

Characterization of Auxin Metabolism in the Ovaries of the Lychee (*Litchi chinensis*) ‘Salathiel’

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Seed size affects the edible portion rate of lychee fruit and is therefore an important trait for fruit quality in lychee (*Litchi chinensis*). Lychee fruits can be classified into four categories in terms of seed properties: normal, small, aborted seed and seedless. ‘Salathiel’ is known to bear a relatively high rate of aborted-seeded (pseudo-parthenocarpy, stenospermocarpy) and seedless (parthenocarpy) fruit regardless of environmental and cultivation conditions. In other horticultural fruit crops, such as tomato, auxin metabolism and signaling is critical for parthenocarpy, although auxin metabolism has not been characterized in relation to (pseudo) parthenocarpy in lychee. The purpose of this study was to characterize the physiological and morphological properties of maternal reproductive organs in ‘Salathiel’ female flowers, with a specific focus on the associated auxin metabolism. Microscopic observations of the internal structures of the ‘Salathiel’ embryo revealed that ‘Salathiel’ reproductive organ differentiation is similar to that of normal-seeded cultivars. However, the obturator was significantly smaller in ‘Salathiel’ than in other normal-seeded cultivars, suggesting that specific developmental characteristics may exist in the maternal reproductive organs of ‘Salathiel’. Our investigation of indole acetic acid (IAA) contents revealed that IAA levels were significantly higher in ‘Salathiel’ than in other cultivars. Moreover, the IAA metabolite contents also differed significantly between ‘Salathiel’ and the normal-seeded and small-seeded cultivars. Specifically, N-3-hydroxy-2-oxindole-3-acetyl glutamic acid contents were significantly higher in ‘Salathiel’ than in ‘Yu Her Pau’ and ‘Hei Ye’. 3-hydroxy-2-oxindole-3-acetic acid was lower in ‘Yu Her Pau’ and ‘Salathiel’ than in ‘Hei Ye’, while indole-acetylaspatic acid was significantly lower in ‘Salathiel’ compared to ‘Hei Ye’. Expression analyses of the genes related to auxin biosynthesis, catabolism, transport, and signaling indicated that the IAA influx-related and efflux-related gene expression levels were respectively higher and lower in ‘Salathiel’ than in the other cultivars, which is consistent with the increased IAA accumulation in the ‘Salathiel’ ovary. The possible involvement of auxin metabolism in the aborted-seeded and seedless fruit production in ‘Salathiel’ is discussed herein.

Key Words: auxin transporter, indole acetic acid (IAA), parthenocarpy, pseudo-parthenocarpy (stenospermocarpy), seed.

Introduction

Lychee (*Litchi chinensis* Sonn.), which is a subtropical fruit tree species belonging to the family Sapindaceae, originated in the region between southern China, northern Vietnam, and Myanmar (Mitra and

Pathak, 2010). In lychee, seed size is an important factor influencing lychee fruit quality and value (Kays, 1999; Menzel, 2002). Almost all common lychee cultivars have one seed per fruit, and seed size greatly affects the amount of edible flesh per fruit as well as how easily it can be eaten. Lychee fruit containing a small aborted seed (i.e., chicken tongue seed) are preferred by consumers and are economically valuable because they have a substantial amount of edible flesh and a desirable taste (Wang et al., 2017). Thus, the production of small aborted-seeded lychee fruit is likely to be economically beneficial for growers. However, the seed abortion that leads to the formation of chicken

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tongue seeds usually adversely affects fruit set and development. Accordingly, small aborted-seeded lychee fruit production is possible only with a few cultivars.

Based on seed development, lychee fruit can be classified into the following four categories: seedless, aborted-, small-, and normal-seeded (Wang et al., 2017). Seedless fruit and aborted-seeded fruit are believed to be produced without and after fertilization, respectively; thus, they are the result of parthenocarpy and pseudo-parthenocarpy (stenospermocarpy), respectively. Lychee cultivars bear fruit with various proportions of aborted seeds with various abortion levels (Menzel et al., 1986; Xie et al., 2019). ‘Hei Ye’ and ‘Chakrapat’, which are the major normal-seeded cultivars, tend to produce a relatively high proportion of normal-seeded fruit (Menzel, 2002; Menzel et al., 1986; Wang et al., 2017), although Matsuda and Higuchi (2019) reported that ‘Chakrapat’ can bear aborted-seeded fruit in greenhouse cultivation conditions. In contrast, ‘No Mai Tsz’, which is also known as ‘Nuomici’, and ‘Salathiel’ usually bear a high proportion (>90%) of aborted-seeded fruit. In particular, although ‘Salathiel’ is categorized as aborted-seeded cultivar (Menzel, 2002; Menzel et al., 2005), it occasionally produce seedless fruit (Koul and Singh, 2017), so it may have both parthenocarpic and pseudo-parthenocarpic ability. Zhang et al. (2018) proposed that in cultivars with a high percentage of aborted-seeded fruit (e.g., ‘Nuomici’), seed abortion is controlled by the mother plant genetic factors. ‘Yu Her Pau’, which is also known as ‘Feizixiao’, and ‘Guiwei’ are often considered to be small-seeded cultivars (Wang et al., 2017; Zhang et al., 2018). These small-seeded cultivars vary in terms of the rates of aborted-, small-seeded (of various sizes), and normal-seeded fruit. In these cultivars, the aborted- and small-seeded fruit rates depend on the year, cultural practices, and location (Menzel et al., 1986; Wang et al., 2017). For example, ‘Yu Her Pau’ was reported to bear a high rate of aborted-seeded fruit in Taiwan (Fang et al., 2018).

Plant hormones have major effects on vegetative and reproductive growth. Accordingly, they are utilized in various types of horticultural production. For example, plant hormones are commonly used to promote high-quality commercial fruit production (Bons and Kaur, 2020). Plant hormones also significantly influence embryo development, seed formation, and fruit set. Auxin is a plant hormone that plays an important role in various plant developmental processes, including embryonic development and seed formation (Möller and Weijers, 2009; Ni et al., 2001; Teale et al., 2006). In tomato (*Solanum lycopersicum*), auxin biosynthesis and metabolism is critical for inducing fruit set (Matsuo et al., 2018). Additionally, auxin inhibitor treatment enhanced tomato seed formation, suggesting auxin significantly modulates fruit set as well as seed abortion (Takisawa et al., 2019). Furthermore, the application of

synthetic auxin (i.e., NAA) is a useful culture practice for increasing fruit set in some lychee orchards in Brazil and Israel (Gonçalves et al., 2014; Stern and Gazit, 1999; Stern et al., 2000). In these studies, synthetic auxin application significantly reduced lychee fruitlet abscission and increased yield. These findings imply that internal auxin metabolism may be related to lychee parthenocarpy and pseudo-parthenocarpy (stenospermocarpy); however, there is a lack of research aimed at characterizing the relationship of endogenous auxin metabolism with seed abortion and fruit retention in lychee.

