

Research Paper

Hydroponic system-dependent variation in phenylpropanoid accumulation and antioxidant activity of greenhouse-grown basil

Sang-Yun Lee^a, Neil Patrick Uy^a, Jong-Suk Park^b, Won-Yong Yang^b, Hoon Kim^c, Sanghyun Lee^{a,d,*} ^a Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea^b Jeonbuk State Agricultural Research & Extension Services, Iksan 54591, Republic of Korea^c Department of Food and Nutrition, Anyang University, Anyang 14028, Republic of Korea^d Natural Product Institute of Science and Technology, Anseong 17546, Republic of Korea

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ABSTRACT

Hydroponic cultivation systems differ fundamentally in root-zone architecture, yet it remains unclear whether these architectural differences can modulate non-volatile phenylpropanoid accumulation and antioxidant-related functional quality in basil. To clarify this uncertainty and determine which hydroponic system is more favorable for improving the functional quality of fresh basil, this study evaluated hydroponic system-dependent variation in phenylpropanoid-derived phenolics and associated antioxidant activity in greenhouse-grown basil (*Ocimum basilicum* L.) under shared environmental and nutrient-solution conditions. Three representative hydroponic systems—an aeroponic culture (APC), a nutrient film technique (NFT) system, and a perlite-based substrate culture (PSC)—were compared. Untargeted liquid chromatography–tandem mass spectrometry profiling tentatively identified twelve metabolites, revealing a largely shared qualitative metabolite profile dominated by phenylpropanoid-derived caffeic acid derivatives. Total phenolic content analysis consistently revealed higher extractable phenolic levels in PSC-grown basil compared with APC- and NFT-grown plants. Subsequent targeted quantification using high-performance liquid chromatography with a photodiode array detector demonstrated clear hydroponic system-dependent differences in the accumulation of caffeic acid (1), caffeic acid (2), chicoric acid (3), and rosmarinic acid (4). PSC cultivation consistently resulted in substantially higher concentrations of compounds 1–4 than the APC and NFT systems. These compositional differences were accompanied by enhanced antioxidant activity, as PSC-grown basil extracts exhibited lower IC₅₀ values in both ABTS and DPPH radical scavenging assays. Under the greenhouse conditions applied in this study, PSC most effectively enhanced phenylpropanoid accumulation and antioxidant capacity without compromising aboveground biomass, indicating its suitability for improving the functional quality of fresh basil.

1. Introduction

Basil (*Ocimum basilicum* L.) is one of the most widely cultivated culinary herbs worldwide and is primarily consumed as fresh leaves, for which quality is determined not only by aroma but also by its nutritional and functional attributes (Nadeem et al., 2022; Du et al., 2023; Spence, 2024). In addition to its well-known essential oil constituents, basil leaves accumulate substantial levels of non-volatile phenolic compounds derived from the phenylpropanoid pathway (Jayasinghe et al., 2003; Larsen et al., 2022). These compounds are consistently associated with

diverse bioactive properties and are considered key contributors to the functional value of basil when consumed as a fresh vegetable rather than solely as a source of volatile oils (Majdi et al., 2020; Kim and Oh, 2024; Park et al., 2024; Rezaie et al., 2020; Song et al., 2020).

With the growing demand for year-round production of fresh herbs with consistent quality, basil cultivation has increasingly shifted toward controlled-environment and soilless systems (Khater et al., 2021; Fussly and Papenbrock, 2022; Chatterjee et al., 2025). Soilless cultivation encompasses a range of approaches, including solution-based hydroponic systems and substrate-mediated cultures, and has been widely adopted

* Corresponding author at: Department of Plant Science and Technology, Chung-Ang University, Anseong, 17546, Republic of Korea.

E-mail addresses: andylysy@naver.com (S.-Y. Lee), jspark88@korea.kr (J.-S. Park), wyang8129@korea.kr (W.-Y. Yang), hkim@anyang.ac.kr (H. Kim), slee@cau.ac.kr (S. Lee).

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in commercial herb production due to its advantages in hygiene, resource-use efficiency, and precise environmental control (Regmi et al., 2024; Tuxun et al., 2025). Accordingly, substantial research efforts have focused on optimizing hydroponic cultivation systems for basil, particularly by refining environmental and management variables within individual systems to improve growth performance, productivity, and yield stability (Ciriello et al., 2021; Sipos et al., 2021). Although hydroponic or soilless systems have been compared in basil and other leafy vegetables, previous studies have mainly focused on growth, yield, nutrient uptake, essential oil-related traits, biofortification, or total phenolic indices, rather than compound-level phenylpropanoid profiles linked to antioxidant capacity (Walters and Currey, 2015; Khater et al., 2021).

While these studies provide valuable guidance for production management, it remains unclear whether the inherent structural characteristics of different hydroponic systems themselves influence the accumulation of non-volatile secondary metabolites in basil leaves, independent of fine-scale parameter optimization. This distinction is important, as growers typically select the hydroponic system architecture prior to adjusting operational conditions, and each system inherently generates a distinct root-zone environment (Palmitessa et al., 2024).

Among hydroponic approaches commonly used for leafy herbs, aeroponic culture (APC) delivers nutrients intermittently as a fine mist to roots suspended in air; nutrient film technique (NFT) systems supply a continuous thin film of nutrient solution over the roots; and perlite-based substrate culture (PSC) provides a buffered root-zone environment through a solid medium with distinct water–air retention properties. These systems represent contrasting yet widely implemented production strategies that differ fundamentally in root exposure, aeration, and moisture dynamics (Tafesse et al., 2021; Levine et al., 2023; Rajaseger et al., 2023). However, their comparative influence on basil leaf chemistry has rarely been evaluated side by side under a shared greenhouse environment, particularly with respect to non-volatile phenylpropanoid-derived phenolic accumulation and its relationship with antioxidant capacity.

Phenylpropanoid-derived phenolic acids are effective chemical indicators for evaluating system-level effects in basil (Kisa et al., 2023; Tateishi et al., 2024). Compounds derived from caffeic acid conjugation pathways, including esterified and structurally related forms, constitute a major fraction of basil leaf phenolics (Lee and Scagel, 2009). Because basil is primarily consumed as a fresh leafy herb, these non-volatile caffeic acid conjugates represent bioactive constituents of direct dietary relevance and contribute to its antioxidant and potential health-promoting properties (Silva and Lopes, 2020). Despite their importance, systematic comparisons of how different hydroponic system architectures influence the accumulation of major phenylpropanoid-derived phenolic acids, integrating untargeted metabolite profiling with validated quantitative analysis using authentic reference standards, remain limited.

Based on these considerations, this study was designed to determine whether hydroponic system architecture itself, rather than fine-scale operational variation, can alter phenylpropanoid-derived phenolic accumulation and antioxidant activity in greenhouse-grown basil, thereby identifying a cultivation system favorable for improving the functional quality of fresh basil. To assess whether system responses were consistent across commercially relevant planting materials, two Genovese-type basil sources were included: a named commercial cultivar and a commercially available Genovese-type seed lot without specific cultivar designation. Three representative hydroponic systems (APC, NFT, and PSC) were compared during a common cultivation period. Total phenolic content (TPC) was first evaluated to assess overall phenolic variation among systems, followed by untargeted liquid chromatography–tandem mass spectrometry (LC–MS/MS) profiling to characterize metabolite features. Targeted high-performance liquid chromatography with photodiode array detection (HPLC/PDA) using

authentic standards was subsequently conducted to quantify major phenylpropanoid-derived phenolic acids, and antioxidant potential was assessed using the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. By focusing on system-level differences rather than fine-scale operational parameters, the novelty of this study lies in linking contrasting hydroponic architectures with compound-level phenylpropanoid accumulation and antioxidant-related functional quality in fresh basil under a shared greenhouse environment.

2. Materials and methods

2.1. Plant materials

Two basil seed sources belonging to the Genovese-type horticultural group were used in this study. One was a named commercial cultivar, *O. basilicum* cv. 'Marian,' supplied by Enza Zaden (Enkhuizen, The Netherlands). The second was an unnamed commercial basil seed lot supplied by Asia Seed Co. (Seoul, Republic of Korea), hereinafter referred to as 'Common.' Seeds were germinated in Oasis® Horticultures® plugs (Smithers-Oasis Korea Co., Ltd., Cheonan, Republic of Korea) at one seed per cell. Seedlings at the two-pair true-leaf stage were transplanted into the hydroponic greenhouse on August 19, 2025.

