

# Quantitative evidence for developmental canalization: CT-metabolome integration reveals a growth-defense axis in coconut fruit development

**Running Title:** CT-metabolome canalization in coconut

Huanning You<sup>1,2</sup>, Huangsheng Ling<sup>3</sup>, Jing Chen<sup>3</sup>, Wenrao Li<sup>1</sup>, Mengxing Huang<sup>✉</sup>, Yu Zhang<sup>✉</sup>, Hongxing Cao<sup>1</sup>, Chengxu Sun<sup>1</sup>

<sup>1</sup> Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences, Wenchang, Hainan 571339, China <sup>2</sup> School of Life Sciences, Henan University, Kaifeng, Henan 475004, China <sup>3</sup> Haikou Affiliated Hospital of Central South University Xiangya School of Medicine, Haikou, Hainan 570208, China <sup>✉</sup> College of Information and Communication Engineering, Hainan University, Haikou, Hainan 570228, China <sup>✉</sup> College of Computer Science and Technology, Hainan University, Haikou, Hainan 570228, China

✉ Co-first authors | \* Corresponding authors: Hongxing Cao (caohx@catas.cn), Chengxu Sun (suncx@catas.cn)

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## Summary

Coconut is a major oil crop in the tropics, yet the structural–metabolic differences in fruit development across varieties remain poorly understood. Here we integrate CT phenomics (7,686 DICOM slices, five varieties, five developmental stages) with widely targeted LC-MS/MS metabolomics (624 metabolites) to dissect the structural–metabolic divergence between the elite dwarf variety W5 and the local tall landrace CK. CT phenotyping revealed that four tissue-specific CT values (Hounsfield Units) constitute a quantitative developmental clock spanning approximately 1,200 HU. Metabolomic profiling uncovered a metabolic bifurcation: W5 was enriched in nucleotides, amino acids and free fatty acids (growth-oriented), whereas CK accumulated flavonoids (42 of 44 species higher, up to 32.5-fold), tannins and phenolic acids (stress-response-oriented). Correlation analysis of 18 CT parameters against 624 metabolites identified 3,376 significant associations in W5 (55 pairs with  $|r| > 0.99$ ) versus none in CK. Permutation tests (10,000 shuffles) and robustness checks (Spearman rank correlation, first-differencing Pearson correlation) confirmed that this asymmetry is not attributable to chance or shared developmental trends. We propose that the inter-variety asymmetry in CT–metabolite association strength may reflect differences in developmental canalization associated with breeding-induced genetic homogenization, rather than breeding having “tightened”™

structuralâ€“metabolic coupling. CT-based germination prediction (AUC = 0.716) demonstrates the translational potential of this integrative framework for non-destructive seed screening.

**Keywords:** Coconut | CT phenomics | Metabolomics | Growthâ€“defense trade-off | Developmental canalization | Multi-omics integration

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## Significance Statement

This study establishes coconutâ€“with its unique ~1,200 HU density range spanning four tissue compartmentsâ€“as an ideal system for CT phenotyping. Integrating 7,686 DICOM slices with 624 metabolites reveals a growthâ€“defense metabolic axis shaped by breeding selection. The striking discovery of 55 near-perfect CTâ€“metabolite correlations ( $|r| > 0.99$ ) in an improved dwarf variety versus none in a local landrace provides the first quantitative statistical signature suggestive of developmental canalization in a fruit tree crop. This integrative framework offers a novel phenotypeâ€“genotype bridge for assessing developmental uniformity and enables non-destructive germination prediction (AUC = 0.716), with broad applicability to breeding programs.

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## 1 | Introduction

Coconut (*Cocos nucifera* L.) is a cornerstone crop of tropical agriculture, with annual production exceeding 60 million tons supporting millions of smallholder farmers across Asia, Africa and the Pacific (Perera et al., 2009). In Hainan, China, systematic breeding programs have developed the Wenye series of dwarf varieties (Lu and Liu, 2021), selected by breeders over decades from local tall landraces for early maturation, dwarf stature, high yield and thin shell (Li et al., 2020). The structural and biochemical differences in fruit development between these improved varieties and their progenitor landraces have not been systematically characterized.

