

Article

Changes in Morphological Characteristics, Endogenous Hormones, Soluble Sugars, and Anthocyanin Content During Fruit Development in Eggplant (*Solanum melongena* L.)

Sung Hyun Park, Faraaz Ahmed Mohammad , Mac Cheryl Sulan Charles Emparang , Sang Rim Kim , Ji Gu Lee , Min Geon Cho , Dae Geun Jeong , Min Jae Kim and Jum Soon Kang * 

Department of Horticultural Bioscience, Pusan National University, Miryang 50463, Republic of Korea; wittyguy010@naver.com (S.H.P.); mdaraazbio1803@gmail.com (F.A.M.); mcchryl@gmail.com (M.C.S.C.E.); tkdflajaid@naver.com (S.R.K.); dlwlrn15@naver.com (J.G.L.); mg6188@naver.com (M.G.C.); daegeunjeong99@gmail.com (D.G.J.); atom0821@pusan.ac.kr (M.J.K.)

* Correspondence: kangjs@pusan.ac.kr; Tel.: +82-10-8516-5395

Abstract

Understanding the coordinated regulation of growth and metabolism during fruit development is essential for improving crop quality and harvest management. This study investigated changes in morphological traits, endogenous phytohormones, soluble sugars, anthocyanins, and water-soluble vitamins in eggplant (*Solanum melongena* L.) fruit from 3 to 15 days after anthesis (DAA). Fruit length, diameter, and fresh weight increased significantly, with fresh weight increasing 16.6-fold. Cellular analysis showed that fruit enlargement was driven predominantly by cell expansion. Total soluble sugars increased 2.8-fold, with glucose and fructose as the predominant sugars. Delphinidin-based anthocyanins increased sharply during mid-development (9–12 DAA) and then declined slightly, whereas water-soluble vitamins decreased progressively. Based on integrated data, fruit development was classified into three phases: early (3 DAA, initiation and defense), mid (6–9 DAA, rapid growth and pigmentation), and late (12–15 DAA, functional maturation). Notably, 15 DAA was identified as a critical candidate stage with maximum sugar content, high anthocyanin levels, and completed seed development, suggesting its utility as a potential indicator of optimal harvest timing under the present conditions. These results provide an integrated understanding of developmental and metabolic processes in eggplant fruit and offer practical insights for optimizing harvest strategies.

Keywords: anthocyanins; eggplant; fruit development; harvest maturity; soluble sugars



Academic Editors: Ana Maria González and Paula Alayón-Luaces

Received: 7 June 2026

Revised: 17 July 2026

Accepted: 22 July 2026

Published: 27 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Eggplant (*Solanum melongena* L.) is one of the most widely cultivated vegetable crops within the Solanaceae and ranks among the most important horticultural commodities worldwide [1]. The species exhibits remarkable phenotypic diversity in fruit shape, size, and skin color, encompassing elongated, oval, and cylindrical forms with purple, white, and green pigmentation [2,3]. In Korea, elongated cultivars with dark purple skin are predominantly grown for fresh-market consumption.

Eggplant fruits are characterized by low caloric content and high nutritional and functional value. They contain abundant phenolic compounds, flavonoids, and anthocyanins, which contribute substantially to antioxidant capacity and anti-inflammatory activity [4–6].

Chlorogenic acid is the predominant phenolic acid in eggplant flesh, whereas delphinidin-derived anthocyanins, including nasunin and delphinidin-3-rutinoside, are responsible for the characteristic purple coloration of the peel and represent major determinants of fruit antioxidant potential [2,4,7–9]. The concentration and composition of these compounds are strongly influenced by genotype and environmental conditions and change dynamically during fruit ontogeny [10].

In addition to secondary metabolites, eggplant fruit contains substantial amounts of mineral nutrients, particularly potassium, calcium, and magnesium, as well as soluble sugars and vitamins that contribute to both flavor and nutritional quality [11]. Soluble sugars, including sucrose, glucose, and fructose, function not only as primary carbon and energy sources but also as essential regulators of osmotic adjustment and cell expansion. During early fruit development, sucrose unloading and cleavage are highly active, whereas the subsequent accumulation of hexoses accompanies rapid fruit enlargement and maturation, thereby influencing sweetness and consumer acceptability [12,13]. These metabolic transitions are tightly coordinated with hormonal signaling and developmental regulation [14,15]. Recent metabolomic studies have identified over 500 primary metabolites and 170 phenolic acids in eggplant fruit, highlighting the complexity of the metabolic network underlying fruit quality [16].

Eggplant fruits are typically harvested at the immature stage before complete seed maturation, generally within 10 to 40 days after anthesis (DAA) depending on cultivar and growing conditions [17,18]. Fruit development proceeds through two major phases: an initial stage dominated by intensive cell division, followed by a prolonged period of cell expansion [19,20]. The duration and intensity of these processes largely determine final fruit size and shape. Morphological traits such as fruit length and diameter are practical indicators of these developmental events and are closely associated with the progression of seed formation.

Developing seeds serve as major sources of endogenous auxins and gibberellins that stimulate pericarp growth and fruit enlargement [21,22]. Recent transcriptomic analyses have identified phytohormone-related genes, including SmARF1, as well as SUN, YABBY, and OVATE family genes, which are potentially involved in early fruit development and shape regulation in eggplant [23]. Accordingly, seed number is frequently positively correlated with fruit size and shape attributes [24,25]. At the hormonal level, indole-3-acetic acid (IAA), gibberellins (GAs), and cytokinins promote cell division and early fruit growth, whereas abscisic acid (ABA), jasmonates (JAs), and salicylic acid (SA) play central roles in maturation, stress responses, and quality regulation [26]. These phytohormones operate within highly integrated signaling networks that are closely linked to carbohydrate metabolism and secondary metabolite biosynthesis, thereby coordinating fruit development and quality formation.

Environmental factors, including temperature, light intensity, and plant water status, exert profound effects on pollen viability, fertilization, and fruit set and consequently influence seed development and fruit enlargement [27]. These factors also modulate the accumulation of anthocyanins, soluble sugars, and vitamins, generating substantial variation in fruit quality even within a single cultivar [10]. Although previous studies have examined fruit morphology, hormone dynamics, and individual quality components, most have addressed these traits in isolation. Consequently, comprehensive studies integrating morphological development with phytohormonal and biochemical changes throughout the entire fruit developmental process remain limited. In particular, the relationships between early developmental events and subsequent metabolic transitions are still not systematically characterized in eggplant.

Therefore, the present study was conducted to characterize stage-specific changes in fruit morphology after anthesis and to simultaneously quantify endogenous phytohormones, soluble sugars, anthocyanins, and water-soluble vitamins during eggplant fruit development. By integrating morphological, physiological, and biochemical analyses, this study aimed to elucidate the coordinated mechanisms underlying fruit growth and quality formation and to provide fundamental information for determining optimal harvest timing and improving fruit quality in eggplant.

2. Materials and Methods

2.1. Plant Materials and Growing Conditions

An eggplant (*Solanum melongena* L.) cultivar, 'Otoking' (Sakata Seed Corporation, Yokohama, Japan), was used in this study. Seeds were sown in 40-cell plug trays filled with a commercial horticultural substrate and grown for approximately 40 d. Uniform seedlings with three to four fully expanded true leaves were selected for transplanting. Seedlings were transplanted into cocopeat growbags (fiber 80% and coir dust 20%; 100 cm × 17 cm × 11 cm; 20 L per bag; Klasmann-Deilmann, Klasmann-Deilmann GmbH, Geeste, Germany.) at a plant spacing of 30 cm. The experiment was conducted from 2 March to 20 September 2024 in a glasshouse located at the Smart Farm Innovation Valley in Miryang. During the cultivation period, air temperature and relative humidity were maintained at 26 ± 2 °C and $80 \pm 5\%$, respectively. Plants were grown under natural solar radiation, and environmental conditions were automatically regulated using an integrated climate-control system (MAGMAPLUS-1000; Green CS Co., Ltd., Damyang, Republic of Korea). Plants were trained to two stems and managed according to standard greenhouse eggplant production practices recommended by the Rural Development Administration (RDA) [28]. Pruning, pest and disease management, and irrigation scheduling were performed uniformly throughout the experiment. A modified Yamazaki standard nutrient solution for fruit vegetables was supplied through a drip irrigation system [29]. The electrical conductivity (EC) of the nutrient solution was maintained at $2.5 \text{ dS} \cdot \text{m}^{-1}$, and the pH of the root-zone solution was adjusted to 5.5–6.0. To account for spatial variation within the glasshouse, three cultivation rows were selected. In each row, five plants were randomly tagged at anthesis (0 DAA) to track fruit age, with one plant assigned to each of the five sampling time points (3, 6, 9, 12, and 15 DAA). Thus, a total of fifteen plants were tagged across the three rows. The cultivar 'Otoking' is facultatively parthenocarpic; under our glasshouse conditions, natural pollination occurred, resulting in the presence of morphologically normal seeds.

2.2. Measurement of Morphological and Physical Characteristics During Fruit Development

To evaluate stage-specific changes in fruit morphology and physical characteristics, at each sampling time point (3, 6, 9, 12, and 15 DAA), one fruit was harvested from each of the three rows (one fruit per row), yielding three fruits per DAA. Thus, each biological replicate ($n = 3$) represents one fruit from a distinct row. Exact fruit position was not recorded; however, fruits were sampled randomly from uniformly pruned two-stem plants, minimizing systematic positional bias, and any remaining positional variation was distributed across all replicates and stages. Measured variables included fruit length, fruit diameter, fresh weight, and fruit firmness. Fruit length was defined as the linear distance from the calyx base to the distal end of the fruit, whereas fruit diameter was measured as the maximum transverse diameter at the equatorial region. Fruit dimensions were determined using a digital caliper (DC150P; Bluetec, Jinhua, China), and fresh weight was recorded immediately after harvest using an electronic balance. Fruit firmness was measured at the equatorial region using a fruit firmness tester (Model FR-5120; Lutron Electronic Enterprise

Co., Ltd., Taipei, Taiwan) equipped with a cylindrical probe 5 mm in diameter. The probe was inserted to a depth of 5 mm at a constant speed. Three measurements were obtained from each fruit, and the mean value was used for statistical analysis.