2.2. Instruments and chemicals

LC–MS/MS analyses were performed using an UltiMate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) coupled to a TripleTOF 5600+ high-resolution mass spectrometer (AB SCIEX, Framingham, MA, USA) and equipped with a CORTECS C18 column (150 mm × 2.1 mm, 1.6 µm; Waters Corporation, Milford, MA, USA). HPLC analyses were conducted using an Alliance e2695 separations module equipped with a 2998 photodiode array detector (Waters Corporation, Milford, MA, USA) and a YMC Pack Pro C18 column (4.6 mm × 250 mm, 5 µm; YMC, Kyoto, Japan). Absorbance for colorimetric assays, including TPC, ABTS, and DPPH radical scavenging assays, was measured using a microplate reader (Epoch, BioTek Instruments, Winooski, VT, USA). Organic solvents were removed using a rotary vacuum evaporator (OSB-2100, Eyela, Tokyo, Japan). Ethanol (EtOH) was purchased from Samchun Pure Chemical (Pyeongtaek, Republic of Korea). HPLC-grade methanol (MeOH), acetonitrile (ACN), dimethyl sulfoxide (DMSO), and ultrapure water were obtained from J.T. Baker (Center Valley, PA, USA). Formic acid (FA; Acros Organics, Geel, Belgium) and trifluoroacetic acid (TFA; Thermo Fisher Scientific, Waltham, MA, USA) were used as mobile-phase modifiers. The Folin–Ciocalteu reagent, sodium carbonate, tannic acid, potassium persulfate, ABTS, and DPPH were obtained from Sigma-Aldrich (St. Louis, MO, USA). Caffeic acid (1), caffeic acid (2), chicoric acid (3), and rosmarinic acid (4) were obtained from the Natural Products Institute of Science and Technology (www.nist.re.kr), Anseong, Republic of Korea (Fig. 1).

2.3. Hydroponic greenhouse cultivation system

Transplanted seedlings were cultivated in a greenhouse at the Jeonbuk State Agricultural Research and Extension Services (Iksan, Republic of Korea) using three hydroponic systems: APC, NFT, and PSC (Fig. 2). Plants were supplied with a Yamazaki nutrient solution maintained at an electrical conductivity of 1.0–1.2 dS m⁻¹ and pH 5.5–5.7. In the APC system, the nutrient solution was delivered as a fine mist for 50 s at 3 min intervals throughout the cultivation period. This intermittent misting regime was applied to maintain root-surface moisture and nutrient contact while preserving root-zone aeration, consistent with the general operating principle of aeroponic cultivation (Eldridge et al., 2020; Tafesse et al., 2021). In the NFT system, the solution was continuously circulated to maintain a thin film depth of approximately 2

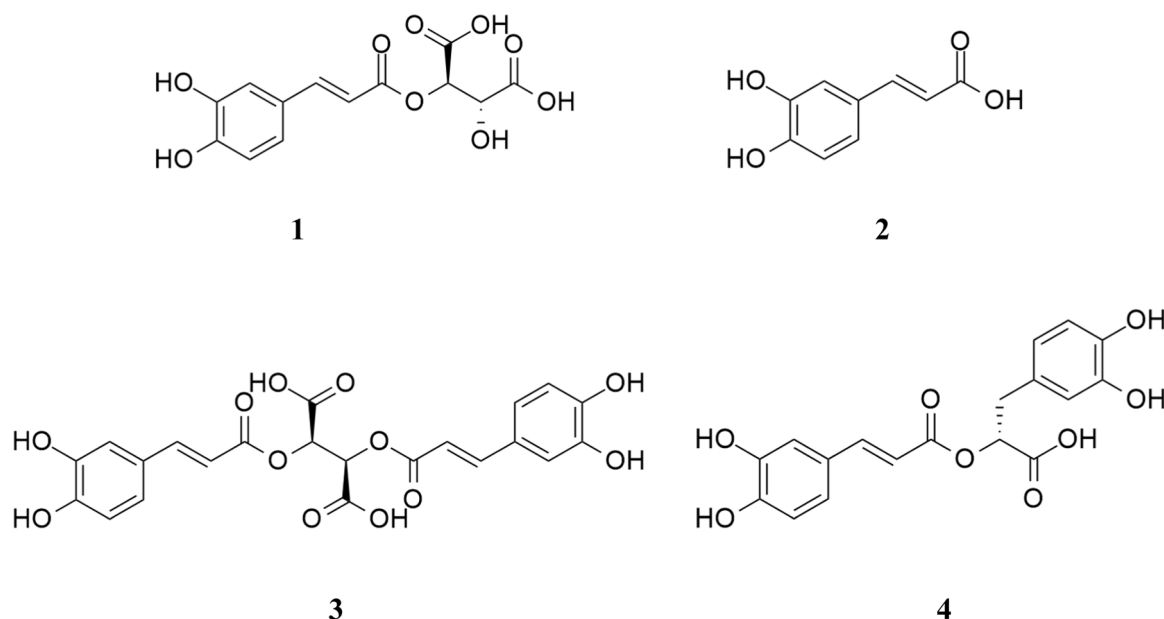


Fig. 1. Chemical structures of compounds 1–4. Compounds: caftaric acid (1), caffeic acid (2), chicoric acid (3), and rosmarinic acid (4).

cm along the cultivation channels. In the PSC system, plants were grown in a perlite substrate (New Pearlshine No. 2; Green Fire Chemicals Co., Ltd., Hongseong, Republic of Korea) using a drip irrigation system. The same nutrient solution was supplied to all systems under stable and standardized irrigation regimes to prevent unintended water stress during cultivation. Plants were grown exclusively under natural light without supplemental lighting. External and internal radiation parameters were continuously monitored using a pyranometer (LI-200R, LI-COR, Inc., Lincoln, NE, USA) and a quantum sensor (LI-190R, LI-COR, Inc., Lincoln, NE, USA), respectively. During the cultivation period, natural day length, calculated from official sunrise and sunset records for Iksan, Republic of Korea, ranged from 13 h 23 min to 12 h 58 min. Mean internal photosynthetic photon flux density and daily light integral were $190.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $16.24 \text{ mol m}^{-2} \text{d}^{-1}$, respectively. Detailed daily environmental data are provided in Table S1. Relative humidity, recorded hourly throughout the cultivation period, averaged 75.0 % and ranged from 45 % to 95 %. The greenhouse was naturally ventilated, with fresh air entering at the front and exhaust fans located at the rear. This configuration created a stable longitudinal temperature gradient along the greenhouse axis. Temperature was not intentionally manipulated as an experimental treatment. However, continuous environmental monitoring during the cultivation period revealed a consistent temperature difference of approximately 2°C between the cooler front zone (T1) and the warmer rear zone (T2) (Fig. 3). Plants were harvested on September 1, 2025, and fully expanded mature leaves were collected for subsequent biochemical analyses. Although temperature was not an independently imposed factor, the consistent spatial gradient enabled its inclusion as a statistical environmental covariate rather than as a controlled treatment variable.

2.4. Extraction of basil from APC, NFT, and PSC

A modified ethanolic reflux extraction procedure was employed based on previously reported methods for basil leaves (Qazizadah et al., 2023). Dried basil leaves were finely pulverized using a mixer to facilitate solvent penetration. For each sample, 0.5 g of powdered material was extracted three times with 300 mL of EtOH under reflux for 3 h per cycle. The combined extracts were filtered to remove insoluble residues and concentrated under reduced pressure using a rotary evaporator. Extraction yield was calculated using the following equation:

$$\text{Yield (\%)} = (\text{weight of extract} / \text{dry weight of plant material}) \times 100$$

2.5. TPC measurement

TPC was determined using the Folin–Ciocalteu assay based on a previously reported method with minor modifications (Qazizadah et al., 2023). Basil extracts were dissolved in deionized water (DIW) and serially diluted to concentrations ranging from 1.25 to 5.0 mg/mL. For analysis, 60 μL of each diluted sample was dispensed into a 96-well plate, followed by the addition of 40 μL of 2 N Folin–Ciocalteu reagent and 100 μL of 7.5 % sodium carbonate solution. The reaction mixtures were agitated using a microplate shaker and incubated in the dark at room temperature for 30 min. The absorbance of the resulting reaction mixtures was measured at 760 nm using a microplate reader, with reagent blanks used for background correction. Tannic acid solutions ranging from 0.015 to 0.25 mg/mL were used to construct the calibration curve. Each sample was analyzed in triplicate, and TPC values were calculated using the following equation and expressed as mg tannic acid equivalents (TAE) per g extract:

TPC = $(C \times V) / M$ where C is the TAE concentration obtained from the calibration curve (mg/mL), V is the volume of the analyzed sample solution (mL), and M is the corresponding mass of extract in the analyzed sample solution (g).

2.6. Sample preparation for LC-MS/MS and HPLC/PDA analysis

Basil extract (20 mg each) was accurately weighed and dissolved in 1 mL of 80 % methanol (v/v) and 1 mL of DMSO/MeOH (1:1, v/v) for LC-MS/MS and HPLC/PDA analyses, respectively. The solutions were then sonicated for 10 min prior to analysis. For standard solutions, compounds 1–4 (1 mg each) were dissolved in 1 mL of DMSO/MeOH (1:1, v/v) and sonicated for 10 min. All sample and standard solutions were filtered through a hydrophilic polyvinylidene difluoride membrane filter (0.20 μm , 25 mm; Hyundai Micro Co., Ltd., Seoul, Republic of Korea) prior to injection.

