

Comparison of Fruit Development, Ripening, and Transcriptome Dynamics in Taiwanese and Japanese Cultivars of Japanese Apricot (*Prunus mume* Sieb. et Zucc.)

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In this study, we compared changes in traits associated with fruit development and ripening in Taiwanese and Japanese cultivars of Japanese apricot (*Prunus mume* Sieb. et Zucc.). We also analyzed transcriptome profiles to comprehensively examine different fruit development and ripening patterns between the two groups in terms of fruit characteristics and gene expression. Early fruit development in Taiwanese cultivars ‘ST’ and ‘Ellching’ and the Japanese cultivar ‘Hakuo’ was ahead of that in other three Japanese cultivars (P1). From late April to early May, around the stone-hardening stage, the developmental differences decreased to the same level. Thereafter, Japanese cultivars showed rapid growth, whereas Taiwanese cultivars showed slower growth, reversing the developmental differences between these lines (P2). Ethylene production was not detected until the full ripening stage and was detected for the first time at this stage in five cultivars, except for ‘Ellching’ (P3). In contrast, no ethylene production was observed during the entire duration of fruit development in ‘Ellching’. A multidimensional scaling plot showed that the overall transcriptome profile changed according to the three stages (P1–P3) of fruit development and ripening. At P1, gene ontologies (GOs) related to cell division, such as the cell cycle and regulation of cyclin-dependent protein serine/threonine kinase activity, were enriched for differentially expressed genes downregulated in Taiwanese cultivars as compared with their expression in Japanese cultivars. At P2, GOs related to fruit development were not enriched, but some genes related to phytohormones, such as auxin, abscisic acid, and cytokinin, which are associated with fruit development and ripening, were differentially expressed. At P3, the expression of genes such as *ACS*, *ACO*, and *PG*, which are involved in ethylene biosynthesis, increased in response to increased ethylene production, but not in ‘Ellching’, which showed no ethylene production. Expression analysis of 115 *NAC* (*NAM-ATAF1/2-CUC2*) family genes, which are related to fruit ripening and ripening date in other fruit species, in the ‘Ellching’ genome revealed changes in expression of *NAC056* and *NAC073* corresponding to fruit development and ripening in Taiwanese and Japanese cultivars. We discuss the differences in fruit development and ripening behaviors between Taiwanese and Japanese cultivars in terms of physiological and transcriptome changes.

Key Words: cell division, ethylene production, *NAC*, phytohormone, stone hardening.

Received; May 14, 2025. Accepted; July 15, 2025.

First Published Online in J-STAGE on November 26, 2025.

This work was partly supported by a donation from Ume Research Association and by JSPS KAKENHI Grant Number 24K01747 to T. K., T. K., and K. U.

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Introduction

The ripening date is one of the most important traits in fruit production. By shifting the harvest period from the main cultivars, high marketing returns can be expected. The length of the harvest period is directly related to the length of time during which the fruit can be processed and consumed, especially for climacteric fruits with a short shelf life (Taiti et al., 2021; Zhou



et al., 2023). Because of these economic advantages, the ripening date is considered one of the main targets in fruit breeding (Çuhacı et al., 2021; Elsadr et al., 2019; Lo Bianco et al., 2010; Saito, 2016).

Fruit development is a major factor contributing to differences in ripening dates. Fruit development exhibits two distinct patterns (Farinati et al., 2017): the single sigmoid pattern, in which fruit growth proceeds continuously, and the double sigmoid pattern, which includes a period of slow growth (stage II) between the early and late rapid-growth stages (stages I and III). The lengths of stages II and III in the double sigmoid pattern have been suggested to correspond to the ripening date in peaches (Bonghi et al., 2011; Farinati et al., 2021; Pavel and DeJong, 1993). Such cases have also been reported for apricot, Japanese plum and sweet cherry (Bolat and Ikinici, 2020; Khan, 2016; Ponce et al., 2021; Velardo-Micharet et al., 2021). Ethylene production is also closely related to ripening date although the causal relationship between them has not been fully characterized. Generally, cultivars with later ripening dates tend to produce ethylene later, and the amount of ethylene produced appears to be lower. ‘Beni Shogun’, a relative of the apple ‘Fuji’, shows an early increase in ethylene production and ripens approximately three weeks earlier than ‘Fuji’ (Kim et al., 2023). For peach and Japanese plum, the amount of ethylene produced has been suggested to correspond to the ripening time (Haji et al., 2004; Milas et al., 2015). Therefore, continuous investigation of fruit development and ethylene production is important to determine differences in the ripening of different varieties.

The Japanese apricot (*Prunus mume* Sieb. et Zucc.) is one of the major fruit trees in Japan, with a production of 96,600 tons in 2022 (FAO, 2022). The fruit is rarely eaten raw, but is processed into ume syrup, ume liqueur, and ume-boshi (pickled fruits) for consumption (Yaegaki, 2013). As Japanese apricot is a climacteric fruit, it must be harvested, distributed, and processed within a short period to maintain fruit quality and/or product value (Oe et al., 2008). Therefore, there is a strong need for breeding to shift the harvest period away from the main cultivars (Yaegaki, 2013). Previous studies on the ripening of Japanese apricot have shown that small-fruit cultivars, with fruit weight lower than 10 g, ripen earlier than other cultivars (Numaguchi et al., 2023). One of these cultivars, ‘Koshusaisho’, showed almost no growth retardation in stage II of fruit development (Yamaguchi et al., 2004), resulting in an early ripening date. Among late-ripening cultivars, “Taiwanese cultivars”, which have a genetic background different from that of major Japanese cultivars (Numaguchi et al., 2020), could be used as promising genetic resources in breeding programs to extend the harvest period of Japanese apricot (Kashiwamoto et al., 2023). However, fruit development and ripening characteristics up to harvest, as well as the associated changes in gene

expression, have not yet been investigated in late-maturing Taiwanese cultivars. In this study, we characterized the differences in fruit development and ethylene production between Taiwanese and Japanese cultivars to obtain basic data to expand the harvest period in Japanese apricot. In addition, we conducted transcriptome analysis during fruit development to gain insights into the transcriptome dynamics associated with different fruit development and ripening patterns between the two groups. Based on our results, we present a comprehensive discussion on the unique characteristics of the late ripening date in Taiwanese cultivars from basic, as well as practical, perspectives.

