Atares, A. and Garcia-Martinez, J.L. (2012) Characterization of the procera tomato mutant shows novel functions of SlDELLA protein in the control of flower morphology, and cell division and expansion and auxin-signalling pathway during fruit-set and development. *Plant Physiol.* 160: 1581–1596.

Chen, J., Feng, J., Qin, Q., Liu, X., Wu, J. and Xie, M. (2006) Characteristics of sugar metabolism and accumulation in GA3 induced parthenocarpic white flesh loquat 'Ninghai Bai' fruit. *Acta Hortic. Sin.* 33: 471–476.

Coenen, C. and Lomax, T.L. (1997) Auxin–cytokinin interactions in higher plants: old problems and new tools. *Trends Plant Sci.* 2: 351–356.

Cosgrove, D.J. (1998) Cell wall loosening by expansins. *Plant Physiol.* 118: 333–339.

Costantini, E., Landi, L., Silvestroni, O., Pandolfini, T., Spena, A. and Mezzetti, B. (2007) Auxin synthesis-encoding transgene enhances grape fecundity. *Plant Physiol.* 143: 1689–1694.

Dangl, J.L., Preuss, D. and Schroeder, J.I. (1995) Talking through walls: signaling in plant development. *Cell* 83: 1071–1077.

Davies, E. and Ozbay, O. (1975) Comparative effects of indoleacetic acid and gibberellic acid on growth of decapitated etiolated epicotyls of *Pisum sativum* cv. Alaska. *Physiol. Plant.* 35: 279–285.

De Jong, A.J., Cordewener, J., Schiavo, F.L., Terzi, M., Vandekerckhove, J., Van Kammen, A. et al. (1992) A carrot somatic embryo mutant is rescued by chitinase. *Plant Cell* 4: 425–433.

de Jong, M., Mariani, C. and Vriezen, W.H. (2009a) The role of auxin and gibberellin in tomato fruit set. *J. Exp. Bot.* 60: 1523–1532.

De Jong, M., Wolters-Arts, M., Feron, R., Mariani, C. and Vriezen, W.H. (2009b) The *Solanum lycopersicum* auxin response factor 7 (SlARF7) regulates auxin signaling during tomato fruit set and development. *Plant J.* 57: 160–170.

Deng, Q., Chen, Z.W. and Kong, W.L. (2012) Machinery used in the production of cucumber seeds. *Jilin Vegetable* 2: 59.

Denissen, C.M. and Den Nijs, A.P.M. (1987) Effects of gamma irradiation on in vitro pollen germination of different *Cucumis* species. *Euphytica* 36: 651–658.

Edenberg, H.J. and Huberman, J.A. (1975) Eukaryotic chromosome replication. *Annu. Rev. Genet.* 9: 245–284.

Eeuwens, C.J. and Schwabe, W.W. (1975) Seed and pod wall development in Pisum sativum, L. in relation to extracted and applied hormones. *J. Exp. Bot.* 26: 1–14.

Feraru, E. and Friml, J. (2008) PIN polar targeting. *Plant Physiol.* 147: 1553–1559.

Ficcadenti, N., Sestili, S., Pandolfini, T., Cirillo, C., Rotino, G.L. and Spena, A. (1999) Genetic engineering of parthenocarpic fruit development in tomato. *Mol. Breed.* 5: 463–470.

Fu, F.Q., Mao, W.H., Yu, J.Q., Zhou, Y.H., Asami, T. and Yu, J.Q. (2008) A role of brassinosteroids in early fruit development in cucumber. *J. Exp. Bot.* 59: 2299–2308.

Fu, F.Q., Mao, W.H., Yu, J.Q., Zhou, Y.H., Yu, J.Q. et al. (2010) Spatio-temporal changes in cell division, endoreduplication and expression of cell cycle-related genes in pollinated and plant growth substances-treated ovaries of cucumber. *Plant Biol.* 12: 98–107.

Garcia, M.R., Asins, M.J. and Carbonell, E.A. (2000) QTL analysis of yield and seed number in Citrus. *Theor. Appl. Genet.* 101: 487–493.

García-Martínez, J.L., Marti, M., Sabater, T., Maldonado, A. and Vercher, Y. (1991b) Development of fertilized ovules and their role in the growth of the pea pod. *Physiol Plantarum* 83: 411–416.

