Transcriptome Profiling Reveals the Regulatory Mechanism Underlying Pollination Dependent and Parthenocarpic Fruit Set Mainly Mediated by Auxin and Gibberellin

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Abstract

Background

Fruit set is a key process for crop production in tomato which occurs after successful pollination and fertilization naturally. However, parthenocarpic fruit development can be uncoupled from fertilization triggered by exogenous auxin or gibberellins (GAs). Global transcriptome knowledge during fruit initiation would help to characterize the molecular mechanisms by which these two hormones regulate pollination-dependent and -independent fruit set.

Principal Findings

In this work, digital gene expression tag profiling (DGE) technology was applied to compare the transcriptomes from pollinated and 2, 4-D/GA3-treated ovaries. Activation of carbohydrate metabolism, cell division and expansion as well as the down-regulation of MADS-box is a comprehensive regulatory pathway during pollination-dependent and parthenocarpic fruit set. The signaling cascades of auxin and GA are significantly modulated. The feedback regulations of Aux/IAAs and DELLA genes which functioned to fine-tune auxin and GA response respectively play fundamental roles in triggering fruit initiation. In addition, auxin regulates GA synthesis via up-regulation of GA20ox1 and down-regulation of KNOX. Accordingly, the effect of auxin on fruit set is mediated by GA via ARF2 and IAA9 down-regulation, suggesting that both pollination-dependent and parthenocarpic fruit set depend on the crosstalk between auxin and GA.

Significance

This study characterizes the transcriptomic features of ovary development and more importantly unravels the integral roles of auxin and GA on pollination-dependent and parthenocarpic fruit set.
Introduction

Fruit set, defined as the shift from the quiescent ovary to fast-growing young fruit, is a key process for fruit production in flowering plants. Owing to its economic importance as a fleshy crop, tomato (*Solanum lycopersicum* L.) has been widely studied as a model for the regulation of these processes. Generally, fruit set occurs after the successful completion of pollination and fertilization, while it is coordinated by signals produced in the developing embryos [1]. Auxin and gibberellins (GAs) are considered the major phytohormones in the control of this process [2], which is supported by the elevated levels of endogenous auxin and GA in pollinated ovaries [1, 3] and parthenocarpy induction via exogenous application of either of the two hormones uncoupling with pollination and fertilization [4], even though others have been shown to be involved in fruit set such as ethylene [5] and cytokinin [6].

Over the years, it has been well established that the fruit developmental program is triggered by the signaling cascades of auxin and GAs. Recent studies have identified several components of the auxin signaling pathway including auxin transport protein PIN4[7], the Aux /IAA protein IAA9 [8], auxin response factor ARF8[9] and ARF7[10] involved in repressing fruit initiation until the fertilization cue in tomatoes. In addition to auxin signaling, fruit set seems to be under the control of GAs biosynthesis and signaling. In pollinated ovaries, the increase of GA content is attributed to higher GA 20-oxidase (GA20ox) activity via elevated GA20ox transcripts [3], hence promotes GA response. DELLA protein has been characterized as a negative regulator of GA signal and thereby prevents ovary growth prior to pollination and fertilization [2]. Although it is suggested that fruit set depends on auxin and GA response by the above-mentioned results, the exact roles of these two hormones are still partially defined, probably due to only a few signaling components involved have been identified to date [2]. Additionally, despite the application of either auxin or GAs can trigger parthenocarpy in tomato, there are several indications that each of them has distinct effects on fruit development such as cell division and cell expansion [1, 11]. Therefore, global transcriptome knowledge during fruit set might help to understand the molecular mechanisms by which these two hormones regulate fruit set and development.

More recently, some advances have brought some insights on their regulatory roles. Vriezen et al. [5] compared the transcriptomes from pollinated and GA-treated ovaries 3 days after treatment. It showed that genes triggered by one treatment were not all triggered by another, which is supported by the fact that pollination appeared to have significant effects on the expression of the auxin signaling genes, such as *Aux/IAAs*, while these genes were hardly influenced by GA treatment. By contrast, a transcriptome analysis underlying pollination-dependent fruit set and parthenocarpy mediated by IAA9 down-regulation revealed that a large number of genes were differentially expressed in common, and only a small subset dependent on IAA9 regulation. This study also showed that some molecular events including the activations of photosynthesis, sucrose metabolism and cell division were considered to be important in both types of fruit set during anthesis to postanthesis transition [12]. Similar results are also revealed by transcriptomic analysis of carpel development in *pat3*/*pat4* mutant with higher GA concentrations [4]. These findings suggest that regardless of the existence of distinctive molecular features, different types of fruit set could be regulated via a similar pathway in partially. Furthermore, the roles of auxin and GAs on fruit set are demonstrated in these studies. Wang et al. [12] shows that the fine-tuning of auxin-related *Aux/IAAs* and *ARFs* are crucial in triggering fruit set, whereas few changes concerning GA appear comparatively. Similarly, auxin biosynthesis and responses were altered in *pat3*/*pat4* mutant [4], indicating the complexity of auxin action in fruit set. Alternatively, it has been reported that auxin-induced parthenocarpic fruit set in tomato is mediated via GAs in partially, which is indicated by the
observation that fruit set induced by 2, 4-D was dramatically repressed by GA biosynthesis inhibitors [13]. The above-mentioned studies demonstrate the complicated and confusing relationship between auxin and GA response during fruit set. It is helpful to clear the crosstalk between these two hormones and the comprehensive mechanisms of fruit set.

In order to characterize the molecular events underlying the fruit set, digital gene expression tag profiling (DGE) technology was applied to compare the transcriptomes from pollinated, 2, 4-D and GA3-treated ovaries in this work. Our study identified the genes modulated throughout carpel development and revealed common and distinct features between pollination-dependent and parthenocarpic fruit set. The signaling cascades of auxin and GAs and potential components of the regulatory mechanism underlying different types of fruit set processes were discussed. A model integrating the role of auxin and gibberellin in tomato fruit set was established in our study.

Results

Transcriptomic analysis of ovary development during fruit set

To characterize transcriptomic profiles of ovary development during pollination-dependent and-independent fruit set, five libraries including ovaries at 2 days ahead of anthesis (2DAA), 4 days post artificial pollination (4DPAP), auxin treatment (4DPAT), GA3 treatment (4DPGT) and untreated ovaries (6DPE) were designed for RNA-Seq analysis (Fig 1). The raw data generated by sequencing for the libraries ranged from 3.49 to 3.68 million reads. After filtering, we
obtained an average of 3.45 million clean data and the number of distinct tags is from 83,342 to 116,726 (Table 1). The results of saturation evaluation and the distribution analysis of RNA-Seq tags showed that sequencing quality was high enough for gene expression analysis (S1 Fig). A SGN unigene database containing 42,257 unigenes was used to tag alignment. Statistics analysis showed that the number of unambiguous tags mapping to gene was between 45,620 and 62,480, accounted for 53.32% to 55.17% of the clean tags (Table 1). In total, 18,705 genes were detected in at least one of the five libraries, of which 11,075 genes were expressed in all the samples. Moreover, 15,111, 13,921, 13,522, 13,499 and 16,446 genes were expressed in ovaries at 2DAA, 4DPAP, 4DPAT, 4DPGT and 6DPE, respectively.

