

Article

Phenotypic and Gene Expression Analysis of Fruit Development of ‘Rojo Brillante’ and ‘Fuyu’ Persimmon (*Diospyros kaki* L.) Cultivars in Two Different Locations

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Abstract: Fruit development and maturation rely on intrinsic genetic programs involving hormone biosynthesis and signalling and environmental cues, integrating phenological cycles and climatic issues encompassing abiotic stresses and climate change. In persimmon trees, environmental inputs strongly influence fitness and agricultural performance, and fruit yield can be severely compromised by them. We have grown two persimmon accessions (‘Rojo Brillante’ and ‘Fuyu’) under contrasting meteorological conditions of two locations in Spain and Japan. Fruit size, colour change, and firmness parameters were followed during fruit development from 30 days after fruit set until commercial ripening, and the expression of genes related to ethylene production and signalling, gibberellin response, carotenoid biosynthesis, cell wall dynamics, and oxidative stress were reported. Genes depending on intrinsic developmental programs (ethylene and ripening variables, mostly) showed common expression trends in both cultivars and locations, whereas gibberellin and abiotic stress-related genes mimicked reduced fruit growth and abiotic stress associated with higher summer temperatures (>35 °C) and lower rainfall reported in the Spanish location. The expression pattern of these genes is consistent with a growth–defence trade-off that explains fruit differential growth through hormonal and stress tolerance mechanisms.

Keywords: fruit development; environmental conditions; gene expression; abiotic stress



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1. Introduction

Persimmon (*Diospyros kaki* L.; Ebenaceae family) is a hexaploid fruit tree species that is of great importance in some Asian countries, especially in China, where it originated [1]. Recently, its cultivation has expanded rapidly in the Mediterranean basin. In particular, Spain has increased its persimmon growth area from 2500 ha in 2004 to almost 15,000 ha in 2015, reaching a total production of 438,819 tons in 2022 [2,3]. Nevertheless, the Asian continent remains the main producer worldwide (90.3%), followed by Europe (6.7%) [2]. According to an FAO report, world persimmon production has duplicated its value in the last 20 years with available data (2002–2022), reaching a total production of 4.43 million tons in the year 2022 [4]. According to both cultivation area and production, the most important cultivar in Spain is ‘Rojo Brillante’; indeed, it is the only one recognized by the Denomination of Origin “Kaki Ribera del Xúquer”, in the productive region of Valencia [5]. On the other hand, cv. ‘Fuyu’ is the most popular pollination-constant non-astringent (PCNA) cultivar in Japan [6].

Persimmon fruit growth fits with a double sigmoidal curve model, and it can be divided into three phases [7]. Phase I and II correspond to two periods of active growth. Phase I starts 10–12 weeks after pollination, and Phase III from 18 weeks after pollination to harvest. These two phases are separated by 6–7 weeks of lower growth rate (Phase II), matching with the summer period, which delays the fruit growth rate because of the typically warmer temperatures of this season [8]. Although it is in Phase II where persimmon fruits experience a lower growth rate, this period has an important role since it has been shown to significantly affect the final size of the fruit [7]. Fruit ripening is regulated by intrinsic factors and environmental cues, leading to a complete modification of phenotypic traits such as fruit colour, aroma, flavour, texture, and other attributes, which finally determine the quality of the fruit [9]. Therefore, it is a continuous and irreversible process that modifies physiological, biochemical, and organoleptic fruit characteristics [10,11], which are controlled by a series of molecular events, starting with the signalling pathway's activation [12]. These pathways regulate specific transcriptional regulators, triggering concerted gene expression that controls biochemical processes [13,14]. In climacteric fruits, such as persimmon, fruit ripening is led by the plant growth regulator (PGR) ethylene [12]. Ethylene is a gaseous plant hormone associated with some other response and developmental processes, including root and flower development, seed germination, senescence, and response to biotic and abiotic stresses [15]. The ethylene signalling cascade ends with the transcriptional activation of transcription factors named Ethylene Response Factors (ERFs) [16]. ERFs directly mediate the ethylene response by repressing or activating the expression of ripening, ethylene biosynthesis, colour change, and fruit softening-related genes [17]. Ethylene response is regulated from the synthesis of this hormone to the signal transduction and transcriptional regulation. [18]. Many studies on the ethylene role in persimmon fruit development have been based on harvested fruits to study traits such as shelf life or the cold storage period [19,20], whereas the transcriptional modulation of ethylene biosynthesis or signal transduction of on-tree fruit ripening mechanisms remains poorly studied. In addition to genetic developmental programs, environmental conditions such as temperature and precipitation have been reported to decisively influence persimmon fruit ripening parameters [21].

Evidence about the hormonal control of developmental and environmental factors on fruit maturation and ripening came from the application of PGRs. A very common agronomic practice is the application of gibberellins (GAs) to modify the stature of many crop species. Bioactive GAs (diterpene phytohormones) modulate growth and development throughout the plant's life cycle. To modulate those processes, the GA pathway is regulated as well by endogenous signals and environmental factors such as light, temperature, or salt stress [22]. Normally, these internal and external signals directly affect GA metabolism and the level of bioactive GA, GA-repressor accumulation (such as DELLA proteins), and GA responses indirectly [23]. Different studies have identified a class of GA receptors and several early elements of the GA signalling pathway [24,25]. Moreover, GAs activate their response pathway by binding to their receptors, GA INSENSITIVE DWARF1s (GID1s) [23].