García-Martínez, J.L., Santes, C., Croker, S.J. and Hedden, P. (1991a) Identification, quantitation and distribution of gibberellins in fruits of Pisum sativum L. cv. Alaska during pod development. *Planta* 184: 53–60.

George, W.L., Scott, J.W. and Splittstoesser, W.E. (1984) Parthenocarpy in tomato. *Hortic. Rev.* 6: 65–84.

Gillaspy, G., Ben-David, H. and Gruissem, W. (1993) Fruits: a developmental perspective. *Plant Cell* 5: 1439–1451.

Goda, H. (2004) Comprehensive comparison of auxin-regulated and brassinosteroid regulated genes in Arabidopsis. *Plant Physiol.* 134: 1555–1573.

Goetz, M., Hooper, L.C., Johnson, S.D., Rodrigues, J.C., Vivian-Smith, A. and Koltenow, A.M. (2007) Expression of aberrant forms of AUXIN RESPONSE FACTOR8 stimulates parthenocarpy in Arabidopsis and tomato. *Plant Physiol.* 145: 351–366.

Goetz, M., Vivian-Smith, A., Johnson, S.D. and Koltenow, A.M. (2006) AUXIN RESPONSE FACTOR8 is a negative regulator of fruit initiation in Arabidopsis. *Plant Cell* 18: 1873–1886.

Gorguet, B., Eggink, P.M., Ocana, J., Tiwari, A., Schipper, D., Finkers, R. et al. (2008) Mapping and characterization of novel parthenocarpy QTLs in tomato. *Theor. Appl. Genet.* 116: 755–767.

Graham, I.A., Leaver, C.J. and Smith, S.M. (1992) Induction of malate synthase gene expression in senescent and detached organs of cucumber. *Plant Cell* 4: 349–357.

Greenboim-Wainberg, Y., Maymon, I., Borochov, R., Alvarez, J., Olszewski, N., Ori, N. et al. (2005) Cross talk between gibberellin and cytokinin: the Arabidopsis GA response inhibitor SPINDLY plays a positive role in cytokinin signaling. *Plant Cell* 17: 92–102.

Gustafson, F.G. (1939) Auxin distribution in fruits and its significance in fruit development. *Amer. J. Bot.* 26: 189–194.

Han, M., Chang, M., Kim, U.J. and Grunstein, M. (1987) Histone H2B repression causes cell-cycle-specific arrest in yeast: effects on chromosomal segregation, replication, and transcription. *Cell* 48: 589–597.

Hartig, K. and Beck, E. (2005) Endogenous cytokinin oscillations control cell cycle progression of tobacco BY-2 cells. *Plant Biol.* 7: 33–40.

Hartig, K. and Beck, E. (2006) Crosstalk between auxin, cytokinins, and sugars in the plant cell cycle. *Plant Biol.* 8: 389–396.

Hartwell, L.H. and Weinert, T.A. (1989) Checkpoints: controls that ensure the order of cell cycle events. *Science* 246: 629–634.

Hirt, M. (1997) Multiple roles of MAP kinases in plant signal transduction. *Trends Biol. Sci.* 2: 11–15.

Huang, S.W., Li, R., Zhang, Z., Li, L., Gu, X., Fan, W. et al. (2009) The genome of the cucumber, *Cucumis sativus* L. *Nat. Genet.* 4: 1275–1281.

Hu, J.B., Li, J.W., Wang, L.J., Liu, L.J., Si, S.W. and Liu, J.Q. (2011) Breeding of new cucumber variety "Xiayan" used for fresh market and slicing. *Acta Agriculturae Jiangxi* 22: 32–36.

Hwang, I. and Sheen, J. (2001) Two-component circuitry in Arabidopsis cytokinin signal transduction. *Nature* 413: 383–389.

James, D.J., Passey, A.J., Mackenzie, K.A.D. and Menhinick, E.C. (1985) The effects of pollen irradiation on the development of the post-fertilization ovule of apple (*Malus pumila* Mill.). *In* Experimental Manipulation of Ovule Tissues. Edited by Chapman, G.P., Mantell, S.H. and Daniels, R.W. pp. 210–224. Longman, London.

Jia, H., Wang, Y., Sun, M., Li, B., Han, Y., Zhao, Y. et al. (2013) Sucrose functions as a signal involved in the regulation of strawberry fruit development and ripening. *New Phytol.* 198: 453–465.