Differentially expressed genes during fruit set

With respect to 2DAA, 2,736, 3,025 and 3,025 genes were differentially expressed in ovaries at 4DPAP, 4DPAT and 4DPGT, respectively (Fig 2A, S1 Table). In addition, with respect to 6DPE, 4,800, 5,315 and 5,077 genes were differentially expressed in ovaries at 4DPAP, 4DPAT and 4DPGT, respectively (Fig 2B, S2 Table). We also found that the majority of differentially expressed genes (DEGs) are down regulated during the process of ovary development (Fig 2). The DEGs were assigned to one of 23 GO terms based on biological process (S2 Fig). The terms “metabolic process” (around 25%), “cellular process” (around 19%), “response to stimulus” (around 10%) and “biological regulation” (around 5.5%) stood for the major proportion. Furthermore, significantly altered KEGG pathways were identified during fruit set, of which the common enriched was plant hormone signal transduction among the six groups (data not shown).

To identify the common features and differences between pollination-dependent and independent fruit set, we compared the sets of DEGs among the comparison groups based on two controls (2DAA and 6DPE). The results showed that 1,615 and 3,361 genes were common differentially expressed in ovaries at 4DPAP, 4DPAT and 4DPGT in contrast with 2DAA and 6DPE, respectively (Fig 3A and 3B, S3 Table). Additionally, there were 1,176 genes differentially expressed in ovaries from 3 different types of fruit set in contrast with both controls, which were considered to be key regulators for fruit set and growth (Fig 3C, S3 Table). Moreover, with respect to 2DAA, 479 genes exhibited differential expression exclusively in ovaries at 4DPAP (S3 Table), indicating a correlation between these genes and seed formation. 2028 genes were identified that are only differentially expressed in parthenocarpic fruit set, of which 968 and 424 displayed auxin-dependent and GA-dependent regulation, respectively (S3 Table). Similar results were obtained based on the control 6DPE.

| Summary                                      | 2DAA   | 4DPAP  | 4DPAT  | 4DPGT  | 6DPE   |
|----------------------------------------------|--------|--------|--------|--------|--------|
| Raw Data Total                               | 3511331| 3582739| 3680921| 3493203| 3560630|
| Raw Data Distinct Tag                        | 208288 | 187850 | 179986 | 180556 | 243899 |
| Clean Tag Total number                       | 339631 | 3478442| 3580876| 3393539| 3431151|
| Clean Tag Distinct Tag number                | 98735  | 86704  | 83342  | 84004  | 116726 |
| Unambiguous Tag Mapping to Gene Distinct Tag number | 53394  | 46234  | 45978  | 45620  | 62480  |
| Unambiguous Tag Mapping to Gene Distinct Tag % of clean tag | 54.08% | 53.32% | 55.17% | 54.31% | 53.53% |
| Unknown Tag Total number                      | 140546 | 139117 | 116197 | 141453 | 147641 |
| Unknown Tag Total % of clean tag              | 4.14%  | 4.00%  | 3.24%  | 4.17%  | 4.30%  |

Differential expression analysis was conducted using DESeq. The full results are summarized in Table 1.
To validate the accuracy of the RNA-Seq data, 19 genes with different functions including carbon assimilation, cell division and hormone signaling were selected for qRT-PCR analysis (Fig 4). There was a good correlation ($R = 0.86$, $P < 0.0001$) between the two methods (Fig 4T), indicating the high reliability of the RNA-Seq data obtained in this study.

Expression of genes involved in photosynthesis and carbohydrate metabolism during fruit set

Photosynthesis-related genes were strongly stimulated during fruit set. Remarkably, the overwhelming majority of these genes (28 out of 29 genes) were up-regulated in ovaries at 4DPAP, 4DPAT and 4DPGT compared with 2DAA and 6DPE, which including 13 chlorophyll a/b-binding proteins (CAB), 3 photosystem I reaction center subunits (psa), 5 photosystem II reaction center proteins (psb) and 2 membrane proteins (S4 Table). The mRNA levels of CAB6A and oxygen-evolving enhancer protein 1 (psbO) were validated by qRT-PCR, which were elevated in ovaries at 4DPAP, 4DPAT and 4DPGT (Fig 4A and 4B), and additionally, their upward trends remained in 10 days after artificial pollination (S3 Fig). Furthermore, 43 DEGs associated with carbohydrate metabolism were identified. The genes encoding carbohydrate assimilation and sugar metabolism enzymes showed increased transcripts in ovaries at 4DPAP, 4DPAT and 4DPGT, e.g. sedoheptulose-1,7-bisphosphatase (SBPase), invertase, hexokinase-1 (HXK1) and fructokinase-2 (Frk2) (Fig 4C, S4 Table). Sugars produced from
assimilation could be further degraded through the glycolysis and TCA cycle to generate energy. Several genes involved in these processes were up-regulated after pollination and hormone supply, e.g. fructose-bisphosphate aldolase (FBA), pyruvate dehydrogenase (PDH), pyruvate kinase (PK) and ATP-citrate synthase (ACLY). In addition, two sugar transporter family proteins including a hexose transporter and an anion transporter 2 (ANTR2) displayed up-regulation during fruit set (S4 Table). The results suggested that photosynthesis and carbohydrate metabolism play important roles in onset of both pollination-dependent and parthenocarpic fruit development.

Expression of genes associated with cell division and cell wall during fruit set

After pollination or hormone application, cell division and cell wall related genes in ovaries were strongly induced (S5 Table). Of cell division related DEGs, 12 (one G2/mitotic-specific cyclin (CYCB1; 2), two cyclin dependent kinases and nine histones) were up-regulated in ovaries at 4DPAP, 4DPAT and 4DPGT. In addition, fruit growth was accompanied by cell wall biosynthesis and expansion. S5 Table listed 21 cell wall associated DEGs during fruit set, which including expansin, xyloglucan glycosyltransferase, pectinesterase and glucanase, among them vast majority (17/21) were up-regulated. Detailed evaluation by qRT-PCR displayed the elevated expression of EXPA15 and XTH16 in ovaries at 4DPAP, 4DPAT and 4DPGT (Fig 4E and 4F), coinciding with the RNA-Seq results. It’s obvious that the changes in cell division and cell wall metabolism are fundamental events during fruit growth.

Hormone metabolism and signaling during fruit set

Up to 59 and 84 DEGs related to hormones were screened during fruit set with respect to 2DAA and 6DPE, respectively (Fig 5A, S4 Fig and S6 Table). These genes were involved in the biosynthesis and signaling of auxin, GA, ethylene, ABA, brassinosteroid and cytokinin, among
which the former three were the most prominent (Fig 5B), indicating the important roles in co-
ordinating fruit set.

As well-known regulator in fruit set, 10 auxin related genes displayed dramatic changes in
their expressions during all the three types of fruit set with respect to 2DAA (S6 Table).

Among them, three IAA-amido synthases (GH3.1, GH3.6), SAUR-AC1, IAA26, and AUX1 were
down-regulated, while anthranilate synthase beta subunit (ASB1), ARG7 and ARF4 genes were up-regulated in ovaries at 4DPAP, 4DPAT and 4DPGT (Figs 5A, 4G and 4H, S6 Table). In particular, the expression of ARF4 was increased gradually until at least 10 days post artificial pollination, while IAA26 decreased sharply from the anthesis stage to 6 DPAP, following a slight ascent (S3 Fig). Of the 59 modulated genes related to hormones, 13 were assigned to GA regulation (Fig 5A). Both pollination and hormones application increased the expression of a GA biosynthesis gene, GA20ox1 and a GA receptor gene, GID1L2 (SGN-U568105). Remarkably, auxin treatment induced the strongest up-regulation of GA20ox1, which exhibiting 10–20 folds higher level of expression than that by pollination or GA3 treatment. Whereas, a GA 2-oxidase
gene (GA2ox4), associated with GA catabolism, was down-regulated dramatically during fruit set, which continued until at least 10 days post artificial pollination (Fig 4J, S3 Fig). Furthermore, some GA responsive proteins were down-regulated e.g. GRAS9 and GRAS6, while others displayed up-regulation e.g. GASA1, RSI-1 and GAST1 during fruit set (S6 Table). Ethylene was known to have potential role during fruit set. RNA-Seq data showed a decrease in the mRNA levels of all ethylene related genes (Fig 5A and 5B). Three ethylene biosynthesis genes (ACC oxidase, ACO, ACO2 and ACO4) and 9 ethylene signaling genes including CTR, ETR, EIN3, EBF and ERFs appeared down-regulation in ovaries at 4DPAP, 4DPAT and 4DPGT (Fig 5A, S6 Table). Validation by qRT-PCR showed that the mRNAs of ACO4 and ERF1B stayed a very low level after fruit initiation (Fig 4K and 4L), suggesting a relationship between the decrease of ethylene response and fruit set.