Regarding environmental determinants, temperature and water availability are key factors affecting plant metabolism at the cellular level. Plants increase the production of many reactive oxygen species (ROS) when they are stressed, leading to the accumulation of ROS-scavenging enzymes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), which contribute to maintaining their normal catalytic state [26]. Previous studies have revealed that persimmon fruit quality parameters such as fruit weight, skin colour, soluble solids, and soluble tannins are affected by temperature [27]. Orchard-dependent differences in temperature range are commonly attributable to variations in latitude, topography, or proximity to large bodies of water [21]. Different authors have reported that hydric stress, together with high temperatures during flower differentiation, induces double or malformed pistils within floral primordia [28]. Also, a deficit of irrigation has been reported as a cause of fruit weight reduction in the cultivar 'Rojo Brillante' [29].

In this study, we compare fruit development and maturation at morphological and molecular levels of two persimmon cultivars grown in two different locations in Spain and Japan, with the goal of finding common and divergent factors involved, respectively, in intrinsic developmental pathways and environmental issues. With this aim, we measured fruit parameters and the differential expression of genes involved in on-tree fruit ripening and stress response from an early stage of fruit development to commercial ripening (CR). Also, climatic data were collected for the whole fruit development process. This information will contribute to deciphering molecular mechanisms related to on-tree persimmon fruit ripening and the influence of the environment on this process.

2. Materials and Methods

2.1. Plant Material

Fruit samples were taken from persimmon cultivars ‘Rojo Brillante’ and ‘Fuyu’, grown in two different locations. The Spanish location (SP) is situated at the IVIA’s germplasm bank in the Valencian Community (39.585346, −0.395117). The Japanese location (JP) corresponds to NARO’s persimmon collection in Hiroshima prefecture (34.3328336674389, 132.823037). Cultivars were selected by their astringency type and genetic background according to a previous work [30]. Three fruits with similar shape, colour, and size were collected at every sampling date (S), with a 21-day interval, from +30 days after fruit set (+30D AFS) until commercial ripening (CR). Once the fruit overpassed the CR stage, sampling was stopped.

2.2. Meteorological Data Collection

Meteorological data were collected from climatic stations at IVIA [31] and NARO (not available online). Values of relative humidity (%), pluviometry (mm), and temperature (°C) were collected to conduct a comparative climate analysis between SP and JP. This data collection can be found in Table S1.

2.3. Phenotypic Evaluation and Sample Collection

Fruit size (mm), weight (g), total soluble solids content (TSS, ° Brix), flesh firmness, and skin colour index (CI) were measured at each sampling date (Table S2). Three measures of height and width per fruit were taken with a calliper to record fruit size. TSS in SP samples was measured with an Atago PR-100 digital refractometer (Atago Co., Shibakoen, Minato-ku, Tokyo, Japan) and with a calibrated refractometer in JP samples (model MASTER-alpha; Atago, Tokyo, Japan), with the last samples of each cultivar/location combination represented along the text. Fruit firmness was measured by the level of force needed to cause tissue break. A Shimadzu EZ-L penetrometer with a cm² probe (Kyoto, Japan) was used in SP samples, and a firmness meter with a 5 mm diameter cylindrical plunger (KM-5; Fujiwara Scientific Co., Ltd., Tokyo, Japan) in JP samples. Firmness values are expressed in percentage with S1 being 100%. The colour of the skin was measured by calculating the mean value from three readings per fruit. A Minolta Chroma Meter CR-300 (Osaka, Japan) was employed in SP samples, and a CR-400 (Konica, Minolta ; Osaka, Japan) was used for JP samples. Colour Index (CI) was calculated as $(1000 \times a)/(L \times b)$ [32], and the CR stage was determined by CI values between 5 and 20 (colour transition from green to orange) as established in a previous study [33]. Whole seedless fruit samples after calyx removal (three biological replicates per accession) were finely ground into powder with liquid nitrogen and stored at −80 °C until RNA extraction.

2.4. RNA Extraction and Gene Expression Measurement

In SP, RNA extraction was performed using a Plant/Fungi Total RNA Purification Kit (Norgen Biotek Corp., Thorold, ON, Canada) from 70 mg of fruit tissue. Contaminant genomic DNA was removed during RNA isolation according to kit recommendations. For the extraction of RNA from persimmon fruits in JP, the hot borate method was employed, as detailed in a previous study [34]. Qubit (Invitrogen, Carlsbad, CA, USA) fluorometry

was used for RNA quantification, and agarose gel electrophoresis was performed to verify RNA integrity. Finally, cDNA was synthesized from RNA in a total volume of 10 µL with the PrimeScript RT Reagent Kit (Takara Bio, Otsu, Japan).

Quantitative real-time PCR (qRT-PCR) was conducted using StepOnePlus Real-Time PCR System (Life Technologies, Carlsbad, CA, USA) with TB GREEN premix Ex Taq (Tli RNaseH Plus) reactive (TAKARA BIO; Kutatsu, Japan) using 1 µL of 10× diluted cDNA. The relative standard curve method was used to calculate relative expression levels. *DkTUA* and *DkACT* were used as housekeeping reference genes [35]. Gene coding sequences were identified using BLASTN and BLASTX tools at PersimmonDB available genome [36,37], *Arabidopsis thaliana* (TAIR) DNA and protein databases (DKA_r1.0.cds, and DKA_r1.0.pep, respectively) [38] and *Diospyros lotus* expressed sequence databases available at NCBI [39]. A reciprocal BLAST was performed in each case to verify putative orthologues. Gene-specific primers employed in gene expression analysis are detailed in Table S3. Primer3 software was used for primer design [40]. The specificity of the qRT-PCR reaction was verified by a single peak in the dissociation curve after the amplification.