2.7. LC-MS/MS conditions

Chromatographic separation was performed with the column maintained at 45°C and a flow rate of 0.25 mL/min. The mobile phases consisted of 0.1 % FA in water (solvent A) and 0.1 % FA in ACN (solvent B). The gradient program was as follows: 3 % B from 0 to 0.5 min; increased to 15 % B at 15 min; linearly ramped to 100 % B by 50 min;

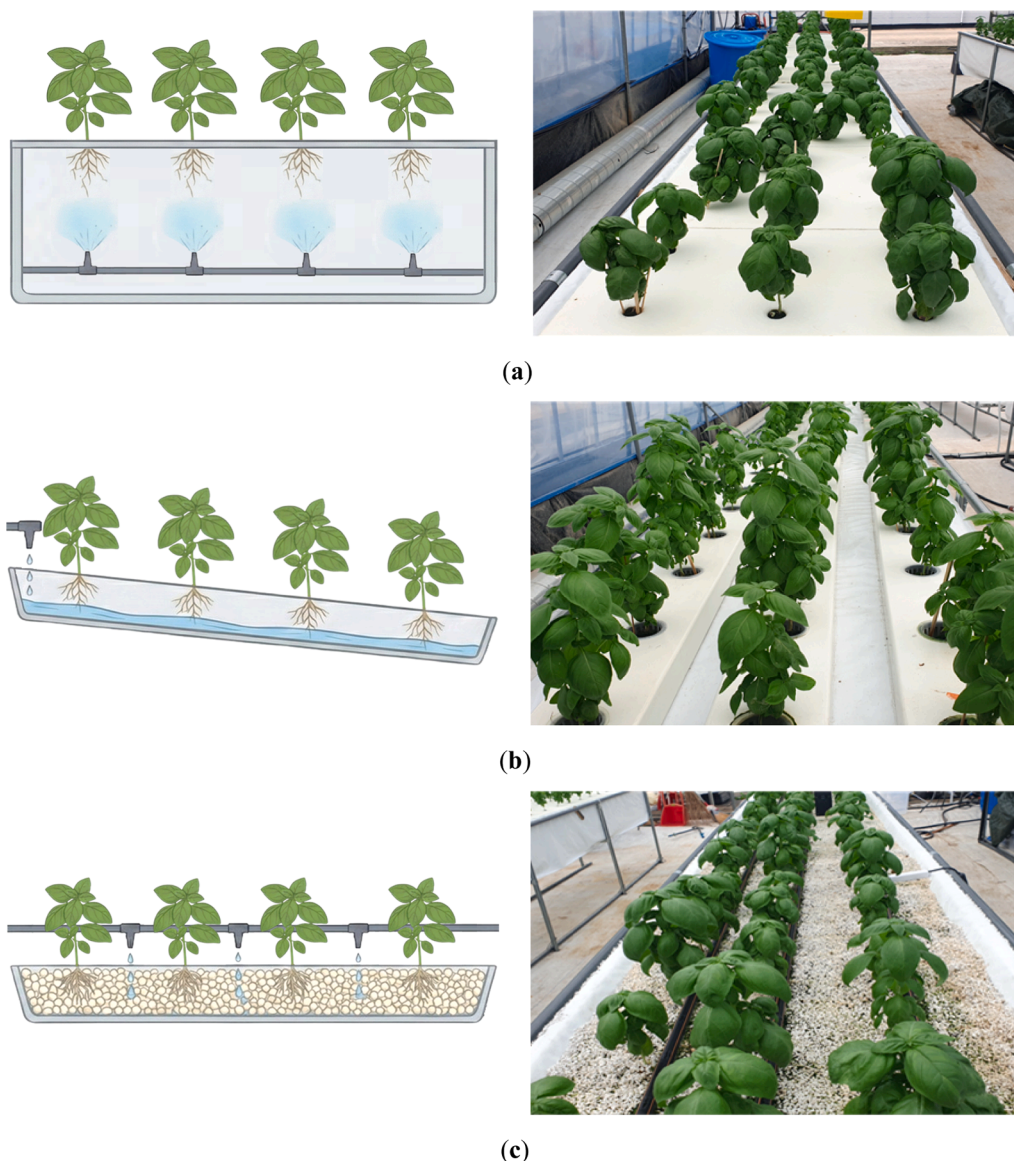


Fig. 2. Schematic illustrations (left) and representative greenhouse photographs (right) of basil cultivated under three hydroponic systems: APC (a), NFT (b), and PSC (c).

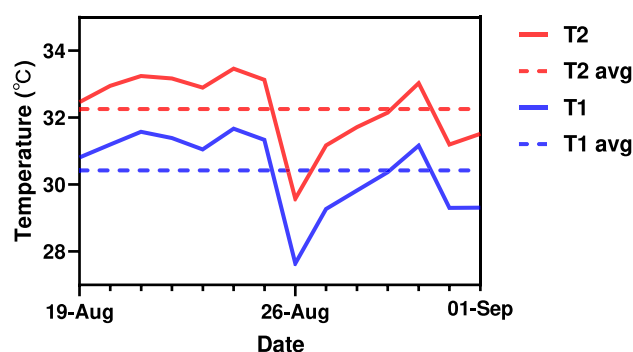


Fig. 3. Daily mean greenhouse temperatures during basil cultivation under two spatial temperature zones. T1 represents the cooler front zone of the greenhouse, whereas T2 represents the warmer rear zone (approximately 2 °C higher than T1). Solid lines represent the daily average temperatures of T1 (blue) and T2 (red), while dashed lines indicate the overall mean temperature for each zone throughout the experimental period.

held at 100 % B until 55 min; then returned to 3 % B at 55.1 min and maintained until 60 min for re-equilibration. The mass spectrometer was operated with an electrospray ionization source in both positive and negative ion modes under information-dependent acquisition. Full-scan MS spectra were acquired over an m/z range of 100–2000, and MS/MS spectra were recorded over an m/z range of 30–2000 using collision-induced dissociation. The ion spray voltages were set to 5.5 kV in positive mode and 4.5 kV in negative mode. Nebulizing and heating gas pressures were both set to 50 psi, and the curtain gas was set to 25 psi. The source temperature was maintained at 500 °C. Collision-induced dissociation was performed using nitrogen as the collision gas, with collision energies set to 35 ± 15 eV.

2.8. HPLC/PDA analysis

Chromatographic separation was performed on a YMC Pack Pro C18 column maintained at 35 °C. The mobile phases consisted of 0.1 % TFA in water (solvent A) and 0.1 % TFA in ACN (solvent B). The gradient program was as follows: 10 % B (0–8 min), 20 % B (16 min), 30 % B (25 min), 50 % B (32 min), and 100 % B (40–48 min); the gradient was then

returned to 10 % B at 53 min and held until 56 min for column re-equilibration. The flow rate was set at 1.0 mL/min. Detection was carried out using a PDA detector at 290 nm, and chromatograms were recorded at this wavelength.

2.9. Calibration curves, limits of detection (LOD), and limits of quantification (LOQ)

Standard solutions were prepared by serial dilution to obtain concentrations ranging from 6.25 to 200 µg/mL. Calibration curves were constructed by plotting peak area (Y, mAU) against concentration (X, µg/mL), and linearity was evaluated using the correlation coefficient (r^2). All measurements were performed in triplicate. The LOD and LOQ were estimated according to the ICH Q2(R2) guideline using the mean slope of the calibration curve (S) and the standard deviation of the y-intercept (σ) obtained from three independent calibration curves, as described in established analytical validation frameworks (ICH, 2023). The corresponding LOD and LOQ values were calculated as follows:

$$\text{LOD} = 3.3 \times \sigma / S, \text{LOQ} = 10 \times \sigma / S$$

2.10. ABTS and DPPH radical scavenging assays

ABTS and DPPH radical scavenging activities were measured based on previously established methods with minor modifications (Napolitano et al., 2022). The ABTS $\bullet^+$ radical cation stock solution was prepared by mixing ABTS (7.4 mM) with potassium persulfate (2.6 mM) in DIW. The mixture was incubated in the dark at 4 °C for 24 h to allow complete formation of the radical cation. The stock solution was subsequently diluted with DIW to obtain an absorbance of 1.00 ± 0.05 at 734 nm. For the DPPH $\bullet$ radical assay, a DPPH $\bullet$ stock solution (2 mM) was prepared by dissolving DPPH $\bullet$ in EtOH. The working solution was then prepared by diluting the stock solution with EtOH to achieve an absorbance of 0.80 ± 0.05 at 514 nm. Plant extracts were serially diluted in DMSO/EtOH (1:1, v/v) to final concentrations ranging from 1 to 30 mg/mL. For both assays, 10 µL of each diluted sample was added to individual wells of a 96-well microplate, followed by the addition of 200 µL of either the ABTS $\bullet^+$ or DPPH $\bullet$ working solution. All assays were conducted in triplicate. The reaction mixtures were thoroughly mixed using a microplate shaker and incubated in the dark at room temperature for 30 min. Absorbance was then measured at 734 nm for the ABTS $\bullet^+$ assay and at 514 nm for the DPPH $\bullet$ assay using a microplate reader. For blank correction, the solvent used to dissolve each sample served as the corresponding blank. Radical scavenging activity (%) was calculated using the following equation for both assays:

$$\text{Radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} represents the absorbance of the radical working solution without test sample, and A_{sample} represents the absorbance measured in the presence of the test sample. IC₅₀ values were determined from dose-response curves using non-linear regression analysis.