X-ray computed tomography (CT) is a non-destructive phenotyping tool in plant science (Piovesan et al., 2021). CT values (Hounsfield Units, HU) provide quantitative readouts of tissue density, enabling in vivo tracking of fruit development. In horticultural crops, CT has been used to detect internal disorders in pomegranate (Khodaei et al., 2023), evaluate internal quality of durian (Saechua et al., 2021) and assess oil palm fruit maturity (Tarmizi et al., 2021). In coconut, a computational CT phenotyping pipeline has been established covering non-invasive 3D quantitative imaging (Zhang et al., 2023), automated tissue-level segmentation via an improved DeepLabV3+ network (Liu et al., 2023) and clinical CT postprocessing for multi-compartment quantification (Lin et al., 2023). Yu et al. (2022) further integrated Micro-CT with DeepLabV3+ for high-precision segmentation, extracting 78 phenotypic parameters. These foundations have been extended to germination dynamics (Lin et al., 2025), automated sprouting stage classification via few-shot generative models (Mehmood et al., 2026), cross-modal association with lignan metabolites (Sun et al., 2024) and location-specific density variation analysis (Lin et al., 2024). Wang et

al. (2024) reviewed the broader application of CT in agricultural non-destructive testing.

Coconut is a favorable system for CT phenotyping. Its fruit comprises four tissue compartments—fiber layer (mesocarp), shell (endocarp), solid endosperm (kernel) and liquid endosperm (coconut water)—spanning a density range from approximately  $-950$  HU (mature fiber) to  $+256$  HU (mineralized shell). This  $\sim 1,200$  HU dynamic range, comparable to the span between lung tissue and cortical bone in medical CT, allows a single non-destructive scan to track four independent developmental processes simultaneously. No other non-destructive modality—RGB imaging (surface only), near-infrared spectroscopy ( $\sim 5$  mm penetration), ultrasound (scattered by hard shells) or MRI (weak signal in dry tissues)—can resolve all four compartments.

Parallel advances in metabolomics have provided high-resolution profiles of the chemical dynamics of fruit development. In coconut, Liang et al. (2021) profiled temporal dynamics of fatty acid biosynthesis in the endosperm; Yang et al. (2022) characterized primary and secondary metabolites across eight developmental stages; Zhao et al. (2023) analyzed variety-specific accumulation across eight varieties; and Widyaningsih et al. (2023) tracked flavonoid dynamics in coconut water with maturity.

The key insight is that CT captures physical outcomes of development (density changes from air-filling, mineralization and lipid deposition), while metabolomics captures the chemical driving forces. The relationship is not coincidental—every HU change results from metabolic activity: programmed cell death and water translocation in the fiber layer, oxalate biosynthesis in the shell, and fatty acid and protein synthesis in the endosperm. By systematically mapping CT—metabolite associations, CT can be transformed from a descriptive tool into a mechanistically interpretable one.

The concept of developmental canalization, first proposed by Waddington (1942), describes the capacity of developmental systems to produce consistent phenotypic outcomes despite genetic or environmental perturbations. This buffering property, often enhanced by genetic homozygosity through directional selection, may leave detectable statistical signatures in population-level phenotypic correlations. While canalization has been extensively studied in model organisms such as *Drosophila* and *Arabidopsis*, its manifestation in fruit tree crops—particularly in the context of breeding-induced genetic homogenization—remains unexplored.

The growth—defense trade-off is a well-established concept in plant biology (Huot et al., 2014), describing resource allocation between biomass accumulation and chemical protection. This has been demonstrated in model species (Zhang et al., 2011) and crop domestication (Schauer et al., 2005; Doebley et al., 2006), but how it operates in fruit trees with multi-month developmental timescales remains unclear. Coconut, with its four-layer structure, breeding history and contrasting variety characteristics, provides an opportunity to investigate how artificial selection shapes structural—metabolic relationships.

Seed germination represents another dimension: thin shells selected for ease of kernel extraction may compromise seed viability. CT-based germination prediction has been validated in oil palm (Tarmizi et al., 2021) and reviewed across multiple species (Goodman et al., 2022), but is unexplored in coconut.

Dwarf varieties subjected to generations of directional selection (such as W5) are expected to possess higher genetic homozygosity, which may canalize developmental programs—buffering trajectories against genetic and environmental variation (Waddington, 1942). This canalization may leave detectable signatures in structural phenotypes and metabolic programs, but its extent has not been quantified in fruit trees.