Materials and Methods

Plant materials and trait investigation

The following cultivars were used in this study: Taiwanese cultivars ‘ST’ and ‘Ellching’, Japanese small-fruit cultivar ‘Hakuo’, and Japanese normal-fruit cultivars ‘Kaidarewase’, ‘Gojiro’, and ‘Nanko’. All cultivars were grown in a field at the Japanese Apricot Laboratory (Minabe, Hidaka, Wakayama, Japan; 33.49°N, 135.21°E). The following traits were investigated in 2019–2020 and 2022: 80% flowering stage, stone hardening stage (50% start and 50% end; only in 2022), and 50% fruit dropping stage. The flowering and fruit dropping stages were determined via direct observation. The stone hardening stage was determined for 10 fruits using the same method as for peaches (Oura et al., 2011). Briefly, each fruit was halved along the suture line, and the stone hardening index was assessed by the phloroglucinol-HCl reaction on the endocarp; 0: no staining, 1: slightly pale pink at the apex side, 2: pink at the apex side, pale pink at the peduncle side, 3: entirely pink, 4: entirely dark pink and 5: entirely dark pink almost reddish purple. Of the 10 fruits collected every two to three days after April 5 (four days after April 1, 4 DAA), the dates when more than five fruits were evaluated as having a score of 1 and a score of 4 were recorded as the 50% start and 50% end dates, respectively. In 2019–2020, the fruit diameter on the horizontal (cheek) side was measured by collecting 10 average-sized fruits from the outside of the trees, approximately every week from April 3 (2 DAA) to the fruit-dropping stage. In 2022, the fruit diameters of the same 10 on-tree fruit were measured every week from April 5 (4 DAA) to fruit dropping. In 2019, the measured fruit were immediately placed in 500 mL sealed containers (Tupperware Brands Japan, Tokyo, Japan) and allowed to stand at 20°C for 1 h, as previously reported (Kashiwamoto et al., 2023). The headspace gas in the container was aspirated with a 1 mL syringe, and ethylene production was measured via gas chromatography (GC-2014ATF/SPL, detector FID, detection temperature 150°C, carrier gas N₂; Shimadzu Corporation, Kyoto, Japan). The 10 fruits were then divided into three to four fruits for three replicates, and the pulpy

parts, excluding the stones, were stored at -80°C and used for RNA extraction.

Library construction and sequencing analysis

Total RNA was extracted from the collected flesh using the hot borate method as described by Wan and Wilkins (1994). The extracted RNA was DNase-treated with deoxyribonuclease (RT Grade) to achieve heat stop (Nippon Gene, Tokyo, Japan) and was used to construct an mRNA-seq library using a KAPA mRNA HyperPrep Kit (NIPPON Genetics, Tokyo, Japan). The 138 prepared libraries were paired-end sequenced on an Illumina HiSeq X platform (Macrogen Japan, Tokyo, Japan). The data sets are available in the DDBJ Sequence Read Archive (DRA) (accession number DRA021741).

Transcriptome analysis during fruit development and ripening

We used the Omics Box software v3.1.11 (BioBam, Valencia, Spain) for subsequent transcriptome data analysis. After quality trimming and adapter removal using Trimmomatic (<http://www.usadellab.org/cms/?page=trimmomatic>), the obtained reads were aligned to the reference genome sequence of ‘Ellching’ assembled and annotated in our laboratory (Kashiwamoto et al., unpublished data) using STAR (Dobin et al., 2013). The whole genome was sequenced using a Nanopore sequencer, assembled with Flye (Kolmogorov et al., 2019), scaffolded using optical mapping (Saphyr System; BioNano Genomics, San Diego, USA), and finally linked with ragtag (Alonge et al., 2022) using the apricot (*P. armeniaca*) and peach (*P. persica*) genomes. Gene prediction was conducted using Braker (Gabriel et al., 2024), and gene function and GO were annotated using Omics Box. The number of mapped reads was counted using RSEM (Li and Dewey, 2011). The read count table obtained was used for the two analyses described below.

1) Multidimensional scaling plot analysis

To characterize the overall changes in transcriptome profiles during fruit development and ripening, a multidimensional scaling (MDS) plot showing similarities and differences in genome-wide expression profiles among the samples was created.

2) Pairwise analysis

We compared the transcriptome profiles of the Taiwanese and Japanese cultivars at characteristic time points (P1, P2, and P3), selected based on fruit development/ripening and the MDS plot. Pairwise differential expression analysis was conducted using edgeR (Robinson et al., 2010) with a false-discovery rate (FDR) < 0.05 , fold change $> |3|$ for P1 and P2, and FDR < 0.05 and fold change $> |5|$ for P3 as the cutoff values. In P1 and P2, up- and down-regulated genes among the differentially expressed genes (DEGs) in six replicates of ‘ST’ and ‘Ellching’ (P1: 2 DAA; P2: 32 DAA) were compared with those in six replicates of

‘Kaidarewase’ and ‘Nanko’ (P1: 2 DAA; P2: 45 DAA) used as controls. In P3, three replicates immediately before ethylene production were used as controls for ‘Kaidarewase’, ‘Nanko’, and ‘ST’ (‘Kaidarewase’: 60 DAA; ‘Nanko’: 73 DAA; and ‘ST’: 80 DAA), respectively. We then obtained the up- and down-regulated genes in three replicates after ethylene production for each cultivar (‘Kaidarewase’: 66 DAA; ‘Nanko’: 80 DAA; and ‘ST’: 92 DAA), and analyzed the commonalities among the three cultivars. Gene ontology (GO) enrichment analysis was performed for the obtained DEGs using g:Profiler (<https://biit.cs.ut.ee/gprofiler/>) with an FDR < 0.05 , using the ‘Ellching’ genome data. Changes in the expression of individual genes were assessed after transcript per million (TPM) normalization.