2.11. Statistical analysis

Statistical analyses and graphical data presentation were performed using GraphPad Prism version 10.2.3 (GraphPad Software, Boston, MA, USA). Analyses were conducted using lane-level composite samples, with three independent lanes per treatment combination. Prior to statistical testing, data were evaluated for normality and homogeneity of variances to confirm the suitability of parametric analyses. Multiple-group comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. A three-way ANOVA was further conducted to assess the effects of cultivar, temperature regime, and hydroponic cultivation method on metabolite contents, with both main and

interaction effects examined. Statistical significance was set at $p < 0.05$. Pearson correlation coefficients were calculated, and the significance of correlations was assessed using two-tailed tests with $p < 0.05$ considered statistically significant. Only correlations with $|r| \geq 0.7$ were visualized in network plots.

3. Results and discussion

3.1. Hydroponic greenhouse cultivation system-dependent variation in TPC

TPC was measured to evaluate overall variation in extractable phenolic pools among the hydroponic greenhouse cultivation systems (Fig. 4). When comparisons were made at the level of cultivation architecture, PSC-grown basil consistently exhibited higher TPC values than APC- and NFT-grown plants across most cultivar $\times$ temperature combinations. Differences among hydroponic systems reached up to approximately 1.9-fold, depending on the treatment combination, whereas APC and NFT frequently did not differ significantly from each other.

The approximately 2 °C longitudinal temperature gradient within the greenhouse did not produce consistent directional changes in TPC, and cultivar-dependent variation was relatively minor compared with hydroponic system effects. Previous studies have reported that substantial changes in phenolic accumulation in basil are typically associated with clearly defined cold or heat stress treatments, rather than with minor temperature fluctuations (Jakovljević et al., 2021). The absence of consistent TPC trends across the approximately 2 °C greenhouse temperature gradient observed in the present study is therefore consistent with the view that mild spatial temperature heterogeneity is insufficient to override system-level cultivation effects. Because the Folin–Ciocalteu assay reflects cumulative reducing capacity rather than specific phenolic subclasses, the observed differences should be interpreted as system-dependent shifts in the overall pool of redox-active metabolites at the extract level (Pérez et al., 2023). Collectively, these findings indicate that hydroponic system architecture exerts a substantial influence on whole-extract phenolic status under shared environmental conditions. The consistent elevation of TPC under perlite-based cultivation therefore warranted further metabolite-level investigation to determine which phenylpropanoid-derived phenolic constituents contributed to the observed system-dependent variation.

3.2. LC–MS/MS-based untargeted profiling of secondary metabolites in basil

Untargeted LC–MS/MS analysis was conducted to obtain a

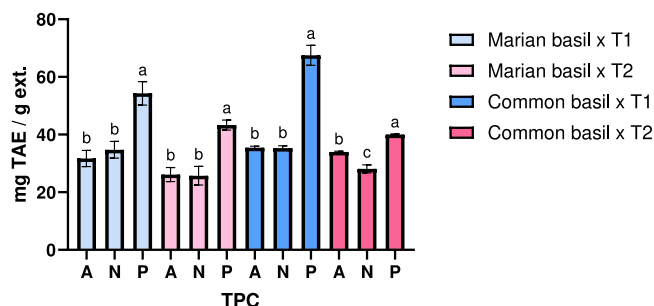


Fig. 4. TPC of basil cultivated under different hydroponic systems (A, APC; N, NFT; P, PSC) under two greenhouse temperature zones. T1 represents the cooler front zone of the greenhouse, whereas T2 represents the warmer rear zone (approximately 2 °C higher than T1). Values are expressed as mg tannic acid equivalents (TAE) per g extract. ^{a–c} Different lowercase letters within each cultivar $\times$ temperature group indicate statistically significant differences among hydroponic systems based on one-way ANOVA followed by Tukey's multiple-comparison test ($p < 0.05$).

comprehensive overview of the secondary metabolite composition of the basil extracts analyzed in this study. Data-dependent acquisition in both negative and positive ionization modes enabled broad metabolite coverage across diverse physicochemical classes, providing an integrated snapshot of the chemical landscape of basil leaves. This untargeted strategy is particularly useful for plant-derived matrices, in which structurally diverse phenolic metabolites may be co-extracted and vary substantially in abundance. In this approach, full-scan MS detection provides broad metabolite coverage without prior selection of specific targets, while data-dependent MS/MS acquisition generates fragmentation spectra that support tentative structural annotation at the metabolite level. Recent LC–MS/MS-based untargeted metabolomics studies have similarly demonstrated the utility of this approach for capturing complex phenolic profiles and resolving metabolite-level variation associated with antioxidant-related properties (Lang et al., 2025).

Based on accurate mass measurements, molecular formula assignments, and mass-to-charge (m/z) ratios, LC–MS/MS analysis of both ‘Marian’ and ‘Common’ basil samples led to the tentative identification of the same set of twelve metabolites in each sample (Table S2). The annotations were supported by acceptable mass errors and interpretable MS/MS fragmentation patterns consistent with spectral libraries and previously reported data (Figures S1, S2, and S3). Nevertheless, metabolite assignments obtained from untargeted LC–MS/MS profiling should be interpreted with caution because accurate mass and MS/MS spectral matching do not always provide definitive structural confirmation, particularly in complex biological matrices where co-eluting or isobaric precursor ions may complicate MS/MS interpretation (Schrimpe-Rutledge et al., 2016). In addition, co-eluting matrix constituents in LC–MS/MS can alter ionization efficiency through ion suppression or ion enhancement, thereby affecting precursor ion abundance and, in some cases, the quality or representativeness of fragmentation spectra used for tentative annotation (Faccin et al., 2016). Accordingly, the present LC–MS/MS dataset was used as a qualitative profiling tool rather than as definitive structural confirmation, and the subsequent quantitative evaluation of the principal phenylpropanoid-derived phenolics was performed by HPLC/PDA using authentic reference standards. The observation of an identical set of tentatively identified metabolites in both seed sources suggests that the major secondary metabolite classes detected by untargeted LC–MS/MS are qualitatively conserved between these Genovese-type materials.

The annotated metabolites were classified into several major chemical groups, including phenylpropanoid-derived phenolics, flavonoids, and triterpenoid-related compounds. Overall, the metabolite profile was dominated by low-molecular-weight phenylpropanoid phenolics, primarily caffeic acid derivatives corresponding to compounds 1–4. Within this group, caffeic acid (2) and rosmarinic acid (4) are well-established phenolic markers of basil, whereas caftaric acid (1) and chicoric acid (3) have also been recognized as characteristic phenolic constituents of basil leaves (Lee and Scagel, 2009; Prinsi et al., 2019).

In addition to phenolic acids, basil also produces *O*-methylated flavones via regioselective *O*-methyltransferases that catalyze position-specific methylation of flavone backbones, generating diverse substitution patterns on the A and B rings (Berim et al., 2012). These flavones have been predominantly reported in surface-associated tissues of basil leaves, including epidermal cells, the cuticular layer, and glandular trichome-rich regions (Grayer et al., 1996). In addition to flavonoids, basil leaves are known to accumulate terpene-related metabolites in glandular trichomes, which function as specialized sites for the biosynthesis and sequestration of isoprenoid-derived compounds (Ciriello et al., 2021; Mahmoud et al., 2021).

Accordingly, the tentative detection of triterpenoid-related features in the present LC–MS/MS dataset is consistent with previous reports indicating active terpene biosynthetic pathways in basil, extending beyond volatile terpenes to include minor non-volatile triterpenoid constituents. Collectively, the untargeted LC–MS/MS results, together

with existing phytochemical evidence, support caffeic acid derivatives as representative marker phenolics of basil leaves. Based on these results, compounds 1–4 were selected as key marker constituents, and their quantitative variation in response to different hydroponic cultivation systems was subsequently evaluated using HPLC/PDA analysis.

3.3. Targeted HPLC/PDA quantification of phenylpropanoid-derived phenolics

Targeted HPLC/PDA analysis was conducted to quantify compounds 1–4, phenylpropanoid-derived phenolics selected based on untargeted LC–MS/MS profiling and previous phytochemical reports (Ma, 2024). HPLC/PDA quantification was performed using authentic reference standards. All calibration curves demonstrated excellent linearity, with coefficients of determination (r^2) exceeding 0.9998 across the tested concentration ranges (Table 1). Representative chromatograms of standards and basil extracts confirm reliable peak assignment and satisfactory chromatographic separation of all target compounds (Figs. 5, S4, and S5).

Three-way ANOVA revealed that hydroponic cultivation method was the primary determinant of phenylpropanoid-derived phenolic acid contents (Fig. 6). Across the four quantified compounds, hydroponic system type accounted for a substantial proportion of the total variance, explaining approximately 58 %–87 %, depending on the compound. For each compound, hydroponic system type explained more than half of the observed variation, indicating that structural differences among cultivation systems are a major driver of phenolic accumulation under the present controlled-environment conditions. Although cultivar and temperature effects were also detected, their contributions were comparatively smaller and exhibited compound-specific patterns, underscoring the dominant role of hydroponic system design in shaping phenylpropanoid profiles.