This study integrates CT phenomics (7,686 DICOM slices, five varieties, five developmental stages) with widely targeted metabolomics (624 metabolites in W5 and CK), asking: (i) Can four tissue-specific CT values constitute a quantitative developmental clock? (ii) Do metabolic differences between W5 and CK exhibit a growth—defense axis pattern? (iii) Is there asymmetry in CT—metabolite association strength between varieties? (iv) Can CT features be used for non-destructive germination prediction?

## 2 | Results

### 2.1 | CT phenotyping reveals variety-specific developmental trajectories

CT phenotyping was performed on 7,686 DICOM slices from five coconut varieties (Wenye 2/3/4/5 and the local tall landrace CK) at five developmental stages (2, 4, 6, 8 and 10 months after pollination). The four tissue-specific CT values exhibited distinct, reproducible developmental trajectories, collectively forming a quantitative —CT developmental clock—™ (Figure 1).

**Fiber layer CT** decreased from ~13 HU at 2 months to ~795 HU at 10 months across all varieties, following a logistic decay curve ( $R^2 > 0.95$ ). The trajectory resolved four phases: (i) initial hydration (2 months, ~13 HU); (ii) rapid decline (2—6 months, ~196 HU/month); (iii) deceleration (6—8 months, ~87 HU/month); and (iv) asymptotic approach to air density (8—10 months). This ~780 HU decline reflects progressive replacement of water by air in mesocarp fiber cells.

**Shell CT** rose from ~0 HU at 2 months to +202 HU (W5) and +256 HU (other varieties) at 10 months, following a sigmoidal curve with rapid deposition (2—6 months, +42 HU/month) and saturation (6—10 months, +8 HU/month) phases. This captures progressive shell mineralization through calcium oxalate deposition (Franceschi and Nakata, 2005). W5 exhibited significantly lower terminal shell CT (202 vs 230—256 HU, Tukey HSD  $p < 0.01$ ), consistent with its thin-shell phenotype. Two-way ANOVA confirmed significant variety — stage interactions for shell CT ( $F_{4,100} = 23.4$ ,  $p < 0.001$ ) and endosperm CT ( $F_{4,100} = 18.7$ ,  $p < 0.001$ ).

**Endosperm CT** increased from 0 HU (no solid endosperm at 2 months) to +37 HU at 10 months. W5 reached positive HU at 4 months—2 months earlier than CK—indicating accelerated solid endosperm development (Figure 1c). This event cannot be detected through external observation.

**Coconut water CT** remained stable at 19—27 HU, with no significant variety or stage effects.

Among the five varieties, W5 maintained the smallest fruit weight (402 to 930 g), CK the largest (1,200 to 2,639 g at 8 months). W5 also showed the thinnest shell, fastest endosperm development and most gradual growth trajectory.

## 2.2 | Metabolomics reveals a growth—defense bifurcation between W5 and CK

Widely targeted metabolomics identified 624 metabolites across 12 major classes: flavonoids (131), phenolic acids (68), amino acids and derivatives (52), lipids (48), nucleotides and derivatives (41), organic acids (39), tannins (28) and terpenoids (14). Volcano plot analysis (Figure 2a) revealed 100 metabolites exceeding  $|\log_{10}FC| > 1$  and  $-\log_{10}(p) > 1.3$ .

**W5—growth-oriented metabolism:** Nucleotide metabolites were enriched in W5. The most differentially abundant metabolite was 2—deoxyinosine-5—monophosphate (34.6-fold,  $\log_{10}FC = +5.11$ ,  $p < 0.001$ ), followed by cytidine ( $\log_{10}FC = +4.45$ ) and adenosine-5—monophosphate ( $\log_{10}FC = +4.12$ ), indicating active nucleic acid synthesis during cell division. Amino acids (88% enriched), free fatty acids (particularly punicic acid C18:3) and the ceramide signaling lipid dihydrosphingosine-1-phosphate ( $\log_{10}FC = +2.92$ ) were also preferentially accumulated in W5 (Figure 2b).

**CK—stress-response-oriented metabolism:** CK accumulated abundant flavonoids: 42 of 44 (95%) differentially abundant flavonoid metabolites were higher in CK. The flavonoid with the greatest difference was acacetin-7-O-galactoside (32.5-fold,  $\log_{10}FC = +5.02$ ,  $p < 0.001$ ), with documented antifungal activity. Other enriched flavonoids included isorhamnetin-3-O-neohesperidoside (17.6-fold) and quercetin-3-O-glucoside ( $\log_{10}FC = +3.87$ ) (Figure 2d). All 28 tannin metabolites were enriched in CK (100%). Phenolic acids (56% of differentially abundant species) including chlorogenic acid, and organic acids including citric and malic acid, were preferentially accumulated in CK (Figure 2e).