Results

Development and ripening traits

The dates of 80% flowering (Full bloom), stone hardening (50% start and 50% end), and 50% dropping (Full ripening) are shown in Table 1. Full bloom dates for Japanese cultivars ranged from January 28 to March 14, whereas those for Taiwanese cultivars were approximately one to two months earlier. Regarding stone hardening (in 2022 only), the dates of 50% start were within one week from April 12 to 19 except for ‘Gojiro’, which was almost the same for both Taiwanese and Japanese cultivars. The 50% end dates were earlier for ‘Hakuo’ and later for ‘Gojiro’, but were almost the same within a week for the other four cultivars. Full ripening dates for ‘Hakuo’ were the earliest (May 27–31) and those for ‘Nanko’ were the latest (June 14–24) among Japanese cultivars. Among Taiwanese cultivars, however, full ripening dates for ‘ST’ were July 1–18 and those for ‘Ellching’ were July 7–27, 10 to 24 days and 23 to 33 days later than those for the leading Japanese cultivar ‘Nanko’, respectively. Days from full bloom to full ripening ranged from 88 to 132 days for Japanese cultivars and from 168 to 193 days for Taiwanese cultivars. These results indicate that the fruit of Taiwanese cultivars starts to develop earlier due to early flowering, but ripens later than Japanese cultivars, resulting in a significantly longer fruit development period on the tree.

The fruit diameter for each cultivar was continuously investigated to compare the fruit developmental patterns. Figure 1 shows relative changes in fruit diameter, with the maximum value set at 100 for each cultivar. In all years, the relative fruit diameter for ‘ST’, ‘Ellching’, and ‘Hakuo’ was larger than that for the other three cultivars at the beginning of this study, and fruit enlargement was ahead of that for the other three cultivars. Subsequently, fruit development slowed in stage II and was almost the same for all cultivars around 30–32 DAA. Thereafter, fruit development restarted in the Japanese cultivars ‘Hakuo’, ‘Kaidarewase’, ‘Gojiro’, and ‘Nanko’ in that order. On the contrary, fruit development

Table 1. Date of flowering, stone hardening, and fruit dropping in Japanese and Taiwanese cultivars in 2019, 2020, and 2022.

Cultivar	Group	Year	80% Flowering (Full bloom)	Stone Hardening ^z		50% Fruit Dropping
				50% Start	50% End	
Hakuo	FS ^y	2019	Feb-12	— ^x	—	May-31
		2020	Feb-8	—	—	May-27
		2022	Mar-3	Apr-12	Apr-22	May-30
Kaidarewase	F	2019	Feb-15	—	—	Jun-6
		2020	Jan-28	—	—	Jun-8
		2022	Feb-22	Apr-15	Apr-28	Jun-11
Gojiro	F	2019	Mar-5	—	—	Jun-12
		2020	Feb-21	—	—	Jun-14
		2022	Mar-14	Apr-25	May-9	Jun-17
Nanko	F	2019	Feb-22	—	—	Jun-21
		2020	Feb-2	—	—	Jun-14
		2022	Mar-2	Apr-19	May-2	Jun-24
ST	T	2019	Dec-24, 2018	—	—	Jul-1
		2020	Jan-15	—	—	Jul-7
		2022	Jan-31	Apr-12	Apr-28	Jul-18
Ellching	T	2019	Jan-4	—	—	Jul-16
		2020	Jan-11	—	—	Jul-7
		2022	Jan-20	Apr-12	Apr-25	Jul-27

^z Stone-hardening stage was determined using the same method as described for peaches (Oura et al., 2011). Briefly, each fruit was halved along the suture line, and the stone hardening index (score 0–5) was assessed by the phloroglucinol-HCl reaction on the endocarp. Of the 10 fruits collected every 2–3 days after April 5, the date when more than five fruits were evaluated to have score 1 and score 4 was recorded as 50% start and 50% end, respectively.

^y FS, F, and T: Japanese cultivars bearing small fruit and Japanese and Taiwanese cultivars bearing normal fruit, respectively (Numaguchi et al., 2020).

^x No data.

in the Taiwanese cultivars ‘ST’ and ‘Ellching’ restarted later and/or at a slower rate than for Japanese cultivars. As a result, fruit development in Taiwanese and Japanese cultivars was reversed, despite the earlier development of Taiwanese cultivars.

Ethylene production was detected for the first time at the fruit dropping stage except for ‘Ellching’ (Fig. 1A). In ‘Ellching’, no ethylene production was observed during the entire period of fruit development.

Relationship between transcriptome dynamics and fruit development

The MDS plot shows the overall changes in the transcriptome profile during fruit development and ripening (Fig. 2). The plot shifted from negative to positive dim1, with a convex downward arc toward negative dim2 for all cultivars. However, the pattern differed among the cultivars, with Taiwanese and Japanese cultivars showing marked differences. We focused on three characteristic points (P1, P2, and P3) that reflect the differences in the expression patterns between Taiwanese and Japanese cultivars. P1 was the first sampling point (2 DAA), when the variation in expression in Taiwanese cultivars progressed earlier than that in Japanese cultivars. Next, between 14 DAA and 32 DAA, which was designated as P2, Taiwanese and Japanese cultivars were once plotted at the same position and then diverged considerably. The five cultivars,

except for ‘Ellching’, changed greatly toward the upper right at the last sampling point, and gathered at the position designated P3. These results were consistent with those of the aforementioned studies on fruit development and ethylene production. In the early stage of fruit development, Taiwanese cultivars were the first to develop (corresponding to P1), but at stage II, the relative fruit diameters for all cultivars were similar, and the fruit growth in Japanese cultivars was reversed compared with that in Taiwanese cultivars (corresponding to P2). In all cultivars except for ‘Ellching’, ethylene was produced from the fruit on the tree during the full ripening stage, and maturation progressed rapidly (corresponding to P3). Thus, a clear correlation was noted between changes in the transcriptome and the progression of fruit development and ripening.