2.3. Histological Analysis and Cell Area Determination

For histological analysis, pericarp tissue samples (approximately $5 \times 5 \times 5$ mm; $l \times b \times h$) were excised from the equatorial region of fruits harvested at 3, 6, 9, 12, and 15 DAA. Samples were fixed in FAA solution consisting of 50% (*v/v*) ethanol, 10% (*v/v*) formalin, and 5% (*v/v*) acetic acid and stored at room temperature until further processing. Fixed tissues were dehydrated through a graded ethanol series, embedded in paraffin wax, and sectioned to a thickness of 10 μm using a rotary microtome (Leica Biosystems, Deer Park, IL, USA). Sections were double-stained with 1% safranin and 0.5% fast green to distinguish cellular structures. Stained sections were observed under a bright-field optical microscope (BX53; Olympus Corporation, Tokyo, Japan), and digital images were analyzed using ImageJ (version 1.54t; National Institutes of Health, Bethesda, MD, USA). For each developmental stage and biological replicate, at least five non-overlapping microscopic fields were analyzed, and a minimum of 30 cells per field were measured to determine mean cell area. Mean cell area was determined by quantitative image analysis. To evaluate the degree of cell packing and structural organization in the fruit pericarp during development, cell density ($\text{cells} \cdot \text{mm}^{-2}$) was estimated from mean cell area values. Following standard plant tissue histomorphometry principles, density was calculated as the reciprocal of mean cell area with a unit conversion factor [30]:

$$\text{Cell density} = \frac{1,000,000}{\text{Mean cell area}} \quad (1)$$

where mean cell area is expressed in μm^2 , and 1,000,000 converts μm^2 to mm^2 ($1 \text{ mm}^2 = 1,000,000 \mu\text{m}^2$).

2.4. Seed Trait Analysis

Fruits harvested at 3, 6, 9, 12, and 15 DAA were used for seed trait analysis. After morphological measurements were completed, fruits were cut longitudinally and seeds were manually separated from the surrounding pericarp tissue. The seeds were thoroughly rinsed with distilled water to remove adhering pulp and dried at room temperature for 24 h before analysis. The seeds were counted and weighed; however, their viability or developmental status (true vs. vestigial) was not assessed. Seed number per fruit was determined by directly counting all seeds in each fruit. At each developmental stage, three fruits were analyzed from the tagged plants described in Section 2.1 (one fruit per row, $n = 3$ biological replicates). The mean number of seeds per fruit was calculated for each sampling stage. Thousand-seed weight (TSW) was determined according to standard seed testing procedures. From each replicate, 100 dried seeds were randomly selected and weighed using an analytical balance (Ohaus Corporation, Parsippany, NJ, USA) with a readability of 0.0001 g. This procedure was repeated three times per replicate, and the mean weight of 100 seeds was multiplied by 10 to obtain TSW, expressed in grams.

2.5. Quantification of Hormones

To quantify endogenous hormones, fruit samples were collected at 3, 6, 9, 12, and 15 DAA. For hormone analysis, the whole pericarp tissues (both peel and flesh) were freeze-dried and ground to a fine powder. Subsamples (50 mg dry weight) were used for hormone extraction. Samples were transferred to 2.0 mL microcentrifuge tubes and extracted with 1.0 mL of 80% (*v/v*) methanol containing 1% (*v/v*) acetic acid and 0.1% (*w/v*) butylated hydroxytoluene (BHT). The mixture was shaken for 2 h at 4 °C and centrifuged

at $15,000\times g$ for 20 min. The supernatant was collected and filtered through a $0.22\text{ }\mu\text{m}$ polytetrafluoroethylene (PTFE) syringe filter before analysis. Hormones were quantified using a high-performance liquid chromatography (HPLC) system (1260 Series; Agilent Technologies, Santa Clara, CA, USA). Separation was performed on an Eclipse Plus C18 column ($4.6\times 50\text{ mm}$, $3.5\text{ }\mu\text{m}$; Agilent Technologies, Santa Clara, CA, USA) maintained at $30\text{ }^{\circ}\text{C}$. The mobile phase consisted of solvent A [water containing 0.1% (*v/v*) formic acid] and solvent B [acetonitrile containing 0.1% (*v/v*) formic acid]. Elution was carried out using a gradient program at a flow rate of $0.5\text{ mL}\cdot\text{min}^{-1}$, and the injection volume was $10\text{ }\mu\text{L}$. Detection was performed using a diode array detector (DAD) at 254 nm for IAA, 280 nm for SA, and 210 nm for ABA, JA, and GAs. The hormones analyzed included abscisic acid (ABA), gibberellic acid (GA_3 and GA_7), indole-3-acetic acid (IAA), jasmonic acid (JA), salicylic acid (SA), and cytokinins (CKs). Authentic standards for all hormones were purchased from Sigma-Aldrich (St. Louis, MO, USA). Quantification was performed using external calibration curves prepared from authentic standards over a concentration range of $0.1\text{--}1000\text{ ng}\cdot\text{mL}^{-1}$, all of which exhibited excellent linearity ($R^2\geq 0.998$). The limits of detection (LOD) and limits of quantification (LOQ) were estimated at signal-to-noise ratios of 3 and 10, respectively. Hormones not detected under the analytical conditions used were reported as not detected. All analyses were conducted in triplicate, and results were expressed as mean $\pm$ standard error (SE).

2.6. Soluble Sugar Analysis

For soluble sugar analysis, the whole pericarp including both peel and flesh was used. Fruit samples were collected at each developmental stage, freeze-dried, and ground to a fine powder. Approximately 0.5 g of powdered tissue was transferred to a 15 mL polypropylene tube, and 10 mL of distilled water was added. Samples were vortexed for 5 min and then incubated in a water bath at $80\text{--}90\text{ }^{\circ}\text{C}$ for 30 min with intermittent shaking. After cooling to room temperature, extracts were centrifuged at $3000\times g$ for 10 min at $4\text{ }^{\circ}\text{C}$. The supernatant was collected and filtered through a $0.22\text{ }\mu\text{m}$ syringe filter. When necessary, samples were diluted appropriately before analysis. Soluble sugars were quantified using an ion chromatography system (ICS-6000; Thermo Fisher Scientific, Waltham, MA, USA) equipped with pulsed amperometric detection (PAD). Sugars were separated on a Dionex CarboPac PA1 analytical column ($4\times 250\text{ mm}$) with a corresponding guard column ($4\times 50\text{ mm}$). The column temperature was maintained at $30\text{ }^{\circ}\text{C}$, and the injection volume was $5\text{ }\mu\text{L}$. The mobile phase consisted of solvent A (ultrapure water) and solvent B (200 mM sodium hydroxide), delivered at a flow rate of $1.0\text{ mL}\cdot\text{min}^{-1}$. The elution program maintained an A:B ratio of 50:50 from 0 to 17 min , followed by a transition to 0:100 from 17.5 to 25 min . Glucose, fructose, and sucrose were identified and quantified using external calibration curves generated from authentic standards. Authentic standards for glucose, fructose, and sucrose ($\geq 99\%$ purity) were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Sugar concentrations were expressed as milligrams per gram dry weight ($\text{mg}\cdot\text{g}^{-1}\text{ DW}$). All analyses were performed with three biological replicates.

2.7. Anthocyanin Analysis

For anthocyanin determination, only the fruit peel was manually separated from the flesh using a scalpel. Fruit samples were collected at 3, 6, 9, 12, and 15 DAA, immediately freeze-dried, and ground to a homogeneous powder. Anthocyanins were extracted using a methanol:water:acetic acid mixture ($50:50:0.5$, *v/v/v*). The residue was re-extracted twice with the same solvent, and the combined supernatants were pooled before analysis to ensure exhaustive extraction. After centrifugation, the supernatant was diluted with acidified methanol and filtered through a $0.22\text{ }\mu\text{m}$ PTFE syringe filter. Anthocyanins were separated

and quantified using an HPLC system (1260 Series; Agilent Technologies, Santa Clara, CA, USA) equipped with a Synergi Max-RP column (2.0 × 150 mm, 4 µm; Phenomenex, Torrance, CA, USA). The mobile phase consisted of solvent A (5% formic acid in water) and solvent B (methanol). Gradient elution was performed at a flow rate of 0.3 mL·min⁻¹ with an injection volume of 5 µL. Compound identification and confirmation were further conducted using tandem mass spectrometry (API 4000 triple quadrupole; SCIEX, Framingham, MA, USA) equipped with an electrospray ionization (ESI) source operating in positive ion mode using multiple reaction monitoring (MRM) of characteristic transitions. Quantification was based on external calibration curves prepared with delphinidin-3-glucoside chloride and delphinidin-3-rutinoside chloride (purity ≥98%; InterPharm, Ness Ziona, Israel). The LOD and LOQ for each anthocyanin were determined at signal-to-noise ratios of 3 and 10, respectively. Anthocyanin concentrations were expressed as milligrams per kilogram dry weight (mg·kg⁻¹ DW). All analyses were performed in triplicate.

2.8. Water-Soluble Vitamin Analysis

Fruit samples were collected at 3, 6, 9, 12, and 15 DAA, immediately freeze-dried, and finely ground. For extraction, 20 mg of freeze-dried powder was mixed with 1 mL of methanol:water (50:50, *v/v*) containing 10 mM ammonium acetate and 0.1% (*w/v*) BHT. Samples were vortexed for 10 min, sonicated for 10 min using 100 W and 42 kHz at room temperature, and centrifuged at 3000 × *g* for 5 min at 4 °C. The supernatant was filtered through a 0.45 µm PTFE syringe filter prior to analysis. Water-soluble vitamins were quantified using an LC–MS/MS system consisting of a 1290 Infinity II liquid chromatograph coupled to a 6470 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Target analytes included thiamine (vitamin B₁), riboflavin (vitamin B₂), pantothenic acid (vitamin B₅), pyridoxine (vitamin B₆), nicotinamide, and ascorbic acid (vitamin C). Detection was performed in positive ion mode using multiple reaction monitoring (MRM) of specific precursor-to-product ion transitions for each vitamin. Authentic standards for all vitamins were purchased from Sigma-Aldrich (St. Louis, MO, USA). Quantification was performed using external calibration curves prepared from authentic standards over a concentration range of 0.5–1000 ng·mL⁻¹. The LOD and LOQ were determined at signal-to-noise ratios of 3 and 10, respectively; compounds with concentrations below the LOQ were reported as '<LOQ', and compounds with no detectable signal were reported as 'not detected'. All analyses were conducted using three biological replicates.

2.9. Statistical Analysis

Univariate statistical analyses were performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). Data presented in figures are expressed as means ± standard errors (SE), whereas data presented in tables are expressed as means ± standard deviations (SD), based on three biological replicates. Differences among developmental stages in fruit morphology, histological characteristics, seed traits, phytohormone concentrations, soluble sugars, anthocyanins, and water-soluble vitamins were evaluated using one-way analysis of variance (ANOVA). Residual normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. When developmental-stage effects were significant at *p* < 0.05, means were separated using Duncan's multiple range test at the 5% significance level.

Multivariate analyses were performed using R software (version 4.5.3) via RStudio (version 2026.05.0; R Foundation for Statistical Computing, Vienna, Austria) based on developmental-stage means for 22 variables measured at 3, 6, 9, 12, and 15 days after anthesis (DAA). Before analysis, all variables were standardized to z-scores to account for differences in measurement units and numerical scales. Principal component

analysis (PCA) was used to summarize developmental variation and identify variables contributing to stage differentiation. Hierarchical cluster analysis was performed using Euclidean distance and Ward's minimum variance method (ward.D2), and the resulting dendrogram was partitioned into three clusters for interpretation. Pearson correlation coefficients were calculated among variables and visualized using a hierarchically clustered heatmap. Because the multivariate analyses were based on five developmental-stage means, the correlations were interpreted as exploratory developmental associations rather than evidence of causal relationships. PCA, clustering, correlation analysis, and visualization were conducted using the stats, ggplot2, factoextra, pheatmap, and ComplexHeatmap packages in R.