Class-level enrichment analysis (Figure 3) confirmed this reciprocal pattern: flavonoids, tannins and phenolic acids dominated CK (stress-response-oriented); nucleotides, amino acids and free fatty acids dominated W5 (growth-oriented). This constitutes a **growth—defense axis** at the metabolomic level.

## 2.3 | Systematic CT–metabolite correlation analysis reveals inter-variety asymmetry

To bridge structural and chemical dimensions, we performed Pearson correlation analysis between 18 CT parameters and 624 metabolites at five developmental time points ( $n = 5$  time-point means per variety,  $df = 3$ ), computed separately by variety. The analysis revealed a striking inter-variety asymmetry: W5 yielded 3,376 significant CT–metabolite associations ( $FDR\ q < 0.05$ ), covering 611 metabolites and all 18 CT parameters, of which 55 pairs achieved near-perfect correlation ( $|r| > 0.99$ ); in stark contrast, CK showed no pair exceeding  $|r| > 0.95$  (Figure 4, Figure S1). These 55 pairs represent 0.49% of the 11,232 total tested pairs and 1.63% of the 3,376 significant associations in W5.

**Methodological caveat:** with  $n = 5$  ( $df = 3$ ), confidence intervals for individual coefficients are wide. We do not treat any single  $r$  value as mechanistic evidence, focusing instead on inter-variety asymmetry as a global pattern. At the uncorrected threshold ( $p < 0.05$ ), W5 had 5,412 significant pairs versus 3,281 in CK, confirming that the asymmetry does not arise from differential FDR correction stringency. To test whether this asymmetry could arise by chance, we performed a permutation test: time-point labels were shuffled 10,000 times. Under permutations, the median number of  $|r| > 0.99$  pairs in W5 was 0 (95% CI 0–1), versus 55 observed ( $p < 0.0001$ ). A leave-one-out sensitivity analysis showed that 48 of the 55 pairs retained  $|r| > 0.95$  after removal of any single time point, indicating that the high correlations are not driven by individual outliers. We further verified that asymmetry is not an artifact of the linearity assumption: Spearman rank correlation and first-differencing Pearson correlation (detrending) both preserved the pattern, with W5 retaining 48 pairs at  $|r| > 0.95$  (Spearman) and 41 pairs at  $|r| > 0.95$  (FD-Pearson), versus 0 and 2 in CK, respectively (Supplementary Table S4).

Three near-perfect associations revealed potential mechanistic links:

- **Shell density** ↔ **13-methyltetradecanoic acid** ( $r = -0.999$ ,  $p < 0.001$ ): this branched-chain fatty acid declined as shell density increased, possibly reflecting down-regulation of membrane lipid metabolism during mineralization.
- **Endosperm density** ↔ **dehydroabietic acid** ( $r = +0.999$ ,  $p < 0.001$ ): this diterpene resin acid, associated with plant defense, accumulated in parallel with endosperm solidification.
- **Shell thickness** ↔ **citric acid** ( $r > +0.99$ ): citric acid serves as both a TCA cycle intermediate and an oxalate precursor for calcium oxalate deposition, possibly reflecting carbon flux coordination between energy metabolism and structural mineralization.

Class-level analysis revealed systematic patterns: flavonoids correlated negatively with fiber density in W5 (mean  $r = -0.83 \pm 0.11$ ), while nucleotides correlated positively with endosperm density (mean  $r = +0.88 \pm 0.09$ ) (Figure 4b). The metabolite co-regulation network further illustrates these class-level interaction patterns (Figure S3).

The most pronounced finding was the asymmetry in correlation strength: CK had no pair exceeding  $|r| > 0.95$ , contrasting with the 55 pairs observed in W5. This asymmetry raises a question: does CTâ€™metabolite association strength reflect direct effects of breeding selection, or a statistical consequence of genetic homogenization?

## 2.4 | CT features predict seed germinationâ€™the dual role of shell thickness

Using nine DICOM-derived CT features from 75 matched CTâ€™germination samples (45 germinated, 30 non-germinated), a random forest classifier achieved a hold-out AUC of 0.716 (Figure 5a) and 5-fold cross-validation AUC of  $0.609 \pm 0.089$  (Figure S2). The discrepancy between hold-out and CV AUC (0.716 vs 0.609) likely reflects the modest sample size ( $n = 75$ ) and the class imbalance; repeated random train-test splits ( $n = 100$ ) yielded a mean hold-out AUC of  $0.678 \pm 0.072$ , closer to the CV estimate. Six alternative algorithms were compared, confirming random forest as the top performer.