Differential gene expression analysis

Three important points were identified in the MDS plot patterns. DEGs were specifically extracted for these points. For P1 and P2, we attempted to detect DEGs between two Taiwanese cultivars (six samples in total) and two standard Japanese cultivars (six samples) using edgeR to detect differences between these lines. In P1, 883 genes were upregulated, and 789 genes were downregulated, in Taiwanese cultivars. GO enrichment analysis revealed that factors related to stone hardening, such as secondary cell wall and lignin, and cell division,

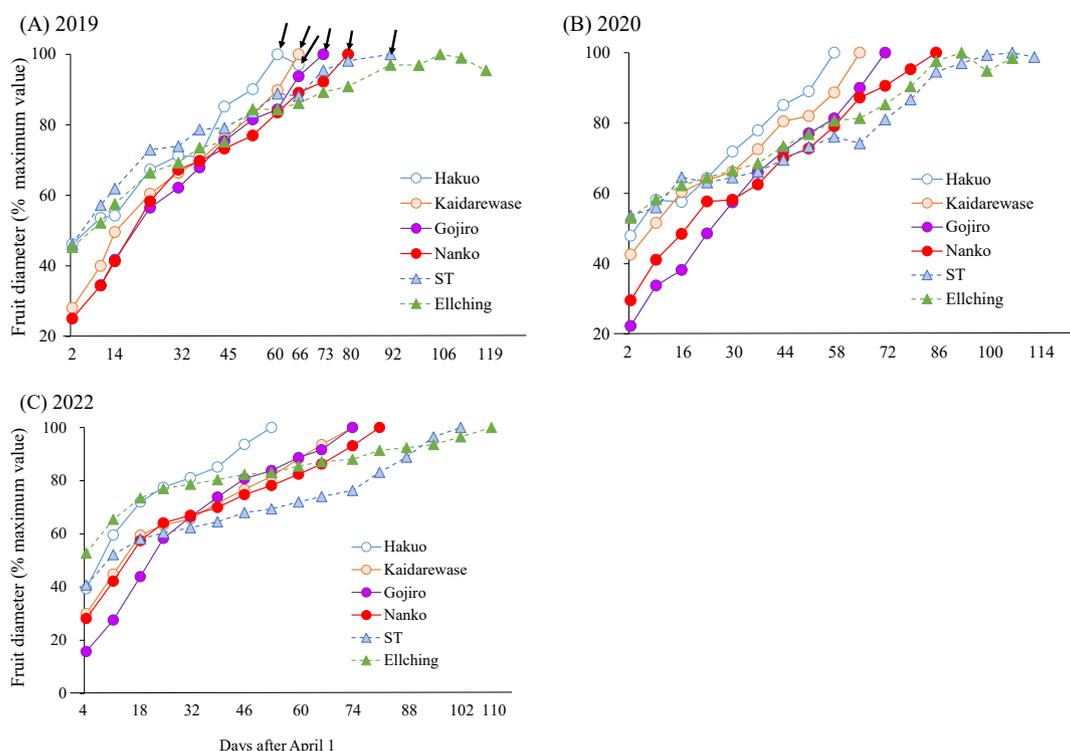


Fig. 1. Changes in relative fruit size during fruit development in Japanese and Taiwanese cultivars in 2019 (A), 2020 (B), and 2022 (C). Diameters of different fruit collected on each sampling date were measured in 2019 and 2020, while those of the same fruit were continuously measured in 2022 ($n = 10$ for all years). Each plot represents the percentage of the mean value of fruit diameter at each sampling/measurement date relative to the maximum mean value during overall fruit development for each cultivar. Arrows indicate the timings when ethylene production was detected in 2019.

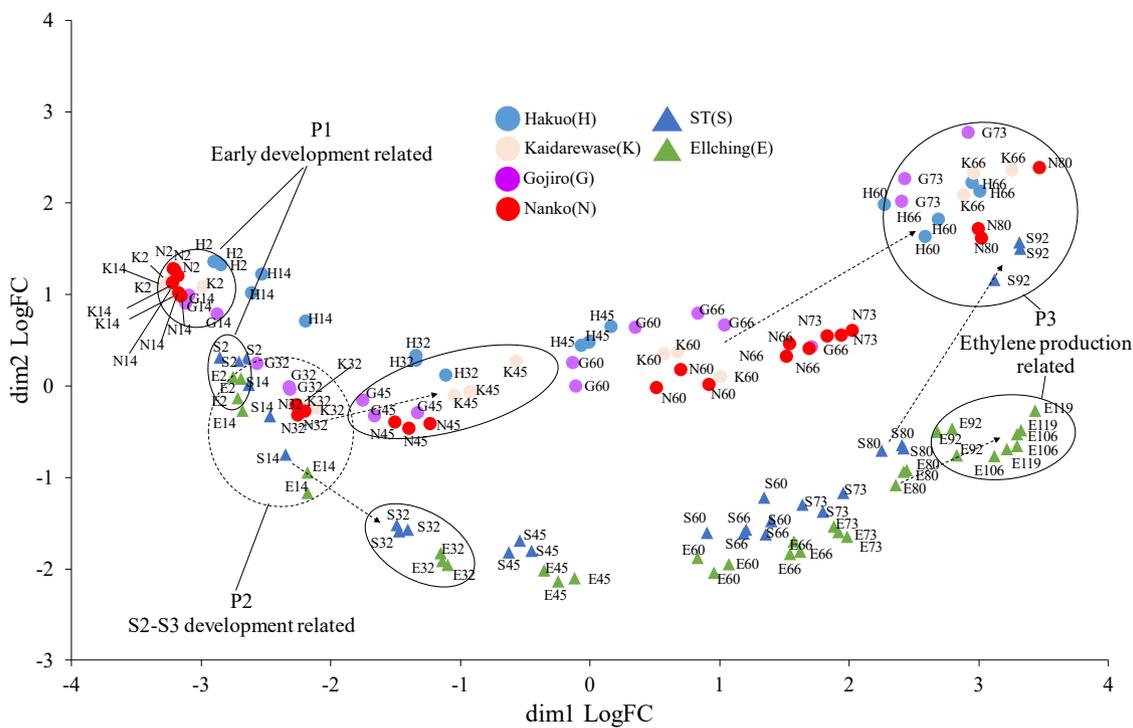


Fig. 2. Multidimensional scaling plot showing changes in transcriptome profiling during fruit development in Japanese and Taiwanese cultivars (2019). The first letter indicates each cultivar, Hakuo (H), Kaidarewase (K), Gojiro (G), Nanko (N), ST (S), and Ellching (E). The numbers 2–119 indicate sampling date calculated as the number of days after April 1 (DAA). P1–P3: Characteristic points that reflect differences in transcriptome profiling between Japanese and Taiwanese cultivars.