3. Results

3.1. Morphological, Physical, and Cellular Changes During Fruit Development

Significant changes in fruit morphology and physical properties were observed from 3 to 15 days after anthesis (DAA) (Figure 1, Table 1). Fruit length increased from 4.60 cm at 3 DAA to 21.30 cm at 15 DAA, representing a 4.6-fold increase. Fruit diameter increased from 1.58 cm to 4.48 cm (2.8-fold). Fruit fresh weight increased from 7.01 g to 116.48 g, a 16.6-fold increase. Fruit firmness decreased from 12.3 N at 3 DAA to 9.1 N at 15 DAA (Table 1). Seed number per fruit decreased slightly but significantly from 642.0 at 3 DAA to 603.0 at 15 DAA, corresponding to a reduction of approximately 6.1%. In contrast, thousand-seed weight increased markedly from 0.11 g at 3 DAA to 1.29 g at 15 DAA, an 11.7-fold increase.

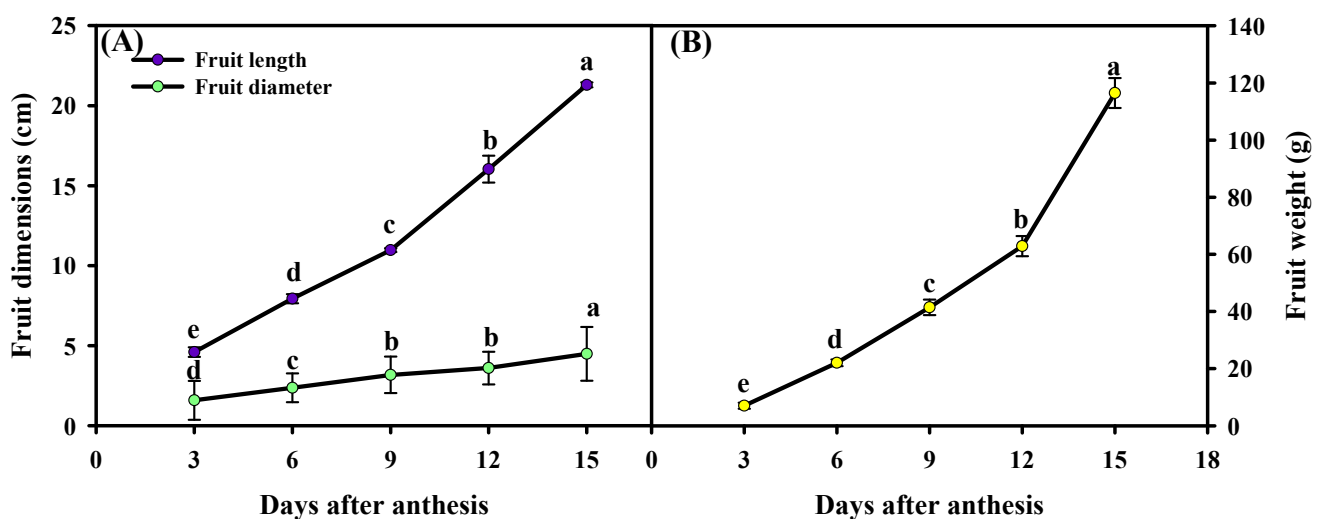


Figure 1. Changes in fruit morphological characteristics during eggplant fruit development. (A) Fruit length and fruit diameter and (B) fruit fresh weight measured at 3, 6, 9, 12, and 15 days after anthesis (DAA). Values are presented as means $\pm$ SE of three biological replicates. Different lowercase letters indicate significant differences among developmental stages within each measured trait according to Duncan's multiple range test at $p < 0.05$.

Histological analysis (Table 2, Figures 2 and 3) showed that mean cell area increased from 1256.5 μm^2 at 3 DAA to 7594.8 μm^2 at 15 DAA, representing more than a sixfold increase. Over the same period, cell density declined sharply from 795.86 to 131.67 cells $\cdot\text{mm}^{-2}$. A strong negative correlation was observed between mean cell area and cell density (Figure 2A), and mean cell area was positively correlated with fruit diameter (Figure 2B). Representative light micrographs (Figure 3) illustrate the progressive changes in cell size and tissue structure during fruit development.

Table 1. Effect of fruit firmness, number of seeds and 1000-seed weight of eggplant during fruit development at different days after anthesis (DAA).

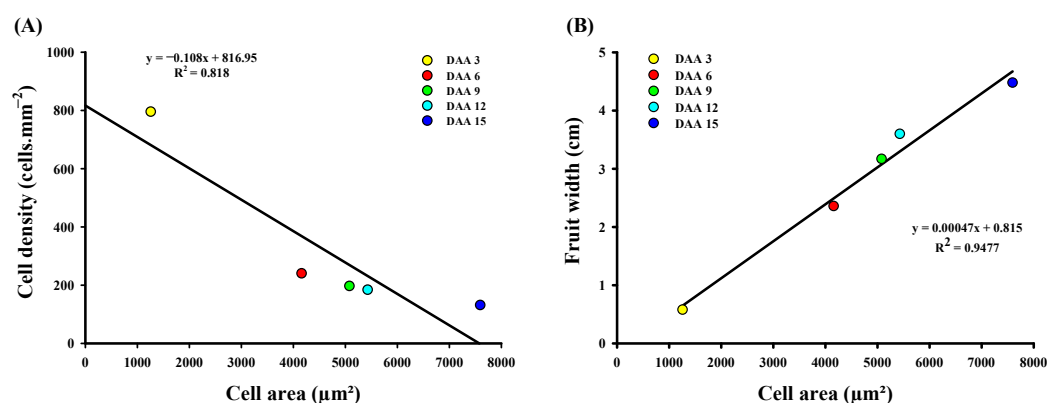
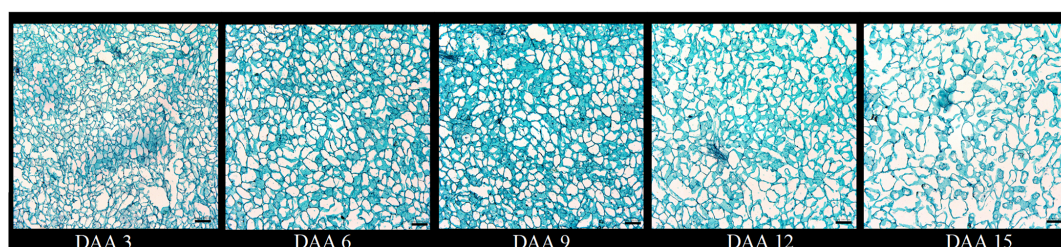
Days After Anthesis	Fruit Firmness (N)	No. of Seeds (per Fruit)	1000-Seed Weight (g)
3	12.3 ± 1.49 a ^z	642.0 ± 3.00 a	0.1200 ± 0.00004 e
6	10.5 ± 0.88 b	621.0 ± 5.57 b	0.4000 ± 0.00001 d
9	9.9 ± 0.83 b	612.0 ± 2.00 bc	0.6000 ± 0.00001 c
12	9.9 ± 0.65 b	611.3 ± 9.02 bc	1.0000 ± 0.00001 b
15	9.1 ± 0.25 b	609.0 ± 3.00 c	1.2998 ± 0.00031 a

^z Values are presented as means ± standard deviations (SD) of three biological replicates. Different lowercase letters within a column indicate significant differences among developmental stages according to Duncan's multiple range test at $p < 0.05$.

Table 2. Effect of mean cell area and cell density in pericarp tissues of eggplant during fruit development at different days after anthesis (DAA).

Days After Anthesis	Cell Area (μm^2)	Cell Density ($\text{Cells}\cdot\text{mm}^{-2}$)
3	1256.5 ± 486.2 c ^z	795.86 ± 307.98 a
6	4158.6 ± 960.9 b	240.47 ± 55.56 b
9	5079.7 ± 1418.8 ab	196.86 ± 54.98 b
12	5430.3 ± 1546.4 ab	184.15 ± 52.44 b
15	7594.8 ± 1926.2 a	131.67 ± 33.39 b

^z Values are presented as means ± standard deviations (SD) of three biological replicates. Different lowercase letters within a column indicate significant differences among developmental stages according to Duncan's multiple range test at $p < 0.05$.

**Figure 2.** Relationships between pericarp cell characteristics and fruit diameter during eggplant fruit development. (A) Relationship between mean cell area and calculated cell density and (B) relationship between mean cell area and fruit diameter at 3, 6, 9, 12, and 15 DAA. Each point represents the developmental-stage mean, and colors indicate the sampling stage. Solid lines represent linear regressions; the regression equations and coefficients of determination (R^2) are shown within each panel.**Figure 3.** Representative light micrographs of eggplant pericarp tissues during fruit development. Longitudinal sections of pericarp tissues collected at 3, 6, 9, 12, and 15 DAA, shown from left to right, were double-stained with Safranin and Fast Green and observed under bright-field microscopy using a 10× objective. The images illustrate the progressive increase in cell size and changes in tissue organization during fruit development. Scale bars located in the lower-right corner of each micrograph represent 100 μm .

3.2. Changes in Soluble Sugars, Peel Anthocyanins, and Water-Soluble Vitamins

Soluble sugars accumulated continuously during development (Figure 4). Total soluble sugar content increased from 111.4 mg·g⁻¹ dry weight (DW) at 3 DAA to 309.3 mg·g⁻¹ DW at 15 DAA, an approximately 2.8-fold increase. Among individual sugars, glucose increased from 61.9 to 170.5 mg·g⁻¹ DW, fructose from 44.8 to 115.3 mg·g⁻¹ DW, and sucrose rose markedly between 6 and 12 DAA, reaching its highest level at 15 DAA. No significant difference in sucrose concentration was detected between 6 and 9 DAA. Peel anthocyanins, dominated by delphinidin-3-rutinoside, showed a distinct stage-dependent accumulation pattern (Table 3). Total anthocyanin content remained low at 3–6 DAA, increased rapidly between 9 and 12 DAA, and declined slightly at 15 DAA. Delphinidin-3-rutinoside was the predominant anthocyanin, accounting for approximately 65.7–76.7% of the total quantified anthocyanins. Its concentration increased from 15.7 mg·kg⁻¹ DW at 3 DAA to 112.3 mg·kg⁻¹ DW at 12 DAA, then decreased to 89.7 mg·kg⁻¹ DW at 15 DAA. Minor delphinidin derivatives (delphinidin-3-glucoside, delphinidin-3-rutinoside-5-galactoside, delphinidin-3-rutinoside-5-glucoside) exhibited similar trends. Water-soluble vitamins generally declined as fruit development progressed (Table 4). Thiamine (vitamin B₁) decreased from 0.99 to 0.57 mg·kg⁻¹ DW, riboflavin (B₂) from 2.13 to 0.83 mg·kg⁻¹ DW, pantothenic acid (B₅) from 14.6 to 9.0 mg·kg⁻¹ DW, and nicotinamide (B₃) from 2.73 to 1.42 mg·kg⁻¹ DW. Pyridoxine (B₆) was detected only at 3 DAA (0.05 mg·kg⁻¹ DW) and was below the limit of quantification (<LOQ) at all subsequent stages. Ascorbic acid (vitamin C) was not detected.