2.5. Data Analysis

Morphological trait measurements were analysed by the non-parametric statistical test Kruskal–Wallis using the IBM SPSS Statistics v.29.0.1.0 software (Armonk, NY, USA). A pairwise comparison was conducted with a significance level of $p < 0.05$. Least-squares regression analysis and 3rd-order polynomial curve fitting of fruit weight were performed using Microsoft Corporation Excel 365 v2017 software (Redmond, WA, USA; Figures S1 and S2, respectively).

Data analysis was carried out using the RStudio integrated development environment for R 2022.02.2 (<https://rstudio.com/>, accessed on 15 November 2023) and Bioconductor framework 3.16 (www.bioconductor.org, accessed on 15 November 2023). The ggfortify R package 0.4.11 [41] was used to perform a principal component analysis (PCA) [42] to plot a two-dimensional projection of normalized gene expression and phenotypic traits correlations. Expression heatmaps were obtained using the pheatmap R package 1.0.12 [43], and gene expression pattern was obtained scaling by row values.

3. Results

3.1. Phenological and Meteorological Differences between Locations

‘Fuyu’ and ‘Rojo Brillante’ cultivars show important differences in fruit astringency, phenology, and genetic background in spite of their common high agronomical relevance in Japanese and Spanish markets, respectively (Table 1). Sampling, from 30 days after fruit set until CR, was performed on different dates in SP and JP (Table 2).

Table 1. Cultivars used in this work.

Accession	Origin	Fruit Astringency	Location	+30D AFS
Rojo Brillante	Spain	PVA *	SP	20 June
Rojo Brillante	Spain	PVA *	JP	27 June
Fuyu	Japan	J-PCNA	SP	20 June
Fuyu	Japan	J-PCNA	JP	27 June

* PVA: pollination variant astringent; J-PCNA: Japanese pollination constant non-astringent.

Regarding environmental conditions, the temperature in the SP location had higher values than the JP location in summer (Figure 1A). This period corresponds to the initial stages of fruit set and development. The SP location registered twenty-two days with temperatures over 35 °C, while the JP location had only one (Table S1). Maximum temperature differences (over 15 °C) between SP and JP were recorded in the S2 sample. Temperatures started to be higher in JP than in SP at the intermediate stages of development, at the beginning of the ripening process. Despite the JP location having warmer autumn and

winter seasons, the temperature did not reach values over 35 °C. The relative humidity was lower in SP in summer (Figure 1B). During fruit early development, the JP location showed 51% of days with RH values over 80%, while SP only showed 2% in the same period. When fruit had reached full development and started to ripen, corresponding to the autumn and winter seasons, RH values were similar in both locations. Finally, precipitation was considerably higher in the JP location throughout the entire fruit development process (Figure 1C). SP showed just one day of high precipitation (>10 mm) during the study (30 September with a rainfall of 12.6 mm per hour). Meanwhile, in the JP location, high rainfall events occurred 15 times, reaching a maximum of 71 mm per hour on 13 October. It should be highlighted that in the early fruit development stages (summer), the JP location had thirty-one times more accumulated rainwater (438 mm) than SP location (14.1 mm).

Table 2. Sampling dates, from 30 days after fruit set until CR, in SP and JP locations.

Sampling	SP	JP
S1	20 June	27 June
S2	11 July	23 July
S3	29 July	8 August
S4	22 August	31 August
S5	12 September	20 September
S6	6 October	17 October
S7	25 October	4 November
S8	14 November (only Fuyu)	16 November (RB)/24 November (Fuyu)
S9	5 December (only Fuyu)	

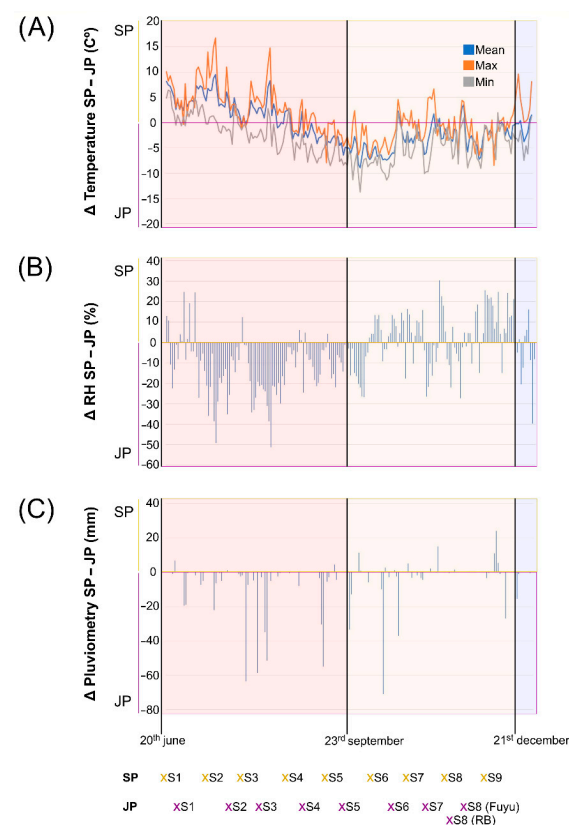


Figure 1. Differential value of climatic variables (Δ = SP values – JP values) on a daily scale during the experimental period. Positive values indicate higher daily scores of SP, and negative ones indicate

higher scores of JP. (A) Differential mean, maximum, and minimum temperature value ($^{\circ}\text{C}$), (B) Differential relative humidity value (RH; %), and (C) Differential pluviometry value (mm). Seasons are labelled with a vertical black line and different light background colour (red: summer; orange: autumn; blue: winter). Sampling dates (XS) are represented below for each location (yellow X belongs to SP and purple X to JP).