Among three plant endogenous auxins, indole-3-acetic acid (IAA), phenyl acetic acid, and 4-chloro-indole acetic acid, IAA is a major auxin, and it is believed to be active as a free acid. The IAA level in organs is regulated by coordinated synthesis, metabolism, and transport. Moreover, IAA metabolites are produced by oxidation, such as with 2-oxindole-3-acetic acid (OxIAA), by conjugation as with indole-acetylaspatic acid (IAA-Asp) and indole-acetylglucose (IAA-Glc), and by a combination of oxidation and conjugation, as with 2-oxindole acetylglucose (OxIAA-Glc). Because the IAA metabolites are generally more abundant and can be more easily monitored (Gonçalves et al., 2008; Kim et al., 2021; Ludwig-Müller, 2011; Osterc et al., 2016), the IAA metabolite contents combined with IAA-related gene expression data may be useful for assessing the auxin status related to tissue growth and development.

In this study, we characterized auxin metabolism in the aborted-seeded cultivar, Salathiel. Auxin and its metabolites contents were compared between ‘Salathiel’ and cultivars other than aborted-seeded ones, the small-seeded ‘Yu Her Pau’, and the normal-seeded ‘Hei Ye’. The expression levels of genes related to IAA biosynthesis, metabolism, transport, and signaling were also analyzed to explore the molecular mechanisms underlying the auxin accumulation in ‘Salathiel’ ovaries. Based on these results, we discuss the relationships among auxin metabolism, seed abortion and fruit retention in lychee.

Materials and Methods

Plant materials

The following five lychee cultivars were used in this study: ‘No Mai Tsz’, ‘Salathiel’, ‘Yu Her Pau’, ‘Hei Ye’, and ‘Chakrapat’. ‘Hei Ye’ (11 years old) and potted ‘Salathiel’ (8 years old) plants were grown at the Subtropical Plant Branch, Miyazaki Agricultural Research Institute, while potted ‘No Mai Tsz’, ‘Yu Her Pau’, and ‘Chakrapat’ plants (all 7 years old) were grown at the Kyoto farmstead of Kyoto University Experimental Farm. In spring 2019, female (functional hermaphroditic female) flowers just after the anthesis period (Fig. 1) were collected to excise their ovaries, which were subsequently analyzed. We removed the

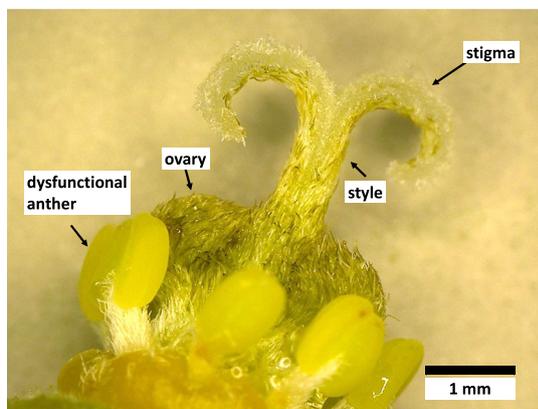


Fig. 1. Photo of a female lychee flower just after the anthesis period. We removed the stigma, style, and anthers just after sampling. The ovary, which includes an ovule and embryo, was examined using a microscope. The auxin content was measured and RNA was extracted.

stigma, style, and anthers just after sampling. Each excised ovary, including an ovule and embryo, was immediately immersed in FAA fixative solution (formaldehyde:acetic acid:50% aqueous ethanol = 5:5:90) and stored at 4°C prior to microscopy analysis. The ovaries of ‘Hei Ye’, ‘Yu Her Pau’, and ‘Salathiel’ were either immediately frozen in liquid nitrogen and stored at -80°C for an analysis of the auxin content or immersed in RNAlater™ Stabilization Solution (Thermo Fisher Scientific, MA, USA) and stored at -20°C for RNA extraction.

Microscopic examination of the internal ovary structure

The FAA fixative solution was replaced with a gradient of sucrose solutions (10%, 20%, and 30%) and then samples were embedded in SCEM medium (Leica Microsystems GmbH, Wetzlar, Germany). Frozen ovaries were cut into 10–15 µm sections using a CM1520 cryostat (Leica) as described by Kawamoto (2003). The longitudinal sections containing the largest ovule section were used for subsequent observations. Sections were stained with 0.1% toluidine blue, embedded in SCEM medium, and examined and photographed using a BX53FL light microscope (Olympus, Tokyo, Japan) equipped with a DP80 digital camera (Olympus). The area and length of specific tissue regions were measured using ImageJ (Abràmoff et al., 2004). At least six samples (either one of two ovule portions were used from six independent florets) were analyzed per cultivar as biological replicates. The Tukey-Kramer test was used to test for significant differences ($P < 0.05$) among the mean values. Percentage data was subjected to arcsine transformation before statistical analysis.

Measurement of endogenous auxin and metabolite contents in ovaries

The frozen ovaries of ‘Hei Ye’, ‘Yu Her Pau’, and

‘Salathiel’ (50–100 mg fresh weight) were homogenized in liquid nitrogen for phytohormone extraction, as described by Kim et al. (2021). Briefly, phytohormones were extracted using an 80% acetone solution containing the isotope-labeled internal standards of IAA and its metabolites (0.1 nmol): $^{13}\text{C}_6$ -IAA, IAA- d_2 , OxIAA- d_2 , 3-hydroxy-2-oxindole-3-acetic acid (DiOxIAA)- d_2 , *N*-2-oxindole-3-acetyl aspartic acid (OxIAA-Asp)- d_2 , *N*-2-oxindole-3-acetyl glutamic acid (OxIAA-Glu)- d_2 , *N*-3-hydroxy-2-oxindole-3-acetyl aspartic acid (DiOxIAA-Asp)- d_2 , and *N*-3-hydroxy-2-oxindole-3-acetyl glutamic acid (DiOxIAA-Glu)- d_2 . The extraction process was repeated and then the extracts were concentrated using an evaporator. After adjusting the pH using 0.1% formic acid, the concentrated extracts were added to Sep-Pak C18 cartridges (Waters, Milford, MA, USA) for solid-phase extraction. The purified samples were eluted using 70% acetonitrile. Following an evaporation step, the contents were determined by LC-ESI-MS/MS using an LCMS-8030 system (Shimadzu, Kyoto, Japan) in the MRM mode. Each metabolite was quantified on the basis of the ratio of its peak area to the peak area of the specified amount of the internal standard. The amount was expressed as nanomoles per gram of fresh weight ($\text{nmol}\cdot\text{g}^{-1}$ FW). For each cultivar, three biological replicates were analyzed. The Tukey-Kramer test was used to test for significant differences ($P < 0.05$) among the mean values.

Analysis of auxin-related gene expression

Total RNA was extracted from the ovaries (‘Hei Ye’, ‘Yu Her Pau’, and ‘Salathiel’) using PureLink Plant RNA Reagent (Thermo Fisher Scientific). mRNA libraries were then constructed and sequenced (Illumina HiSeq, 150-bp paired-end reads) according to the manufacturer’s instructions (GENEWIZ, NJ, USA). For each cultivar, three biological replicates were prepared for the mRNA sequencing (mRNA-seq) analysis, with one replicate comprising two ovaries from one female flower.