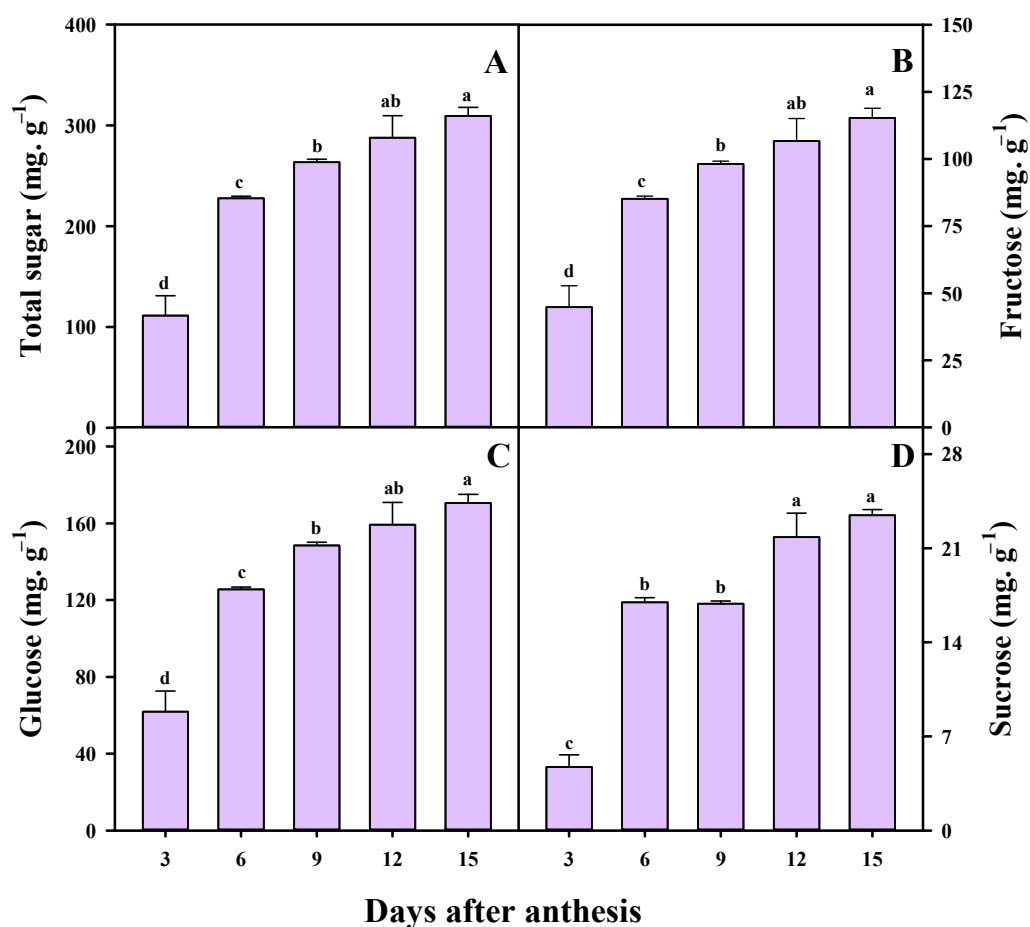


Figure 4. Changes in total sugar (A), fructose (B), glucose (C), and sucrose (D) content of eggplant fruit at 3, 6, 9, 12, and 15 days after anthesis (DAA). Values represent means $\pm$ standard error (SE) of three replicates ($n = 3$). Different letters indicate significant differences among developmental stages according to Duncan's multiple range test at $p < 0.05$.

Table 3. Changes in delphinidin-based anthocyanin contents in eggplant peel at different days after anthesis (DAA).

Days After Anthesis	Delphinidin-3 -Glucoside (mg·kg ⁻¹)	Delphinidin-3 -Rutinoside (mg·kg ⁻¹)	Delphinidin-3 -Rutinoside -5-Galactoside (mg·kg ⁻¹)	Delphinidin-3 -Rutinoside -5-Glucoside (mg·kg ⁻¹)
3	4.1 ± 0.05 e ^z	15.7 ± 0.4 e	0.29 ± 0.01 c	0.38 ± 0.02 c
6	7.6 ± 0.10 d	25.9 ± 0.2 d	0.28 ± 0.01 c	0.39 ± 0.03 c
9	13.1 ± 0.20 c	40.7 ± 0.8 c	0.31 ± 0.04 c	0.40 ± 0.02 c
12	53.6 ± 2.80 a	112.3 ± 4.3 a	1.31 ± 0.09 a	1.79 ± 0.12 a
15	44.9 ± 0.90 b	89.7 ± 8.1 b	0.78 ± 0.07 b	1.19 ± 0.09 b

^z Values are presented as means ± standard deviations (SD) of three biological replicates and are expressed on a dry-weight basis. Different lowercase letters within a column indicate significant differences among developmental stages according to Duncan's multiple range test at $p < 0.05$.

Table 4. Changes in water-soluble vitamin contents in eggplant fruit development at different days after anthesis (DAA).

Days After Anthesis	Vitamin B ₁	Vitamin B ₂	Vitamin B ₅	Vitamin B ₆	Vitamin B ₃	Vitamin C
Concentration (mg·kg ⁻¹)						
3	0.99 ± 0.16 a ^z	2.13 ± 0.28 a	14.6 ± 1.70 a	0.05 ± 0.04	2.73 ± 0.18 a	ND
6	0.83 ± 0.07 ab	1.49 ± 0.17 b	12.9 ± 0.49 b	<LOQ	2.41 ± 0.24 a	ND
9	0.77 ± 0.06 b	1.32 ± 0.06 b	10.0 ± 0.55 c	<LOQ	2.48 ± 0.25 a	ND
12	0.53 ± 0.04 c	0.66 ± 0.04 c	8.5 ± 0.79 c	<LOQ	1.00 ± 0.11 c	ND
15	0.57 ± 0.06 c	0.83 ± 0.09 c	9.0 ± 0.41 c	<LOQ	1.42 ± 0.13 b	ND

^z Values are presented as means ± standard deviations (SD) of three biological replicates and are expressed on a dry-weight basis. Different lowercase letters within a column indicate significant differences among developmental stages according to Duncan's multiple range test at $p < 0.05$. <LOQ, below the limit of quantification; ND, not detected.

3.3. Endogenous Hormone Concentrations and Integrated Developmental Stage Data

Endogenous hormone concentrations changed markedly during development (Table 5). IAA decreased from 0.21 ng·mg⁻¹ DW at 3 DAA to 0.10 ng·mg⁻¹ DW at 15 DAA. ABA decreased from 0.57 to 0.29 ng·mg⁻¹ DW, JA from 1.80 to 0.25 ng·mg⁻¹ DW, and SA from 3.73 to 0.60 ng·mg⁻¹ DW. The highest concentrations of all four detected hormones occurred at 3 DAA, followed by generally declining trends during subsequent development. Gibberellins (GA₃ and GA₇) and cytokinin's were not detected under the analytical conditions used. Integration of the morphological, cellular, hormonal, and metabolic data revealed clear temporal patterns across the developmental window. During the earliest period (3–6 DAA), concentrations of IAA, ABA, JA, and SA were highest, while soluble sugars and anthocyanins remained at relatively low levels and water-soluble vitamins were most abundant. Between 6 and 12 DAA, fruit fresh weight increased most rapidly, pericarp cell area expanded substantially, and thousand-seed weight rose markedly. This same period saw sharp accumulation of glucose, fructose, and delphinidin-based anthocyanins, along with progressive declines in all four measured hormones. From 12 to 15 DAA, soluble sugar concentrations reached their maximum, anthocyanins peaked at 12 DAA then declined slightly, fruit growth rate slowed, and seed development was essentially completed. These patterns are visually summarized in Figure 5, which shows fruit photographs at each stage.

Table 5. Changes in endogenous plant hormone contents of eggplant fruit during fruit development at different days after anthesis (DAA).

DAA	ABA (ng·mg ⁻¹)	IAA (ng·mg ⁻¹)	JA (ng·mg ⁻¹)	SA (ng·mg ⁻¹)	CK (ng·mg ⁻¹)	GA ₃ (ng·mg ⁻¹)	GA ₇ (ng·mg ⁻¹)
3	0.57 ± 0.02 a ^z	0.21 ± 0.05 a	1.80 ± 0.07 a	3.73 ± 0.14 a	ND	ND	ND
6	0.50 ± 0.01 b	0.15 ± 0.01 b	1.45 ± 0.14 b	1.04 ± 0.03 b	ND	ND	ND

Table 5. Cont.

DAA	ABA (ng·mg ^{−1})	IAA (ng·mg ^{−1})	JA (ng·mg ^{−1})	SA (ng·mg ^{−1})	CK (ng·mg ^{−1})	GA ₃ (ng·mg ^{−1})	GA ₇ (ng·mg ^{−1})
9	0.44 ± 0.01 c	0.15 ± 0.01 b	1.14 ± 0.03 c	0.82 ± 0.03 c	ND	ND	ND
12	0.41 ± 0.02 d	0.12 ± 0.01 bc	0.58 ± 0.04 d	0.58 ± 0.02 d	ND	ND	ND
15	0.29 ± 0.01 e	0.10 ± 0.01 c	0.25 ± 0.06 e	0.60 ± 0.02 d	ND	ND	ND

^z Values are expressed on a dry-weight basis. Values represent means ± standard deviations (SD) of three biological replicates. Different lowercase letters within a column indicate significant differences among developmental stages according to Duncan’s multiple range test at *p* < 0.05. ND, not detected under the analytical conditions used; ABA, abscisic acid; IAA, indole-3-acetic acid; JA, jasmonic acid; SA, salicylic acid; CKs, cytokinins; GA₃ and GA₇, gibberellins.