such as cell cycle and cyclin-dependent protein kinase (*CDK*), were overrepresented for the up- and down-regulated genes in Taiwanese cultivars, respectively (Table S2). The ‘Ellching’ genome contains 48 *CDKs* and cyclins, of which nine were detected as DEGs. When the actual expression patterns of these DEGs were confirmed in the six cultivars used in this study, expression levels of most of them were clearly low in Japanese ‘Hakuo’, as well as in Taiwanese cultivars, in early fruit development (Fig. 3). Generally, phytohormones affect cell division and expansion during the early stages of fruit development. Therefore, we extracted genes annotated with GO terms (Table S1) related to phytohormones for the 1,672 DEGs in P1. In total, 150 DEGs were identified. Among the nine phytohormones, 14 DEGs were most frequently annotated with auxin-related genes, including *IAA* and *SAUR32*, for which expression is known to change in response to auxin, and were high in Taiwanese cultivars in P1 (Fig. 4). Cytokinin (CK) is a hormone related to cell division, and genes involved in CK synthesis (*IPT* and *LOG*) or metabolism (*cytokinin dehydrogenase*) were included in the DEGs in P1. The expression of CK synthesis-related genes was low in Taiwanese cultivars, whereas that of CK metabolism-related genes was high (Fig. 4).

Among the DEGs in P2, 790 genes were upregulated, and 581 genes were downregulated, in Taiwanese cultivars (Table S3). The GO terms enriched in these DEGs were likely systemic acquired resistance (GO:

0009627), defense response (GO: 0006952), and floral whorl development (GO: 0048438). However, no clear GOs related to fruit development were enriched (Table S4). When hormone-related genes were extracted as in P1, 157 DEGs were identified. Among these, 43 DEGs were lineage-specific, such as Taiwanese/Japanese cultivars, and were possibly related to fruit development and ripening. Seventeen of these DEGs were related to auxin, and 9 DEGs each were related to ABA and CK. Auxin-related genes include *SAUR* and *GH3*, the expression levels of which vary upon auxin stimulation. *GH3* is an enzyme that regulates auxin metabolism, whereas *zeatin O-glucosyltransferase*, *cytokinin dehydrogenase 5*, and *abscisic acid 8'-hydroxylase 3* are CK and ABA metabolism-related enzymes. Among the 43 DEGs, some genes encoding these enzymes exhibited characteristic expression patterns (Fig. 5). In particular, the expressions of *GH3* and *zeatin O-glucosyltransferase* increased rapidly from P2 to late fruit development and ripening, and their expression patterns were similar despite their involvement in the metabolism of different phytohormones.

In P3, the results of the MDS plot suggested that the presence or absence of ethylene synthesis on the tree was related to lineage-specific differences, such as in Taiwanese and Japanese cultivars. Therefore, we adopted a different approach to detect DEGs than that used for P1 and P2. First, DEGs were detected immediately before and after ripening for three cultivars, ‘ST’,

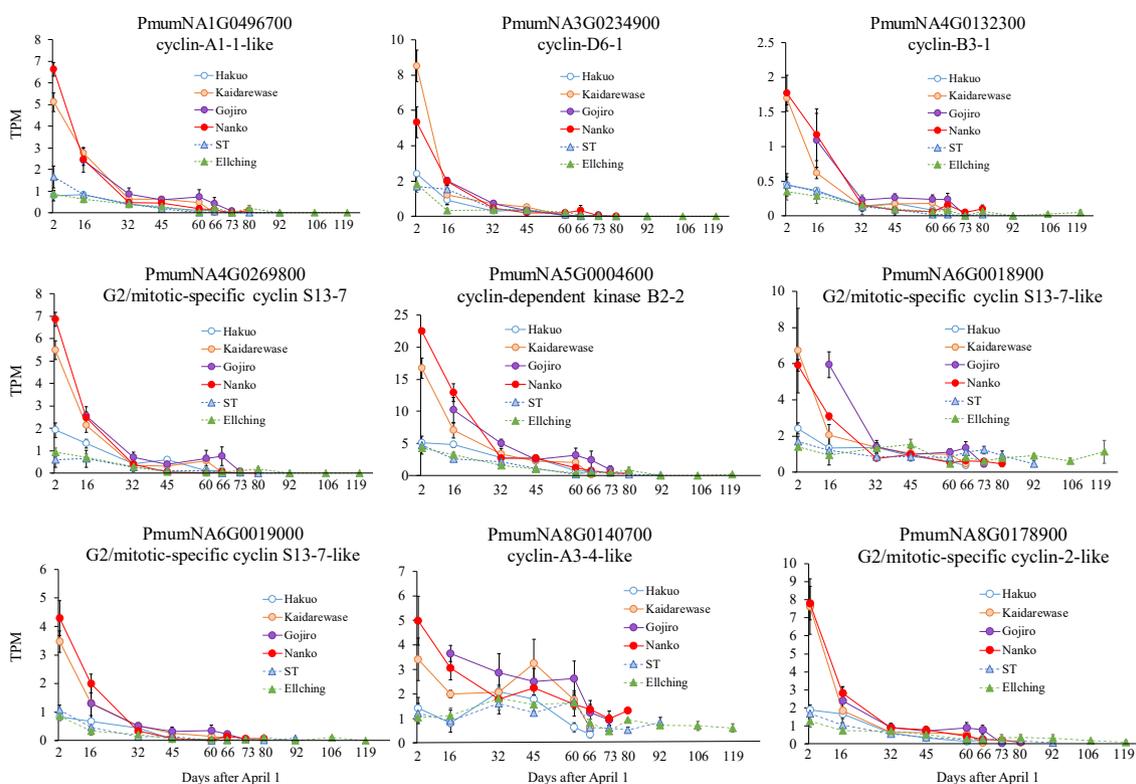


Fig. 3. Expression patterns of genes associated with cell division in Table S1. Changes in their expression during fruit development are shown as transcripts per million (TPM) for each cultivar ($n = 3$).