The raw sequencing data were preprocessed by removing adapter sequences using fastp (Chen et al., 2018). The remaining ‘Hei Ye’ reads were used for the *de novo* sequence assembly using Trinity (Grabherr et al., 2011), with a read-length cut-off of 35 bp. After clean reads were mapped to the *de novo* assembled contigs by bowtie2 (Langmead and Salzberg, 2012; Langmead et al., 2019) and samtools (Li, 2011; Li et al., 2009), the sam/bam file created was used to calculate the TPM values of each contig using Salmon (Patro et al., 2017). A principal component analysis (PCA) of the TPM values of all contigs was performed using R (version 4.1.0) (R Core Team, 2021) to confirm similarities and differences in the sequencing data among cultivars.

The coding sequences and encoded amino acid sequences of the auxin-related genes in *Arabidopsis*

thaliana, *Oryza sativa*, *Citrus sinensis*, *Vitis vinifera*, and *Prunus persica* were obtained from the NCBI and Phytozome databases (Goodstein et al., 2012). Twelve auxin-related gene homologs, including 3 IAA biosynthesis-related

TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA), *YUCCA (YUC)*, *AMIDASE (AMI)*, 3 IAA metabolism-related *DIOXYGENASE (DAO)*, *GRETCHEN HAGEN3 (GH3)*, *UDP-GLYCOSYLTRANSFERASE (UGT)*, 3 auxin signaling-related *AUX/IAA*, *AUXIN RESPONSE FACTOR (ARF)*, *TRANSPORT INHIBITOR RESPONSE (TIR1)*, and 3 auxin transporters, *PIN-FORMED (PIN)*, *ATP-BINDING CASSETTE B (ABCB)*, and *AUXIN RESISTANT/LIKE AUX (AUX/LAX)*, were extracted by a local BLAST search from the contigs of the *de novo* assembly using *A. thaliana* coding sequences as the query (e-value < 1E-10). A phylogenetic analysis was conducted to confirm the sequence similarity and conservation between the extracted contigs and the sequences in *A. thaliana*, *O. sativa*, *C. sinensis*, *V. vinifera*, and *P. persica*. Protein sequences were aligned using MAFFT (version 7) (Katoh and Standley, 2013). The phylogenetic tree was constructed according to the neighbor-joining method using MEGA X (Kumar et al., 2018). The TPM values of each contig encoding auxin-related genes were compared between cultivars. The data are herein presented as a heatmap, which was produced on the basis of the Z-score using the gplots package in R.

Results and Discussion

In this study, we categorized five cultivars into the aborted-seeded cultivars, ‘No Mai Tsz’ and ‘Salathiel’, the small-seeded cultivar ‘Yu Her Pau’, and the normal-seeded cultivars, ‘Hei Ye’ and ‘Chakrapat’.

The mean seed size of ‘Salathiel’ and ‘Chakrapat’ fruits from the trees grown at Miyazaki Agricultural Research Institute, were reported to be 0.5 g and 3.3 g, respectively (Hirahara, 2018), supporting that the ‘Salathiel’ trees used in this study were an aborted-seeded cultivar. Although the seed size of ‘No Mai Tsz’, ‘Chakrapat’, and ‘Yu Her Pau’ fruits from the trees grown at Kyoto University were not investigated, they are considered to be aborted-, normal-, and small-seeded cultivars, respectively, based on previous reports (Menzel, 2002; Menzel et al., 1986; Wang et al., 2017; Zhang et al., 2018).

Ovule, inner and outer integuments, and obturator differentiation and development in ‘Salathiel’

Abnormal ovule development and integument deficiency lead to pseudo-parthenocarpy (stenospermocarpy), resulting in abnormal seed development and seedless fruit formation in *A. thaliana* and *Annona squamosa* (Dos Santos et al., 2014; Lora et al., 2011). Our microscopic observations of the female reproductive organs of several lychee cultivars revealed that the differentiation of the embryo sac, inner and outer integuments, and obturator, in the aborted-seeded cultivars, ‘Salathiel’ and ‘No Mai Tsz’, were similar to those in normal-seeded and small-seeded cultivars (Fig. 2). This suggests that the relatively high proportion of aborted seeds in ‘Salathiel’ fruit is not due to the abnormal differentiation of the organs in the ovary during the flowering period. This is consistent with the findings of a previous study that demonstrated the normal differentiation of female reproductive organs in another aborted-seeded cultivar (‘No Mai Tsz’) (Zhu, 2008).

We then compared the development of these organs among the examined cultivars. The degree of the development of the ovary, ovule, embryo sac, integuments, and obturator varied significantly among the cultivars (Table 1). The ovary, ovule and embryo sac were

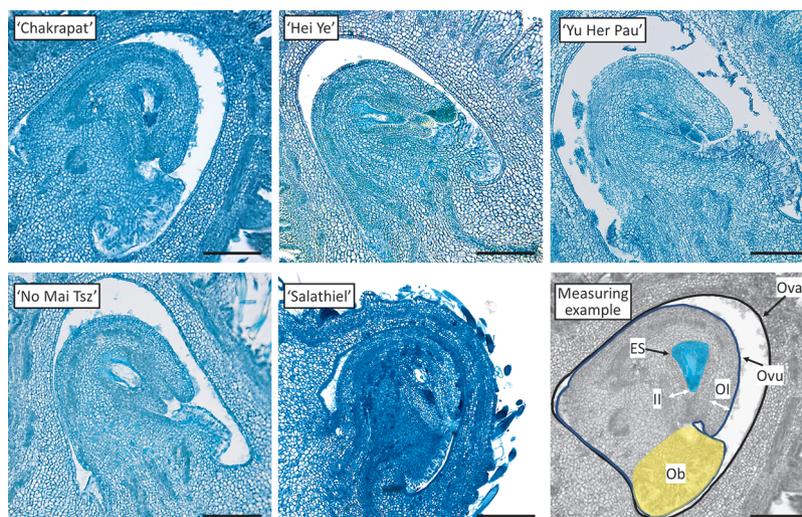


Fig. 2. Longitudinal section of an ovary just after flowering. Ova: ovary, Ovu: ovule, ES: embryo sac, Ob: obturator, II: inner integument, OI: outer integument. Sections containing a clearly visible embryo sac were subjected to observation and size evaluations. Bar indicates 200 μ m.

Table 1. Development of female reproductive organs in various lychee cultivars.