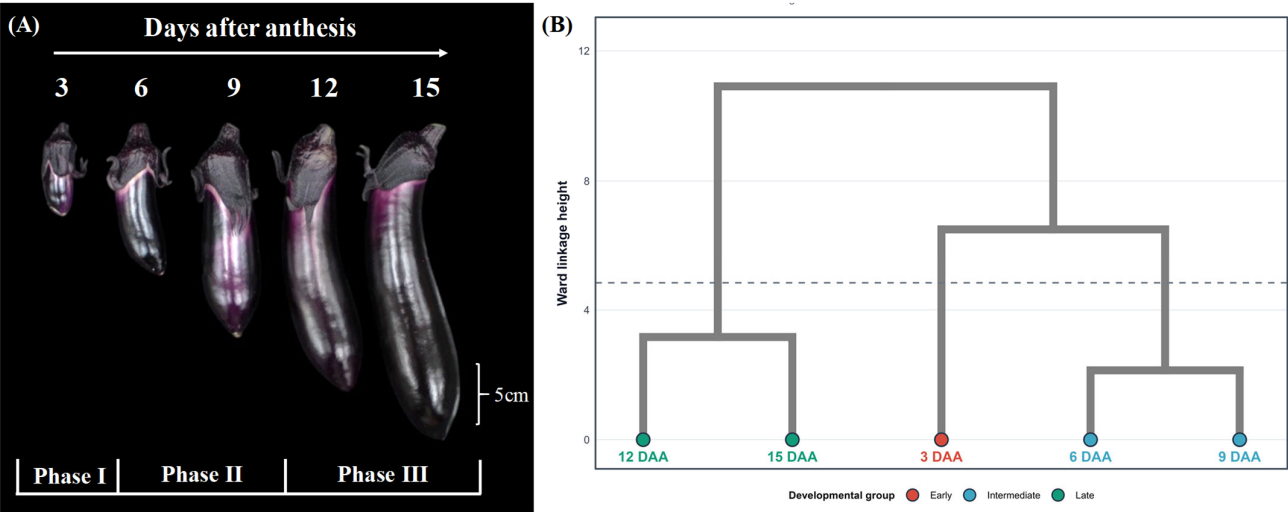


Figure 5. Morphological progression and hierarchical clustering of eggplant fruit developmental stages. **(A)** Representative fruits of ‘Otoking’ eggplant harvested at 3, 6, 9, 12, and 15 days after anthesis (DAA). Developmental groups are indicated as Phase I, early stage (3 DAA); Phase II, intermediate stage (6–9 DAA); and Phase III, late stage (12–15 DAA). Scale bar = 5 cm. **(B)** Hierarchical clustering dendrogram of the five developmental stages based on 22 standardized morphological, cellular, seed, phytohormonal, soluble-sugar, anthocyanin, and vitamin variables. The dashed horizontal line indicates the cut-off used to partition the dendrogram into three clusters corresponding to the early, intermediate, and late developmental groups. Lower linkage heights indicate greater similarity among developmental-stage profiles.

3.4. Multivariate Analysis of Integrated Developmental Traits

Principal component analysis (PCA), hierarchical cluster analysis (HCA), and Pearson correlation analysis were conducted to provide additional stage-level multivariate support for the proposed developmental framework. The analyses included 22 standardized morphological, cellular, seed, phytohormonal, soluble-sugar, anthocyanin, and vitamin variables measured at 3, 6, 9, 12, and 15 DAA. The first two principal components explained 96.0% of the total variance, with PC1 and PC2 accounting for 86.9% and 9.1%, respectively (Figure 6). The five developmental stages followed a clear trajectory along PC1. The 3 DAA stage was positioned on the negative side of PC1 and was associated with higher fruit firmness, seed number, phytohormone concentrations (IAA, ABA, JA, SA), and vitamin contents (B₁, B₂, B₃, B₅). The 6 and 9 DAA stages occupied intermediate positions. The 12 and 15 DAA stages were positioned on the positive side of PC1 but were differentiated along PC2; the 12 DAA stage was more strongly associated with delphinidin-based anthocyanins, whereas the 15 DAA stage was associated with fruit dimensions, fruit weight, cell area, thousand-seed weight, and soluble sugars (glucose, fructose, sucrose). Hierarchical clustering was performed using Euclidean distance and Ward’s minimum variance method (Figure 5B). At the selected three-cluster cut-off, the developmental stages were

partitioned into an early group comprising 3 DAA, an intermediate group comprising 6 and 9 DAA, and a late group comprising 12 and 15 DAA. The correspondence between the HCA-derived groups and the developmental trajectory shown by PCA provided additional multivariate support for the proposed early, intermediate, and late developmental framework. The clustered Pearson correlation matrix (Figure 7) revealed two broad groups of developmentally associated traits. Fruit firmness, seed number, phytohormones, and water-soluble vitamins were generally positively associated with one another and negatively associated with fruit growth, cell area, thousand-seed weight, soluble sugars, and anthocyanins. Conversely, fruit dimensions, fruit weight, cell area, thousand-seed weight, soluble sugars, and anthocyanins showed predominantly positive associations. Because the correlation analysis was based on five developmental-stage means, these relationships were interpreted as exploratory developmental associations rather than evidence of causal or independent physiological relationships. Collectively, the PCA, HCA, and correlation analysis were consistent with a stage-level transition from the early stage at 3 DAA, through the intermediate stage at 6–9 DAA, to the late stage at 12–15 DAA.

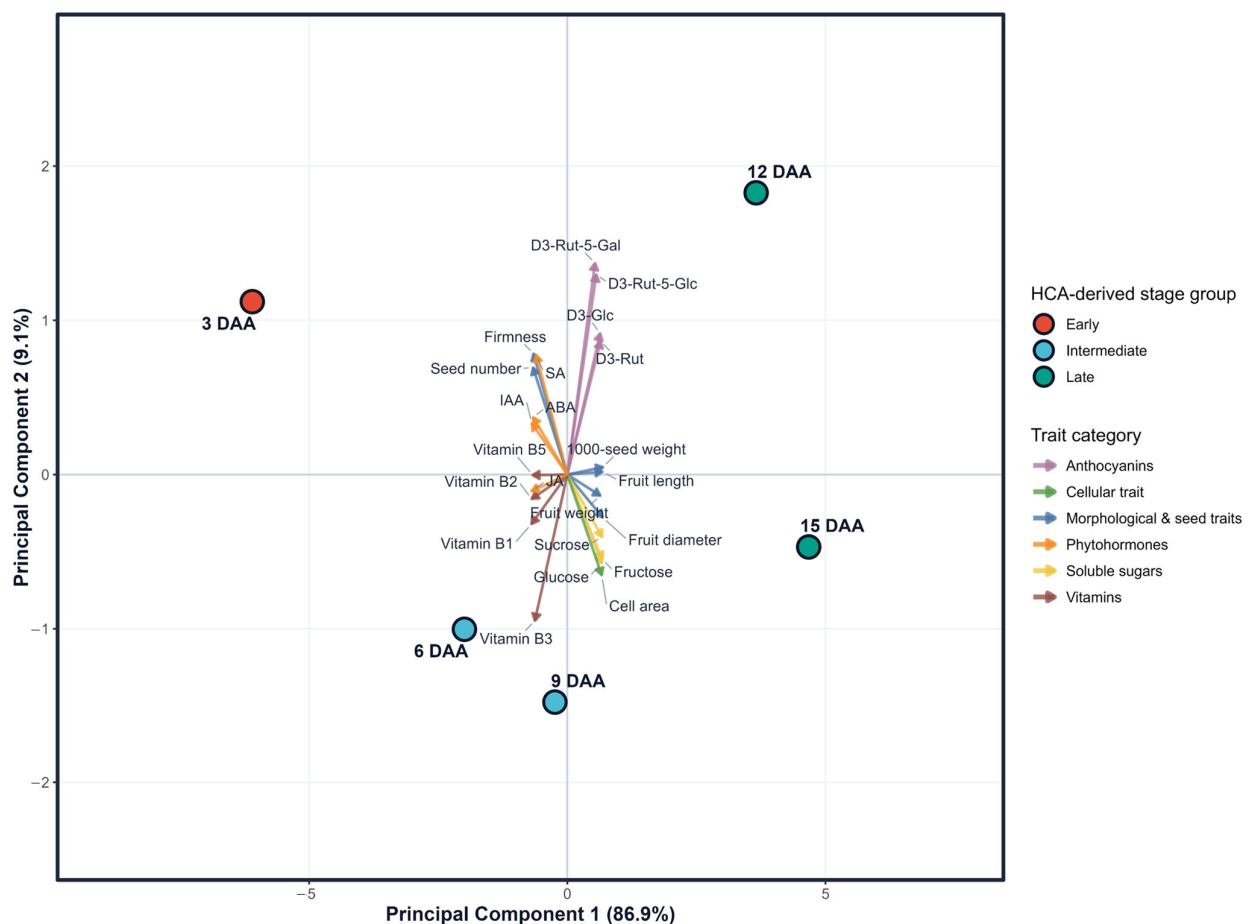


Figure 6. Principal component analysis of morphological, cellular, seed, phytohormonal, soluble-sugar, anthocyanin, and vitamin traits during eggplant fruit development. Principal component analysis was performed using standardized developmental-stage means for 22 variables measured at 3, 6, 9, 12, and 15 DAA. The first and second principal components explained 86.9% and 9.1% of the total variance, respectively. Points represent developmental stages and are colored according to the groups identified by hierarchical cluster analysis. Arrows represent variable loadings, with their direction and relative length indicating associations with the principal components. D3-Glc, delphinidin-3-glucoside; D3-Rut, delphinidin-3-rutinoside; D3-Rut-5-Gal, delphinidin-3-rutinoside-5-galactoside; D3-Rut-5-Glc, delphinidin-3-rutinoside-5-glucoside.