Based on these statistical results, phenolic acid contents were further examined with emphasis on differences among hydroponic systems (Table 2 and Fig. 7). Across all cultivar × temperature combinations, PSC samples consistently exhibited higher levels of all quantified phenylpropanoid-derived phenolic acids compared with APC and NFT samples. However, the magnitude of increase varied among compounds, indicating differential responsiveness of individual phenylpropanoid-derived phenolics to hydroponic system variation. Caffeic acid (2) showed relatively moderate responsiveness to hydroponic system variation, typically within an approximately 2–3-fold range. In contrast, chicoric acid (3) and rosmarinic acid (4) displayed greater responsiveness, with increases ranging from several-fold up to approximately 8-fold and 13-fold, respectively, depending on the treatment condition. Caftaric acid (1) exhibited intermediate behavior. These findings indicate that the increase in total phenolic acid content reflects coordinated but non-uniform accumulation of multiple caffeic acid derivatives, rather than a uniform upregulation of the entire phenylpropanoid

Table 1
Calibration curves for compounds 1–4.

Compounds	t_R (min)	Calibration equation	Correlation factor (r^2)	LOD ($\mu\text{g}/\text{mL}$)	LOQ ($\mu\text{g}/\text{mL}$)
1	11.404	$Y = 2864,684 X - 817$	0.9999	1.481	4.486
2	16.123	$Y = 15,847,329 X + 6276$	0.9999	1.360	4.121
3	24.820	$Y = 10,930,973 X + 3509$	0.9999	1.344	4.074
4	26.712	$Y = 4352,897 X + 5710$	0.9998	2.234	6.771

t_R , retention time; Y, peak area; X, concentration of the standard ($\mu\text{g}/\text{mL}$); r^2 , correlation coefficient calculated from six calibration points. Compounds: caftaric acid (1), caffeic acid (2), chicoric acid (3), and rosmarinic acid (4).

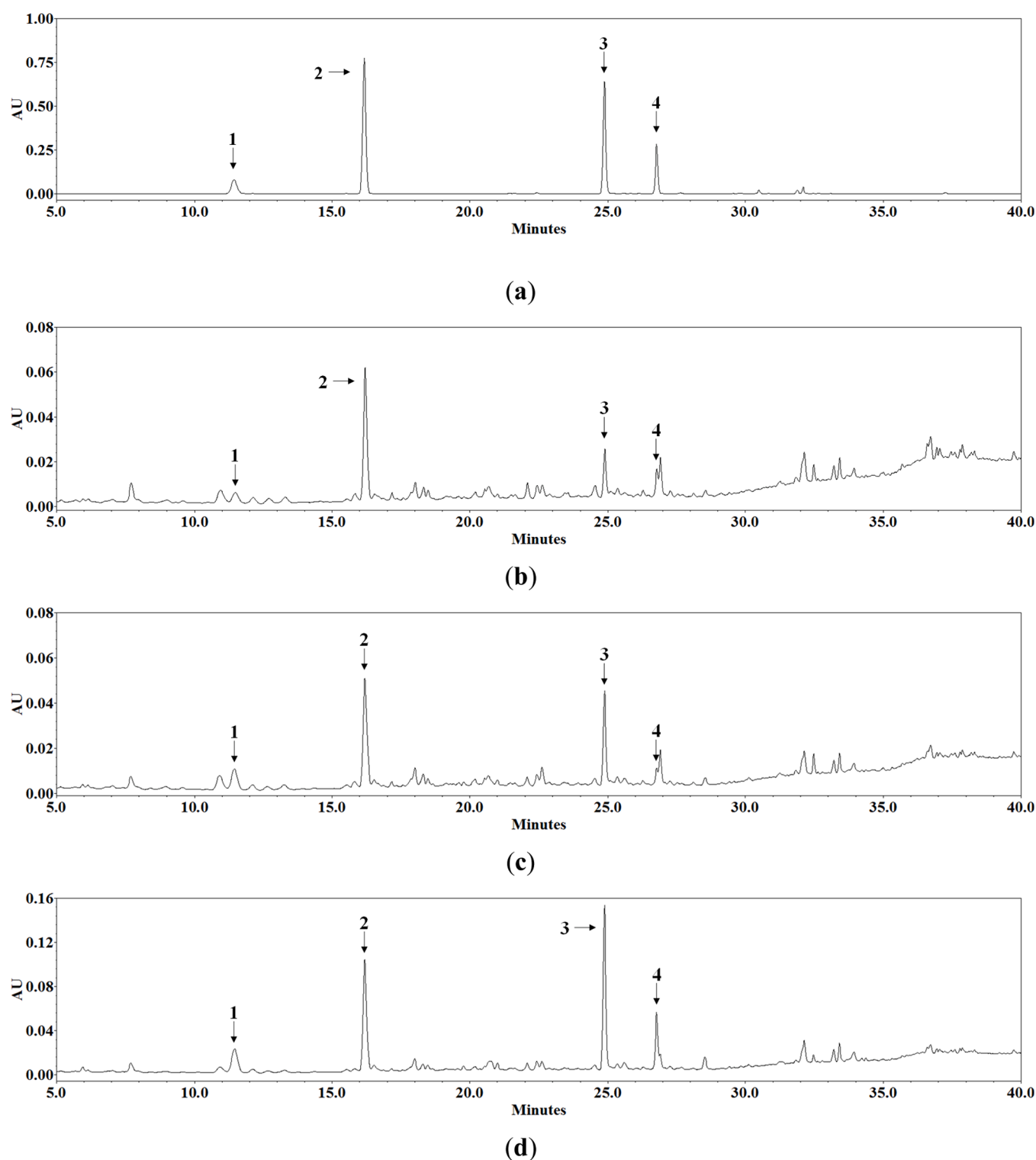


Fig. 5. HPLC/PDA chromatograms of compounds 1–4 (a) and Marian basil cultivated under the T1 condition (cooler front zone) using APC (b), NFT (c), and PSC (d). Compounds: caftaric acid (1), caffeic acid (2), chicoric acid (3), and rosmarinic acid (4).

pathway.

The discrepancy between the moderate response of caffeic acid and the more pronounced accumulation of chicoric and rosmarinic acids may be explained by their different metabolic positions within the phenylpropanoid-derived caffeic acid conjugation network (Petersen and Simmonds, 2003). Free caffeic acid can be interpreted as a hydroxycinnamic acid unit contributing to multiple esterified derivatives, whereas rosmarinic acid is a conjugated caffeic acid ester and

chicoric acid is a dicaffeoyltartaric acid derivative (Lee and Scagel, 2013; Guan et al., 2022). Rosmarinic acid biosynthesis has been extensively studied in Lamiaceae and related taxa and involves the coupling of hydroxycinnamoyl and hydroxyphenyllactate-derived branches, ultimately yielding an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (Trócsányi et al., 2020). In *O. basilicum*, genes associated with rosmarinic acid biosynthesis have also been characterized, supporting the metabolic relevance of this pathway in basil (Kwon et al., 2019). By

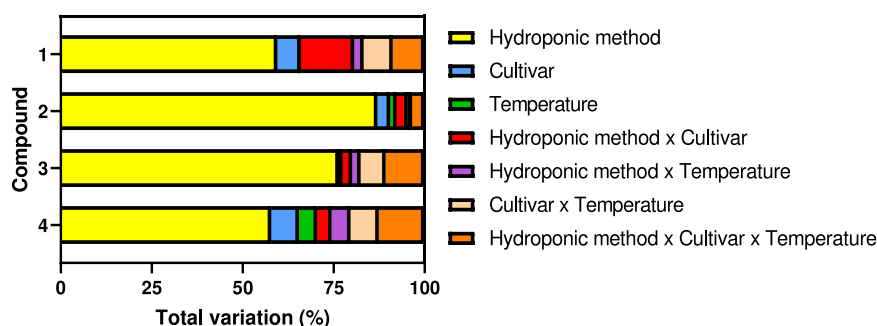


Fig. 6. Basil samples were cultivated under two temperature regimes (T1, cooler front zone; T2, warmer rear zone, approximately 2 °C higher than T1), two cultivars (Marian basil and Common basil), and three hydroponic systems (APC, NFT, and PSC). Stacked bar charts show the relative contribution of each factor to the total variation in individual phenolic compounds based on three-way ANOVA. All main effects and interaction terms were statistically significant ($p < 0.05$). Compounds: caftaric acid (1), caffeic acid (2), chicoric acid (3), and rosmarinic acid (4).

Table 2

Quantification of compounds 1–4 in basil samples grown under different hydroponic systems.