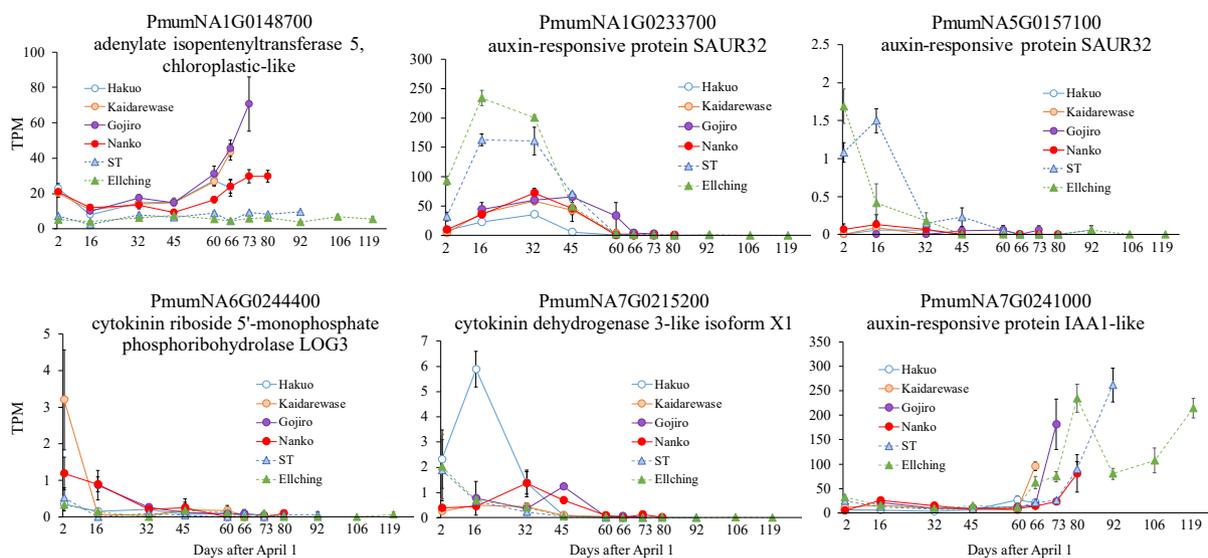


Fig. 4. Expression patterns of genes associated with the hormone synthesis or metabolism in Table S1. Changes in the expression levels of genes during fruit development are shown as transcripts per million (TPM) for each cultivar ($n = 3$).

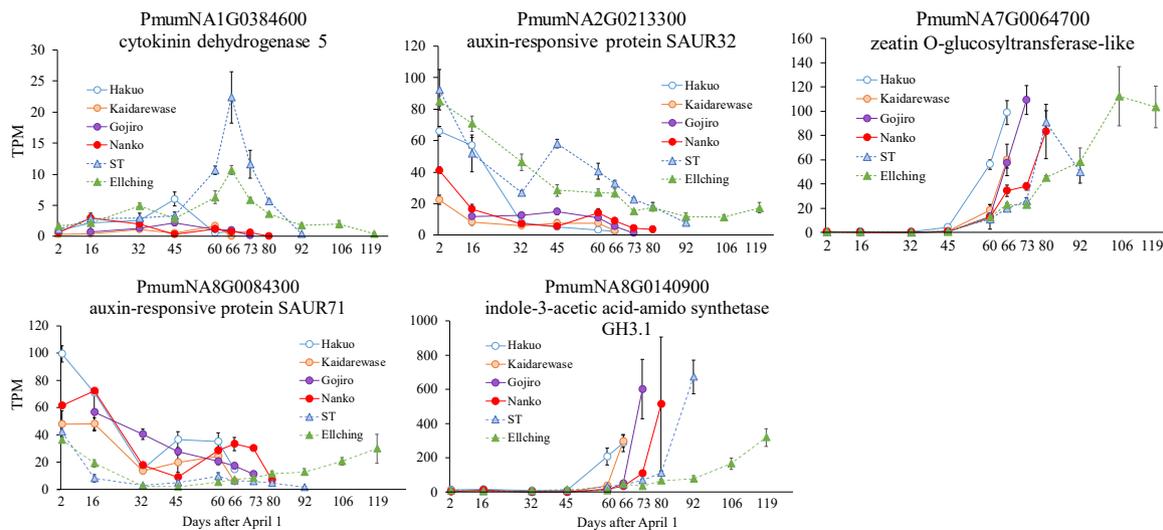


Fig. 5. Expression patterns of genes associated with the hormone synthesis or metabolism in Table S3. Changes in the expression levels of the genes during fruit development are shown as transcripts per million (TPM) for each cultivar ($n = 3$).

‘Kaidarewase’, and ‘Nanko’, which were considered to be strongly affected by ethylene. Genes that were commonly up- or downregulated in the three cultivars were selected as DEGs in P3. For ‘ST’, ‘Kaidarewase’, and ‘Nanko’, 143 genes were commonly upregulated and 182 genes were commonly downregulated after ethylene production (Table S5). The GO terms enriched in the highly expressed genes after ethylene production included fruit ripening (GO: 0009835) (Table S6). Genes with this GO term included *1-aminocyclopropane-1-carboxylate synthase (ACS)*, *1-aminocyclopropane-1-carboxylate oxidase (ACO)*, and *polygalacturonase (PG)*, which are characteristic of fruit maturation. When the expression levels of these genes were verified in ‘Ellching’, no increase in expression was observed (Fig. 6).