3.2. Differential Growth of Persimmon Cultivars in SP and JP Locations

The evolution of fruit size during the development process is shown in Figure 2A. Diameter and weight were noticeably higher in fruits of both varieties when they were grown in the JP location, with the only exception being S1 samples. Particularly, ‘Fuyu’ fruit’s average weight in JP (approx. 350 g) doubled the one recorded in SP. At this location, the maximum average weight was around 150 g in the latest samplings. Accordingly, ‘Rojo Brillante’ fruits collected in JP showed about 100 g more weight on average than SP ones. Interestingly, TSS analysis of the last samples revealed higher values in fruits collected in the location country from where each cultivar originated (Figure 2B). On the other side, Figure 2C,D illustrate the change of fruit firmness (expressed as a percentage of initial firmness) and CI, respectively. A similar pattern evolution of fruit firmness and skin colour is observed in both locations and accessions. Regarding fruit skin colour, values ranged from negative to positive values (corresponding to green and orange skin colour, respectively) during fruit development progression and reached values > 5 in the CR stage (Figure 2D). All phenotypic values are summarized in Table S2. A non-parametric statistical test was performed with phenotypic parameters analysed in Figure 2, and they revealed significative differences between samplings (Figure S1). Growth data were fitted to a polynomial function (Figure S2).

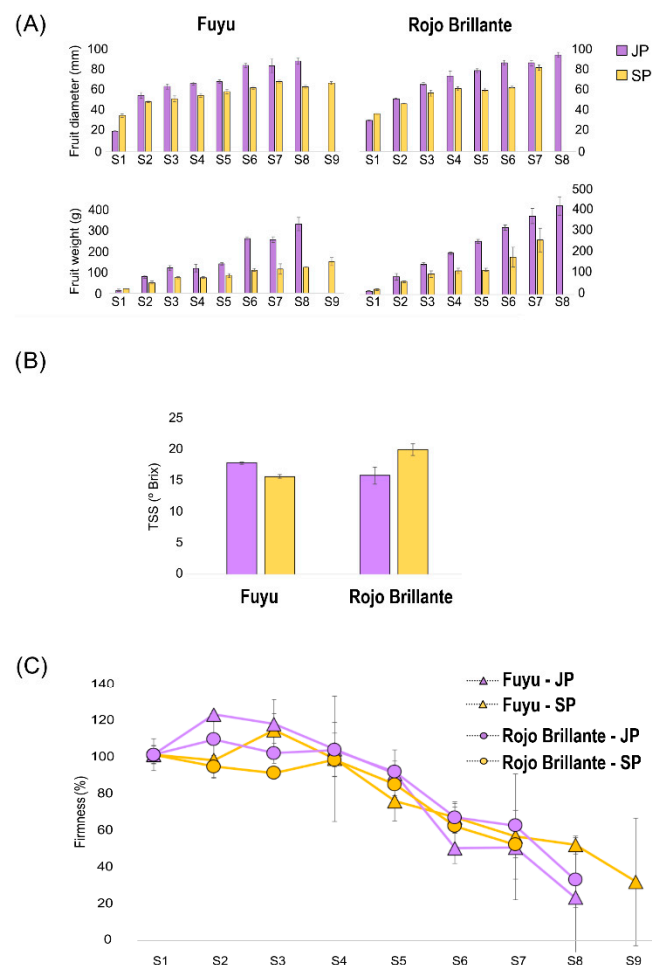


Figure 2. Cont.

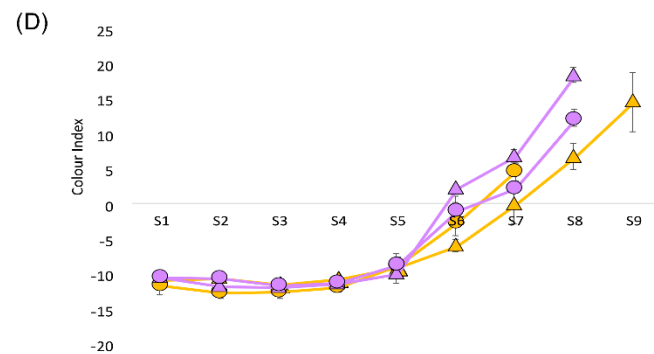


Figure 2. Phenotypical parameters across fruit development of ‘Fuyu’ and ‘Rojo Brillante’ cultivars in JP and SP. (A) Fruit diameter and fruit weight. (B) Last sample TSS value. (C) Fruit firmness (expressed in percentage of S1 sample). (D) Fruit skin colour. Standard deviations (SD) are represented by vertical error bars.

Visual evidence of differential growth between cultivars and locations at the CR stage is shown in Figure 3.