Samples		Contents (mg/g DW)						
		1	2	3	4	Total		
Marian basil	T1	A	0.37 ± 0.01	0.45 ± 0.00	0.17 ± 0.00	0.21 ± 0.00	1.208	
		N	0.70 ± 0.00	0.41 ± 0.00	0.34 ± 0.00	0.12 ± 0.01	1.568	
		P	1.61 ± 0.00	0.81 ± 0.00	1.21 ± 0.01	0.97 ± 0.01	4.588	
	T2	A	0.56 ± 0.00	0.29 ± 0.00	0.21 ± 0.00	0.10 ± 0.00	1.155	
		N	0.98 ± 0.01	0.36 ± 0.00	0.44 ± 0.01	0.12 ± 0.00	1.908	
		P	2.17 ± 0.00	0.77 ± 0.01	1.68 ± 0.00	1.37 ± 0.01	5.976	
	Common basil	T1	A	0.58 ± 0.00	0.34 ± 0.00	0.37 ± 0.00	0.25 ± 0.00	1.530
			N	0.68 ± 0.00	0.44 ± 0.00	0.61 ± 0.00	0.68 ± 0.00	2.402
			P	1.52 ± 0.01	1.12 ± 0.01	1.82 ± 0.02	3.23 ± 0.02	7.688
T2		A	0.54 ± 0.00	0.43 ± 0.00	0.33 ± 0.00	0.31 ± 0.00	1.606	
		N	0.87 ± 0.00	0.45 ± 0.00	0.65 ± 0.00	0.28 ± 0.00	2.246	
		P	0.61 ± 0.01	0.88 ± 0.00	0.74 ± 0.01	0.99 ± 0.02	3.220	

DW, dry weight; A, APC; N, NFT; P, PSC; T1, cooler front zone; T2, warmer rear zone (approximately 2 °C higher than T1). Values are expressed as mean ± SD. Compounds: caftaric acid (1), caffeic acid (2), chicoric acid (3), and rosmarinic acid (4).

contrast, the enzymatic basis of chicoric acid biosynthesis has been less clearly resolved in basil; however, studies in chicoric-acid-accumulating species have shown that caffeoyltartaric acid derivatives, including caftaric acid, can participate in downstream chicoric acid formation (Fu et al., 2021). Therefore, the stronger response of chicoric and rosmarinic acids under PSC cultivation may indicate preferential accumulation of esterified caffeic acid derivatives rather than proportional accumulation of the free caffeic acid pool. Because enzyme activities, gene expression, and metabolic fluxes were not directly measured in the present study, this interpretation should be regarded as a plausible metabolic explanation for the compound-specific response pattern rather than direct mechanistic evidence.

In contrast, cultivar and temperature effects did not exhibit consistent or directional patterns across compounds or hydroponic systems. Although minor differences were observed under specific conditions, these effects were not reproducible across treatments and did not produce stable trends. Overall, phenylpropanoid-derived phenolic acid

profiles were comparable between the two Genovese-type basil sources and between temperature zones, indicating that cultivar- and temperature-associated variation was limited relative to the dominant influence of hydroponic system type. Collectively, these results demonstrate that, under the cultivation conditions examined, phenolic acid profiles were shaped predominantly by hydroponic system design, with cultivar and temperature contributing only marginal and non-systematic variation. The compound-specific responsiveness observed here is consistent with previous reports in Lamiaceae, which describe differential sensitivity of individual phenolic compounds to cultivation- and environment-related factors, often associated with abiotic stress responses rather than uniform metabolic shifts (Shahhat et al., 2025).

From a physiological perspective, the system-dependent differences in phenolic acid accumulation are more plausibly attributed to inherent differences in root-zone physical environments than to specific nutritional factors. All systems received the same Yamazaki nutrient solution under comparable EC and pH ranges. Therefore, the observed metabolic differences are more likely associated with system-dependent differences in nutrient-solution delivery, retention, and root exposure than with nutrient formulation itself. PSC provides a physically buffered and mechanically supported root environment with higher water-holding capacity and relatively stable aeration. In contrast, APC and NFT expose roots to unsupported conditions characterized by rapid fluctuations in water availability, oxygen status, and mechanical stability (Bengough et al., 2011; Jacobsen et al., 2021; Kou et al., 2022; Tomobe et al., 2023). Under such stabilized substrate conditions, plants may adopt a more compact root architecture, potentially reducing carbon investment in root elongation and altering carbon allocation toward shoot-level secondary metabolism (Strock et al., 2017; Mundim and Pringle, 2018; Rouina et al., 2025).

Consistent with previous reports suggesting that root-zone moisture buffering and mechanical support can modulate phenylpropanoid biosynthesis in leafy crops, the higher phenolic acid contents observed under perlite-based cultivation in the present study support the hypothesis that a stable, mechanically supported root-zone environment favors the accumulation of non-volatile phenolic metabolites (Hasibeder et al., 2014; Dong and Lin, 2021).

3.4. ABTS and DPPH-based evaluation of antioxidant activity of basil

The antioxidant activities of basil extracts cultivated under different hydroponic systems were assessed using ABTS and DPPH radical scavenging assays (Fig. 8). Across all cultivar and temperature combinations, PSC samples consistently exhibited lower IC₅₀ values than APC and NFT samples, indicating stronger antioxidant capacity, with differences of up to approximately two-fold depending on the assay and cultivation condition. Comparable trends were observed in both assays despite their distinct reaction mechanisms, suggesting that the differences reflect

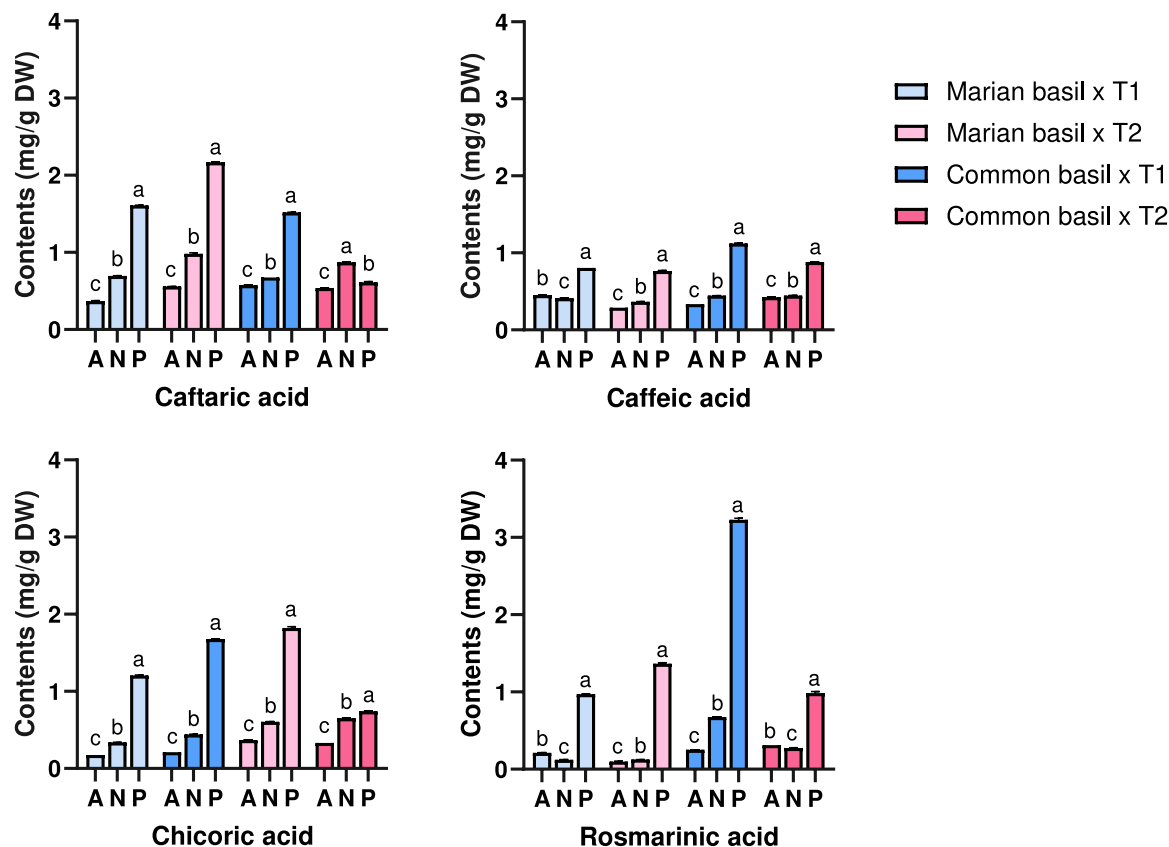


Fig. 7. Contents of caftaric acid, caffeic acid, chicoric acid, and rosmarinic acid in basil cultivated in three hydroponic systems (A, APC; N, NFT; P, PSC) across two greenhouse temperature zones (T1, cooler front zone; T2, warmer rear zone, approximately 2 °C higher than T1). ^{a-c} Different lowercase letters within each cultivar × temperature group indicate statistically significant differences among hydroponic systems based on one-way ANOVA followed by Tukey's multiple-comparison test ($p < 0.05$).

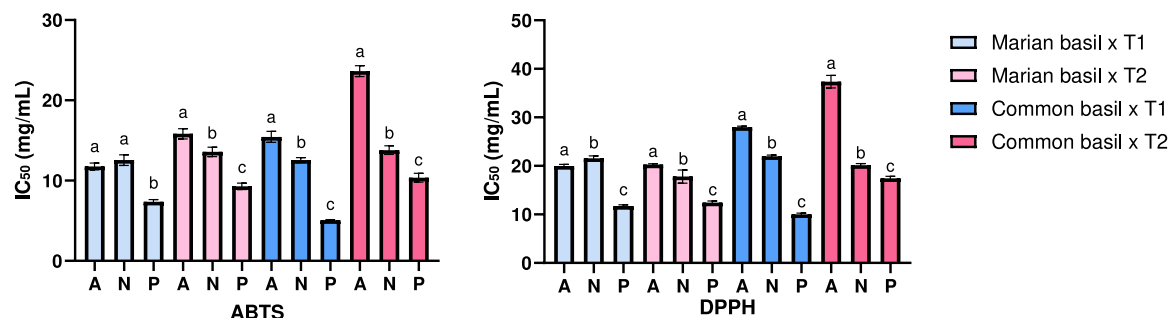


Fig. 8. IC_{50} values of basil samples cultivated in different hydroponic systems (A, APC; N, NFT; P, PSC) across two greenhouse temperature zones (T1, cooler front zone; T2, warmer rear zone, approximately 2 °C higher than T1), as determined by ABTS and DPPH radical-scavenging assays. ^{a-c} Different lowercase letters within each cultivar × temperature group indicate statistically significant differences among hydroponic systems based on one-way ANOVA followed by Tukey's multiple-comparison test ($p < 0.05$).

intrinsic extract properties rather than assay-specific effects.