Identification of transcription factors associated with fruit set

RNA-seq data showed that 64 and 99 transcription factors displayed differential expression with respect to 2DAA and 6DPE respectively, and the vast majority of them were down-regulated during fruit set (Fig 6A, S5 Fig). These TFs were classified into several families including MADS-box, HB (HD-Zip and TALE), AP2/ERF, bZIP, MYB, bHLH, WRKY and GRAS, among which MADS-box, HB and AP2/ERF family recruited the major members (Fig 6B). 13 MADS-box genes displayed down-regulation in ovaries at 4DPAP, 4DPAT and 4DPGT during fruit set (Table 2). QRT-PCR analysis showed that the transcripts of four MADS-box genes, Agamous1 (AG1), Agamous-like 1 (AGL1), Deficiens (Def) and FBP24, were in dramatic declines after pollination or auxin/GA3 treatments (Fig 4O–4R). Meanwhile, there were 9 HB superfamily members (6 for HD-Zip and 3 for TALE) differentially expressed and the overwhelming majority (8/9) was down-regulated. Further evaluated by qRT-PCR showed that a TALE family member, BEL1-like homeodomain protein 9 (BLH9), underwent a down-regulation during fruit set (Fig 4S). It can be noted that these TFs, especially MADS-box and HB family may play key roles in both pollination-dependent and independent fruit set and function via down-regulating their transcription.

Strongly regulated auxin and GA signaling genes revealed differences between pollination-dependent and parthenocarpic fruit set

Considering the prominent role of auxin in the process of fruit set, we sought to identify the expression profiles of Aux/IAAs and ARFs in 4-day-old ovaries after pollination and 2,4-D /GA3 treatments with respect to 2DAA (Table 3). Throughout pollinated-dependent fruit set, some ARFs and Aux/IAAs displayed dramatic shifts in their expressions. These genes including ARF4, ARF9-1, ARF9-2, IAA2, IAA12, IAA13 and IAA14 were up-regulated and the enhanced expressions of Aux/IAAs could be needed to create a negative feedback loop[2]. Notably, 2, 4-D induction resulted in a similar regulation of these ARFs and Aux/IAAs. Others including ARF2, ARF5 and IAA26 underwent down-regulation in pollinated and GA3-treated ovaries. Interestingly, pollination and GA3 treatment inhibited the mRNA levels of IAA9, which were more strongly suppressed with respect to 6DPE than 2DAA (Table 3, S2 Table). In the case of IAA9 transcripts were higher in ovaries at 6DPE, it showed flat in 2,4-D induced ovaries (S2 Table).

In contrast with ovaries at 2DAA or 6DPE, DEGs associated with GA signaling were screened in ovaries 4 days after pollination or 2,4-D /GA3 treatment (Table 3). In particular, several genes were suppressed by GA treatment alone, such as SCL14 and GRAS7. Two GA receptors, GID1-Like (SGN-U581650 and SGN-U585573), were significantly down-regulated during pollination and GA induced fruit set. In addition, a number of genes were modulated
by both GA and auxin treatment, e.g. \textit{SCL13} and \textit{GRAS1} with down-regulation and \textit{GAI} with up-regulation, indicating that these genes may be associated with parthenocarpy (Table 3).

### Differentially expressed genes associated with parthenocarpy

RNA-Seq data indicated that throughout the process of auxin induced fruit set, a subset of genes displayed dramatic changes in their expressions (Table 4). Briefly, auxin treatment resulted in a strong up-regulation of an auxin transporter \textit{LAX2} and 3 \textit{Aux/IAAs} (\textit{IAA19}, \textit{IAA6} and \textit{IAA7}), while sharply reduced the transcripts of a MADS-box protein \textit{AGL66} and a KNOX gene \textit{KNAT3}. Also, DEGs related to parthenocarpy only assigned to the GA treatment were screened (Table 4). GA application decreased the expression of several genes including \textit{GA2ox1}, \textit{GID2} and \textit{GRAS}. Moreover, it’s interesting that the transcription level of \textit{ARF8B} was
inhibited by GA application alone, which showed no changes in ovaries at 4DPAP and 4DPAT with respect to 2DAA (S1 Table). A functionally unknown Aux/IAA gene, IAA36, was identified to be up-regulated in both auxin and GA induced ovaries. Furthermore, two argonaute genes (AGO5 and AGO10) and a transcription factor BLH1 were significantly down-regulated, implying their potential involvement in the process of parthenocarpic fruit set (Table 4).

**Discussion**

Recently, several studies have been focused on fruit set, however, the regulatory mechanisms associated with pollinated-dependent and-independent fruit set are still plausible [4, 5, 12]. Our aim is to identify transcriptomic changes occurring during the ovary development after fertilization and hormone application including auxin and GA3, in order to perceive the common features and differences between pollination-dependent and parthenocarpic fruit set.

**The common molecular features between pollination-dependent and parthenocarpic fruit set**

Global transcriptome profiling identified a great many genes showed differential expression in the developing ovaries under different treatments. Among them, approximately half were common to both fertilization-dependent and parthenocarpic fruit set, which revealing that some conserve mechanisms occur in varies fruit set processes.

Photosynthesis and carbohydrate metabolism related genes showed strongly accumulation during both pollinated-dependent and-independent fruit set (S4 Table). Although recent study

### Table 2. DEGs involved in MADS-box and homeobox family during fruit set.

| SGN accession number | TF family | 4DPAP/2DAA | 4DPAT/2DAA | 4DPGT/2DAA | Gene annotation |
|----------------------|-----------|------------|------------|------------|-----------------|
| SGN-U578526          | MADS-box  | -3.55      | -2.54      | -3.17      | AG1             |
| SGN-U568929          | MADS-box  | -1.32      | -2.16      | -1.25      | TDR6            |
| SGN-U581481          | MADS-box  | -2.04      | -3.14      | -2.00      | TM29            |
| SGN-U568823          | MADS-box  | -1.73      | -2.08      | -1.14      | Def             |
| SGN-U578103          | MADS-box  | -9.13      | -9.13      | -9.13      | JOINTLESS       |
| SGN-U577632          | MADS-box  | -1.56      | -2.28      | -1.82      | MADS11          |
| SGN-U579381          | MADS-box  | -4.37      | -5.14      | -5.36      | MADS-box protein 6 |
| SGN-U577553          | MADS-box  | -1.21      | -1.59      | -1.47      | SEPALLATA3      |
| SGN-U578128          | MADS-box  | -2.29      | -2.54      | -3.73      | fruitfull1      |
| SGN-U581068          | MADS-box  | -1.48      | -1.69      | -1.36      | AGL1            |
| SGN-U585391          | MADS-box  | -1.74      | -3.57      | -4.05      | AGL11           |
| SGN-U566000          | MADS-box  | -2.16      | -2.37      | -2.79      | MADS3           |
| SGN-U581981          | MADS-box  | -4.18      | -10.6      | -10.6      | MADS29          |
| SGN-U562997          | TALE      | -3.69      | -9.52      | -2.64      | BLH9            |
| SGN-U571928          | TALE      | -1.42      | -1.91      | -1.29      | LeT12           |
| SGN-U578026          | TALE      | -1.76      | -2.09      | -2.99      | LeT6            |
| SGN-U563285          | HD-zip    | -2.2       | -3.03      | -2.58      | HB-40           |
| SGN-U565659          | HD-zip    | -1.57      | -2.69      | -2.61      | HDG2            |
| SGN-U578039          | HD-zip    | -10.3      | -3.91      | -3.42      | HAT5            |
| SGN-U574960          | HD-zip    | -4.06      | -4.09      | -4.44      | HB-13           |
| SGN-U572955          | HD-zip    | 2.439      | 3.548      | 2.001      | HB-15           |
| SGN-U575946          | HD-zip    | -2.21      | -2.8       | -1.97      | GLABRA2         |