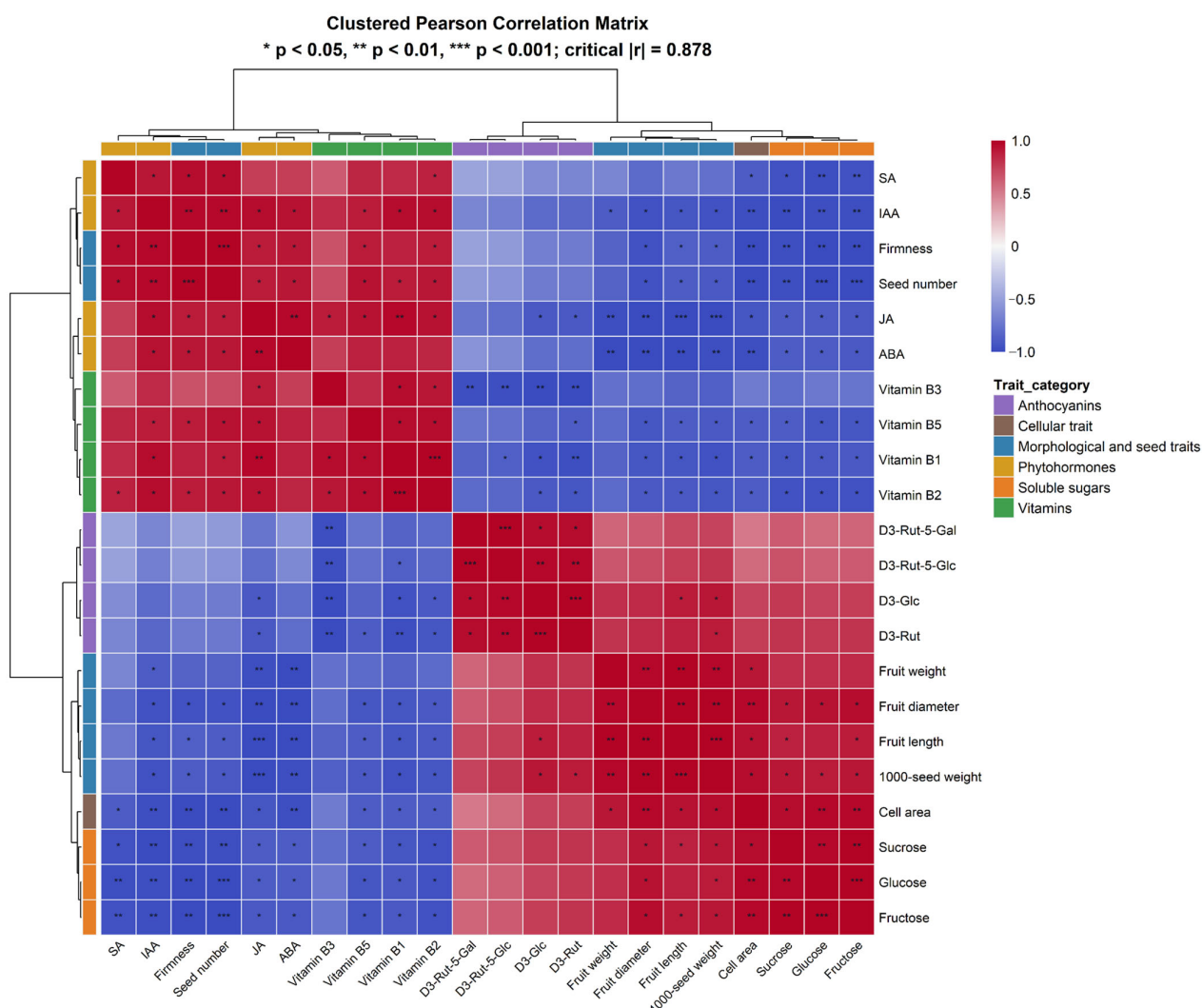


Figure 7. Clustered Pearson correlation matrix of morphological, cellular, seed, phytohormonal, soluble-sugar, anthocyanin, and vitamin traits during eggplant fruit development. Pearson correlation coefficients were calculated using developmental-stage means at 3, 6, 9, 12, and 15 days after anthesis. Variables were ordered by hierarchical clustering, and trait categories are indicated by the colored annotation bars. Red and blue cells represent positive and negative correlations, respectively, with color intensity proportional to the correlation coefficient. Asterisks indicate significant correlations at $* p < 0.05$, $** p < 0.01$, and $*** p < 0.001$; the critical correlation coefficient at $p < 0.05$ was $|r| = 0.878$. D3-Glc, delphinidin-3-glucoside; D3-Rut, delphinidin-3-rutinoside; D3-Rut-5-Gal, delphinidin-3-rutinoside-5-galactoside; D3-Rut-5-Glc, delphinidin-3-rutinoside-5-glucoside.

4. Discussion

4.1. Morphological, Physical, and Cellular Determinants of Fruit Growth

The substantial increase in fruit fresh weight (16.6-fold) was proportionally greater than the increases in length and diameter, indicating that developing eggplant fruits function as strong sink organs, continuously importing photoassimilates, water, and mineral nutrients from source tissues [12]. This growth pattern is consistent with the general developmental model of fleshy fruits, in which an early phase of active cell division is followed by a prolonged period of cell expansion that largely determines final fruit size and weight [19,22]. The disproportionately greater increase in fresh weight compared with fruit dimensions suggests that pericarp enlargement was driven primarily by cellular expansion and associated water accumulation rather than by increases in cell number alone.

The decline in fruit firmness during early development suggests that cell wall loosening and increased tissue elasticity occurred concurrently with rapid fruit enlargement, facilitating cell expansion and allowing the fruit to accommodate substantial volume increases during active growth [31]. The relatively small decrease in the number of seeds (6.1%) suggests that most ovules remained morphologically present, although their viability was not assessed [24]. The dramatic 11.7-fold increase in thousand-seed weight reflects progressive embryo and endosperm development accompanied by active deposition of storage compounds. Because seeds are major sources of endogenous auxins and gibberellins that stimulate pericarp growth and maintain fruit sink strength [17,20], increasing seed maturity likely contributed to sustained fruit enlargement.

The histological data demonstrate that pericarp cell expansion was the principal anatomical process underlying fruit enlargement. The rapid increase in cell area and concomitant decrease in cell density at 3 DAA suggest that cell division and cell enlargement overlapped during this transitional stage. From 6 to 15 DAA, cell area continued to increase, although the rate of expansion became more gradual, indicating that cell enlargement remained the dominant process while approaching developmental stabilization. The strong negative correlation between mean cell area and cell density reflects the expected inverse relationship between cell size and cell packing density within a fixed tissue area [24]. In eggplant, SmCIP7 has been shown to positively regulate fruit growth and anthocyanin accumulation, providing a recent molecular example of developmental control over fruit enlargement [32]. The positive correlation between mean cell area and fruit diameter demonstrates that radial fruit growth was closely associated with enlargement of individual pericarp cells rather than with increased cell proliferation. Similar relationships have been reported in tomato and other solanaceous fruits, where final fruit size is strongly determined by the extent of cell expansion [24]. Cell expansion is regulated by water uptake, cell wall loosening, and osmotic adjustment, with soluble sugars serving as major osmotic regulators [12]. Endoreduplication may also enhance cellular metabolic capacity and promote cell enlargement [33].

4.2. Metabolic Dynamics: Sugars, Anthocyanins, and Vitamins

The continuous increase in total soluble sugars (2.8-fold) confirms that eggplant fruit is a strong assimilate sink. Glucose and fructose were the predominant sugars throughout development, indicating that hexoses constitute the principal carbohydrate pool and likely play a central role in determining sweetness, osmotic potential, and consumer acceptability at harvest [13]. Sucrose was present at comparatively lower concentrations during early development but increased significantly during the mid to late stages, with no significant difference between 6 and 9 DAA, suggesting that sucrose accumulation and turnover were relatively stable during this transitional period. This pattern supports the widely accepted model in which sucrose is actively unloaded from source leaves into developing fruit and subsequently cleaved by invertases to generate glucose and fructose [12,34]. The progressive accumulation of hexoses coincides with substantial increases in fruit fresh weight and mean cell area, supporting the interpretation that carbohydrate accumulation is mechanistically linked to pericarp expansion and rapid fruit growth. The inverse relationship between declining IAA/ABA and increasing sugars suggests a shift from hormonal to metabolic control of growth [12]. Moreover, soluble sugars are known to stimulate anthocyanin biosynthesis through transcriptional regulation of phenylpropanoid pathway genes [8].

The stage-dependent accumulation of delphinidin-based anthocyanins, with a sharp increase between 9 and 12 DAA followed by a slight decline at 15 DAA, indicates that anthocyanin biosynthesis is tightly coordinated with fruit developmental progression. The peak at 12 DAA coincides with the period of most active fruit enlargement and sugar accu-

mulation, suggesting that anthocyanin biosynthesis is closely associated with increasing sink strength and carbohydrate availability [8]. Delphinidin-3-rutinoside accounted for 69–88% of total anthocyanins, confirming it as the principal pigment responsible for the characteristic purple coloration of eggplant peel, consistent with previous reports [2,7,35]. The decline in anthocyanin concentration at 15 DAA may be attributed to a dilution effect (pigment concentration decreases as tissue biomass increases more rapidly than pigment synthesis) and/or pigment degradation during maturation. Similar reductions have been reported in eggplant and other anthocyanin-rich fruits [15]. Although we did not examine gene expression, the developmental increase in peel pigmentation is consistent with earlier studies showing that anthocyanin biosynthesis in eggplant is regulated by structural genes and transcription factors [36–39]. Anthocyanins serve multiple physiological functions beyond peel coloration, including UV protection, ROS scavenging, and defense against stresses [8], making them important indicators of both visual quality and functional value.

The progressive decline of water-soluble vitamins (B_1 , B_2 , B_5 , B_3 , and the loss of detectable B_6 after 3 DAA) can be attributed primarily to a growth dilution effect: fruit fresh weight increased 16.6-fold while vitamin biosynthesis did not increase proportionally. Similar declines have been reported in tomato and other fleshy fruits [40,41]. In addition to dilution, metabolic reprioritization likely shifts carbon and energy resources from cofactor-rich primary metabolism toward cell expansion, cell wall biosynthesis, and secondary metabolite accumulation. B-complex vitamins are essential cofactors in glycolysis, the TCA cycle, and nucleotide biosynthesis; their high concentrations at early stages reflect elevated metabolic demand for rapid cell division and differentiation. As tissues transition to structural enlargement, the relative requirement per unit dry mass decreases, resulting in lower measured concentrations [42,43]. The absence of detectable ascorbic acid likely reflects analytical limitations rather than biological absence alone. Ascorbic acid is highly susceptible to oxidation during aqueous extraction, and its recovery is poor without stabilizing agents such as metaphosphoric acid. This interpretation is consistent with eggplant being a modest vitamin C source [6]; however, fresh-tissue extraction with appropriate stabilizers would be required for accurate quantification in future studies.

4.3. Hormonal Regulation and a Three-Stage Developmental Model for Harvest Timing

The hormonal profiles show that IAA, ABA, JA, and SA were highest at 3 DAA and declined progressively thereafter. IAA's highest concentration at 3 DAA is consistent with its well-established role in initiating fruit set and stimulating cell division immediately after fertilization [22,44]. The progressive decrease in IAA suggests that auxin-mediated regulation is most critical during early fruit development and becomes less dominant as fruit enlargement shifts from cell proliferation to cell expansion. ABA also decreased steadily; although ABA is known as a stress hormone, increasing evidence indicates it participates in early fruit development by modulating cell differentiation, osmotic adjustment, and cell wall metabolism [45,46]. The elevated ABA at 3 DAA may reflect active regulation of tissue organization and water uptake during rapid fruit growth. JA and SA, both associated with defense signaling, were highest in young fruits, indicating activation of protective mechanisms when tissues are most susceptible to environmental and biotic stresses. As fruit development progressed and structural integrity increased, the decline in JA and SA likely reflected a metabolic transition from defense-oriented signaling toward growth- and sink-driven processes [47]. The non-detection of GA_3 , GA_7 , and CKs under our HPLC-DAD conditions should be interpreted cautiously. DAD has lower sensitivity for these analytes compared to LC-MS/MS, and the simple methanolic extraction without solid-phase purification may have contributed to matrix interference and poor recovery. It is also plausible that these hormones peaked transiently before our first sampling point

(0–3 DAA), as both GAs and CKs are known to act immediately after pollination [23,48]. The cultivar ‘Otoking’ is facultatively parthenocarpic, and parthenocarpic fruit set in eggplant is associated with hormone signaling, transcription factor regulation, and secondary metabolism [49]; similar cultivars have been developed for commercial production [50]. Targeted LC-MS/MS protocols with optimized extraction are needed to confirm their developmental profiles. The multivariate patterns were broadly consistent with the proposed developmental framework, but their main value lies in showing that early-stage hormone- and vitamin-related traits, and late-stage growth- and sugar-related traits, changed in a coordinated manner rather than independently. This suggests a developmental transition from regulatory and protective metabolism toward expansion and quality formation. Because these analyses were based on stage means, they should be interpreted as exploratory support for the model rather than as independent proof. Integration of all findings is consistent with a division of eggplant fruit development into three physiologically distinct stages. The first stage, 3 DAA, is the initiation and defense stage, characterized by the highest concentrations of IAA, ABA, JA, and SA, active cell division (high cell density), lowest sugars and anthocyanins, and highest water-soluble vitamins. During this period, metabolic resources are preferentially allocated to primary metabolism, organogenesis, and stress protection, making it critical for fruit set and early tissue differentiation. The second stage, 6–9 DAA, is the rapid growth and pigmentation stage, the most dynamic phase. Fruit fresh weight increases most rapidly, cell area expands sharply, cell density declines, and thousand-seed weight rises substantially. Glucose, fructose, and delphinidin anthocyanins accumulate rapidly, while all four hormones decline progressively. This stage represents a metabolic reprioritization from hormone-dominated signaling toward carbon allocation, cell expansion, and secondary metabolism. Fruit enlargement, sugar accumulation, and color development occur simultaneously and determine much of the final fruit quality. The third stage, 12–15 DAA, is the functional maturation stage. Fruit growth rate slows, soluble sugar concentrations reach their maximum, anthocyanins peak at 12 DAA then decline slightly, thousand-seed weight continued to increase, and vitamins continue to decline. Fruit appearance, sweetness, and functional properties approach commercially desirable levels, and quality attributes are consolidated as physiological maturity is attained.