These functional differences closely paralleled variations in phenylpropanoid-derived phenolic acid contents among hydroponic systems. Phenolic acids such as compounds 1–4, which contain multiple hydroxyl groups and exhibit strong electron- and hydrogen-donating capacities, are well-established contributors to radical scavenging activity in both ABTS and DPPH assays (Yi et al., 2020; Moazzen et al., 2022; Nguyen et al., 2024). From a structural antioxidant perspective, recent work on plant-derived antioxidants further supports that molecular architecture can influence antioxidant behavior by facilitating proton or electron transfer and stabilizing reactive intermediates (Quan et al., 2025). Accordingly, the enhanced accumulation of these

compounds under the PSC system provides a chemically plausible explanation for the lower IC_{50} values observed. In particular, rosmarinic acid (4), which showed the most pronounced increase under PSC cultivation, is widely recognized as a major antioxidant constituent of basil due to its dual catechol structure and high electron-donating potential. Previous studies indicate that rosmarinic acid (4) can contribute disproportionately to total extract-level antioxidant capacity, potentially accounting for the marked differences observed among hydroponic systems (Nguyen and Niemeyer, 2008).

In addition to the quantified phenylpropanoid-derived phenolic acids, basil leaves contain a diverse array of additional non-volatile phenolic compounds and flavonoids that may also contribute to

antioxidant activity (Teofilović et al., 2021; Ullah et al., 2022; Ulewicz-Magulska and Wesolowski, 2023). Minor or co-occurring phenolics have been reported to exert additive or synergistic effects in radical scavenging assays (Kaurinovic et al., 2011; Ahmed et al., 2019). Therefore, although the quantified compounds provide a robust chemical basis for the observed trends, contributions from other non-quantified constituents should be considered as part of the overall antioxidant response.

Collectively, these results demonstrate that hydroponic system architecture substantially influences not only the accumulation of phenylpropanoid-derived phenolic acids but also antioxidant activity, with differences of up to approximately two-fold among cultivation systems. Given that non-volatile phenolic compounds are widely recognized as key dietary antioxidants associated with the mitigation of oxidative stress-related disorders, the enhanced radical scavenging capacity observed in PSC-grown basil may confer additional functional value when consumed as a fresh herb (Zhang and Tsao, 2016; Chandimali et al., 2025).

In a broader context, plant-derived bioactive compounds are increasingly recognized not only for their chemical radical-scavenging capacity but also for their potential to modulate antioxidant-related physiological processes at the organism level (Chen et al., 2023). Although the present study focuses on non-volatile phenolic compounds in basil and their in vitro antioxidant activity, this perspective underscores the broader physiological relevance of plant-derived phytochemicals in oxidative stress regulation.

Within this framework, the present findings demonstrate that differences among commonly adopted hydroponic systems for basil production, including APC, NFT, and PSC, can markedly affect both secondary metabolite abundance and associated antioxidant activity. Hydroponic system selection should therefore be considered an important agronomic factor for growers aiming to optimize the functional quality of fresh basil.

3.5. Integrative analysis of growth performance and metabolite-antioxidant relationships

In addition to metabolite and antioxidant variation, growth-related traits were evaluated to provide a more comprehensive assessment of hydroponic system effects. Although numerical differences were observed among systems, most aboveground biomass parameters were not clearly separated by Tukey's test, indicating that overall vegetative productivity was not substantially altered by system architecture (Table 3). However, consistent directional tendencies were evident in root morphology. Root length was generally greatest under APC conditions, lowest under PSC cultivation, and intermediate under NFT across seed sources and temperature zones. These findings suggest that hydroponic configuration exerts a stronger influence on belowground

development than on shoot biomass accumulation. Although the relatively shorter root length observed under PSC cultivation does not provide direct evidence of nutrient uptake limitation under the present short-term cultivation conditions, it may have physiological relevance if the cultivation period is extended or if basil is managed under repeated-harvest production (Formisano et al., 2021). Under such conditions, further evaluation would be needed to determine whether the relatively shorter root system observed under PSC affects nutrient uptake stability during repeated harvests, particularly under variable root-zone conditions.

This pattern is consistent with previous reports demonstrating enhanced root growth under aeroponic systems (Khater et al., 2021). The pronounced root elongation observed under APC conditions can be mechanistically attributed to the distinctive root-zone environment provided by aeroponics. Elevated oxygen availability and the absence of mechanical impedance are known to stimulate root elongation rates and root hair proliferation in aeroponic systems (Eldridge et al., 2020). Reduced substrate resistance facilitates rapid cell expansion mediated by auxin–ethylene–reactive oxygen species signaling networks, thereby promoting longitudinal root growth (Jacobsen et al., 2021). This signaling framework provides a plausible mechanistic link to phenylpropanoid metabolism. Mechanical impedance can alter root growth demand and cell-wall remodeling, while the phenylpropanoid pathway supplies precursors for both soluble phenolic compounds and cell-wall-associated polymers such as lignin (Vogt, 2010). Thus, differences in mechanical resistance among hydroponic architectures may affect carbon partitioning between root structural growth and soluble phenolic acid accumulation.

Such enhanced primary growth may, in turn, influence carbon allocation patterns. Carbon partitioning theory predicts a trade-off between structural biomass production and phenylpropanoid-derived secondary metabolism under conditions of rapid vegetative growth (Dong and Lin, 2021). Accelerated root development may preferentially channel assimilated carbon toward primary metabolic processes and structural expansion, potentially constraining carbon flux into secondary metabolite biosynthesis. Isotopic pulse-labeling studies in aeroponic systems further support the rapid allocation of newly fixed carbon to expanding root tissues (Mannerheim et al., 2020), reinforcing the concept that growth sinks can dominate carbon demand under highly aerated conditions. In line with this framework, our results showed that, despite largely comparable shoot biomass among hydroponic systems, PSC-grown basil accumulated substantially higher levels of phenylpropanoid-derived phenolic acids and exhibited enhanced antioxidant activity. This apparent growth–metabolite dissociation likely reflects system-dependent plasticity in carbon partitioning under distinct hydroponic architectures.

Pearson correlation network analysis further supported this interpretation by revealing system-specific coordination among growth

Table 3
Growth performance parameters of basil cultivated under different hydroponic systems.

Samples			Plant length (cm)	Root length (cm)	No. of leaf	Leaf length (cm)	Leaf width (cm)	Fresh weight (g)	Dry weight (g)	Dry matter rate (%)
Marian basil	T1	A	26.6 ± 4.3 ^{ab}	22.5 ± 1.9 ^{bd}	60.3 ± 21.2 ^{ab}	6.7 ± 0.1 ^b	4.9 ± 0.1 ^b	23.7 ± 8.6 ^a	1.8 ± 0.8 ^a	7.6 ± 0.7 ^{cd}
		N	25.2 ± 3.1 ^{ab}	17.6 ± 0.6 ^{df}	62.7 ± 15.5 ^{ab}	7.2 ± 0.1 ^b	4.8 ± 0.1 ^b	23.5 ± 6.0 ^a	1.7 ± 0.5 ^a	7.1 ± 0.4 ^{cd}
		P	27.8 ± 2.7 ^{ab}	12.7 ± 1.2 ^f	60.7 ± 15.5 ^{ab}	6.7 ± 0.5 ^b	4.4 ± 0.3 ^b	19.9 ± 5.4 ^a	1.8 ± 0.5 ^a	8.8 ± 0.3 ^{ab}
	T2	A	29.8 ± 3.2 ^a	28.9 ± 2.7 ^{ab}	83.7 ± 22.2 ^a	6.7 ± 0.4 ^b	4.6 ± 0.5 ^b	34.2 ± 3.9 ^a	2.6 ± 0.3 ^a	7.6 ± 0.1 ^{cd}
		N	26.5 ± 3.5 ^{ab}	15.6 ± 1.2 ^{df}	68.3 ± 16.1 ^{ab}	7.0 ± 0.3 ^b	4.6 ± 0.1 ^b	21.8 ± 5.9 ^a	1.5 ± 0.5 ^a	7.0 ± 0.4 ^{cd}
		P	26.5 ± 3.3 ^{ab}	12.9 ± 2.5 ^f	60.7 ± 12.9 ^{ab}	6.9 ± 0.5 ^b	4.5 ± 0.5 ^b	17.9 ± 5.1 ^a	1.5 ± 0.5 ^a	8.0 ± 0.4 ^{abc}
Common basil	T1	A	20.6 ± 0.2 ^b	26.9 ± 1.2 ^{abc}	48.0 ± 2.6 ^{ab}	10.5 ± 0.3 ^a	8.2 ± 0.0 ^a	27.2 ± 1.6 ^a	2.2 ± 0.1 ^a	8.0 ± 0.4 ^{bc}
		N	18.8 ± 2.2 ^b	16.7 ± 2.2 ^{df}	49.0 ± 4.6 ^{ab}	10.4 ± 0.3 ^a	8.6 ± 1.1 ^a	29.9 ± 2.6 ^a	2.1 ± 0.1 ^a	6.9 ± 0.3 ^d
		P	19.3 ± 3.1 ^b	12.6 ± 1.0 ^f	35.0 ± 4.6 ^b	9.1 ± 0.7 ^a	7.0 ± 1.2 ^a	17.1 ± 5.7 ^a	1.6 ± 0.5 ^a	9.2 ± 0.2 ^a
	T2	A	24.0 ± 1.7 ^{ab}	32.9 ± 6.5 ^a	47.0 ± 9.6 ^{ab}	10.2 ± 0.4 ^a	7.5 ± 0.9 ^a	25.7 ± 8.0 ^a	2.0 ± 0.6 ^a	7.8 ± 0.4 ^{bd}
		N	20.1 ± 1.2 ^b	20.4 ± 1.1 ^{cde}	44.0 ± 7.5 ^b	10.0 ± 0.9 ^a	7.3 ± 0.3 ^a	23.7 ± 5.6 ^a	1.7 ± 0.5 ^a	7.1 ± 0.5 ^{cd}
		P	22.1 ± 5.1 ^{ab}	14.8 ± 0.2 ^{ef}	43.7 ± 8.0 ^b	10.4 ± 1.0 ^a	7.8 ± 0.7 ^a	24.4 ± 8.2 ^a	2.0 ± 0.7 ^a	8.0 ± 0.3 ^{bd}