The fold-change value is represented by the Log2 Ratio.

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holds the opinion that metabolites for fruit growth are predominantly imported from source tissues [14], the green fruit pericarp of tomato is photosynthetically active[15]. Kolotilin et al [16] showed that the induction of genes associated with photosynthesis positively correlate with cell size in tomato pericarp cells. Our result shares the viewpoint that fruit photosynthesis plays an important role in fruit establishment [12]. Plant reproduction depends largely on a sufficient import of photoassimilates, mainly sucrose [17]. In tomato plants, the major sugars including sucrose, glucose and fructose were found accumulated in ovaries after fertilization [12]. Previous reports showed that higher levels of sucrose and starch accumulated via overexpression of \textit{SBPase} in plants[18]. Accordingly, up-regulation of \textit{SBPase} displayed after pollination and hormone application in our study (Fig 4C). Indeed, maintaining the sucrose supply and utilization, that is, degradation into hexoses is a key to sustain the fruit set developmental process. The role of invertase in regulating sucrose metabolism has long been postulated[19]. RNAi-mediated silencing of an invertase gene, \textit{Lin5}, aggravated fruit abortion in tomato [20], and vice versa [21]. Here, we reported that an \textit{invertase} gene (SGN-U579353) was up-regulated in ovaries after pollination and hormone treatments (S4 Table). The results seemingly confirm the theory that glucose generated by invertase serves as a signal to repress programmed cell

| SGN accession number | 4DPAP/2DAA | 4DPAT/2DAA | 4DPGT/2DAA | Gene annotation |
|----------------------|------------|------------|------------|----------------|
| SGN-U568849          | -1.50      | 1.21       | -0.58      | IAA9           |
| SGN-U569639          | 2.26       | 2.73       | 2.85       | ARF4           |
| SGN-U571269          | -1.90      | -          | -5.25      | ARF5           |
| SGN-U573372          | -2.69      | -1.82      | -3.51      | IAA26          |
| SGN-U574088          | -          | -          | -1.04      | ARF8B          |
| SGN-U576826          | 4.85       | 4.35       | -          | ARF9-1         |
| SGN-U577682          | -          | 1.15       | -          | IAA6           |
| SGN-U579354          | 2.97       | 4.57       | -          | IAA13          |
| SGN-U579607          | -          | 1.64       | -          | IAA19          |
| SGN-U579618          | 1.92       | 3.21       | -          | IAA14          |
| SGN-U579795          | 1.49       | 2.78       | -          | IAA12          |
| SGN-U579928          | -1.33      | -          | -1.69      | ARF2           |
| SGN-U580599          | 9.30       | 8.61       | -          | ARF9-2         |
| SGN-U586760          | -2.34      | 1.92       | -          | IAA36          |
| SGN-U592947          | -          | 9.26       | -          | IAA7           |
| SGN-U599474          | 3.96       | 5.30       | -          | IAA2           |
| SGN-U566795          | -          | -1.53      | -3.24      | SCL13          |
| SGN-U568105          | 1.82       | 1.76       | 2.12       | GID1L2         |
| SGN-U568385          | -          | -          | -1.6       | SCL14          |
| SGN-U569734          | -1.6       | -2.19      | -2.85      | GRAS6          |
| SGN-U572192          | -2.79      | -3.42      | -4.15      | GID1L2         |
| SGN-U575114          | -          | 1.27       | 1.03       | GAI            |
| SGN-U575808          | -          | 1.00       | 2.03       | GID1L3         |
| SGN-U580033          | -          | -          | -1.45      | GRAS7          |
| SGN-U581650          | -2         | -          | -2.97      | GID1B-like     |
| SGN-U581883          | -          | -3.77      | -2.7       | GID1-like      |
| SGN-U583815          | -2.01      | -1.25      | -1.91      | GRAS9          |
| SGN-U585152          | -          | -2.08      | -1.19      | GRAS1          |
| SGN-U585573          | -1.77      | -          | -1.23      | GID1L3         |

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death (PCD) and to promote cell division which lead to fruit set [17]. Fructokinase (Frk), as a limited enzyme in sucrose metabolism, was significantly up-regulated during fruit set (S4 Table), which is in accordance with a recent study that Frk gene suppression resulted a reduction in flower development and fruit setting in tomato[22], implying its significant role in fruit development. Moreover, sugar metabolism may be regulated by the up-regulation of ARF4 (Fig 4G) during fruit set [23].

Down-regulation of a large number of transcription factors is another striking feature during fruit set (Fig 6A, S5 Fig). Among them, MADS-box and homeobox genes were the most represented members, especially the former (Table 2). Notably, several MADS-box genes identified by RNA-Seq have been functionally characterized. Mazzucato et al.[24] indicated a role for SlDef in the control of fruit set. Down-regulation of AG1 displays the replacement of carpels with pseudocarpels bearing indeterminate floral meristems and strong reduction of seed production [25]. Similar result occurs in FBP24 that the knockdown line produces a few seeds [26]. In addition, down-regulation of TM29 produces infertile pistil coupled with parthenocarpic fruit formation[27]. In total, we agree with the hypothesis that the low expression of MADS-box promotes fruit set even in the absence of fertilization[12]. However, we are as yet unable to exclude this possibility that other MADS-box genes have roles in early fruit development (Table 2). Two Knotted-like homeobox (KNOX) genes, which can repress GA biosynthesis by the repression of GA20ox genes[28], LeT6 and LeT12, were found down-regulation after

Table 4. DEGs associated with parthenocarpy induced by auxin and GA.