Implications for harvest timing: Among the developmental stages, 15 DAA represents a useful physiological reference point under the present greenhouse conditions and for the cultivar studied. At this stage, fruits exhibit maximum soluble sugar concentrations, near-maximum anthocyanin accumulation, fully developed seeds, and substantial physical enlargement. These characteristics suggest that 15 DAA may be a useful harvest reference; however, sensory evaluation and postharvest studies would be required to confirm consumer-driven optimal harvest timing. However, an important observation is the contrasting behavior of nutritional and quality-related compounds: water-soluble vitamins decline throughout development, whereas sugars and anthocyanins increase markedly. This divergence suggests that fruit quality is determined by a balance between nutritional value and sensory/functional attributes, and that the optimal harvest stage may vary depending on the intended use of the fruit [6]. The three-stage framework offers practical guidance for optimizing harvest timing and production strategies aimed at improving fruit size, appearance, nutritional composition, and functional quality in greenhouse-grown eggplant. Recent multi-omics approaches have elucidated the regulatory networks underlying quality traits in eggplant, revealing that fruit quality changes stem from alterations in key metabolites, and that the ratio of soluble sugars to organic acids significantly impacts taste [51]. This study was limited to one cultivar, one growing season, one greenhouse environment, five developmental time points, and three biological replicates per trait. In addition, the destructive and assay-specific sampling design prevented individual fruits

from being matched across all analytical categories; therefore, the integrated multivariate analyses were conducted using developmental-stage means. The proposed three-stage framework and the identification of 15 DAA as a candidate late-stage harvest reference should consequently be interpreted as condition-specific and exploratory. Validation across additional cultivars, seasons, production environments, and later developmental stages, together with sensory and postharvest evaluations, will be required before broader harvest recommendations can be established.

5. Conclusions

This study integrated morphological, cellular, hormonal, and metabolic analyses to characterize eggplant fruit development from 3 to 15 days after anthesis (DAA). Fruit enlargement was driven predominantly by post-mitotic cell expansion, as mean cell area increased >6-fold while cell density declined sharply. Soluble sugars (especially glucose and fructose) accumulated continuously, whereas delphinidin-based anthocyanins peaked at 12 DAA then declined slightly. In contrast, water-soluble vitamins decreased progressively, likely due to dilution and metabolic reprioritization. Endogenous IAA, ABA, JA, and SA were highest at 3 DAA and declined thereafter, indicating that hormonal regulation dominates early development before shifting to carbon allocation and secondary metabolism. Based on integrated data, three physiologically distinct stages are proposed: Stage I (3 DAA)—initiation and defense (high hormones, cell division, high vitamins); Stage II (6–9 DAA)—rapid growth and pigmentation (cell expansion, sugar and anthocyanin accumulation); Stage III (12–15 DAA)—functional maturation (maximum sugars, advanced seed development, quality consolidation). Among these, 15 DAA emerged as a promising harvest benchmark for greenhouse-grown ‘Otoking’ eggplant. The proposed stage-based framework provides a useful reference for interpreting fruit development and harvest-related quality changes in eggplant.

Author Contributions: Conceptualization, S.H.P. and J.S.K.; methodology, S.H.P., F.A.M. and S.R.K.; software, M.G.C.; validation, J.G.L., M.C.S.C.E., D.G.J. and M.J.K.; formal analysis, S.H.P. and F.A.M.; investigation, S.H.P., F.A.M., S.R.K., J.G.L., M.C.S.C.E., M.G.C., D.G.J. and M.J.K.; resources, J.S.K.; data curation, J.G.L. and M.J.K.; writing—original draft preparation, S.H.P. and F.A.M.; writing—review and editing, S.R.K., M.C.S.C.E., M.G.C., D.G.J. and J.S.K.; visualization, S.H.P. and M.G.C.; supervision, J.S.K.; project administration, J.S.K.; funding acquisition, J.S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was carried out with the support of “Cooperative Research Program for Agriculture Science and Technology Development (Project No. RS-2025-02303116)”, funded by Rural Development Administration, Republic of Korea.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors thank the Smart Farm Innovation Valley in Miryang for providing greenhouse facilities and technical support during the experiment. During the preparation of this manuscript, the authors used the following AI-assisted tools: (1) Perplexity AI (free version, accessed via web interface in June 2026) for grammar corrections, language refinement, and manuscript formatting suggestions; (2) DeepSeek (web interface, accessed June 2026) for generating R code to produce the graphical abstract. The authors have reviewed, edited, and tested all outputs, including the generated R code and graphical abstract and take full responsibility for the final content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ABA	Abscisic acid
ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
CKs	Cytokinins
DAA	Days after anthesis
DW	Dry weight
EC	Electrical conductivity
FAA	Formalin-acetic acid-alcohol
GA	Gibberellin/gibberellic acid
GAs	Gibberellins
HPLC	High-performance liquid chromatography
IAA	Indole-3-acetic acid
JA	Jasmonic acid
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
mg·g ^{−1} DW	Milligrams per gram dry weight
mg·kg ^{−1} DW	Milligrams per kilogram dry weight
mL·min ^{−1}	Milliliters per minute
MS/MS	Tandem mass spectrometry
PAD	Pulsed amperometric detection
PTFE	Polytetrafluoroethylene
RDA	Rural Development Administration
SA	Salicylic acid
SE	Standard error
TSW	Thousand-seed weight
UV	Ultraviolet
w/v	Weight/volume
v/v	Volume/volume

References

- Knapp, S.; Vorontsova, M.S.; Prohens, J. Wild relatives of the eggplant (*Solanum melongena* L.: Solanaceae): New understanding of species names in a complex group. *PLoS ONE* **2013**, *8*, e57039. [[CrossRef](#)] [[PubMed](#)]
- Matsubara, K.; Kaneyuki, T.; Miyake, T.; Mori, M. Antiangiogenic activity of nasunin, an antioxidant anthocyanin, in eggplant peels. *J. Agric. Food Chem.* **2005**, *53*, 6272–6275. [[CrossRef](#)] [[PubMed](#)]
- Munoz-Falcón, J.E.; Prohens, J.; Vilanova, S.; Nuez, F. Characterization, diversity, and relationships of the Spanish striped (Listada) eggplants: A model for the enhancement and protection of local heirlooms. *Euphytica* **2008**, *164*, 405–419. [[CrossRef](#)]
- Stommel, J.R.; Whitaker, B.D. Phenolic acid content and composition of eggplant fruit. *J. Am. Soc. Hortic. Sci.* **2003**, *128*, 704–710. [[CrossRef](#)]
- McCullough, M.L.; Feskanich, D.; Stampfer, M.J.; Giovannucci, E.L.; Rimm, E.B.; Hu, F.B.; Spiegelman, D.; Hunter, D.J.; Colditz, G.A.; Willett, W.C. Diet quality and major chronic disease risk in men and women: Moving toward improved dietary guidance. *Am. J. Clin. Nutr.* **2002**, *76*, 1261–1271. [[CrossRef](#)] [[PubMed](#)]
- Raigón, M.D.; Prohens, J.; Muñoz-Falcón, J.E.; Nuez, F. Comparison of eggplant landraces and commercial varieties for fruit content of phenolics, minerals, and antioxidant activity. *J. Food Compos. Anal.* **2008**, *21*, 370–376. [[CrossRef](#)]
- Wu, X.; Prior, R.L. Identification and characterization of anthocyanins by high performance liquid chromatography–electrospray ionization–tandem mass spectrometry in common foods in the United States: Vegetables, nuts, and grains. *J. Agric. Food Chem.* **2005**, *53*, 3101–3113. [[CrossRef](#)] [[PubMed](#)]
- Jaakola, L. New insights into the regulation of anthocyanin biosynthesis in fruits. *Trends Plant Sci.* **2013**, *18*, 477–483. [[CrossRef](#)] [[PubMed](#)]
- Zhou, X.; Liu, S.; Yang, Y.; Liu, J.; Zhuang, Y. Integrated metabolome and transcriptome analysis reveals a regulatory network of fruit peel pigmentation in eggplant (*Solanum melongena* L.). *Int. J. Mol. Sci.* **2022**, *23*, 13475. [[CrossRef](#)] [[PubMed](#)]