Cultivar	Ovary (mm ²)	Ovule (mm ²)	Embryo Sac (mm ²)	Obturator (mm ²)	Inner Integument (μm)	Outer Integument (μm)	Embryo Sac/Ovule (%)	Obturator/Ovule (%)
Chakrapat	0.36±0.02 b*	0.27±0.02 b	0.010±0.01 a	0.041±0.02 a	74.0±2.0 a	75.6±2.0 b	3.9±0.1 ab	15.5±0.6 a
Hei Ye	0.23±0.01 c	0.20±0.01 c	0.008±0.01 b	0.024±0.01 b	49.6±1.2 c	88.0±2.6 b	3.9±0.2 ab	11.5±0.2 b
Yu Her Pau	0.55±0.01 a	0.34±0.01 a	0.012±0.01 a	0.043±0.02 a	52.0±0.7 c	109.8±2.0 a	3.6±0.2 b	12.7±0.5 b
No Mai Tsz	0.36±0.01 b	0.25±0.08 b	0.011±0.00 a	0.017±0.01 c	63.2±2.5 b	105.9±4.7 a	4.6±0.3 a	6.2±0.4 d
Salathiel	0.22±0.01 c	0.20±0.05 c	0.006±0.01 b	0.018±0.01 bc	71.6±2.0 ab	78.4±2.2 b	3.2±0.4 b	9.3±0.4 c

Data are presented as the mean±SE.

* Different letters in a column indicate significantly different means ($P < 0.05$), as determined by the Tukey-Kramer test. The percentages were subjected to arcsine transformation before the statistical analysis.

significantly larger in ‘Yu Her Pau’ and smaller in ‘Hei Ye’ and ‘Salathiel’, which likely reflects the differences in whole flower size itself (data not shown). The width of the inner integument was greatest in ‘Chakrapat’ while that of the outer integument was significantly longer in ‘Yu Her Pau’ and ‘No Mai Tsz’. The embryo sac to ovule size ratio was significantly larger in ‘No Mai Tsz’ than ‘Salathiel’ and ‘Yu Her Pau’. These results suggest that there is no clear relationship between ovary, ovule, integument developmental degree and cultivar-dependent seed size characteristics. On the contrary, the obturator to ovule size ratio was significantly smaller in the aborted-seeded cultivars ‘No Mai Tsz’ and ‘Salathiel’ than in the other cultivars. The obturator mediates pollen tube growth and the subsequent fertilization (Arbeloa and Herreo, 1987; Cousin and El Maataoui, 1998; Losada and Herrero, 2017). It has been suggested that pollination positively affects fruiting and seed size in lychee (Chu et al., 2015). Thus, we speculated that the differences in the obturator to ovule size ratio may be related to the altered fertilized embryo or seed development of ‘No Mai Tsz’ and ‘Salathiel’, which ultimately leads to increased seed abortion in these cultivars. However, the potential relationship between the small obturator of aborted-seeded cultivars and seed abortion will need to be experimentally verified.

High level of IAA in the ‘Salathiel’ ovaries

We analyzed auxin metabolism because it has been well-documented that auxin is critical for fruit set, seed development, and parthenocarpy in other plants, including tomato (Matsuo et al., 2018; Möller and Weijers, 2009; Teale et al., 2006). The active auxin IAA and its metabolites (OxIAA, DiOxIAA, IAA-Asp, OxIAA-Asp, DiOxIAA-Asp, DiOxIAA-Asp, IAA-Glu, and DiOxIAA-Glu) were detected in the aborted-seeded ‘Salathiel’, small-seeded ‘Yu Her Pau’, and normal-seeded ‘Hei Ye’ cultivars (Fig. 3). However, OxIAA-Glu and other amino acid and glucose conjugates were not detected (data not shown), which is consistent with the results of our recent study on lychee flower auxin contents (Kim et al., 2021). Additionally, the IAA,

DiOxIAA, IAA-Asp, and DiOxIAA-Glu levels were significantly different among cultivars. More specifically, the IAA and DiOxIAA-Glu contents were significantly higher in ‘Salathiel’ than in the other two cultivars examined. DiOxIAA was lower in ‘Yu Her Pau’ and ‘Salathiel’ than in ‘Hei Ye’, while IAA-Asp was significantly lower in ‘Salathiel’ compared to ‘Hei Ye’.

Our study revealed that the ‘Salathiel’ ovary contains a higher level of IAA than the ovaries of normal-seeded and small-seeded cultivars. Auxin has important effects on the stability of fruit set in fruit tree species, as well as in various other horticultural crops (Alabadi et al., 1996; Bons and Kaur, 2020; Tiwari et al., 2012). In fact, exogenous auxin application is one conventional lychee crop management strategy used to improve fruit set in Southeast Asia (Anand et al., 2003; Arora and Singh, 2017; Menzel, 2002). In tomato and eggplant (*Solanum melongena*), high IAA levels are associated with fruit retention and the induction of parthenocarpic fruit production (Matsuo et al., 2020; Takisawa et al., 2019). Moreover, auxin promotes fruit retention in some other fruit crops (Else et al., 2004; Kurnlawati and Hamim, 2009). Therefore, high IAA levels in reproductive organs, including the ovary, may contribute to flower and fruit retention. Because the strong sink activities of seeds help trees retain fruit (Ohsugi, 2003; Zhang et al., 2020), normal-seeded or small-seeded fruit are likely retained on trees more than aborted-seeded fruit. Poor embryo development reportedly increases physiological fruit drop in peach (Fukuda et al., 2006). Furthermore, it has been suggested that appropriate pollination, fertilization, and seed development may increase the lychee fruiting percentage (Chu et al., 2015). Therefore, we hypothesize that the high IAA level in the ‘Salathiel’ ovaries may contribute to fruit retention and prevent early flower shattering or aborted-seeded and seedless fruit drop.

‘Hei Ye’, which is a normal-seeded cultivar, had high IAA metabolite contents (Fig. 3). Among the IAA metabolites detected in this study, OxIAA and DiOxIAA, which are oxidized IAA metabolites, were the most abundant (Fig. 3B, C). ‘Yu Her Pau’ had a

