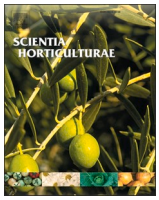
Scientia Horticulturae 359 (2026) 114766

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Research Paper

Metabolomics insights into the shared and cultivar-related mechanisms controlling postharvest cold tolerance in *Cucurbita pepo* L

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ARTICLE INFO

Keywords:
Natural genetic variability
Foodomics
Postharvest
Chilling stress
Metabolomics
Breeding markers

ABSTRACT

Zucchini (Cucurbita pepo L.) is highly sensitive to chilling injury during cold storage, which leads to surface damage, quality loss, and reduced nutraceutical value. Fruits were stored at 4 °C, and samples were collected at harvest (T0), 3 days (T3), and 14 days (T14) of cold storage. To elucidate the biochemical determinants of cold tolerance, we selected five cold-tolerant and five cold-sensitive cultivars from a previous screening of 126 accessions based on differences in chilling injury index (CI), weight loss (WL), and physiological indicators of oxidative stress. Metabolomic profiling was performed on fruit exocarp tissue using an untargeted LC–MS approach. Despite genotypic variability, all cultivars displayed a shared metabolic response to cold storage characterized by the accumulation of N6,N6,N6-trimethyl-L-lysine and 6-hydroxymethyl-7,8-dihydropterin, intermediates in carnitine and folate biosynthesis. Notably, tolerant cultivars maintained higher levels of these metabolites throughout storage, suggesting the presence of a metabolic signature associated with improved cold tolerance. Beyond this shared response, tolerant cultivars exhibited distinct metabolic adjustments involving nucleotides, phenolic compounds, amino acids, phytohormones, vitamins, and cucurbitacins. Overall, these results provide new insights into metabolic responses associated with postharvest cold tolerance in zucchini and highlight candidate metabolic markers that may be useful for breeding programs aimed at improving postharvest performance and shelf life.

1. Introduction

Several methodologies are being investigated to extend the shelf life of fruits while preserving their quality and nutraceutical properties during the postharvest period, with cold storage being the most widely used technique. However, zucchini fruits (*Cucurbita pepo* L.) often develop a physiological disorder known as chilling injury (CI), which is attributed to their subtropical origin (Valenzuela et al., 2017). Chilling injury in horticultural crops is a complex physiological disorder involving multiple interconnected processes. Previous studies in different fruits and vegetables have associated cold tolerance with mechanisms such as membrane lipid remodeling, maintenance of cellular energy metabolism, regulation of reactive oxygen species (ROS) and antioxidant defenses, hormonal signaling, and osmotic and cell-wall adjustments (Wu et al., 2024). However, the relative contribution of these processes may vary among species and cultivars, and the metabolic networks underlying genotype-dependent cold tolerance remain poorly understood. In zucchini, although several physiological responses to cold stress have been described, the metabolic pathways associated with contrasting levels of chilling tolerance among cultivars are still largely unexplored. *C. pepo* is one of the most genetically diverse species cultivated globally, and its postharvest tolerance to cold stress is

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https://doi.org/10.1016/j.scienta.2026.114766
Received 17 December 2025; Received in revised form 8 March 2026; Accepted 18 March 2026
Available online 25 March 2026

cultivar-dependent, as observed in both gene bank accessions and commercial zucchini varieties (Carvajal et al., 2011; García et al., 2024; Megías et al., 2016a). A wide range of mechanisms have been investigated to enhance the postharvest cold tolerance of zucchini fruit, including the activation of antioxidant defense systems mediated by nitric oxide, abscisic acid, and riboflavin (Benítez et al., 2022; Castro-Cegrí et al., 2023, 2024a; Jiménez-Muñoz et al., 2021). In contrast, stress-related events, such as the induction of ethylene production (Megías et al., 2016b), have been linked to heightened sensitivity to cold and, consequently, accelerated postharvest quality deterioration. Given that the reduction of food waste, particularly postharvest losses, has become a global priority aligned with Sustainable Development Goal (SDG) 12, "Responsible Consumption and Production" (FAO) (Food loss estimation, 2023), developing novel strategies and cultivars with improved cold tolerance is critical for extending fruit shelf life. Achieving this goal requires a comprehensive understanding of the molecular and physiological mechanisms underlying cold tolerance in fruits, including the key regulatory networks, genes, and metabolite crosstalk involved.