10. Zaro, M.Z.; Keunchkarian, S.; Chaves, A.R.; Vicente, A.R.; Concellón, A. Changes in bioactive compounds and response to postharvest storage conditions in purple eggplants as affected by fruit developmental stage. *Postharvest Biol. Technol.* **2014**, *96*, 110–117. [\[CrossRef\]](#)
11. Savvas, D.; Lenz, F. Influence of NaCl concentration in the nutrient solution on mineral composition of eggplants grown in sand culture. *Gartenbauwissenschaft* **1996**, *61*, 124–127.
12. Koch, K.E. Sucrose metabolism: Regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Curr. Opin. Plant Biol.* **2004**, *7*, 235–246. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Harsha, S.G.; Girish, B.; Bindu Shekhar, M.G.; Dinsha, M.; Manjugouda, I.P.; Laxman, R.H.; Singhe, T.H.; Shivashankara, K.S.; Prathibha, M.D. Comparative study on physiological intricacies and sugar accumulation dynamics in brinjal (*Solanum melongena* L.) under drought stress. *Sci. Hortic.* **2024**, *325*, 112633. [\[CrossRef\]](#)
14. Wang, H.; Schauer, N.; Usadel, B.; Frasse, P.; Zouine, M.; Hernould, M.; Latche, A.; Pech, J.C.; Fernie, A.R.; Bouzayen, M. Regulatory features underlying pollination-dependent and -independent tomato fruit set revealed by transcript and primary metabolite profiling. *Plant Cell* **2009**, *21*, 1428–1452. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Wang, X.; Lio, S.; Li, Q.; Song, L.; Zhang, W.; Yu, P.; Xuan, S.; Wang, Y.; Zhao, J.; Chen, X.; et al. Delphinidins and naringenin chalcone underlying the fruit color changes during maturity stages in eggplant. *Agronomy* **2022**, *12*, 1036. [\[CrossRef\]](#)
16. Zhou, X.; Obel, H.O.; Liu, S.; Yang, Y.; Liu, J.; Zhuang, Y. Comparative analysis of metabolic variation in eggplant fruit of different varieties reveals metabolites important for quality traits. *Foods* **2023**, *12*, 4383. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Jha, S.N.; Matsuoka, T. Surface stiffness and density of eggplant during storage. *J. Food Eng.* **2002**, *54*, 23–26. [\[CrossRef\]](#)
18. Gajewski, M.; Arasimowicz, A. Sensory quality of eggplant fruits in relation to cultivar and maturity stage. *Acta Sci. Pol. Hortorum Cultus* **2004**, *3*, 67–73.
19. Gillasp, G.; Ben-David, H.; Gruissem, W.; Darwin, C. Fruits: A developmental perspective. *Plant Cell* **1993**, *5*, 1439–1451. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Serrani, J.C.; Sanjuán, R.; Ruiz-Rivero, O.; Fos, M.; García-Martínez, J.L. Gibberellin regulation of fruit set and growth in tomato. *Plant Physiol.* **2007**, *145*, 246–257. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Pascual, L.; Blanca, J.M.; Canizares, J.; Nuez, F. Transcriptomic analysis of tomato carpel development reveals alterations in ethylene and gibberellin synthesis during pat3/pat4 parthenocarpic fruit set. *BMC Plant Biol.* **2009**, *9*, 67. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Mounet, F.; Moing, A.; Kowalczyk, M.; Rohrmann, J.; Petit, J.; Garcia, V.; Maucourt, M.; Yano, K.; Deborde, C.; Aoki, K.; et al. Down-regulation of a single auxin efflux transport protein in tomato induces precocious fruit development. *J. Exp. Bot.* **2012**, *63*, 4901–4917. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Shi, S.; Li, D.; Li, S.; Wang, Y.; Tang, X.; Liu, Y.; Ge, H.; Chen, H. Comparative transcriptomic analysis of early fruit development in eggplant (*Solanum melongena* L.) and functional characterization of *SmOVATE5*. *Plant Cell Rep.* **2023**, *42*, 321–336. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Bertin, N. Analysis of the tomato fruit growth response to temperature and plant fruit load in relation to cell division, cell expansion and DNA endoreduplication. *Ann. Bot.* **2005**, *95*, 429–447. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Valerga, L.; Darré, M.; Zaro, M.J.; Arambarri, A.; Vicente, A.R.; Lemoine, M.L.; Concellón, A. Micro-structural and quality changes in growing dark-purple eggplant (*Solanum melongena* L.) as affected by the harvest season. *Sci. Hortic.* **2019**, *244*, 22–30. [\[CrossRef\]](#)
26. Davies, P.J. (Ed.) *Plant Hormones: Biosynthesis, Signal Transduction, Action!* 3rd ed.; Springer Science & Business Media: Dordrecht, The Netherlands, 2004.
27. Uzun, S. The effect of light and temperature on the phenology and maturation of the fruit of eggplant (*Solanum melongena*) grown in greenhouses. *J. Appl. Biol. Sci.* **2007**, *1*, 5–10. [\[CrossRef\]](#)
28. Rural Development Administration (RDA). Available online: <https://www.nongsaro.go.kr/portal/ps/psb/psbl/workScheduleDtl.ps?menuId=PS00087&cntntsNo=30770&sKidofcomdtySeCode=VC%20> (accessed on 2 June 2026).
29. Zuo, Z.; Lü, T.; Sun, J.; Peng, H.; Yang, D.; Song, J.; Ma, G.; Mao, H. Dynamic Water and Fertilizer Management Strategy for Greenhouse Tomato Based on Morphological Characteristics. *Agriculture* **2025**, *15*, 304. [\[CrossRef\]](#)
30. Lu, X.; He, N.; Anees, M.; Yang, D.; Kong, W.; Zhang, J.; Yuan, L.; Luo, X.; Zhu, H.; Liu, W. A Comparison of Watermelon Flesh Texture across Different Ploidy Levels Using Histology and Cell Wall Measurements. *Horticulturae* **2024**, *10*, 112. [\[CrossRef\]](#)
31. Carpita, N.; McCann, M. The cell wall. In *Biochemistry and Molecular Biology of Plants*; Buchanan, B.B., Wilhelm, G., Jones, R.L., Eds.; American Society of Plant Physiologists: Rockville, MD, USA, 2000; pp. 52–108.
32. Li, Y.; Xing, M.; Yang, Q.; Wang, Y.; Jiang, J.; Zhao, Y.; Zhao, X.; Shen, A.; Feng, Y.; Zhao, X.; et al. *SmCIP7*, a COP1 interactive protein, positively regulates anthocyanin accumulation and fruit size in eggplant. *Int. J. Biol. Macromol.* **2023**, *234*, 123729. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Lemaire-Chamley, M.; Petit, J.; Garcia, V.; Just, D.; Baldet, P.; Germain, V.; Fagard, M.; Mouassite, M.; Cheniclet, C.; Rothan, C. Changes in transcriptional profiles are associated with early fruit tissue specialization in tomato. *Plant Physiol.* **2005**, *139*, 750–769. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Boo, H.; Kim, H.; Lee, H. Changes in sugar content and sucrose synthase enzymes during fruit growth in eggplant (*Solanum melongena* L.) grown on different polyethylene mulches. *HortScience* **2010**, *45*, 775–777. [\[CrossRef\]](#)

35. Azuma, K.; Ohyama, A.; Ipoushi, K.; Ichiyanagi, T.; Takeuchi, A.; Saito, T.; Fukuoka, H. Structures and antioxidant activity of anthocyanins in many accessions of eggplant and its related species. *J. Agric. Food Chem.* **2008**, *56*, 10154–10159. [[CrossRef](#)] [[PubMed](#)]
36. Yu, X.; Cao, S.; Wang, J.; Li, D.; He, Y. Comprehensive genomic analysis of *SmbHLH* genes and the role of *SmbHLH93* in eggplant anthocyanin biosynthesis. *Plant Cell Rep.* **2025**, *44*, 36. [[CrossRef](#)] [[PubMed](#)]
37. Docimo, T.; Francese, G.; Ruggiero, A.; Batelli, G.; Palma, M.D.; Bassolino, L.; Toppino, L.; Rotino, G.; Mennella, G.; Tucci, M. Phenylpropanoids accumulation in eggplant fruit: Characterization of biosynthetic genes and regulation by a MYB transcription factor. *Front. Plant Sci.* **2016**, *7*, 1233. [[CrossRef](#)] [[PubMed](#)]
38. Lloyd, A.; Brockman, A.; Aguirre, L.; Campbell, A.; Bean, A.; Cantero, A.; Gonzalez, A. Advances in the MYB–bHLH–WD repeat (MBW) pigment regulatory model: Addition of a WRKY factor and co-option of an anthocyanin MYB for betalain regulation. *Plant Cell Physiol.* **2017**, *58*, 1431–1441. [[CrossRef](#)] [[PubMed](#)]
39. Liu, Y.; Tikunov, Y.; Schouten, R.E.; Marcelis, L.F.M.; Visser, R.G.F.; Bovy, A. Anthocyanin biosynthesis and degradation mechanisms in Solanaceous vegetables: A review. *Front. Chem.* **2018**, *6*, 52. [[CrossRef](#)] [[PubMed](#)]
40. Davey, M.W.; Van Montagu, M.; Inzé, D.; Sanmartin, M.; Kanellis, A.; Smirnoff, N.; Benzie, I.J.J.; Strain, J.J.; Favell, D.; Fletcher, J. Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. *J. Sci. Food Agric.* **2000**, *80*, 825–860. [[CrossRef](#)]
41. Raffo, A.; La Malfa, G.; Fogliano, V.; Maiani, G.; Quaglia, G. Seasonal variations in antioxidant components of cherry tomatoes (*Solanum lycopersicum* L.). *J. Food Compos. Anal.* **2006**, *19*, 11–19. [[CrossRef](#)]
42. Howard, L.R.; Talcott, S.T.; Brenes, C.H.; Villalon, B. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* spp.) as influenced by maturity. *J. Agric. Food Chem.* **1999**, *47*, 3165–3170. [[CrossRef](#)] [[PubMed](#)]
43. Lee, S.K.; Kader, A.A. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol.* **2000**, *20*, 207–220. [[CrossRef](#)]
44. Vriezen, W.H.; Feron, R.; Maretto, F.; Keijman, J.; Mariani, C. Changes in tomato ovary transcriptome demonstrate complex hormonal regulation of fruit set. *New Phytol.* **2008**, *177*, 60–76. [[CrossRef](#)] [[PubMed](#)]
45. Zhang, M.; Yuan, B.; Leng, P. The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. *J. Exp. Bot.* **2009**, *60*, 1579–1588. [[CrossRef](#)] [[PubMed](#)]
46. Leng, P.; Yuan, M.; Guo, P. The role of abscisic acid in fruit ripening and responses to abiotic stress. *J. Exp. Bot.* **2014**, *65*, 4577–4588. [[CrossRef](#)] [[PubMed](#)]
47. Wasternack, C.; Hause, B. Jasmonates: Biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *Annals of Botany*. *Ann. Bot.* **2013**, *111*, 1021–1058. [[CrossRef](#)] [[PubMed](#)]
48. Li, J.; Yang, Y.; Chai, M.; Ren, M.; Yuan, J.; Yang, W.; Dong, Y.; Liu, B.W.; Jian, Q.; Wang, S.; et al. Gibberellins modulate local auxin biosynthesis and polar auxin transport by negatively affecting flavonoid biosynthesis in the root tips of rice. *Plant Sci.* **2020**, *298*, 110545. [[CrossRef](#)] [[PubMed](#)]
49. Zhou, S.; Yang, Y.; Zou, M.; Tao, T.; Tang, X.; Wang, Y.; Tian, S. The molecular mechanism of eggplant parthenocarpy revealed through a combined analysis of the transcriptome and the metabolome. *Ind. Crops Prod.* **2023**, *193*, 116168. [[CrossRef](#)]
50. Saito, T.; Yoshida, T.; Monma, S.; Matsunaga, H.; Sato, T.; Saito, A.; Yamada, T. Development of the parthenocarpic eggplant cultivar ‘Anominori’. *JARQ* **2009**, *43*, 123–127. [[CrossRef](#)]
51. Deng, W.; Yan, X.; Peng, J.; Xiao, D.; Su, K.; Huang, J.; Fang, H.; Zhang, J.; Chen, X.; Liang, Y.; et al. Multi-omics analysis reveals the regulatory network of quality traits in green-skinned eggplant (*Solanum melongena* L.). *Food Chem.* **2026**, *523*, 150152. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.