Kano, Y. and Asahira, T. (1981) Roles of cytokinin and abscisic acid in the maturing of strawberry fruits. *J. Jpn Soc. Hortic. Sci.* 50: 31–36.

Kiernan, J.A. (2008) Histological and Histochemical Methods: Theory and Practice, 4th edn. Scion Press.

Kim, I.S., Okubo, H. and Fujieda, K. (1992) Endogenous levels of IAA in relation to parthenocarpy in cucumber (*Cucumis sativus* L.). *Sci. Hortic.* 52: 1–8.

Laplaze, L. (2007) Cytokinins act directly on lateral root founder cells to inhibit root initiation. *Plant Cell* 19: 3889–3900.

Li, J., Li, Z., Tang, L., Yang, Y., Zouine, M. and Bouzayen, M. (2012) A conserved phosphorylation site regulates the transcriptional function of ETHYLENE-INSENSITIVE3-like1 in tomato. *J. Exp. Bot.* 63: 427–439.

Li, R., Yu, C., Li, Y., Lam, T.W., Yiu, S.M., Kristiansen, K. et al. (2009) SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics* 25: 1966–1967.

Li, X.X., Kobayashi, F., Ikeura, H. et al. (2011) Chlorophenoxyacetic acid and chloropyridylphenylurea accelerate translocation of