Feature importance analysis (Figure 5b) identified **shell thickness** (8.7% relative importance) and **embryo size** (8.2%) as the most informative predictors. Shell thickness simultaneously served as a central node in the CTâ€™metabolite correlation network ( $r > 0.99$  with citric acid) and as the strongest germination predictor. The two most important features together contributed less than 17%, with predictive signal distributed across multiple features.

## 3 | Discussion

### 3.1 | The CT developmental clock: a quantitative framework for coconut fruit maturation

The four tissue-specific CT trajectories provide a quantitative, non-destructive framework for in vivo coconut fruit maturation. The fiber layer CT trajectory captures progressive mesocarp air-filling, unique to coconut among the Arecaceaeâ€™oil palm mesocarp accumulates oil rather than losing water (Tarmizi et al., 2021). The shell CT trajectory captures a conserved biomineralization program shared among hard-shelled fruits (Schoeman et al., 2021). The endosperm CT trajectory provides a non-destructive measure of solid endosperm formation.

The accelerated endosperm development and lower terminal shell CT of W5 are consistent with its breeding history of early maturation and thin shell. The ~1,200 HU density span makes coconut a well-suited system for CT phenotyping.

### 3.2 | The growth–defense axis: a metabolomic imprint of coconut breeding

The reciprocal metabolic profile between W5 and CK constitutes the first metabolomic evidence of a growth–defense trade-off in a fruit tree crop. The scale of bifurcation is substantial: 95% of differentially abundant flavonoids were higher in CK, with the most abundant compound—acacetin-7-O-galactoside (32.5-fold)—having documented antifungal and photoprotective roles (Agati and Tattini, 2010; Falcone Ferreyra et al., 2012). This pattern is consistent with reduced secondary metabolites accompanying increased fruit size in domesticated tomato (Schauer et al., 2005) and co-selection of starch and cell wall pathways at the expense of defense metabolites during maize selection (Doebley et al., 2006).

### 3.3 | Inter-variety CT–metabolite association asymmetry: a statistical signal consistent with breeding canalization

The central observation in this study—55 pairs with  $|r| > 0.99$  in W5 versus none exceeding  $|r| > 0.95$  in CK—requires careful interpretation, as two competing explanations are plausible.

**Hypothesis A (–tightened coupling™):** Breeding selection that targeted specific phenotypes (shell thickness, fruit weight, maturation timing) may have incidentally tightened structural–metabolic coordination for the selected traits, producing stronger CT–metabolite correlations in W5. Under this view, the asymmetry is a byproduct of multivariate phenotypic selection.

**Hypothesis B (developmental canalization):** W5, as a genetically homozygous variety developed through generations of directional selection, exhibits canalized developmental programs—trajectories buffered against environmental fluctuations and genetic background variation (Waddington, 1942). All W5 individuals follow nearly identical trajectories, producing tight statistical correlations at the population level. CK, as a heterogeneous landrace, possesses substantial genetic variation that dilutes correlation strength.

We find Hypothesis B more parsimonious for three reasons. **First**, the asymmetry is global rather than trait-specific: the 55 near-perfect correlations span diverse parameter combinations—from endosperm density to branched-chain fatty acids, from shell thickness to TCA cycle intermediates. If selection targeted only specific traits (shell thickness, fruit size), the global scope of the asymmetry—covering all 18 CT parameters and 611 metabolites—would require the coordinated selection of an implausibly large number of independent pathways. **Second**, metabolic variation is broader in CK than W5 (median CV: 1.13 vs 0.98,  $p = 0.017$ , Wilcoxon test), ruling out the possibility that CK’s weaker correlations result from insufficient metabolic variation. **Third**, the observation that CK maintains denser inter-metabolite correlations (mean  $|r| = 0.493$  vs 0.441 in the co-regulation network) suggests that the weaker CT–metabolite

coupling in CK reflects genuine developmental decoupling rather than statistical noise.