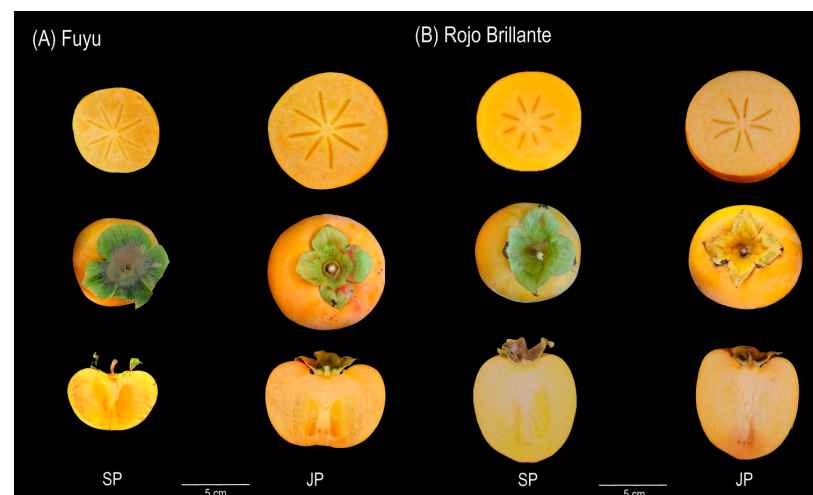


Figure 3. Fruit size and shape of (A) ‘Fuyu’ and (B) ‘Rojo Brillante’ at CR in both locations.

3.3. Gene Expression Measurements Associate with Phenotypic Data

We selected fourteen persimmon genes for gene expression studies based on previous reports on fruit development processes in persimmon [33] or similar to genes formerly described as hormone and oxidative stress-responsive factors in the model plant *Arabidopsis thaliana*. These are genes related to ethylene synthesis and response, GA signalling, fruit colour and development, and oxidative stress (Table 3). We studied the relative expression of these genes in ‘Fuyu’ and ‘Rojo Brillante’ fruit samples in SP and JP locations.

Table 3. List of selected genes involved in fruit development, gibberellin pathway, and oxidative stress.

Gene Name	Accession Number	Protein Name	Function
<i>DkACO1</i>	AB073008	1-aminocyclopropane-1-carboxylate oxidase 1	Key enzyme in ethylene biosynthesis.
<i>DkACO2</i>	AB073009	1-aminocyclopropane-1-carboxylate oxidase 2	Key enzyme in ethylene biosynthesis.
<i>DkERF16</i>	KJ170916	Ethylene response factor 16	Regulator of ethylene biosynthesis. Possible promoter of fruit ripening by cell wall modification.

Table 3. Cont.

Gene Name	Accession Number	Protein Name	Function
<i>DkERF18</i>	KJ170918	Ethylene response factor 18	Regulator of ethylene biosynthesis.
<i>DkERF8</i>	JN256078	Ethylene response factor 8	Regulator of ethylene biosynthesis. Possible promoter of fruit ripening by cell wall modification.
<i>DkEXP3</i>	XM_052336116 (<i>D. lotus</i>)	Expansin 3	Cell wall modifying and loosening enzyme.
<i>DkEXP4</i>	XM_052320777 (<i>D. lotus</i>)	Expansin 4	Cell wall modifying and loosening enzyme.
<i>DkPSY</i>	FJ713744	Phytoene synthase	Catalysis in the first committed step of the carotenoid biosynthesis pathway.
<i>DkZDS</i>	XM_052336035 (<i>D. lotus</i>)	Zeta-carotene desaturase	Involved in the biosynthesis of carotenes.
<i>DkPL1</i>	XM_052333091 (<i>D. lotus</i>)	Pectate-lyase 1	Cell wall degradation enzyme.
<i>DkGID1B</i>	XM_052314796 (<i>D. lotus</i>)	Gibberellin receptor GID1B-like	Triggers ubiquitination of DELLA proteins, enhancing transcription of gibberellin response genes.
<i>DkRGA1</i>	XM_052332049 (<i>D. lotus</i>)	RGA-like protein	DELLA subfamily member that acts as a negative regulator of GA signalling.
<i>DkCAT</i>	XM_052346323 (<i>D. lotus</i>)	Catalase 1	Catalyzes the reduction of hydrogen peroxide, protecting cells against oxidative stress.
<i>DkGR</i>	XM_052313957 (<i>D. lotus</i>)	Glutathione reductase	Protect cells against oxidative stress damage.

A joint PCA analysis of fruit development parameters and gene expression data was performed in order to assess their contribution to observed variability. After analysis, the first two and three principal components (PCs) explained, respectively, 48.7 and 60.4% of the total variability of the dataset. A two-dimensional plot of the first two PCs is shown in Figure 4.

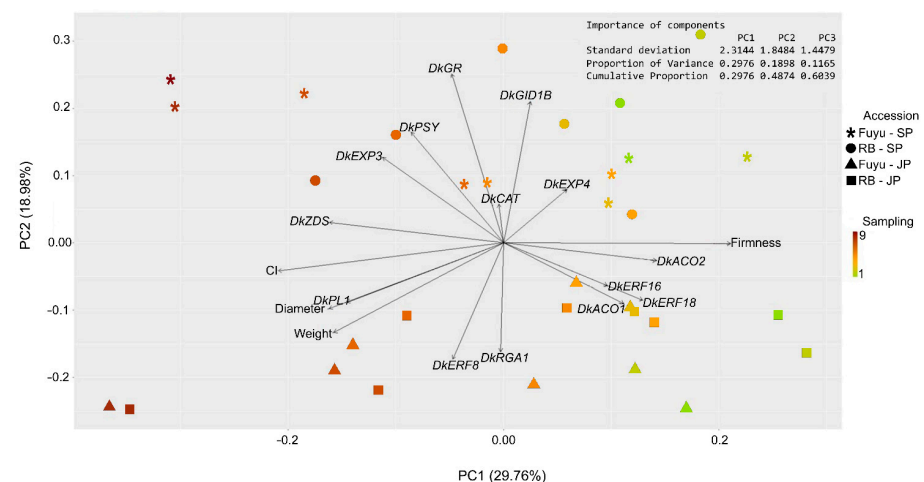


Figure 4. Phenotypic and genotypic expression data PCA of each cultivar across fruit development samplings in SP and JP. Early fruit samplings are coloured light green, and it turns dark red as the fruits reach the CR stage. The importance of the first three principal components is detailed in the upper-right corner of this two-dimensional plot.