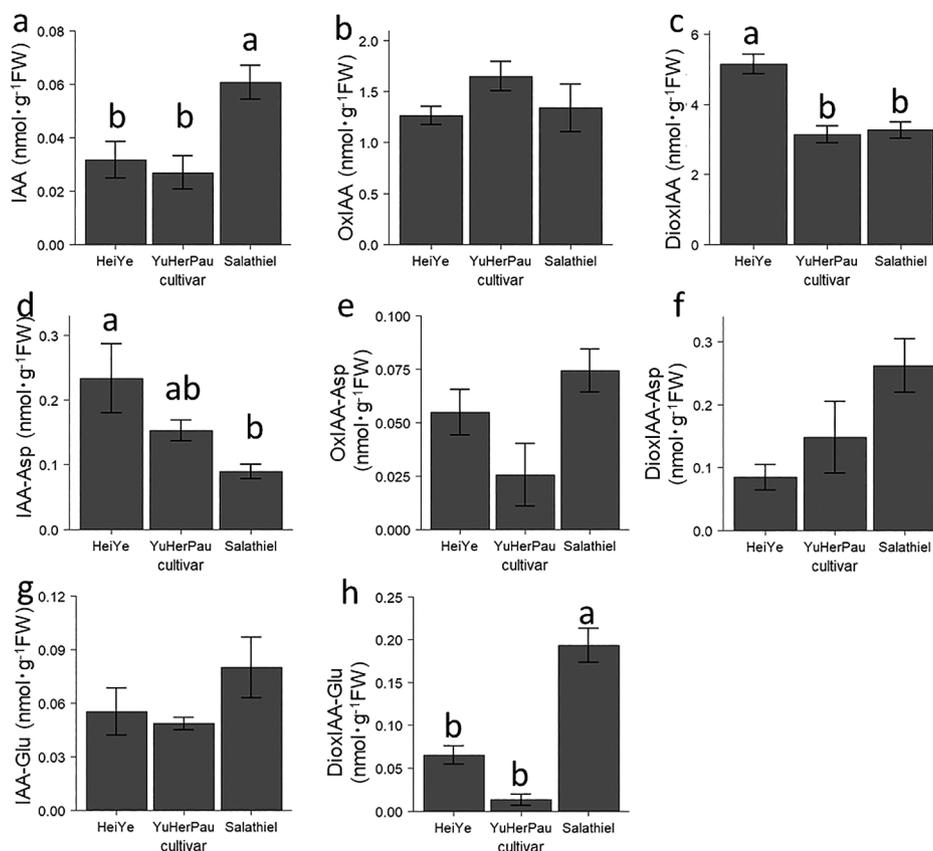


Fig. 3. Endogenous IAA contents in lychee ovaries just after flowering. The amount was expressed as nanomoles per gram of fresh weight ($\text{nmol}\cdot\text{g}^{-1}\text{FW}$). For each cultivar, three biological replicates were analyzed. a: IAA, b: OxIAA, c: DiOxIAA, d: IAA-Asp, e: OxIAA-Asp, f: DiOxIAA-Asp, g: IAA-Glu, h: DiOxIAA-Glu. Different letters above the bars in each graph indicate significant differences ($P < 0.05$), as determined by the Tukey-Kramer test.

lower endogenous IAA level than ‘Salathiel’, but it tended to have more or equal amounts of OxIAA. ‘Hei Ye’ contained significantly more DiOxIAA than the other cultivars. Several studies have confirmed that IAA metabolites are derived from IAA (Gonçalves et al., 2008; Kim et al., 2021; Ludwig-Müller, 2011; Osterc et al., 2016). Therefore, these results may indicate that IAA levels peaked in ‘Hei Ye’ and ‘Yu Her Pau’ before flowering, and that the IAA level peaked later in ‘Salathiel’ than in the other two cultivars. Auxin contents and homeostasis greatly affect plant organ development (Cao et al., 2020). In this study, the abundance of IAA metabolites varied between cultivars with diverse seed traits. We hypothesize that differences in auxin levels during female flower development may be related to the variability in female organ development and subsequent seed and fruit development.

IAA efflux and influx may be the primary factors mediating the high auxin content in ‘Salathiel’

We tried to clarify the mechanisms underlying the increased IAA accumulation in the ‘Salathiel’ ovaries by conducting mRNA-seq analysis. Our mRNA-seq analysis generated a total of 106,840,115 reads (32.05 Gb). All the obtained sequences from Illumina

sequencing reads are available from the NCBI/DBJ Sequence Read Archive under the following accession number, DRA012830. The PCA results revealed a clear separation of read components among cultivars and a high similarity between biological replicates (Supplementary Fig. 1). Moreover, PC1 and PC2, respectively, accounted for 29.3% and 24.2% of the variance, and each sample formed three clusters depending on the cultivar. This implies that the expression pattern in the ovary just after flowering may differ among the cultivars, even though the samples were collected at the same development stage.

The expression levels of genes related to IAA biosynthesis, metabolism, transport, and signaling were compared among the cultivars (Fig. 4). In *A. thaliana*, the indole-3-pyruvic acid (IPA) pathway is a major IAA biosynthetic pathway (Mashiguchi et al., 2011). In this pathway, TAA converts tryptophan to IPA, which is then oxidized by YUC to produce IAA. Several studies indicated IAA can also be synthesized in a pathway mediated by AMI, in which indole acetamide is converted to IAA (Abu-Zaitoon et al., 2012; Mano et al., 2010; Mashiguchi et al., 2011). We identified several homologs of the corresponding genes and the subsequent gene expression analysis indicated that

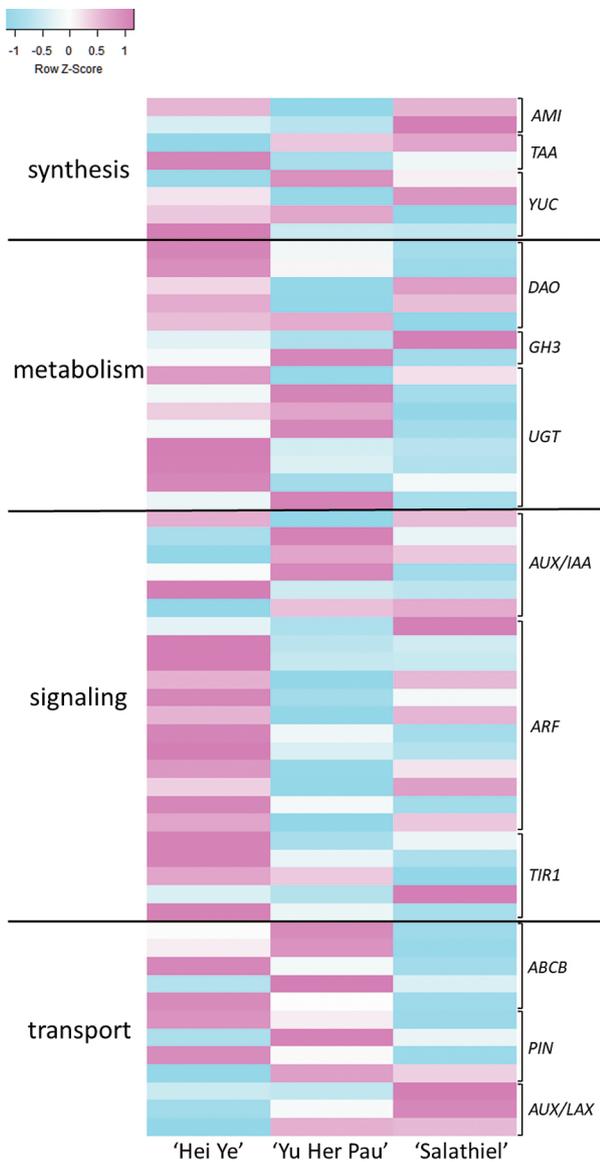


Fig. 4. Heatmap presenting the expression tendencies of IAA-related genes on the basis of the mRNA-seq analysis. Each TPM value was converted to a Z-score before constructing the heatmap.