Under this interpretation, CT's metabolite association strength measures not the tightness of coupling at the individual level, but developmental canalization at the population level—a statistical signature of breeding-induced genetic homogenization (Doebley et al., 2006). If validated at the DNA level (by correlating canalization metrics with genomic diversity indices such as nucleotide diversity  $\pi$ , runs of homozygosity or fixation index  $F_{st}$ ), CT's metabolite association strength could become a practical indicator for assessing developmental uniformity in coconut breeding populations.

### **3.4 | CT-based germination prediction**

The two strongest predictors—shell thickness and embryo size—correspond to known germination biology. Shell thickness is the primary physical barrier for radicle emergence (Tarmizi et al., 2021); the coconut haustorium plays a key role in mobilizing endosperm reserves during germination (Sugimura and Murakami, 1990). Embryo size reflects growth potential and storage reserves. This method reduces germination assessment from 2–3 months to approximately 15 minutes, providing a practical screening tool for coconut nurseries.

### **3.5 | Limitations and future directions**

The metabolomic analysis was limited to two varieties (W5 and CK). Extension to all five varieties would allow comprehensive characterization of the growth–defense axis across a broader genetic range. CT's metabolite correlations are associative rather than causal; functional validation experiments (e.g., CT monitoring following targeted inhibition of flavonoid biosynthesis) are needed. Our interpretation of CT's metabolite asymmetry as developmental canalization requires DNA-level validation—specifically, comparing genomic diversity indices across varieties and correlating them with CT's metabolite association strength. Integrating transcriptomics could identify the regulatory genes underlying selection-driven canalization.

At a descriptive level, this study provides a systematic CT phenomic record of multi-variety coconut fruit development. At a mechanistic level, it reveals a growth–defense axis through cross-modal correlation. At a hypothesis-generating level, it proposes that CT's metabolite association strength may serve as a candidate quantitative phenotype for developmental uniformity, with testable predictions for genetic validation.

Beyond validation, future research should explore whether CT's metabolite association strength can serve as a predictive indicator for breeding population uniformity. If validated across diverse genetic backgrounds, this metric could be integrated into breeding programs as a non-destructive, high-throughput screening tool for developmental consistency. Additionally, extending this integrative framework to other fruit tree crops with hard shells (e.g., walnut, macadamia) could reveal whether the growth–defense

axis and canalization signatures are conserved features of breeding selection.

## **4 | Experimental Procedures**

### **4.1 | Plant Materials and Experimental Design**

Five dwarf coconut varieties: Wenye 2 (W2), Wenye 3 (W3), Wenye 4 (W4), Wenye 5 (W5) “improved varieties bred by the Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences” and CK (Hainan Lvgao, local tall landrace). Fruits were collected from the Wenchang Experimental Station, Hainan (19°32′N, 110°47′E), harvested at 2, 4, 6, 8 and 10 months after pollination, with 5 biological replicates per variety × month (total  $n \approx 100$  fruits). Metabolomics was performed on W5 and CK at the same five time points with three replicates each ( $n = 30$ ). For germination prediction, 100 fruits were tracked for 6 months, of which 75 had matched CT records.

### **4.2 | CT Scanning and Computational Phenotyping**

SOMATOM Definition AS+ (Siemens Healthineers, Germany) clinical CT scanner (120 kV, 200 mAs, 0.6 mm slice thickness, 512 × 512 matrix, B30f reconstruction kernel), yielding 7,686 DICOM slices. The pipeline included automated equatorial slice selection, tissue-level density profiling and DICOM-derived feature extraction, implemented in Python 3.11 (pydicom, OpenCV, scikit-learn). See Supplementary Methods for details.

### **4.3 | Metabolomics Analysis**

Freeze-dried endosperm samples (100 mg) were extracted with 70% methanol/water and analyzed by UHPLC-MS/MS (Q-Exactive Orbitrap, Thermo Fisher Scientific) in MRM mode. Metabolites were identified against an in-house standard library, mzCloud and HMDB databases, yielding 624 metabolites. Metabolite identification confidence followed MSI guidelines (Sumner et al., 2007): 178 metabolites (28.5%) were confirmed at Level 1 (authentic standard match on retention time and MS/MS), and the remaining 446 (71.5%) at Level 2 (database spectral match).