Samples were distributed throughout PC1 according to their stage of development, with PC1 values between 0.1 and 0.3 corresponding to the earliest developmental stages and gradually lower values as they reached more advanced stages. Samples closer to the CR stage showed PC1 values lower than -0.3 . On the other hand, PC2 grouped samples according to the location, irrespective of the cultivar, acquiring SP- and JP-located samples positive and negative values of PC2, respectively.

Regarding phenotypic and gene expression variables, firmness, *DkACO1*, *DkACO2*, *DkERF16*, and *DkERF18* had more weight at early development stages (positive PC1), whereas CI, fruit weight and diameter, *DkZDS*, and *DkPL1* were associated with later development stages (negative PC1). A third group of variables (*DkEXP3*, *DkEXP4*, *DkERF8*, *DkPSY*, *DkGID1B*, *DkRGA1*, *DkCAT*, and *DkGR*) distributed samples along PC2 defined space, grouping them according to the location in which they were cultivated.

3.4. Fruit Maturation Genes Are Similarly Expressed in Both Locations

Genes related to ethylene biosynthesis and signalling and fruit ripening processes, such as cell wall loosening and fruit colour change, led to similar expression patterns with slight differences in both cultivars and locations, as shown in heatmaps representing qRT-PCR data (Figure 5).

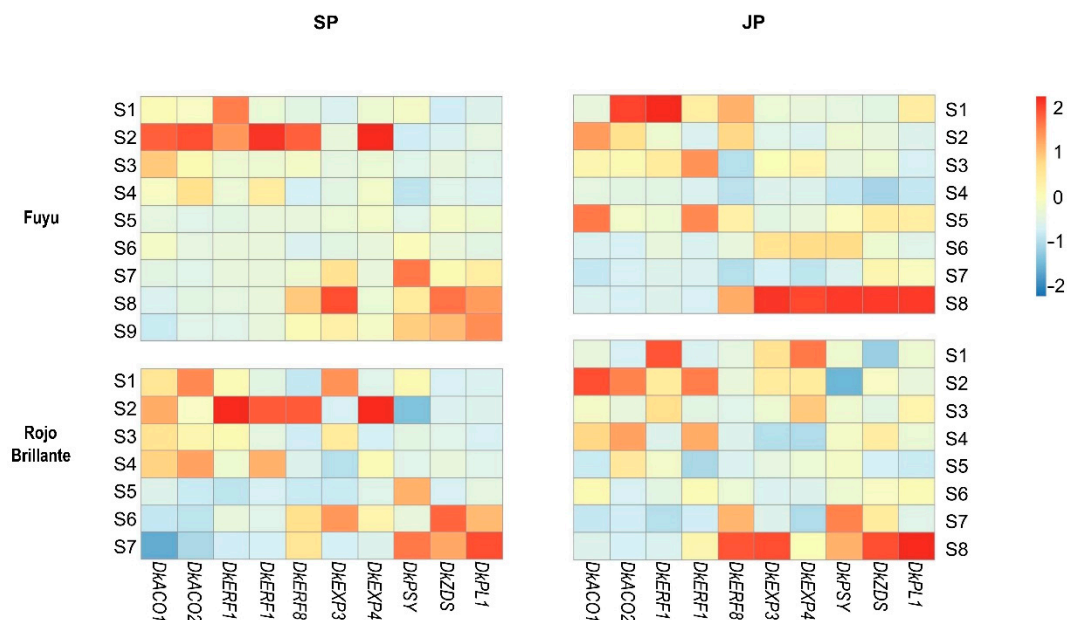


Figure 5. Gene expression heatmap of fruit maturation and ripening genes across fruit development in ‘Fuyu’ and ‘Rojo Brillante’ cultivars in SP and JP.

Genes codifying for ethylene biosynthesis enzymes *DkACO1* and *DkACO2* and ethylene signal transduction factors *DkERF16* and *DkERF18* were highly expressed at early fruit development stages (S1–S4). However, they were downregulated in the advanced stages of fruit maturation and ripening (S5–S9). On the contrary, genes *DkPSY* and *DkZDS*, involved in the carotenoid biosynthesis pathway required for fruit colour change, and *DkPL1*, involved in fruit softening, increased their expression in the final steps of fruit development, around CR. Interestingly, the ethylene response factor *DkERF8* showed a dual expression peak, increasing both after fruit set (S1–S2) and in the final stages, concomitantly with fruit ripening. Expansins *DkEXP3* and *DkEXP4* did not follow a consistent pattern, as their expression was activated at different stages along fruit development.

3.5. GA and Oxidative Stress Gene Expression Patterns Differ between Locations

Genes related to the GA response (*DkGID1B* and *DkRGA1*) and oxidative stress (*DkCAT* and *DkGR*) showed different expression patterns depending on the location (Figure 6).