Recently, the postharvest quality parameters of 126 zucchini accessions, representing the global genetic diversity conserved in gene banks, and 10 pre-commercial and inbred lines of *C. pepo* were evaluated (García et al., 2024). The large-scale screening of zucchini accessions, together with previous genetic analyses identifying loci associated with cold tolerance, provides a valuable framework to investigate the metabolic basis underlying these phenotypic differences. The analysis revealed significant diversity in fruit cold tolerance across cultivars, and genome-wide association analysis (GWAS) identified relevant genomic regions linked to cold tolerance (García et al., 2024). In this context, metabolomic profiling of contrasting cultivars can help reveal the biochemical pathways associated with cold tolerance and identify metabolic markers that may support breeding strategies aimed at improving postharvest performance. The cultivars that were phenotypically most contrasting in terms of postharvest cold tolerance were selected for further investigation. In this work, these cultivars underwent a comprehensive untargeted metabolic analysis to unravel the biochemical mechanisms underlying cold tolerance. Metabolomics is a powerful tool for investigating compositional changes that occur during the postharvest period (Yun et al., 2022). This approach has been successfully applied to several Cucurbitaceae species, including *Cucumis melo* L. (Zainal et al., 2019), and more recently to *Cucurbita pepo* L., to elucidate the metabolomic alterations associated with postharvest cold tolerance induced by abscisic acid and riboflavin treatments (Castro-Cegrí et al., 2024b, 2025a).

Despite increasing knowledge on chilling injury in horticultural crops, the metabolic mechanisms underlying cultivar-dependent cold tolerance in zucchini remain poorly understood. In particular, it is unclear which metabolic pathways are consistently associated with improved tolerance to cold storage and which responses are cultivar-specific. Based on current knowledge of chilling injury in horticultural crops, pathways related to antioxidant metabolism, membrane lipid remodeling, and energy metabolism are expected to play important roles in cold tolerance. Therefore, the objective of this study was to characterize the metabolic responses of contrasting zucchini cultivars during cold storage using untargeted metabolomics, with the aim of identifying metabolic pathways associated with cold tolerance and distinguishing shared and genotype-specific metabolic adjustments. The results will support the development of metabolite-informed strategies to enhance postharvest quality traits, which could contribute to the breeding of new cultivars with improved quality maintenance and extended shelf life.

2. Materials and methods

2.1. Fruit material and experimental design

In autumn 2022, 126 Cucurbita pepo accessions with conserved natural variability in germplasm banks and 10 pre-commercial and inbred lines of *C. pepo* were evaluated for postharvest quality under cold storage conditions (García et al., 2024). Accessions were obtained from the Germplasm Bank of the United States Department of Agriculture (National Plant Germplasm System, NPGS; https://npgsweb.ars-grin.gov/gringlobal/search), and subsequently propagated and preserved at the University of Almería's Germplasm Bank (BSUAL). The inbred line 4.7 was provided by Green Breeding Biotech S.L. PI 222786, PI 174187, PI 174188, PI 599994 accessions, and the inbred line 4.7 were selected as cultivars with a higher tolerance against cold stress (T-cultivars), while PI 525176, PI 525166, PI 169442, PI 172860, and PI 357955 were selected as cold-sensitive (S-cultivars). The origin of these accessions was from Iran (1), Turkey (4), Egypt (2), Macedonia (1), the United States (1). Fruits from 12 plants per cultivar were harvested at a commercial immature stage, weighing between 250–350 g, with an approximate length of 20 cm. These fruits were harvested daily and stored in a temperature-controlled chamber at 4 °C, 85–90 % relative humidity (RH), and permanent darkness. Each biological replicate consisted of a pooled sample of three fruits from the same cultivar. Three biological replicates were established for each storage duration (0, 3, and 14 days). In each biological replicate, the fruit exocarp was entirely removed, frozen in liquid nitrogen, ground to a powder, lyophilized, and stored at room temperature in complete darkness.