APC, A; NFT, N; PSC, P; T1, cooler front zone; T2, warmer rear zone (approximately 2 °C higher than T1). Values are expressed as mean ± SD. ^{a-f} Different lowercase letters within the same column indicate statistically significant differences based on one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

traits, phenolic accumulation, and antioxidant function (Fig. 9). Under the substrate-free APC and NFT systems, growth-related variables showed stronger interconnections and were linked to several metabolic traits, suggesting that phenolic variation in these systems remained more closely associated with overall vegetative growth patterns. This structure may reflect the greater contribution of primary growth processes, particularly root elongation and shoot development, to whole-plant carbon demand under APC and NFT cultivation. In contrast, the PSC network showed a more coherent metabolite–function module, characterized by close correlations among individual phenolic acids and TPC, together with inverse associations between these phenolic variables and the IC_{50} values obtained from the ABTS and DPPH assays. Because lower IC_{50} values indicate stronger radical-scavenging activity, this network structure suggests that the coordinated accumulation of phenylpropanoid-derived phenolics was more directly reflected in antioxidant performance under PSC cultivation. Therefore, the structural differences among the three networks imply that hydroponic system architecture altered the internal coordination between growth-related carbon demand and phenylpropanoid-derived antioxidant metabolism, rather than merely changing individual trait values. However, because correlation networks do not demonstrate causal relationships, these patterns should be interpreted as integrative evidence of system-dependent trait coordination rather than direct mechanistic proof.

Collectively, these findings extend beyond conventional yield-oriented evaluations of hydroponic systems. Despite comparable aboveground biomass across treatments, substantial differences in phenylpropanoid-derived phenolic acid accumulation and antioxidant capacity were observed, demonstrating that hydroponic architecture modulates secondary metabolism independently of gross vegetative productivity. This architecture-driven metabolic regulation suggests that root-zone physical configuration acts as a regulatory layer influencing carbon allocation dynamics and phenylpropanoid pathway activity.

From an operational perspective, these quality-related gains should be weighed against the practical requirements of each cultivation system. For PSC, the observed improvement in functional quality is accompanied by substrate-related costs and handling requirements, including substrate purchase, replacement or reuse, disposal, and drainage management (Vinci and Rapa, 2019; Kennard et al., 2020). In contrast, the substrate-free APC and NFT systems reduce solid-substrate inputs and handling burden but require reliable misting or nutrient-solution flow continuity during operation. Therefore,

hydroponic system selection should balance functional quality targets with substrate-related inputs, handling requirements, and system-operation stability.

To our knowledge, this study provides one of the first integrative demonstrations that commonly adopted hydroponic system designs differentially reshape the metabolic–functional landscape of basil under a shared greenhouse environment. Accordingly, hydroponic system selection should be considered not only an agronomic decision for yield optimization, but also a strategic tool for tailoring phytochemical composition and functional quality in controlled-environment herb production.

4. Conclusions

This study demonstrates that hydroponic system architecture significantly influences both the phytochemical profile and antioxidant capacity of fresh basil leaves under controlled-environment cultivation. PSC consistently produced higher concentrations of major phenylpropanoid-derived phenolic acids, including caffeic acid (1), caffeic acid (2), chicoric acid (3), and rosmarinic acid (4), and correspondingly lower IC_{50} values in both ABTS and DPPH assays compared with APC and NFT systems. In contrast, APC promoted greater root elongation while maintaining comparable aboveground biomass across treatments. These results indicate that hydroponic configuration can differentially modulate belowground morphology and secondary metabolite accumulation without substantially compromising vegetative productivity. Although the underlying physiological mechanisms were not directly examined, the findings suggest that root-zone conditions associated with substrate-based systems may favor phenolic enrichment in basil. Collectively, our findings indicate that hydroponic system selection represents a practical cultivation strategy for enhancing functional quality while sustaining stable biomass production in fresh basil under greenhouse conditions. Building on these findings, future studies could extend this system-level evaluation to repeated-harvest and commercial-scale greenhouse production and integrate broader profiling of volatile and non-volatile bioactive constituents with physiological analyses of nutrient uptake, root-zone conditions, and phenylpropanoid-related gene expression to clarify how hydroponic system architecture regulates functional quality in basil.

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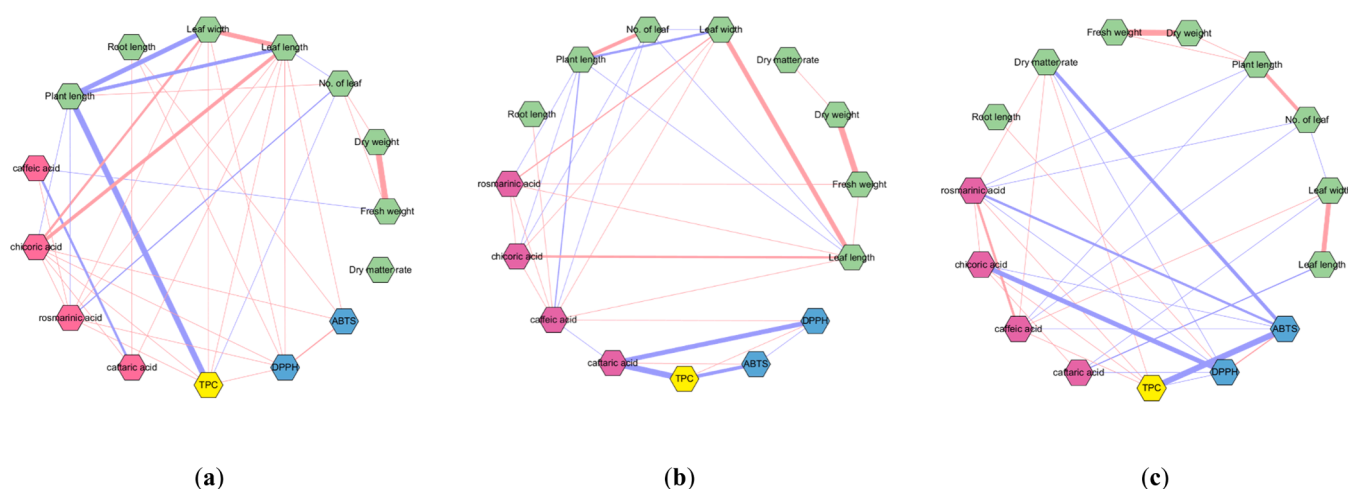


Fig. 9. Pearson correlation coefficient networks ($|r| \geq 0.7$) illustrating relationships among growth parameters, phenylpropanoid-derived phenolic acids, TPC, and antioxidant activity under APC (a), NFT (b), and PSC (c) cultivation systems. Nodes represent measured variables and are color-coded by category: green, growth-related traits; pink, individual phenolic compounds; yellow, total phenolic content; blue, antioxidant indicators. Red edges indicate positive correlations, whereas blue edges indicate negative correlations. Edge thickness reflects the strength of the correlation coefficient (r) between variables.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

CRediT authorship contribution statement

Sang-Yun Lee: Writing – original draft, Investigation, Formal analysis, Data curation. **Neil Patrick Uy:** Methodology, Investigation, Data curation. **Jong-Suk Park:** Resources, Methodology, Investigation. **Won-Yong Yang:** Resources, Investigation, Data curation. **Hoon Kim:** Investigation, Formal analysis. **Sanghyun Lee:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.scienta.2026.114910](https://doi.org/10.1016/j.scienta.2026.114910).

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