DkGID1B and *DkRGA1* increased their expression at early stages of fruit development (S1–S2) in the SP location, whereas in JP, they were mostly upregulated after S4, with the exception of *DkRGA1* in ‘Fuyu’, with an additional peak in S1 (Figure 6A,B). Regarding the *DkCAT* expression profile, its activity was higher in the early phases of fruit development when both cultivars were grown in SP. While in the JP location, *DkCAT* expression increased as fruits reached CR (Figure 6C). Finally, the expression of the *DkGR* gene, encoding a glutathione reductase supposedly involved in protection against oxidative stress, is represented in a bar diagram rather than a heatmap in order to highlight the absolute differences in the expression found in JP and SP, two to ten times higher in this last location (Figure 6D).

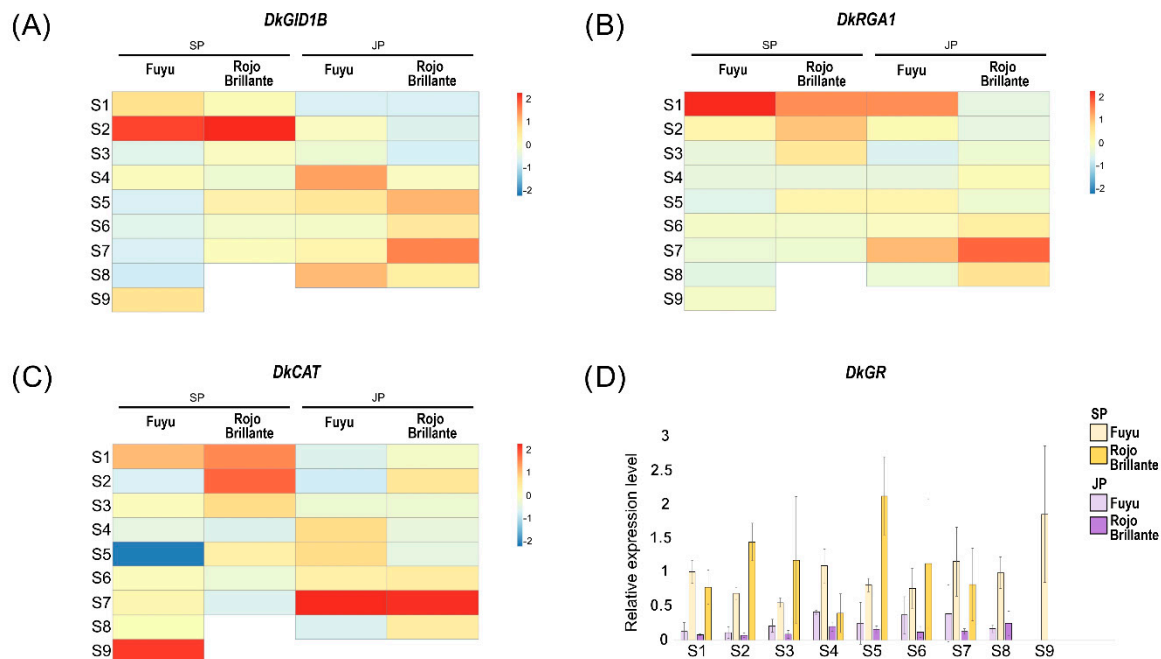


Figure 6. Expression of gibberellin signalling and oxidative stress genes. (A) *DkGID1B* heatmap for both cultivars across fruit development in SP and JP, (B) *DkRGA1* heatmap, (C) *DkCAT* heatmap, and (D) *DkGR* relative expression level. Standard deviations (SD) are represented by vertical error bars.

4. Discussion

4.1. Fruit Maturation and Ripening Genes Show Common Expression Patterns in JP and sp.

Deciphering the constituents of molecular networks modulating fruit ripening, including hormone response and transcriptional and epigenetic regulation, are of great importance for understanding and improving fruit quality and the post-harvest behaviour of fresh fruit [9]. In this work, the expression of certain ethylene biosynthesis and response genes, previously related to fruit development and on-tree maturation in persimmon [33], have been found to be associated with specific stages of fruit development irrespective of the persimmon cultivar and location.

Among them, the expression pattern of ethylene response factors *DkERF16* and *DkERF18* correlated well in this work with the early expression of *DkACO1* and *DkACO2*, coding for conserved 1-aminocyclopropane-1-carboxylic (ACC) oxidase (ACO) enzymes involved in a principal ethylene biosynthesis step under developmental and environmental control [44]. In a previous work, it was demonstrated that the *DkERF18* expression pattern is synchronized with ethylene biosynthesis in persimmon due to its role in ACC synthase (ACS) activation through the ACCGAC motif [45]. Other related *ERF* genes from tomatoes and bananas have been similarly described to affect ethylene production through the control of specific *ACO* genes [46,47]. Thus, these data suggest the participation of *DkERF16*

and *DkERF18* genes in the control of ethylene production in the early developmental stages of persimmon fruit.

On the other side, in this work, *DkERF8* expression showed a late fruit development peak in the four possible combinations of cultivars and locations, upregulated concomitantly with genes presumably involved in fruit colour change and firmness drop associated with the ripening process: the carotenoid biosynthesis genes *DkPSY* and *DkZDS*, the expansin coding genes *DkEXP3* and *DkEXP4*, and the pectate-lyase *DkPL1*. A similar up-regulation of *DkERF8* in late stages of on-tree fruit maturation and ripening, in concordance with the expression of *DkPSY* and *DkXTH9*, coding for a xyloglucan endotransglucosylase with a role in fruit softening has been reported elsewhere [33,48]. Moreover, previous post-harvest studies analysing the regulation of hypoxia treatments to eliminate astringency have revealed that both *DkERF8* and *DkERF16* genes activated *DkEXP4* and *DkXTH9* promoters and other cell wall-related genes by dual-luciferase transient expression assays [45,49]. These results and our findings support the participation of *DkERF8* among other *ERF* genes in fruit softening and fruit colour change processes during natural on-tree ripening in persimmon, as proposed in other species [17].