Expression patterns of transcription factors

Compared with P1 and P3, no clear GO enrichment was observed for P2. Therefore, we investigated the expression levels of *NAC* family genes, which encode transcription factors involved in fruit ripening. Most of the 115 *NAC* family genes in the ‘Ellching’ genome were only slightly expressed or did not differ between Taiwanese and Japanese cultivars. Among the *NACs* that apparently differed between Taiwanese and Japanese cultivars, *NAC056* and *NAC073* had high TPM values (Fig. 7). The expression of *NAC056* was higher in Taiwanese cultivars in the early stage, but exhibited a reversal in the late stage of development, between Taiwanese and Japanese cultivars. The expression of *NAC056* showed a more rapid increase after ethylene production, but not in ‘Ellching’, which showed

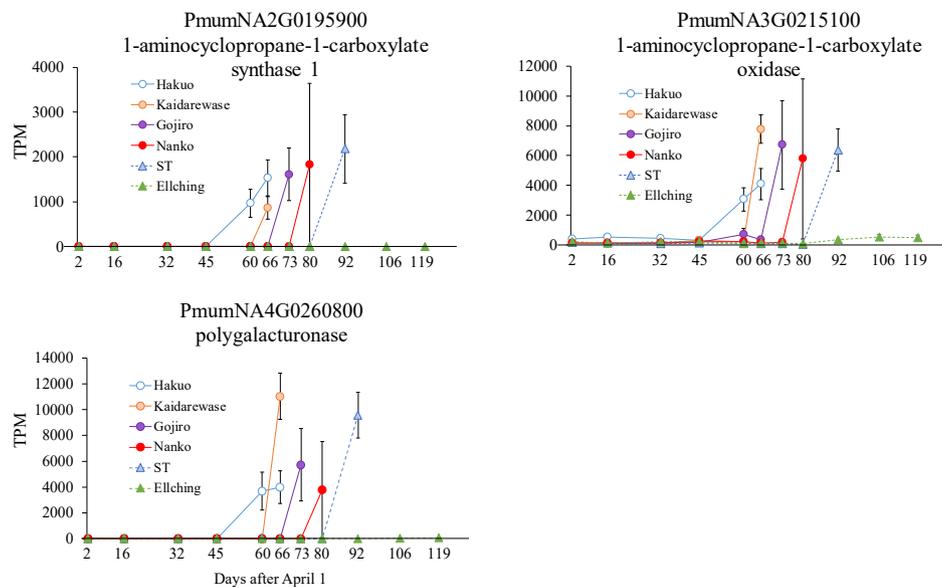


Fig. 6. Expression patterns of genes associated with fruit maturation in Table S5. Changes in the expression levels of genes during fruit development are shown as transcripts per million (TPM) in each cultivar ($n = 3$).

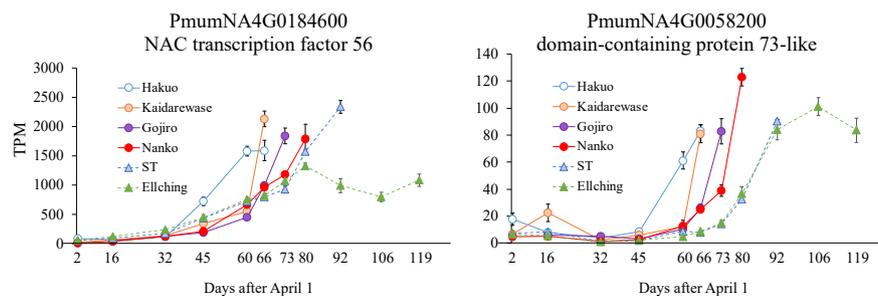


Fig. 7. Expression patterns of genes associated with the fruit maturation in Japanese and Taiwanese cultivars. Genes of NAC family in ‘Ellching’ genome were selected, and changes in their expression levels during fruit development are shown as transcripts per million (TPM) for each cultivar ($n = 3$).

no ethylene production. The expression of *NAC073* changed corresponding to early or late ripening, with an early increase in Japanese cultivars and a late increase in Taiwanese cultivars. At the ripening stage, the expression of *NAC073* increased rapidly in ‘Ellching’, as well as in ‘ST’, showing a different pattern compared to *NAC056*.

Discussion

In this study, to identify physiological characteristics affecting the harvest period of Japanese apricot, we investigated changes in fruit development and ethylene production in Taiwanese and Japanese cultivars at different ripening dates and analyzed the transcriptomes associated with fruit development. We then compared the ripening patterns of each line from both fruit trait and molecular biological perspectives.

First, at the beginning of this study (P1; 2 DAA), early fruit enlargement was observed in Taiwanese cultivars and the Japanese small-fruit ‘Hakuo’ (Fig. 1). Fruit development in Japanese apricot occurs due to cell

division in the first half of S1, and the expansion of cells proceeds as the cell division decreases (Yamaguchi et al., 2004). Taiwanese cultivars have a lower chilling requirement for flower buds (Yamane et al., 2006), and ‘ST’ and ‘Ellching’ used in this study also reached the full-bloom stage on average more than one month earlier than ‘Nanko’ (Table 1), which may have resulted in earlier cessation of cell division and earlier cell expansion. In addition, the Japanese small-fruit ‘Koshusaisho’ showed an earlier cessation of cell division than other Japanese cultivars (Yamaguchi et al., 2004), indicating that cell expansion in ‘Hakuo’ was progressing as in the case of Taiwanese cultivars. After the stone-hardening stage began in mid-April (Table 1), the development of all cultivars slowed down to a similar level (P2; 14 DAA to 32 DAA), and Japanese cultivars developed rapidly and ripened earlier, whereas Taiwanese cultivars developed more slowly and ripened later (Fig. 1). The beginning of the stone-hardening stage (stage II) was almost the same for Taiwanese and Japanese cultivars except for ‘Gojiro’, indicating that the length of stage II

to III was longer in Taiwanese cultivars. The development of stone fruit shows a double sigmoid pattern, whereby enlargement is stagnant during stage II and then restarts during stage III (Farinati et al., 2017). The transition period from stage II to stage III is longer in late-ripening peach cultivars compared with that in early-ripening ones (Pavel and DeJung, 1993). The same trend was observed in apricot, Japanese plum, and sweet cherry (Bolat et al., 2020; Khan, 2016; Velardo-Micharet et al., 2021). Therefore, the Taiwanese cultivars used in this study were also considered to have a developmental pattern of later-ripening cultivars compared with the Japanese cultivars.