| SGN accession number | 4DPAT/2DAA | 4DPGT/2DAA | Gene annotation |
|----------------------|------------|------------|-----------------|
| SGN-U579607          | 1.64       | -          | AUX/IAA family protein, IAA19 |
| SGN-U577682          | 1.15       | -          | AUX/IAA family protein, IAA6 |
| SGN-U592947          | 9.26       | -          | AUX/IAA family protein, IAA7 |
| SGN-U583242          | 8.61       | -          | Auxin transporter-like protein, LAX2 |
| SGN-U569018          | 9.39       | -          | SAUR, auxin-induced protein 6B |
| SGN-U573534          | 4.91       | -          | Probable IAA-amido synthetase GH3.1 |
| SGN-U585261          | 1.1        | -          | Probable IAA-amido synthetase GH3.1 |
| SGN-U572630          | 2.12       | -          | homeobox protein knotted-1 like 1 (KN1) |
| SGN-U571929          | -2.2       | -          | homeobox protein knotted-1 like 3 (KNAT3) |
| SGN-U569575          | -2.1       | -          | MADS-box protein AGL66 |
| SGN-U574088          | -          | -1.04      | auxin-responsive factor (ARF8B) |
| SGN-U572513          | -          | -1.2       | SLEEPY1 protein, GID2 |
| SGN-U597732          | -          | -1.38      | gibberellin 2-oxidase, GA2ox1 |
| SGN-U572163          | -          | -1.53      | Gibberellin-regulated protein 6 |
| SGN-U580033          | -          | -1.45      | GRAS family, GRAS7 |
| SGN-U568385          | -          | -1.56      | GRAS family, SCL14 |
| SGN-U604674          | -          | -8.78      | homeobox family protein, HDG5 |
| SGN-U563343          | -8.78      | -8.8       | Argonaute protein, AGO10 |
| SGN-U562884          | -1.09      | -1.2       | Argonaute protein, AGO5 |
| SGN-U586760          | 2.34       | 1.92       | AUX/IAA family protein, IAA36 |
| SGN-U578015          | -2.74      | -1.3       | BEL1-like homeodomain protein 1, BLH1 |
| SGN-U575114          | 1.27       | 1.03       | DELLA protein, GAI |
| SGN-U581883          | -3.77      | -2.7       | gibberellin receptor, GID1-like |
| SGN-U575808          | 1          | 2.03       | gibberellin receptor, GID1L3 |
| SGN-U585152          | -2.08      | -1.2       | GRAS family, GRAS1 |
| SGN-U566795          | -1.53      | -3.2       | GRAS family, SCL13 |

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fertilization and auxin/GA$_3$ treatments (Table 2), which is in agreement with the higher expression of GA20ox1. Previous study in wild-type tomato and the pat mutant also acquired the same results [29]. It’s indicating that LeT6 and LeT12 might act as key negative regulators of fruit set.

A large number of genes involved in hormone biosynthesis and signaling were identified to be altered during fruit set (Fig 5A). Besides auxin and GA, ethylene was the most represented (Fig 5B). Previous study in Arabidopsis ethylene mutants showed that ethylene is involved in the modulation of the ovule senescence and the determination of the pistil fate during fruit set [30]. In our study, genes involved in ethylene biosynthesis (ACOs) and signaling (ETR, EIN3, ERFs) were down-regulated after fertilization and auxin/GA$_3$ treatments (S6 Table). It is in accordance with the results in tomato performed by Vriezen [5] and in zucchini squash performed by Martínez [31]. Recently, Switzenberg et al. [32] reported that inhibition of ethylene perception ETR1 resulted in earlier fruit set and greater number of fruits. We also found that parthenocarpy occurred in EIN3/EIL1 co-suppression tomato lines (data not published). Taken together, ethylene is considered to be a key regulator in coordinating the process of fruit set.

Regulation of pollination-dependent and parthenocarpic fruit set by auxin and GA

In the present study, we found that pollination and 2,4-D application can bring about the changes in the expression of the auxin signaling components, Aux/IAAs and ARFs, while these genes were hardly regulated by the GA$_3$ treatment e.g. ARF9 (Table 3). Conversely, a GA synthesis gene, GA20ox1 was strongly elevated by 2,4-D application. Moreover, GA signaling genes can be modulated by auxin (Table 3). These findings support the observation that auxin may act prior to GA during natural fruit initiation and auxin-induced fruit set is mediated in part by GA [2]. It has been reported that down-regulation of IAA9 leads to parthenocarpy [12]. In accordance with this, IAA9 transcript levels were decreased after pollination and GA$_3$ treatment, but not in 2,4-D treated ovaries (Table 3, S1 Table and S2 Table). Serrani et al. [13] also reported that increase in IAA9 transcript level was exhibited in 2, 4-D treated ovaries. It is possible that 2, 4-D induced parthenocarpy is not mediated by IAA9 down-regulation [13] and the parthenocarpic fruit set by IAA9 silence is mediated by modified GA responses through auxin/GA crosstalk [8]. ARF2, identified to be involved in crosstalk between auxin and GA mediated by GID1, was suppressed after GA$_3$ treatment (Table 3). Similarly, Richter et al. [33] reported that GA treatment promotes ARF2: GFP abundance, but with a decrease in ARF2 transcript level, which is explained by the hypothesis that ARF2 protein may repress its own transcription as part of a negative feedback regulatory loop. Therefore, we speculate that ARF2 plays a central role in fruit initiation, which is supported by our previous study that overexpression of ARF2 results in an increase of fruit number and reduction of seed production (data not published). Interestingly, ARF8B was only modulated by GA$_3$ application, with a decrease in its expression (Table 4, S1 Table). It’s in agreement with the fact that seedless fruits were obtained in ARF8 loss of function mutants [9]. These findings indicate that GA-induced parthenocarpy may be mediated by ARF2 and ARF8 down-regulation.

A subset of genes associated with GA signaling was identified to be altered during ovary development. Six GID1-like genes, encoding putative GA receptors, were modulated during fruit set. While previous studies also showed GID1 transcripts were regulated in response to pollination or GA application [6, 13]. GID2, an F-box protein that interacts directly with DELLA and mediates its degradation, is a positive regulator of GA signaling [34, 35]. Here, we reported that GID2 transcript was repressed only by GA$_3$ application (Table 4), and same result was recruited by Vriezen [5]. It seems that GID2 expression is subject to GA feedback in order to sustain GA.
homeostasis. We also speculated that down-regulation of GID2 may be involved in GA mediated parthenocarpy. DELLA, act as a GA repressor, is a key member in GA signaling. Reduction of SIDELLA mRNA levels induced parthenocarpy [36]. A member of DELLA gene family, GAI, was up-regulated after GA3 and 2, 4-D application in our dataset, which is in agreement with the results described by Serrani [13], suggesting feedback regulation. This result achieves equal outlook that DELLA proteins mediate repression of DELLA transcription[37]. In addition, other GRAS family members without DELLA domain draw attention regarding their significant down-regulation (Table 3). However, it is noted that the roles of them on GA response and fruit development are largely unknown.

Pagnussat et al.[38] reported that the interaction of BLH1 and KNAT3 was considered to compose functional complexes regulating normal development and cell specification in the Arabidopsis embryo sac. In the present study, auxin application resulted in a dramatic decline in the expression of KNAT3. And BLH1 displayed down-regulation during both auxin-and GA-mediated fruit set (Table 4). Moreover, parthenocarpy was induced in BLH1 loss of function mutants [39]. Hence, auxin/GA induced parthenocarpy might be mediated by BLH1–KNAT3 interaction. In addition, AGL66 transcript was found to be repressed (Table 4), which was also occurred in parthenocarpy lines with PIN4 silence, indicating its potential role in the formation of seedless fruits[7]. We also found that AGO10 and AGO5 were significantly down-regulated (Table 4). AGO10, as a critical regulator of SAM maintenance, functions by interacting miR165/166. It has been reported that miR166 levels are increased in ago10 loss of function mutants [40]. We hypothesis that down-regulation of AGO10 causes miR166 accumulation in early developing ovaries and subsequent regulation of fruit growth driven by target genes. Furthermore, it has been revealed that overexpression of miR166 by a fruit specific promoter p2A11 results in a remarkable shift in tomato carpel development, that is production of secondary fruits at the apex and seedless fruits (data not published). Therefore, the results indicate the major roles of these genes in parthenocarpic fruit set.