Further investigations are needed to unravel the intriguing expression of *ERFs* and ethylene biosynthetic genes in the early stages of fruit development in persimmon, which has been proposed to trigger the autocatalytic production of ethylene when young fruits are detached from the tree [50]. The study of *ERFs* and their orchestration during persimmon fruit development and ripening process will be the subject of future research works.

4.2. GA and Oxidative Stress-Related Genes Are Divergently Expressed in JP and SP.

Environmental and hormonal cues exert a strong impact on fruit development and maturation. The evaluation of meteorological variables during this study showed a warmer range of temperatures in SP compared with the JP location from fruit set until early September. Temperatures higher than 35 °C were reached in SP during this period. It has been reported that high whole-tree temperatures (30 °C) inhibit fruit growth and ripening in the ‘Fuyu’ cultivar [51]. This direct effect of temperature on fruit ripening has also been reported in grapes and mandarins [52,53]. These observations indicate that temperature might have exerted an important role in fruit growth, size, and ripening during the experiment. In addition, water availability in the SP location was lower than in JP, as indicated by precipitation and RH values during this period. This suggests that JP’s environmental conditions were more favorable for optimal fruit growth. Results on final fruit size in both locations and cultivars reinforce this idea, especially in ‘Fuyu’. At the early stages of development, fruit growth was similar in both locations, but at the later stages, the fruit growth rate became lower in the SP location.

Previous studies have already demonstrated the role that GAs have in different aspects of plant development, such as germination, tissue elongation, flowering, fruit set, and fruit development [54], with a relevant impact on cell division and plant growth, which makes GA response a putative candidate for mediating growth differences observed in this study. In this work, genes encoding a GA receptor (*DkGID1B*) and a DELLA-related GA signalling regulator (*DkRGA1*) showed location-dependent expression. In SP, these genes had lower expression after the early stages of fruit development, while in JP, an expression peak was observed in the late developmental stages. These data suggest that fruit growth differences found in SP and JP are associated with hormonal signalling mechanisms.

Reactive oxygen species (ROS) produced by the metabolic activity during plant development are detoxified by an antioxidant defence system, including catalase (CAT) and glutathione reductase (GR) enzymes [55–58]. Indeed, ROS production and the corresponding detoxifying machinery are highly activated during the fruit maturation process [59,60]. This phenomenon has been observed in different climacteric fruits, such as mango, pear, apple, tomato, and peach [60–65]. In close agreement with these reports, *DkCAT* was specifically upregulated in a late developmental stage (S7) in the JP location. However, in the SP location, *DkCAT* was also highly expressed in the early stages of fruit development

(S1–S2). Furthermore, *DkGR* expression levels were higher in SP than in JP throughout the whole development process. Such an increase in *DkCAT* and *DkGR* antioxidant activity is indicative of a higher accumulation of ROS during fruit development and ripening in the SP location. Since plants under drought stress undergo secondary oxidative stress due to ROS production [66–69], environmental conditions with higher temperatures and lower water availability could determine the increased expression of antioxidant proteins in SP.

Former studies have confirmed that in addition to their effect on plant growth, low levels of endogenous GA and attenuated GA signalling increase plant tolerance to drought stress [70,71]. This statement has been corroborated by several authors in both cereals and tomatoes [72,73]. On the other side, drought stress reduces cell division and expansion and alters the hormonal signalling metabolism, directly affecting plant growth [74]. These are constitutive elements of a trade-off between growth and stress resistance that optimizes plant survival by limiting growth and activating stress responses under deleterious conditions [75]. Such a growth–defence trade-off consistently explains the differential fruit growth reported in SP and JP locations through hormonal and stress tolerance mechanisms. More exhaustive studies, including additional hormone and stress-related genes, should be carried out in order to better understand the influence of environmental conditions and climate change on persimmon cultivation.

5. Conclusions

We performed a two-location agronomical study set in orchards in Spain and Japan to assess persimmon fruit development at physiological and molecular levels. Whereas ethylene-associated factors involved in conserved fruit maturation pathways were similarly expressed in both locations, diverging environmental conditions led to differential patterns of fruit growth and GA and oxidative stress-related gene expression. While validation of these results in additional locations is still needed, this study provides data consistent with a growth–defence trade-off explaining persimmon fruit differential growth and development through hormonal and stress response mechanisms.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy14071555/s1>. Table S1: Climatic data measured during the trial in Spain and Japan locations. Relative values of humidity (%), pluviometry (mm), and temperature (°C) were recorded; Table S2: Phenotypical data of ‘Rojo Brillante’ and ‘Fuyu’ fruits measured during the trial in Spain and Japan locations. Fruit size (mm), weight (g), total soluble solids content (TSS, ° Brix), flesh firmness, and skin colour index (CI) were measured at each sampling date; Table S3: Gene-specific primers used for expression analysis; Figure S1: Kruskal–Wallis statistical test of ‘Rojo Brillante’ and ‘Fuyu’ fruits’ phenotypical data; Figure S2: Least-squares regression analysis and 3rd order polynomial curve fitting of ‘Rojo Brillante’ and ‘Fuyu’ fruit weight.

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