### **4.4 | Statistical Analysis**

Developmental trajectories: two-way ANOVA (variety × month) with Tukey HSD post-hoc tests. Differential metabolites: two-tailed Welch t-test with Benjamini-Hochberg FDR correction ( $q < 0.05$ ). CT × metabolite correlations: Pearson correlation was computed separately by variety using  $n = 5$  time-point means ( $df = 3$ ). To control for multiple testing, p-values were adjusted using the Benjamini-Hochberg false discovery rate (FDR) procedure across all 11,232 pairs (18 CT parameters × 624 metabolites) per variety, with significance threshold set at  $q < 0.05$ . Permutation test: time-point labels shuffled 10,000 times. Robustness checks: Spearman rank correlation and first-differencing Pearson correlation (detrending).

Coefficient of variation analysis: two-sided Wilcoxon rank-sum test. Metabolite co-regulation networks (194,376 pairs) constructed from 15 individual samples per variety. Random forest classifier: nine DICOM-derived CT features,  $n\_estimators = 200$ ,  $max\_depth = 5$ ,  $class\_weight = \text{“balanced”}$ , 75:25 train-test split, 5-fold cross-validation, AUC evaluation. Hyperparameters were selected based on a preliminary grid search ( $n\_estimators$ : 100/200/500;  $max\_depth$ : 3/5/7/10), with the combination maximizing mean CV AUC. Six alternative algorithms (logistic regression, SVM, k-NN, decision tree, gradient boosting, XGBoost) were compared under identical conditions.

## 4.5 | Data and Code Availability

All data supporting this study are publicly available. Metabolomics raw data and CT DICOM archives (7,686 slices) are available from the corresponding authors without restriction. Processed abundance tables, CT feature matrices and analysis scripts are accessible at [palm.suncx.top](http://palm.suncx.top) (DOI pending upon Zenodo deposition). Interactive tools (CT viewer, metabolic network browser, germination predictor) are hosted at the same URL for community use.

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## Author Contributions

Huanning You: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing “original draft. Huangsheng Ling: Data curation, Formal analysis, Investigation, Methodology. Jing Chen: CT technical guidance, Data fitting, Resources. Wenrao Li: Investigation, Resources. Mengxing Huang: Methodology, Supervision, Writing “review & editing. Yu Zhang: Methodology, Software, Validation. Hongxing Cao: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing “review & editing. Chengxu Sun: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing “original draft, Writing “review & editing.

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## Conflicts of Interest

The authors declare no conflicts of interest.

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## Figure Legends

**Figure 1.** CT developmental trajectories. (a) Representative equatorial CT slices of W2–W5 with tissue compartments annotated (F = fiber, S = shell, E = endosperm, W = water cavity); (b) fruit weight trajectories; (c) endosperm CT values—W5 reaches positive HU at 4 months, 2 months earlier than CK; (d) shell CT values—reflecting progressive mineralization. Error bars = SD from 3–5 biological replicates.

**Figure 2.** Metabolomic comparison. (a) Volcano plot of 624 metabolites; (b) top 10 enriched in W5—nucleotide-dominated; (c) top 10 enriched in CK—flavonoid-dominated; (d) metabolite class distribution—up vs. down; (e) mean log<sub>2</sub>FC by class.

**Figure 3.** Metabolite class heatmap. Flavonoids/tannins/phenolic acids dominate CK (stress-response-oriented); nucleotides/amino acids/fatty acids dominate W5 (growth-oriented). Z-score normalized.

**Figure 4.** CT–metabolite association framework. (a) Network of 3,376 significant associations, with 55 pairs at  $|r| > 0.99$  (W5); (b) class-level patterns—flavonoids negatively correlated with fiber density, nucleotides positively correlated with endosperm density; (c) asymmetric association strength between W5 and CK.

**Figure 5.** Germination prediction. (a) ROC curve (AUC = 0.716; n = 75 samples: 45 germinated, 30 non-germinated); (b) feature importance—shell thickness and embryo size are the top two predictors.

**Figure S1.** Complete list of 3,376 significant CT–metabolite associations (FDR  $q < 0.05$ ) in W5. Heatmap of Pearson correlation coefficients between 18 CT parameters and 624 metabolites across five developmental stages.

**Figure S2.** Five-fold cross-validation AUC distribution for the random forest germination classifier. Box plot showing individual fold AUC values and mean  $\hat{A} \pm \text{SD}$ .

**Figure S3.** Metabolite co-regulation network based on 194,376 Pearson correlation pairs across 624 metabolites in W5 samples. Node color indicates metabolite class; edge thickness reflects correlation strength. Only  $|r| > 0.95$  pairs are shown for visual clarity.