2.2. Phenotypic assessment

To evaluate the phenotypic responses of the selected cultivars, fruit images were captured at the onset and conclusion of the cold storage period (days 0 and 14). Additionally, weight loss and chilling injury were assessed at three time points: 0, 3, and 14 days of storage. Changes in the percentage of weight loss (WL) along cold storage for each fruit were calculated following Eq. (1), being Wi the initial and Wf the final fruit weights.

$$\%\ Weight\ loss\ = \frac{(Wi - Wf)}{Wi} x\ 100 \quad (1)$$

To evaluate the chilling injury index (CI), the subjective scale of visual symptoms proposed by Carvajal et al. (2011) was used, according to the following scale: 0, no pitting; 1, mild pitting ($\leq$ 10 % of pitting in the fruit surface); 2, medium pitting (10 – 20 % of pitting in the fruit surface); and 3, severe pitting ($>$ 20 % of pitting in the fruit surface).

2.3. Antioxidant capacity and oxidative damage evaluation

The antioxidant capacity of the samples was evaluated by the Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain, 1996), with some modifications: 10 mg of lyophilized exocarp material was extracted with 4 mL of 80 % (*v/v*) acetone and shaken for 2 h at 4 °C. The extracts were then centrifuged at 5000 × *g* for 15 min at 4 °C. Subsequently, the reaction mixture consisted of 0.1 mL of supernatant and 0.9 mL of FRAP reagent [0.3 M acetate buffer (pH 3.6), 0.01 M TPTZ (2,4,6-tripyridyl-s-triazine) in 0.04 M HCl, and 0.02 M $FeCl_3$ $6H_2O$ (10:1:1, v/v/v)], which was incubated for 30 min at 37 °C in permanent darkness. Absorbance was measured at 595 nm, and the results were quantified using a $FeSO_4$ calibration curve mg kg^{-1} DW and expressed as the percentage change at days 3 (T3) and 14 (T14) relative to the measurements at day 0 (T0) for each cultivar.

Oxidative damage was assessed by determining the malondialdehyde (MDA) content using the thiobarbituric acid reactive substances (TBARS) assay (Heath and Packer, 1968), with some modifications. Briefly, 20 mg of lyophilized exocarp material was homogenized in 1 mL of 20 % trichloroacetic acid (TCA, *w/v*) and 0.2 mL of 4 % butylated hydroxytoluene (BHT, *w/v*). The homogenate was centrifuged for 15 min at 10,000 × g and 4 °C. The reaction mixture consisted of 0.25 mL of supernatant and 0.75 mL of 0.5 % thiobarbituric acid (TBA, *w/v*). Samples were incubated at 94 °C for 30 min, followed by rapid cooling

on ice for 10 min, and centrifuged again at 10,000 × g for 10 min at 4 °C. Lipid peroxidation was quantified using an MDA calibration curve. Absorbance was measured at 532 nm, with non-specific absorbance corrected at 600 nm. Results were expressed as the percentage change at days 3 (T3) and 14 (T14) relative to the measurements at day 0 (T0) for each cultivar.

2.4. Sample extraction and metabolomics analysis

For metabolomic analysis, 50 mg of lyophilized material was homogenized in 2 mL of methanol/H_2O/formic acid solution (80.0/19.9/0.1; *v/v/v*) and subjected to ultrasound-assisted extraction using a sonication bath for 15 min. Samples were then centrifuged at 8000 × g for 10 min at 4 °C, and the supernatants were collected and syringe-filtered (cellulose membrane, 0.22 μm pore size) into analytical vials. In parallel, quality control (QC) samples were prepared by pooling 20 μL of each sample into the same vial.