Conclusions

In summary, regulatory events underlying pollination-induced and parthenocarpic fruit set are revealed in our work. The model presented in Fig 7 highlights the roles of auxin and GA on fruit set and the crosstalk between the two hormones. We proposed that the effect of auxin on pollinated dependent and parthenocarpic fruit set is mediated by GAs to some extent. Auxin can elevate GA biosynthesis gene GA20ox1, which may be partially attributed to the down-regulation of KNOX genes that act as negative regulators on GA20ox1, thus control GA responses. Alternatively, the crosstalk between auxin and GA involves in ARF2 and IAA9 down-regulation. In contrast to pollination, a much stronger activation of Aux/IAAs and feedback of GID2 and DELLA in their transcripts displayed after 2, 4-D and GA3 application respectively, suggesting their roles in fine-tuning hormone response and regulating parthenocarpic fruit set.

It is likely that whilst auxin and GA-mediated parthenocarpy act in partially distinct cascades, some pathways are common to pollination and hormones induced fruit set. Of particular note was the concerted activation of carbohydrates metabolism, cell division and expansion as well as the down-regulation of transcription factors especially in MADS-box after pollination or hormone application, suggesting that they may functioned as key components in regulating fruit initiation and ovary development. In addition, ethylene is considered to be a key regulator in coordinating the process of fruit set. In the further work, metabolomics tools would be employed to analysis the metabolic changes during fruit set, which is coupled with our transcriptome data in order to elucidate the mechanism of seed formation in pollinated ovaries and differences in morphology between auxin and GA triggered seedless fruits.
Fig 7. Model for regulatory events underlying fruit set mainly mediated by auxin and gibberellin. (A) After pollination, the increase of auxin and GA biosynthesis and response will trigger fruit initiation. And the elevated auxin and GA would inhibit ethylene biosynthesis and response which negatively regulate fruit set. Auxin stimulates GA biosynthesis gene GA20ox1 via down-regulating KNOX (LeT6, LeT12) in partially, subsequently influences GA response by regulating GID1 and GRAS. Some Aux/IAAs may be degraded by the 26S proteasome and in turn, the transcripts are increased via feedback regulation to fine-tune auxin response. Some ARFs (ARF4, ARF9) are up-regulated which could promote auxin signaling cascades. In addition, auxin response may be mediated by GA via down-regulation of IAA9 and ARF2 during fruit set. Accordingly, events that the activation of carbohydrate metabolism, cell division and expansion as well as the down-regulation of MADS-box occur to promote fruit set process. (B) After 2, 4-D application, KNAT3 displayed down-regulation, which would interact with BLH1, thus negatively regulating parthenocarpy. In the present of GA, the transcripts of DELLa and GID2 are regulated via feedback to fine-tune GA response. GA application decreased the transcripts of IAA9 and ARF8B, and then led to parthenocarpy. Moreover, other
Materials and Methods

Plant materials and growth conditions

Tomato plants (*Solanum lycopersicum* L. cv micro-tom) were used in the experiments. Seeds were germinated in 1/2 MS medium, and then transplanted to 6 L pots containing a mixture of peat: sludge: perlite(6:1:1). Plants (four per pot) were cultured in a greenhouse under standard conditions (25°C/20°C day/night temperature, 80% humidity and natural light was supplemented with lamps to get a 14 h photoperiod) and supplied with 500 mL of Hoagland solution every 7 days.

Flower emasculation was carried out 2 days before anthesis to avoid self-pollination. Artificial pollination was performed on the day just equivalent to anthesis. The ovaries were collected as samples for RNA-seq at the 2 days ahead of anthesis (2DAA, control) and 4 days after artificial pollination (4DPAP). Besides, the ovaries at 0, 2, 6, 8 and 10 days after artificial pollination were collected (0 DPA, 2 DPAP~10 DPAP).

Application of auxin and gibberellin

According to Serrani et al. [11], unpollinated ovaries responded to different auxins (IAA, NAA and 2,4-D), 2,4-D being the most efficient. Serrani et al.[3] also revealed that unpollinated ovaries developed parthenocarpically in response to GA₃ > GA₁ = GA₄ > GA₂₀. Therefore, 2,4-D and GA₃ (Sigma Aldrich, USA) were used in this study. Briefly, 2,4-D and GA₃ were dissolved in ethanol as storage solution, then diluted with distilled water including 5% ethanol and 0.1% Tween 80 when worked to the concentration of 2,4-D and GA₃ at 0.05mM and 0.3mM, respectively. The stigmas were treated with 2,4-D and GA₃ in 10μL solution on the day just equivalent to anthesis. After 4 days, the uniform ovaries were collected and named as 4DPAT and 4DPGT which represented ovaries obtained from 4 days after auxin and GA₃ treatment, respectively. Meanwhile, the untreated ovaries (6DPE) were collected as another control. For samples collection, three biological replicates were performed (40 ovaries on controls and 15 ovaries on the other treatments approximately in each replicate). All collected samples were frozen in liquid N₂ and stored at -80°C until RNA-Seq experiment.

RNA-Seq analysis

Total RNA from ovary samples including 2DAA, 6DPE, 4DPAP, 4DPAT and 4DPGT was extracted using Trizol reagent (Invitrogen, USA) according to manufacturer’s instruction. Poly A RNA purification, cDNA synthesis, tag preparation and RNA-Seq were performed by technicians in line with specified experimental process at Beijing Genome Institute (BGI) (Shenzhen, China). Briefly, mRNA are enriched through beads of Oligo (d'T) and transferred into double-stranded cDNA via reverse transcription. cDNA is digested with *NlaIII* which cut off CATG sites and Illumina adaptor 1 is ligated to the 5’ end of fragments. Then, *Mmel* is used to digest at 17 bp downstream of CATG site and Illumina adaptor 2 is ligated at 3’ end. Finally, 95 bp fragments produced by PCR amplification are purified through 6% TBE PAGE and sequenced using Illumina HiSeq 2000 system.
Sequence assembly and gene annotation

The raw sequences were filtered by removing 3' adaptor fragments as well as low quality sequences (tags with unknown sequence "N") and several types of impurities which are too long or too short and with a copy number of 1. After that, raw reads were transformed into clean tags. To identify the gene expression signatures from the four types of tomato libraries, the SGN unigene database (ftp://ftp.sgn.cornell.edu/unigene_builds/combined_species_assemblies/tomato_species/curr/tomato_species_unigenes.seq.gz) was used as a reference database. All clean tags were aligned to the reference sequences using SOAP [41], only allowing 1 bp mismatch. Clean tags with multiple matches were excluded. The number of unambiguous clean tags for each gene was calculated and then normalized to TPM (number of transcripts per million clean tags) [42, 43].

Screening of differentially expressed genes

R package DESeq2 was applied to identify differentially expressed genes with random sampling model [44]. A P-value could denote its expression difference between two libraries, and false discovery rates (FDRs) were used to determine the threshold of P value. We set "FDR\leq0.001 and the absolute value of \log2Ratio\geq1" as the threshold to judge the significance of gene expression difference according to described method [45]. Cluster analysis of differentially expressed genes was performed using "cluster" [46] and "Java Treeview" [47] software. The value of log2 ratio was used for the hierarchical clustering analysis. Gene ontology (GO) terms of differentially expressed genes were identified by the software Blast2GO (version 2.3.4) [48] using the default parameters. The hypergeometric test was applied to perform GO enrichment analysis of functional significance when all differentially expressed genes were mapped to terms in GO database. Blast2GO was also employed to identify significantly altered pathways during fruit set. Pathways with Q value \leq0.05 are considered significantly enriched in DEGs.