'Salathiel' had relatively high *AMI* expression levels. Regarding the IAA biosynthesis-related genes, although *AMI* was more highly expressed in 'Salathiel' than in the other cultivars, the expression levels of *TAA* and *YUC*, which are key genes in the main IAA biosynthesis pathway, were not different from the others overall. Therefore, there was no clear association between IAA biosynthesis activity and the high IAA content in 'Salathiel'. Regarding IAA catabolism, the three major catabolic pathways (oxidation, amino acid conjugation, and glycosylation) have been well characterized in Arabidopsis; these pathways involve *DAO*, *GH3*, and *UGT*, respectively. The expression data for these IAA catabolism-related genes revealed that *UGT* expression levels were lower in 'Salathiel' than in the

other cultivars (Fig. 4). Regarding IAA metabolism-related genes, in 'Hei Ye', which had a relatively low IAA content, the *DAO* expression level was generally higher than that in the other cultivars, which is in accordance with the highest accumulation of DiOxIAA in 'Hei Ye'. The *UGT* expression levels were lower in 'Salathiel' than in 'Hei Ye' and 'Yu Her Pau'. However, IAA glycosides were undetectable in our analysis of IAA metabolites, which is similar to the lack of IAA glycosides detected in lychee flowers during our previous investigation (Kim et al., 2021). Therefore, similar to IAA biosynthesis genes, IAA metabolism-related genes may not be the primary factors responsible for the high IAA contents in 'Salathiel'.

The AUX/IAA family proteins negatively regulate auxin signaling by inactivating the ARFs that promote auxin-responsive gene expression. In the presence of high auxin levels, auxin binds to the TIR1–AUX/IAA complex, leading to the ubiquitination and degradation of AUX/IAA and the activation of ARFs (Dharmasiri et al., 2005; Guilfoyle and Hagen, 2007; Wang et al., 2005). In our study, the magnitudes of *ARF* expression levels were in the order 'Hei Ye' > 'Salathiel' > 'Yu Her Pau' and *TIR1* expression levels were substantially higher in 'Hei Ye' than the others (Fig. 4). *AUX/IAA* expressions tended to be higher in 'Yu Her Pau' compared to the other cultivars because three out of six *AUX/IAA* genes showed higher expression intensities. In tomato, the molecular roles of the IAA signaling genes *ARF* and *TIR1* on parthenocarpy have been thoroughly characterized. The suppressed expression of *SIARF5* alters ovary growth and results in the formation of seedless fruit (Liu et al., 2018), whereas the overexpression of *SITIR1* induces abnormal seed development and stimulates stenosperrmocarpy fruit formation in tomato (Ren and Wang, 2016). Because no clear difference was found in the overall expression patterns of *ARF*, *TIR1*, and *AUX/IAA* genes between 'Salathiel' and the others, the relationship between altered IAA signaling activity and genetic regulation of seed abortion in 'Salathiel' was unclear. Actually, our data indicated the IAA signaling genes, especially *ARF* and *TIR1*, were highly expressed in 'Hei Ye', which can produce more fruit per tree than the other cultivars (Chang et al., 2017). Although the relationship between auxin signaling genes expressions (i.e. *ARF* and *TIR1*) and fruit retention have not been examined in lychee, it is possible that the enhanced expressions of IAA signaling genes in 'Hei Ye' could be related to its relatively high fruit set.

In contrast to the genes related to IAA biosynthesis and metabolism, the IAA transporter gene expression levels were closely correlated with the increased IAA accumulation in 'Salathiel'. Three major auxin transporter families have been reported, including the auxin efflux carriers (*PIN* and *ABCB*) and the auxin influx carrier (*AUX/LAX*) (Cho and Cho, 2013; Péret et al.,

2012; Vieten et al., 2005). Compared with the gene expression in ‘Hei Ye’ and ‘Yu Her Pau’, the *ABCB* and *PIN* expression levels were lower in ‘Salathiel’, while the *AUX/LAX* expression levels were higher in ‘Salathiel’ (Fig. 4). Because *ABCB* and *PIN* encode auxin efflux carriers, whereas *AUX/LAX* encodes an auxin influx carrier, lower expressions of *ABCB* and *PIN* and higher expressions of *AUX/LAX* may help to accumulate IAA more in the cytoplasm rather than extracellular tissues, leading to the localization of IAA inside the ovary rather than transportation of IAA from the ovary. Thus, this expression pattern is consistent with the higher endogenous IAA levels in the ‘Salathiel’ ovaries. Auxin transport helps facilitate auxin homeostasis and localization in plants, as well as auxin metabolism (Cho and Cho, 2013; Péret et al., 2012; Robert et al., 2015; Vieten et al., 2005). The regulation of IAA contents and the maintenance of auxin homeostasis via IAA transport are reportedly vital for embryonic and subsequent development (Robert et al., 2015; Vieten et al., 2005).

In conclusion, we revealed that ‘Salathiel’, which can produce a high proportion of aborted-seeded fruit, shows specific reproductive organ developmental characteristics and accumulates a relatively large amount of IAA in the ovary in the anthesis period. High accumulation of IAA could be due to altered auxin transport polarity and intensity. On the basis of the results of previous studies and the current study, we hypothesize that the accumulation of endogenous auxin and the timing of peak accumulation of auxin in ‘Salathiel’ may contribute to the retention of aborted-seeded and seedless fruit. Our study also raises the possibility that auxin metabolism could be related to lychee seed abortion; however, future studies will need to verify this possibility.