photoassimilates to parthenocarpic and seeded fruits of muskmelon (*Cucumis melo*). *J. Plant Physiol.* 168: 920–926.
Li, Z., Zhang, Z., Yan, P., Huang, S., Fei, Z and Lin, K. (2011) RNA-Seq improves annotation of protein-coding genes in the cucumber genome. *BMC Genomics* 12: 540.
Mapelli, S., Frova, C., Torti, G. and Soressi, G.P. (1978) Relationship between set, development and activities of growth regulators in tomato fruits. *Plant Cell Physiol.* 19: 1281–1288.
Martí, C., Orzáez, D., Ellul, P., Moreno, V., Carbonell, J. and Granell, A. (2007) Silencing of DELLA induces facultative parthenocarpy in tomato fruits. *Plant J.* 52: 865–876.
Mezzetti, B., Landi, L., Pandolfini, T. and Spena, A. (2004) The DefH9-iaaM auxin-synthesizing gene increases plant fecundity and fruit production in strawberry and raspberry. *BMC Biotechnol.* 4: 4.
Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L. and Wold, B. (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods* 5: 621–628.
Miller, C., Skoog, F., Von Saltza, M.H. and Strong, F.M. (1955) Kinetin, a cell division factor from deoxyribonucleic acid. *J. Amer. Chem. Soc.* 77: 1392.
Miyatake, K., Saito, T., Negoro, S., Yamaguchi, H., Nunome, T., Ohyama, A. et al. (2012) Development of selective markers linked to a major QTL for parthenocarpy in eggplant (*Solanum melongena* L.). *Theor. Appl. Genet.* 1–11.
Murray, A.W. (1992) Creative blocks: cell-cycle checkpoints and feedback controls. *Nature* 359: 599–604.
Murray, A.W. (1995) The genetics of cell cycle checkpoints. *Curr. Opin. Genet. Dev.* 5: 5–11.
Nemhauser, J.L., Mockler, T.C. and Chory, J. (2004) Interdependency of brassinosteroid and auxin signaling in *Arabidopsis*. *PLoS Biol.* 2: E258.
Nishiyama, I. and Uematsu, S. (1967) Radiobiological studies in plants XIII. Embryogenesis following X-irradiation of pollen in *Lycopersicum pimpinellifolium*. *Radiat. Biol.* 7: 481–489.
Pagnussat, G.C., Yu, H.J., Ngo, Q.A., Rajani, S., Mayalagu, S., Johnson, C.S. et al. (2005) Genetic and molecular identification of genes required for female gametophyte development and function in *Arabidopsis*. *Development* 132: 603–614.
Pandey, K.K. and Phung, M. (1982) 'Hertwig effect' in plants: induced parthenogenesis through the use of irradiated pollen. *Theor. Appl. Genet.* 62: 295–300.
Pandolfini, T., Rotino, G.L., Camerini, S., Defez, R. and Spena, A. (2002) Optimization of transgene action at the post-transcriptional level: high quality parthenocarpic fruits in industrial tomatoes. *BMC Biotechnol.* 2: 1.
Paulovich, A.G. and Hartwell, L.H. (1995) A checkpoint regulates the rate of progression through S phase in *S. cerevisiae* in response to DNA damage. *Cell* 82: 841–847.
Pezzotti, M., Feron, R. and Mariani, C. (2002) Pollination modulates expression of the PPAL gene, a pistil-specific β-expansin. *Plant Mol. Biol.* 49: 187–197.
Pharis, R.P. and King, R. (1985) Gibberellins and reproductive development in higher plants. *Annu. Rev. Plant Physiol.* 36: 517–568.
Phillips, J.M. and Hayman, D.S. (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55: 158–161.
Picken, A.J.F. (1984) A review of pollination and fruit-set in the tomato (*Lycopersicon esculentum* Mill.). *J. Hortic Sci.* 59: 1–13.
Ren, Z.X., Li, Z.G., Miao, Q., Yang, Y., Deng, W. and Hao, Y. (2011) The auxin receptor homologue in *Solanum lycopersicum* stimulates tomato fruit set and leaf morphogenesis. *J. Exp. Bot.* 62: 2815–2826.
Rodrigo, M.J., García-Martínez, J.L., Santes, C.M., Gaskin, P. and Hedden, P. (1997) The role of gibberellins A1 and A3 in fruit growth of Pisum sativum L. and the identification of gibberellins A4 and A7 in young seeds. *Planta* 201: 446–455.
Rotino, G.L., Perri, E., Zottini, M., Sommer, H. and Spena, A. (1997) Genetic engineering of parthenocarpic plants. *Nat. Biotechnol.* 15: 1398–1401.
Schubert, V., Weißleder, A., Ali, H., Fuchs, J., Lermontova, I., Meister, A. et al. (2009) Cohesin gene defects may impair sister chromatid alignment and genome stability in Arabidopsis thaliana. *Chromosoma* 118: 591–605.
Serrani, J.C., Fos, M, Atarés, A. and García-Martínez, J.L. (2007) Effect of gibberellin and auxin on parthenocarpic fruit growth induction in the cv. *Micro-Tom* of tomato. *J. Plant Growth Regul.* 26: 211–221.
Sheen, J. (1994) Feedback control of gene expression. *Photosynth. Res.* 39: 427–438.
Sheen, J., Zhou, L. and Jang, J.C. (1999) Sugars as signaling molecules. *Curr. Opin. Plant Biol.* 2: 410–418.
Silverman, R.B. (2002) Organic Chemistry of Enzyme-Catalyzed Reactions. Elsevier, Amsterdam.
Sniezko, R. and Visser, T. (1987) Embryo development and fruit-set in pear induced by untreated and irradiated pollen. *Euphytica* 36: 287–294.
Spolaore, S., Trainotti, L. and Casadoro, G. (2001) A simple protocol for transient gene expression in ripe fleshy fruit mediated by Agrobacterium. *J. Exp. Bot.* 52: 845–850.
Sponsel, V.M. (1983) The localization, metabolism and biological activity of gibberellins in maturing and germinating seeds of Pisum sativum cv. Progress No. 9. *Planta* 159: 454–468.
Steeves, T.A. and Sussex, I.M. (1989) Patterns in Plant Development. Cambridge University Press, Cambridge.
Sun, T. (2000) Gibberellin signal transduction. *Curr. Opin. Plant Biol.* 3: 374–380.
Sun, Z., Staub, J.E., Chung, S.M. and Lower, R.L. (2006) Identification and comparative analysis of quantitative trait loci associated with parthenocarpy in processing cucumber. *Plant Breed.* 125: 281–287.
Talon, M., Hedden, P. and Primo-Millo, E. (1990) Gibberellins in Citrus sinensis: a comparison between seeded and seedless varieties. *J. Plant Growth Regul.* 9: 201–206.
Talon, M., Zacarias, L. and Primo-Millo, E. (1992) Gibberellins and parthenocarpic ability in developing ovaries of seedless mandarins. *Plant Physiol.* 99: 1575–1581.
To, J.P. and Kieber, J.J. (2008) Cytokinin signaling: two-components and more. *Trends Plant Sci.* 13: 85–92.
Ünligil, U.M. and Rini, J.M. (2000) Glycosyltransferase structure and mechanism. *Curr. Opin. Struct. Biol.* 10: 510–517.
Varga, A. and Bruinsma, J. (1986) Tomato. *In* CRC Handbook of Fruit Set and Development. Edited by Monselise, S.P. pp. 461–480. CRC Press, Boca Raton, FL.
Vassileva-Dryanovska, O.A. (1966a) Fertilization with irradiated pollen in *Tradescantia*. *Radiat. Bot.* 6: 469–479.
Vassileva-Dryanovska, O.A. (1966b) The induction of embryos and tetraploid endosperm nuclei with irradiated pollen in lilium. *Hereditas* 55: 160–165.
Vassileva-Dryanovska, O.A. (1966c) Induced chromosomal aberrations and other abnormalities in the embryo sac following pollen irradiation in *Melandrium rubrum*. *Hereditas* 55: 149–159.