Ethylene production was not detected in any of the cultivars until the fruit-dropping stage, and was detected for the first time in five cultivars at that stage, but not in ‘Ellching’. Ethylene production in Japanese apricot fruits remained below 0.1 until the beginning of fruit yellowing, and then increased rapidly, leading to rapid ripening and fruit drop (Inaba and Nakamura, 1981). In this study, the same trend was observed for the five cultivars, but the trend was different for ‘Ellching’. ‘Ellching’ did not produce ethylene on the tree until the end of this survey, resulting in an average ripening date nine days later compared with that in Taiwanese ‘ST’ (Table 1).

Considering the changes in fruit developmental stage and transcriptome profiling, P1 and P2 were considered important factors determining the fruit maturation rate of Taiwanese and Japanese cultivars (Fig. 2). Three points (P1–3) were analyzed in the transcriptome, and P3 was believed to capture changes in gene expression caused by climacteric ethylene signals. Typical ethylene-related genes, such as *ACS*, *ACO*, and *PG* (Houben and Van de Poel, 2019; Tucker et al., 2017) were identified as DEGs (Fig. 6). In P1, GO factors such as cell cycle and cell division, were enriched in DEGs downregulated in Taiwanese cultivars (Table S2). In fact, cell cycle-related genes, such as *cyclin A1-1-like*, *B3-1*, *D6-1*, and *S13-7-like* (Koepp et al., 1999), were barely expressed in Taiwanese cultivars at P1, suggesting that cell division had stopped (Fig. 3). In general, fruit enlargement is believed to be caused by cell division in the early stage, followed by cell expansion (Cheniclet et al., 2005), and phytohormones like CK and auxin are involved in these changes (Farinati et al., 2017). Generally, CKs control cell division. The fact that CK synthesis genes, including *IPT* and *LOG* (Kamada-Nobusada and Sakakibara, 2009), were expressed in Japanese cultivars indicated that the fruits were still in the cell division phase in P1. In contrast, the fact that *CKX*, a gene involved in CK metabolism (Schmülling et al., 2003), was expressed at a higher level in Taiwanese cultivars suggested that they had already transitioned from the cell division to the cell expansion phase (Fig. 4). The flowering of Taiwanese cultivars, which have low cold requirements (Yamana

et al., 2006), occurs one month or more earlier than that of Japanese cultivars. Cell division begins immediately after fruit set and is followed by cell expansion (Cheniclet et al., 2005). Therefore, it is highly plausible that the difference between Taiwanese and Japanese cultivars in P1 reflects this difference in flowering time. Because this study only investigated gene expression, it is believed that investigating hormone levels, as well as other factors, will be necessary to support this hypothesis.

During P2, fruit development temporarily stalled and then resumed (Fig. 1). After resumption, the development of Japanese cultivars was rapid, whereas that of Taiwanese cultivars was delayed, resulting in a reversal in the degree of development. We were unable to identify the genes or transcriptome changes that caused differences in fruit development between Taiwanese and Japanese cultivars during this period (Table S4). When we focused on hormone-related genes in the same way as in the analysis of P1, we found that auxin-related *SAUR* and *GH3* and CK-related *cytokinin dehydrogenase* and *zeatin O-glucosyltransferase-like* genes were expressed in parallel with fruit development (Fig. 5). *SAUR* and *GH3* are directly regulated by auxin signaling, indicating that auxin signaling may be activated (Hagen and Guilfoyle, 2002; Ren and Gray, 2015). However, considering that some *SAUR* genes showed contradictory expression patterns between Japanese and Taiwanese cultivars (Fig. 5), that *SAUR* is a large gene family (Hagen and Guilfoyle, 2002), and that *GH3* is involved in metabolism (Mellor et al., 2016), it is difficult to confirm whether auxin is involved in fruit development at this stage, if it promotes or inhibits development, or whether it is involved in other mechanisms. The expression of *zeatin O-glucosyltransferase-like*, a CK-related gene (Polanská et al., 2007), also increased in response to fruit development (Fig. 5). Both *GH3* and *zeatin O-glucosyltransferase-like* showed a sharp increase in expression in the late developmental stage rather than in the P2 stage. This expression pattern may indicate that these genes function in the late developmental stage rather than in the P2 stage. However, if it is assumed that these genes reflect the activity of hormone signaling, it is implied that these hormones may function at this time. As we did not measure plant hormones other than ethylene, it is difficult to discuss the relationship between this phenomenon and plant hormones. In addition, sampling for transcriptome analysis was performed once every one or two weeks, which is considered too long an interval for such analysis. However, we were able to clarify the differences between Taiwanese and Japanese cultivars and identify the importance of the P2 stage. Although the cause could not be identified, this is an important finding for future Japanese apricot research.

Finally, because no characteristic GOs were detected in P2, which is associated with fruit development and ripening, we investigated changes in the expression

of individual genes among the *NAM-ATAF1/2-CUC2* (*NAC*) family genes. The representative *SINOR* is an important regulator of fruit ripening, and its mutants do not ripen normally (Gao et al., 2019). *MaNAC18.1* and *PavNAC056* are highly associated with ripening date and fruit hardness in apple and sweet cherry (Migicovsky et al., 2021; Qi et al., 2022). In peach, a close relative of Japanese apricot, the fruit ripening date is controlled by *PpNAC1* and *PpNAC5* haplotypes (Zhang et al., 2024). Therefore, we selected the *NAC* family for analysis in this study. We investigated the expression of 115 *NAC* family genes in the ‘Ellching’ genome and found that two *NAC* orthologs showed expression patterns related to fruit development (Fig. 7). In particular, *NAC056* showed a reversal in expression in Taiwanese and Japanese cultivars in the latter half of development, and the functions of its orthologs in tomato, apple and peach suggest that it may be involved in fruit development and ripening in Japanese apricot. The analysis in this study was limited to the expression pattern; therefore, further studies will be required to prove gene function and identify the genes that regulate expression.

In this study, we identified differences in fruit development between Taiwanese and Japanese cultivars and found a significant difference at P2 based on transcriptome profiling. Although we could not confirm the cause of these differences, we identified several hormone-related genes and transcription factors for which expression changed in response to fruit development. These results could provide important insights for future studies on fruit development and ripening in Japanese apricot.

Acknowledgements

We are grateful to researchers and technicians at the Japanese Apricot Laboratory for maintaining the plant materials used in this study. We would like to thank Editage (www.editage.jp) for English language editing.

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