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Literature Cited

- Abramoff, M. D., P. J. Magalhães and S. J. Ram. 2004. Image processing with imageJ. *Biophotonics Int.* 11: 36–42.
- Abu-Zaitoon, Y. M., K. Bennett, J. Normanly and H. M. Nonhebel. 2012. A large increase in IAA during development of rice grains correlates with the expression of tryptophan aminotransferase *OsTAR1* and a grain-specific *YUCCA*. *Physiol. Plant.* 146: 487–499.
- Alabadi, D., M. S. Agüero, M. A. Pérez-Amador and J. Carbonell. 1996. Arginase, arginine decarboxylase, ornithine decarboxylase, and polyamines in tomato ovaries (Changes in unpollinated ovaries and parthenocarpic fruits induced by auxin or gibberellin). *Plant Physiol.* 112: 1237–1244.
- Anand, M., P. S. Kahlon and B. V. C. Mahajan. 2003. Effect of exogenous application of growth regulators on fruit drop, cracking and quality of litchi (*Litchi chinensis* sonn.) cv. Dehradun. *Agric. Sci. Digest* 23: 191–194.
- Arbeloa, A. and M. Herrero. 1987. The significance of the obturator in the control of pollen tube entry into the ovary in peach (*Prunus persica*). *Ann. Bot.* 60: 681–685.
- Arora, R. and N. Singh. 2017. Growth regulators for yield and quality enhancement in litchi (*Litchi chinensis* L)—a review. *Int. J. Curr. Microbiol. App. Sci.* 6: 2152–2159.
- Bons, H. K. and M. Kaur. 2020. Role of plant growth regulators in improving fruit set, quality and yield of fruit crops: a review. *J. Hortic. Sci. Biotechnol.* 95: 137–146.
- Cao, J., G. Li, D. Qu, X. Li and Y. Wang. 2020. Into the seed: Auxin controls seed development and grain yield. *Int. J. Mol. Sci.* 21: 1662. DOI: 10.3390/ijms21051662.
- Chang, J. W., P. A. Chen and I. Z. Chen. 2017. Litchi breeding and plant management in Taiwan. p. 31–58. In: M. Kumar, V. Kumar, R. Prasad and A. Varma (eds.). *The Lychee Biotechnology*. Springer Nature, Singapore.
- Chen, S., Y. Zhou, Y. Chen and J. Gu. 2018. Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34: i884–i890.
- Cho, M. and H. T. Cho. 2013. The function of ABCB transporters in auxin transport. *Plant Signal. Behav.* 8: e22990. DOI: 10.4161/psb.22990.
- Chu, Y. C., T. S. Lin and J. C. Chang. 2015. Pollen effects on fruit set, seed weight, and shriveling of ‘73-S-20’ litchi- with special reference to artificial induction of parthenocarpy. *HortScience* 50: 369–373.
- Cousin, M. and M. El Maataoui. 1998. Female reproductive organs in self-compatible almond (*Prunus dulcis* (Mill.) D.A. Webb) Lauranne and fertilization patterns. *Sci. Hortic.* 72: 287–297.
- Dharmasiri, N., S. Dharmasiri and M. Estelle. 2005. The F-box protein TIR1 is an auxin receptor. *Nature* 435: 441–445.
- Dos Santos, R. C., L. M. Ribeiro, M. O. Mercadante-Simões, M. R. Costa, S. Nietzsche and M. C. T. Pereira. 2014. Stenospermy and seed development in the “Brazilian seedless” variety of sugar apple (*Annona squamosa*). *An. Acad. Bras. Cienc.* 86: 2101–2108.
- Else, M. A., A. P. Stankiewicz-Davies, C. M. Crisp and C. J. Atkinson. 2004. The role of polar auxin transport through pedicels of *Prunus avium* L. in relation to fruit development and retention. *J. Exp. Bot.* 55: 2099–2109.
- Fang, H. H., W. L. Lee, S. H. Yang, C. C. Hsu and Y. S. Liang. 2018. Investigation on composition changes of sour and sweet substances during the fruit development of lychee (*Litchi chinensis* Sonn.). *J. Taiwan Soc. Hortic. Sci.* 64: 137–146 (In Chinese with English abstract).
- Fukuda, F., R. Yoshimura, H. Matsuoka and N. Kubota. 2006. Relationship between fruit enlargement and seed development with respect to physiological fruit drop in ‘Shimizu Hakuto’ Peach. *J. Japan. Soc. Hort. Sci.* 75: 213–218.
- Gonçalves, J. C., G. Diogo, M. T. Coelho, N. Vidal and S. Amâncio. 2008. Quantitation of endogenous levels of IAA, IAAsp and IBA in micro-propagated shoots of hybrid chestnut pre-treated with IBA. *In Vitro Cell. Dev. Biol. Plan.* 44: 412–418.
- Gonçalves, V. D., M. C. Pires and O. K. Yamanishi. 2014. Synthetic auxin 3-5-6 TPA increased fruit size and retention of “Bengal” lychee in Brazil. *Acta Hortic.* 1042: 65–72.
- Goodstein, D. M., S. Shu, R. Howson, R. Neupane, R. D. Hayes, J. Fazo, T. Mitros, W. Dirks, U. Hellsten, N. Putnam and D. S. Rokhsar. 2012. Phytozome: A comparative platform for green plant genomics. *Nucleic Acids Res.* 40: 1178–1186.

- Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, Z. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind, F. Di Palma, B. W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman and A. Regev. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* 29: 644–652.
- Guilfoyle, T. J. and G. Hagen. 2007. Auxin response factors. *Curr. Opin. Plant Biol.* 10: 453–460.
- Hirahara, Y. 2018. Project for the creation of new fruit production areas for the future: 1. Research on the characteristics of lychee cultivars. *Bull. Subtropic. Plant Branch Miyazaki Agric. Res. Inst.* 2018: 148–149 (In Japanese).
- Katoh, K. and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30: 772–780.
- Kawamoto, T. 2003. Use of a new adhesive film for the preparation of multi-purpose fresh-frozen sections from hard tissues, whole-animals, insects and plants. *Arch. Histol. Cytol.* 66: 123–143.
- Kays, S. J. 1999. Preharvest factors affecting appearance. *Post-harvest Biol. Technol.* 15: 233–247.
- Kim, R., Y. Osako, H. Yamane, R. Tao and H. Miyagawa. 2021. Quantitative analysis of auxin metabolites in lychee flowers. *Biosci. Biotechnol. Biochem.* 85: 467–475.
- Koul, B. and J. Singh. 2017. Lychee biology and biotechnology p. 137–192. In: M. Kumar, V. Kumar, R. Prasad and A. Varma (eds.). *The Lychee Biotechnology*. Springer Nature, Singapore.
- Kumar, S., G. Stecher, M. Li, C. Nknyaz and K. Tamura. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35: 1547–1579.
- Kurnlawati, B. and Hamim. 2009. Physiological responses and fruit retention of carambola fruit (*Averrhoa carambola* L.) induced by 2,4-D and GA3. *HAYATI J. Biosci.* 16: 9–14.
- Langmead, B. and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9: 357–359.
- Langmead, B., C. Wilks, V. Antonescu and R. Charles. 2019. Scaling read aligners to hundreds of threads on general-purpose processors. *Bioinformatics* 35: 421–432.
- Li, H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* 27: 2987–2993.
- Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis and R. Durbin. 2009. The sequence alignment/map format and SAMtools. *Bioinformatics* 25: 2078–2079.
- Liu, S., Y. Zhang, Q. Feng, L. Qin, C. Pan, A. T. Lamin-Samu and G. Lu. 2018. Tomato AUXIN RESPONSE FACTOR 5 regulates fruit set and development via the mediation of auxin and gibberellin signaling. *Sci. Rep.* 8: 2971. DOI: 10.1038/s41598-018-21315-y.
- Lora, J., J. I. Hormaza, M. Herrero and C. S. Gasser. 2011. Seedless fruits and the disruption of a conserved genetic pathway in angiosperm ovule development. *Proc. Natl. Acad. Sci. USA* 108: 5461–5465.
- Losada, J. M. and M. Herrero. 2017. Pollen tube access to the ovule is mediated by glycoprotein secretion on the obturator of apple (*Malus × domestica*, Borkh). *Ann. Bot.* 119: 989–1000.
- Ludwig-Müller, J. 2011. Auxin conjugates: Their role for plant development and in the evolution of land plants. *J. Exp. Bot.* 62: 1757–1773.
- Mano, Y., K. Nemoto, M. Suzuki, H. Seki, I. Fujii and T. Muranaka. 2010. The *AMII* gene family: Indole-3-acetamide hydrolase functions in auxin biosynthesis in plants. *J. Exp. Bot.* 61: 25–32.
- Mashiguchi, K., K. Tanaka, T. Sakai, S. Sugawara, H. Kawaide, M. Natsume, A. Hanada, T. Yaeno, K. Shirasu, H. Yao, P. McSteen, Y. Zhao, K. I. Hayashi, Y. Kamiya and H. Kasahara. 2011. The main auxin biosynthesis pathway in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 108: 18512–18517.
- Matsuda, H. and H. Higuchi. 2019. Effects of the pollen parent on the fruit set and seed type of ‘Bengal’, ‘Chakrapat’, and ‘Tai So’ lychee cultivars. *Trop. Agr. Develop.* 63: 79–82.
- Matsuo, S., K. Kikuchi, K. Nagasuga, H. Ueno and S. Imanishi. 2018. Transcriptional regulation of auxin metabolic-enzyme genes during tomato fruit development. *Sci. Hortic.* 241: 329–338.
- Matsuo, S., K. Miyatake, M. Endo, S. Urashimo, T. Kawanishi, S. Negoro, S. Shimakoshi and H. Fukuoka. 2020. Loss of function of the *Pad-1* aminotransferase gene, which is involved in auxin homeostasis, induces parthenocarp in Solanaceae plants. *Proc. Natl. Acad. Sci. USA* 117: 12784–12790.
- Menzel, C. 2002. The lychee crop in Asia and the Pacific. *RAP Publ. FAO*. Bangkok.
- Menzel, C. M., K. R. Chapman, B. F. Paxton and D. R. Simpson. 1986. Growth and yield of lychee cultivars in subtropical queensland. *Aust. J. Exp. Agric.* 26: 261–265.
- Menzel, C. M., X. Huang and C. Liu. 2005. Cultivars and plant improvement. p. 59–87. In: C. M. Menzel and G. K. Waite (eds.). *Litchi and longan: botany, production and uses*. CABI Publishing, Oxfordshire.
- Mitra, S. K. and P. K. Pathak. 2010. Litchi production in the Asia-Pacific region. *Acta Hortic.* 863: 29–36.
- Möller, B. and D. Weijers. 2009. Auxin control of embryo patterning. *Cold Spring Harb. Perspect. Biol.* 1: a001545. DOI: 10.1101/cshperspect.a001545.
- Ni, D. A., L. J. Wang, C. H. Ding and Z. H. Xu. 2001. Auxin distribution and transport during embryogenesis and seed germination of *Arabidopsis*. *Cell Res.* 11: 273–278.
- Ohsugi, R. 2003. Sink-source relationship and crop yield. *Kagaku To Seibutsu* 41: 366–373 (In Japanese).
- Osterc, G., M. M. Petkovšek and F. Stampar. 2016. Quantification of IAA metabolites in the early stages of adventitious rooting might be predictive for subsequent differences in rooting response. *J. Plant Growth Regul.* 35: 534–542.
- Patro, R., G. Duggal, M. I. Love, R. A. Irizarry and C. Kingsford. 2017. Salmon provides fast and bias-aware quantification of transcript expression. *Nat. Methods* 14: 417–419.
- Péret, B., K. Swarup, A. Ferguson, M. Seth, Y. Yang, S. Dhondt, N. James, I. Casimiro, P. Perry, A. Syed, H. Yang, J. Reemmer, E. Venison, C. Howells, M. A. Perez-Amador, J. Yun, J. Alonso, G. T. S. Beemster, L. Laplaze, A. Murphy, M. J. Bennett, E. Nielsen and R. Swarup. 2012. *AUX/LAX* genes encode a family of auxin influx transporters that perform distinct functions during *Arabidopsis* development. *Plant Cell* 24: 2874–2885.
- R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <<https://www.R-project.org/>>.
- Ren, Z. and X. Wang. 2016. SITIR1 is involved in crosstalk of phytohormones, regulates auxin-induced root growth and stimulates stenopermocarpy fruit formation in tomato. *Plant Sci.* 253: 13–20.
- Robert, H. S., W. Grunewald, M. Sauer, B. Cannoot, M. Soriano, R. Swarup, D. Weijers, M. Bennett, K. Boutilier and J. Friml. 2015. Plant embryogenesis requires AUX/LAX-mediated auxin influx. *Development* 142: 702–711.

- Stern, R. A. and S. Gazit. 1999. The synthetic auxin 3,5,6-TPA reduces fruit drop and increases yield in “Kaimana” litchi. *J. Hort. Sci. Biotechnol.* 74: 203–205.
- Stern, R. A., D. Stern, M. Harpaz and S. Gazit. 2000. Applications of 2,4,5-TP, 3,5,6-TPA, and combinations thereof increase lychee fruit size and yield. *HortScience* 35: 661–664.
- Takisawa, R., S. Koeda and T. Nakazaki. 2019. Effects of the *pat-2* gene and auxin biosynthesis inhibitor on seed production in parthenocarpic tomatoes (*Solanum lycopersicum* L.). *Hort. J.* 88: 481–487.
- Teale, W. D., I. A. Paponov and K. Palme. 2006. Auxin in action: Signalling, transport and the control of plant growth and development. *Nat. Rev. Mol. Cell Biol.* 7: 847–859.
- Tiwari, A., R. Offringa and E. Heuvelink. 2012. Auxin-induced fruit set in *Capsicum annuum* L. requires downstream gibberellin biosynthesis. *J. Plant Growth Regul.* 31: 570–578.
- Vieten, A., S. Vanneste, J. Wiśniewska, E. Benková, R. Benjamins, T. Beeckman, C. Luschig and J. Friml. 2005. Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. *Development* 132: 4521–4531.
- Wang, H., B. Jones, Z. Li, P. Frasse, C. Delalande, F. Regad, S. Chaabouni, A. Latché, J. C. Pech and M. Bouzayen. 2005. The tomato *Aux/IAA* transcription factor IAA9 is involved in fruit development and leaf morphogenesis. *Plant Cell* 17: 2676–2692.
- Wang, H. C., B. Lai and X. M. Huang. 2017. Litchi fruit set, development, and maturation. p. 1–30. In: M. Kumar, V. Kumar, R. Prasad and A. Varma (eds.). *The Lychee Biotechnology*. Springer Nature, Singapore.
- Xie, D. R., X. S. Ma, M. Z. Rahman, M. C. Yang, X. M. Huang, J. G. Li and H. C. Wang. 2019. Thermo-sensitive sterility and self-sterility underlie the partial seed abortion phenotype of *Litchi chinensis*. *Sci. Hortic.* 247: 156–164.
- Zhang, C., D. Xie, T. Bai, X. Luo, F. Zhang, Z. Ni and Y. Chen. 2020. Diversity of a large collection of natural populations of mango (*Mangifera indica* Linn.) revealed by agromorphological and quality traits. *Diversity* 12: 27. DOI: 10.3390/d12010027.
- Zhang, J., Z. Wu, F. Hu, L. Liu, X. Huang, J. Zhao and H. Wang. 2018. Aberrant seed development in *Litchi chinensis* is associated with the impaired expression of cell wall invertase genes. *Hortic. Res.* 5: 39. DOI: 10.1038/s41438-018-0042-1.
- Zhu, Y. J. 2008. Seed development and fruit-set enhancement by girdling in ‘No Mai Tsz (73-S-20)’ Litchi (*Litchi chinensis* Sonn.). Master Thesis. National Taiwan Univ., Taipei (In Chinese with English abstract).