Sequence deposition

The raw transcriptome reads reported here have been deposited in the NCBI Short Read Archive under accession Nos. SRX850785 (2DAA), SRX850788 (4DPAP), SRX850793 (4DPAT), SRX850794 (4DPGT) and SRX850795 (6DPE).

Validation of RNA-seq data by quantitative real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, USA) from different ovary samples. cDNA was synthesized from 2 μg of total DNA-free RNA using RevertAid First Strand cDNA Synthesis Kit (Fermentas, USA) according to the manufacturer’s instruction and diluted five folds as the template. Gene-specific primers for selected genes were designed by online software (https://www.genscript.com/ssl-bin/app/primer) (S7 Table). And melt curve analysis was used to confirm the specificity. Quantitative real-time PCR (qRT-PCR) were carried out using SsoFast EvaGreen Supermix (Bio-Rad, USA) on an Bio-Rad CFX96 real-time PCR detection system by the three-step method, which was incubated at 95°C for 5 min, then followed by 40 cycles of 95°C for 5 s, 60°C for 5 s and 72°C for 5 s. For qPCR experiments, three biological replicates were performed. Relative expression levels were calculated based on the $2^{-\Delta\Delta Ct}$ method using actin as reference gene.
Supporting Information

S1 Fig. Saturation evaluation and distribution analysis of the RNA-Seq tags in the five libraries.
(TIF)

S2 Fig. Functional categorization of DEGs during fruit set based on biological process of Gene Ontology.
(TIF)

S3 Fig. The expressions of selected genes in ovaries after artificial pollination.
(TIF)

S4 Fig. Cluster analysis of DEGs related to hormone during fruit set.
(TIF)

S5 Fig. Cluster analysis of DEGs related to transcription factors.
(TIF)

S1 Table. The DEGs in ovaries during fruit set with respect to 2DAA in tomato.
(XLSX)

S2 Table. The DEGs in ovaries during fruit set with respect to 6DPE in tomato.
(XLSX)

S3 Table. The common and distinct DEGs during the three types of fruit set in tomato.
(XLSX)

S4 Table. DEGs involved in photosynthesis and carbohydrate metabolism during fruit set.
(XLSX)

S5 Table. DEGs involved in cell division and cell wall metabolism during fruit set.
(XLSX)

S6 Table. DEGs involved in hormone biosynthesis and signaling during fruit set.
(XLSX)

S7 Table. Primers used for qRT-PCR and the accession numbers of the relevant genes.
(XLSX)

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Author Contributions

Conceived and designed the experiments: NT ZGL. Performed the experiments: NT. Analyzed the data: NT GJH. Contributed reagents/materials/analysis tools: NT NH. Wrote the paper: NT. Helped draft and revise the manuscript: WD.

References

1. Gillaspy G, Ben-David H, Gruissem W. Fruits: a developmental perspective. The Plant Cell. 1993; 5 (10):1439. PMID: 12271039
2. De Jong M, Mariani C, Vriezen WH. The role of auxin and gibberellin in tomato fruit set. Journal of experimental botany. 2009; 60(5):1523–32. doi: 10.1093/jxb/erp094 PMID: 19321650
3. Serrani JC, Sanjuán R, Ruiz-Rivero O, Fos M, García-Martínez JL. Gibberellin regulation of fruit set and growth in tomato. Plant physiology. 2007; 145(1):246–57. PMID: 17660355

4. Pascual L, Blanca JM, Cañizares J, Nuez F. Transcriptomic analysis of tomato carpel development reveals alterations in ethylene and gibberellin synthesis during pat3/pat4 parthenocarpic fruit set. BMC plant biology. 2009; 9(1):67.

5. Vriezen WH, Feron R, Maretto F, Keijman J, Mariani C. Changes in tomato ovary transcriptome demonstrate complex hormonal regulation of fruit set. New Phytologist. 2008; 177(1):60–76. PMID: 18028300

6. Ding J, Chen B, Xia X, Mao W, Shi K, Zhou Y, et al. Cytokinin-induced parthenocarpic fruit development in tomato is partly dependent on enhanced gibberellin and auxin biosynthesis. PLoS one. 2013; 8(7): e70080. doi: 10.1371/journal.pone.0070080 PMID: 23922914

7. Mounet F, Moing A, Kowalczyk M, Rohrmann J, Petir J, Garcia V, et al. Down-regulation of a single auxin efflux transport protein in tomato induces precocious fruit development. Journal of experimental botany. 2012; 63(13):4901–17. doi: 10.1093/jxb/ers167 PMID: 22844095

8. Wang H, Jones B, Li Z, Frasse P, Delalande C, Regad F, et al. The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. The Plant Cell Online. 2005; 17(10):2676–92. PMID: 16126837

9. Goetz M, Hooper LC, Johnson SD, Rodrigues JCM, Vivian-Smith A, Koltunow AM. Expression of aberrant forms of AUXIN RESPONSE FACTOR8 stimulates parthenocarpy in Arabidopsis and tomato. Plant Physiology. 2007; 145(2):351–66. PMID: 17766399

10. De Jong M, Wolters-Arts M, Feron R, Mariani C, Vriezen WH. The Solanum lycopersicum auxin response factor 7 (SIARF7) regulates auxin signaling during tomato fruit set and development. The Plant Journal. 2009; 57(1):160–70. doi: 10.1111/j.1365-313X.2008.03671.x PMID: 18778404

11. Serrani JC, Fos M, Atarés A, García-Martínez JL. Effect of gibberellin and auxin on parthenocarpic fruit growth induction in the cv Micro-Tom of tomato. Journal of Plant Growth Regulation. 2007; 26(3):211–21.

12. Wang H, Schauer N, Usadel B, Frasse P, Zouine M, Hernould M, et al. Regulatory features underlying pollination-dependent and-independent tomato fruit set revealed by Plant Cell Online and primary metabolome profiling. The Plant Journal. 2009; 21(5):1428–52.

13. Serrani JC, Ruiz-Rivero O, Fos M, García-Martínez JL. Auxin-induced fruit-set in tomato is mediated in part by gibberellins. The Plant Journal. 2008; 66(6):922–34. doi: 10.1111/j.1365-313X.2008.03654.x PMID: 18702668

14. Do PT, Prudent M, Sulpice R, Causse M, Fernie AR. The influence of fruit load on the tomato pericarp metabolome in a Solanum chmielewskii introgression line population. Plant physiology. 2010; 154(3):1128–42. doi: 10.1104/pp.110.163030 PMID: 20841452

15. Piechulla B, Glick RE, Bahl H, Melis A, Gruissem W. Changes in photosynthetic capacity and photosynthetic protein pattern during tomato fruit ripening. Plant physiology. 1987; 84(3):911–7. PMID: 16665543

16. Kolotilin I, Koltau H, Tadmor Y, Bar-Or C, Reuveni M, Meir A, et al. Transcriptional profiling of high pigment-2dg tomato mutant links early fruit plastid biogenesis with its overproduction of phytosterols. Plant physiology. 2007; 145(2):389–401. PMID: 17704236

17. Ruan Y-L, Patrick JW, Bouzayen M, Osorio S, Fernie AR. Molecular regulation of seed and fruit set. Trends in plant science. 2012; 17(11):656–65. doi: 10.1016/j.tplants.2012.06.005 PMID: 22776090

18. Lefebvre S, Lawson T, Fryer M, Zakhleniuk OV, Lloyd JC, Raines CA. Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. Plant Physiology. 2005; 138(1):451–60. PMID: 15863701

19. Carrari F, Baxter C, Usadel B, Urbanczyk-Wochniak E, Zanor M-I, Nunes-Nesi A, et al. Integrated analysis of metabolite and transcript levels reveals the metabolic shifts that underlie tomato fruit development and highlight regulatory aspects of metabolic network behavior. Plant Physiology. 2006; 142(4):1380–96. PMID: 17071647