Vivian-Smith, A. and Koltunow, A.M. (1999) Genetic analysis of growth regulator-induced parthenocarpy in arabidopsis. *Plant Physiol.* 121: 437–451.

Wang, H., Jones, B., Bouzayen, M., Frasse, P., Delalande, C., Regad, F. et al. (2005) The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. *Plant Cell* 17: 2676–2692.

Weiler, E.W., Jourdan, P.S. and Conrad, W. (1981) Levels of indole-3-acetic acid in intact and decapitated coleoptiles as determined by a specific and highly sensitive solid-phase enzyme immunoassay. *Planta* 153: 561–571.

Weinert, T.A., Kiser, G.L. and Hartwell, L.H. (1994) Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. *Genes Dev.* 8: 652–665.

Wirth, M. and Withner, C.L. (1959) Embryology and development in the Orchidaceae. *In* The Orchids: A Scientific Survey. Edited by Withner, C.L. pp. 155–188. The Ronald Press Company, New York.

Withner, C.L., Nelson, P.K. and Wejksnora, P.J. (1974) The anatomy of orchids. *In* The Orchids. Scientific Studies. Edited by Withner, C.L. pp. 267–347. John Wiley and Sons, New York.

Wobus, U. and Weber, H. (1999) Sugars as signal molecules in plant seed development. *Biol. Chem.* 380: 937–944.

Xu, Z., Escamilla-Treviño, L., Zeng, L., Lalgondar, M., Bevan, D., Winkel, B. et al. (2004) Functional genomic analysis of Arabidopsis thaliana glycoside hydrolase family 1. *Plant Mol. Biol.* 55: 343–367.

Xue, Y. (1985) Handbook of Plant Physiological Experiments. pp. 134–139. Shanghai Science and Technology Press, Shanghai.

Yan, L.Y., Lou, L.N. and Chen, J.F. (2008) Inheritance of parthenocarpy in gynoecious cucumber. *Acta Hortic. Sin.* 35: 1441–1446.

Yan, L.Y., Lou, L.N., Li, X.L., Feng, Z.H., Lou, Q.F. and Chen, J.F. (2009) Evaluation of parthenocarpy in cucumber germplasm. *Acta Hortic. Sin.* 36: 975–982.

Yan, L.Y., Lou, L.N., Li, X.L., Feng, Z.H., Lou, Q.F. and Chen, J.F. (2010) Inheritance of parthenocarpy in monoecious cucumber. *Sci. Agric. Sin.* 6: 26.

Yang, J., Zhang, J., Wang, Z., Zhu, Q. and Wang, W. (2001) Hormonal changes in the grains of rice subjected to water stress during grain filling. *Plant Physiol.* 127: 315–323.

Yang, T. (1996) Genetic dissection of the relative roles of auxin and gibberellin in the regulation of stem elongation in intact lightgrown peas. *Plant Physiol.* 110: 1029–1034.

Yennawar, N.H., Li, L.C., Dudzinski, D.M., Tabuchi, A. and Cosgrove, D.J. (2006) Crystal structure and activities of EXPB1 (Zea m 1), a β-expansin and group-1 pollen allergen from maize. *Proc. Natl Acad. Sci. USA* 103: 14664–14671.

Yin, Z., Malinowski, R., Ziolkowska, A., Sommer, H., Plcader, W. and Malepszy, S. (2006) The DefH9-iaaM-containing construct efficiently induces parthenocarpy in cucumber. *Cell Mol. Biol.* 11: 279–290.

Zhang, Y.X. and Lespinasse, Y. (1991) Pollination with gamma-irradiated pollen and development of fruits, seeds and parthenogenetic plants in apple. *Euphytica* 54: 101–109.