20. Zanor MI, Osorio S, Nunes-Nesi A, Carrari F, Lohse M, Usadel B, et al. RNA interference of LIN5 in tomato confirms its role in controlling Brix content, uncovers the influence of sugars on the levels of fruit hormones, and demonstrates the importance of sucrose cleavage for normal fruit development and fertility. Plant Physiology. 2009; 150(3):1204–18. doi: 10.1104/pp.109.136598 PMID: 19439574

21. Jin Y, Ni D-A, Ruan Y-L. Posttranslational elevation of cell wall invertase activity by silencing its inhibitor in tomato delays leaf senescence and increases seed weight and fruit hexose level. The Plant Cell Online. 2009; 21(7):2072–89. doi: 10.1105/tpc.108.063719 PMID: 19574437

22. Odanaka S, Bennett AB, Kanayama Y. Distinct Physiological Roles of Fructokinase Isozymes Revealed by Gene-Specific Suppression of Frk1 and Frk2 Expression in Tomato. Plant physiology. 2002; 129(3):1119–26. PMID: 12114566
23. Sagar M, Chervin C, Mila I, Hao Y, Roustan J-P, Benichou M, et al. SIARF4, an auxin response factor involved in the control of sugar metabolism during tomato fruit development. Plant physiology, 2013; 161(3):1362–74. doi: 10.1104/pp.113.213843 PMID: 23341361

24. Mazzucato A, Olimpieri I, Siligato F, Picarella ME, Soressi GP. Characterization of genes controlling stamen identity and development in a parthenocarpic tomato mutant indicates a role for the DEFICIENS ortholog in the control of fruit set. Physiologia plantarum. 2008; 132(4):526–37. doi: 10.1111/j.1399-3054.2007.01035.x PMID: 18334005

25. Penuel L, Hareven D, Rounsley SD, Yanofsky MF, Lifschitz E. Isolation of the tomato AGAMOUS gene TAG1 and analysis of its homeotic role in transgenic plants. The Plant Cell Online. 1994; 6(2):163–73.

26. De Folter S, Shchennikova AV, Franken J, Baskar M, Grossniklaus U, et al. A Bsister MADS-box gene involved in ovule and seed development in petunia and Arabidopsis. The Plant Journal. 2006; 47(6):934–46. PMID: 16925602

27. Ampomah-Dwamena C, Morris BA, Sutherland P, Veit B, Yao J-L. Down-Regulation of TM29, a Tomato SEPALLATA Homolog, Causes Parthenocarpic Fruit Development and Floral Reversion. Plant Physiology. 2002; 130(2):605–17. PMID: 12376628

28. Hay A, Kaur H, Phillips A, Hedden P, Hake S, Tsiantis M. The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body plans. Current Biology. 2002; 12(18):1557–65. PMID: 12372247

29. Olimpieri I, Siligato F, Caccia R, Soressi GP, Mazzucato A, Mariotti L, et al. Tomato fruit set driven by pollination or by the parthenocarpic fruit allele are mediated by transcriptionally regulated gibberellin biosynthesis. Planta. 2007; 226(4):877–88. PMID: 17503074

30. Carbonell-Bejerano P, Urbez C, Granell A, Carbonell J, Perez-Amador MA. Ethylene is involved in pistil fate by modulating the onset of ovule senescence and the GA-mediated fruit set in Arabidopsis. BMC plant biology. 2011; 11(1):84.

31. Martínez C, Manzano S, Megías Z, Garrido D, Pico B, Jamilena M. Involvement of ethylene biosynthesis and signalling in fruit set and early fruit development in zucchini squash (Cucurbita pepo L.). BMC plant biology. 2013; 13(1):139.

32. Switzenberg JA, Beaudry RM, Grumet R. Effect of CRC::etr1-1 transgene expression on ethylene production, sex expression, fruit set and fruit ripening in transgenic melon (Cucumis melo L.). Transgenic research. 2014; 1–11. doi: 10.1007/s10722-014-9843-7 PMID: 25344849

33. Richter R, Behringer C, Zourelidou M, Schwechheimer C. Convergence of auxin and gibberellin signalling on the regulation of the GATA transcription factors GNC and GNL in Arabidopsis thaliana. Proceedings of the National Academy of Sciences. 2013; 110(32):13192–7. doi: 10.1073/pnas.1304250110 PMID: 23878229

34. Griffiths J, Murase K, Rieu I, Zentella R, Zhang Z-L, Powers SJ, et al. Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. The Plant Cell Online. 2006; 18(12):3399–414. PMID: 17194763

35. Sasaki A, Itoh H, Gomi K, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, et al. Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. Science. 2003; 299(5614):1896–8. PMID: 12649483

36. Martí C, Orzáez D, Ellul P, Moreno V, Carbonell J, Granell A. Silencing of DELLA induces facultative parthenocarpy in tomato fruits. The Plant Journal. 2007; 52(5):865–76. PMID: 17883372

37. Middleton AM, Úbeda-Tomás S, Griffiths J, Holman T, Hedden P, Thomas SG, et al. Mathematical modeling elucidates the role of transcriptional feedback in gibberellin signaling. Proceedings of the National Academy of Sciences. 2012; 109(19):7571–6. doi: 10.1073/pnas.1304250110 PMID: 22523240

38. Pagnussat GC, Yu H-J, Sundaresan V. Cell-fate switch of synergid to egg cell in Arabidopsis eostre mutant embryo sacs arises from misexpression of the DELLA-like homeodomain gene BLH1. The Plant Cell Online. 2007; 19(11):3578–92. PMID: 18055603

39. Vivian-Smith A. The molecular basis for the initiation of fruit development and parthenocarpy: The University of Adelaide; 2000.

40. Zhu H, Hu F, Wang R, Zhou X, Sze S-H, Liou LW, et al. Arabidopsis Argonaute10 Specifically Sequesters miR166/165 to Regulate Shoot Apical Meristem Development. Cell. 2011 Apr 15; 145(2):242–56. PMID: WOS:000289549900013. doi: 10.1016/j.cell.2011.03.024

41. Li R, Li Y, Kristiansen K, Wang J. SOAP: short oligonucleotide alignment program. Bioinformatics. 2008; 24(5):713–4. doi: 10.1093/bioinformatics/btn025 PMID: 18227114

42. Morrissy AS, Morin RD, Delaney A, Zeng T, McDonald H, Jones S, et al. Next-generation tag sequencing for cancer gene expression profiling. Genome research. 2009; 19(10):1825–35. doi: 10.1101/gr.094482.109 PMID: 19541910
43. AC't Hoen P, Ariyurek Y, Thygesen HH, Vreugdenhil E, Vossen RH, de Menezes RX, et al. Deep sequencing-based expression analysis shows major advances in robustness, resolution and inter-lab portability over five microarray platforms. Nucleic acids research. 2008; 36(21):e141–e. doi: 10.1093/nar/gkn705 PMID: 18927111

44. Wang L, Feng Z, Wang X, Wang X, Zhang X. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. Bioinformatics. 2010; 26(1):136–8. doi: 10.1093/bioinformatics/btp612 PMID: 19855105

45. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. Annals of statistics. 2001:1165–88.

46. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proceedings of the National Academy of Sciences. 1998; 95(25):14863–8. PMID: 9843981

47. Saldanha AJ. Java Treeview—extensible visualization of microarray data. Bioinformatics. 2004; 20(17):3246–8. PMID: 15180930

48. Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics. 2005; 21(18):3674–6. PMID: 16081474