Metabolic profiling was conducted as proposed by Castro-Cegrí et al., 2025b through ultra-high-performance liquid chromatography coupled to quadrupole-time-of-flight high-resolution mass spectrometry (UHPLC/QTOF-HRMS). The analysis was performed using a 1290 UHPLC system equipped with an electrospray ionization source (ESI)-equipped QTOF G6550 iFunnel mass spectrometer (Agilent Technologies®, Santa Clara, CA, USA). Briefly, 6 μL of each sample was injected into an Agilent® 1200 series liquid chromatograph equipped with a ZORBAX RRHD Eclipse Plus C18 column (2.1 × 100 mm, 1.8 μm), and the solvent flow rate was set at 0.2 mL/min with a binary gradient elution (6–94 % of eluent B in 32 min), prepared with eluent A (water) and eluent B (acetonitrile), both containing 0.1 % (*v/v*) formic acid. The Agilent 6560 mass spectrometer was operated in SCAN and positive ionization mode (ESI+). Additional parameters were set as follows: nitrogen was used as both the sheath gas (12 L/min and 315 °C) and drying gas (14 L/min and 250 °C), nebulizer pressure was 45 psi, nozzle voltage was 350 V, and capillary voltage was 4 kV. The mass acquisition of samples was performed in MS-only mode set in the *m/z* 100–1200 range (1 spectra/s) with a mass resolution of 30,000 full width at half maximum (FWHM) at 200 *m/z*. The data-dependent acquisition mode was performed for precursor fragmentation (10, 20, and 40 eV) and acquisition of MS/MS data from QC samples, with a mass resolution of 30,000 (FWHM), selecting 8 top precursors per cycle (1 Hz, *m/z* 80–1200, and active exclusion after 2 spectra). Chromatographic data processing was performed using the MassHunter Qualitative Analysis software (version B.06.00, Agilent Technologies®).

2.5. Data processing

After feature detection, the acquired raw data were processed using MS-DIAL software (v. 4.90), incorporating peak detection, feature identification *via* spectral library matching, gap filling, and alignment. Initially, the raw '.d' format files were converted into '.abf' files by the Reifycs Abf Converter. These '.abf' data files were imported into MS-DIAL, searching the features in the retention time range of 1 – 32 min, *m/z* range 100–1200 for MS-only data and 80–1200 for MS/MS data, setting a minimum of 3000 counts for peak detection. Feature annotation was conducted using the FooDB database (https://foodb.ca/) with mass tolerance thresholds of 0.01 Da and 0.05 Da for MS and MS/MS, respectively, and an identification score cut-off >60 %. Identification was based on mass accuracy, isotopic patterns, and spectral matching. Features detected in <83.33 % of replicates per group were excluded from further analysis. Furthermore, the software MS-Finder (Tsugawa et al., 2016) was used for in-silico fragmentation of the not-annotated mass compounds, using the FooDB database. According to the Metabolomics Standards Initiative (MSI) guidelines (Salek et al., 2013), a level 2 confidence in features' annotation was achieved, conferring the assessment of putatively annotated compounds further involved in multivariate statistical analysis.

2.6. Statistical analysis

The experiments were conducted in a randomized design. Statistical analysis of zucchini fruit quality parameters was performed using one-way analysis of variance (ANOVA) in SPSS 28.0 (SPSS Inc.), with mean comparisons conducted using Duncan's least significant differences test, assuming significant differences at $p < 0.05$.

Raw metabolomics data were first $\log_2$-transformed and normalized at the 75th percentile by the software Mass Profiler Professional 15.1 (Agilent Technologies®), before applying multivariate statistical analysis. An unsupervised hierarchical cluster analysis (HCA; Euclidean distance, Ward's linkage method) was then conducted to provide a metabolome-wide overview of sample clustering based on cultivar differences and their modulation during cold storage. In parallel, supervised multivariate data analysis was carried out in terms of orthogonal projection to latent structures discriminant analysis (OPLS-DA) by SIMCA® 16 software (Sartorius®, Umeå, Sweden), building independent models to discriminate the effect of cold stress by comparing in each cultivar samples of T3 vs T0, and T14 vs T0. Model quality was evaluated using goodness-of-fit (R^2Y) and predictive ability (Q^2), with a Q^2 threshold > 0.5 indicating good predictability. OPLS-DA models were validated through cross-validation analysis of variance (CV-ANOVA, $p < 0.01$), and overfitting was ruled out using a permutation test ($n = 100$). Finally, the models were combined with the variable importance in the projection (VIP) approach to identify the compounds showing the highest contribution to the discriminating effect of cold stress in T- and S-cultivars. Common VIP markers over time were investigated through a Venn diagram by jvenn tool (https://jvenn.toulouse.inrae.fr/app/index.html).

Differentially accumulated metabolites (DAMs) were identified using volcano analysis based on fold change (FC) thresholds (cut-off = ±3), in combination with one-way ANOVA followed by Bonferroni family-wise error rate (FWER) *post hoc* test ($p < 0.01$). These analyses were conducted using Mass Profiler Professional software version 15.1. Comparisons were made between time points T3 vs T0 and T14 vs T0 for each cultivar. Subsequently, the identified DAMs were subjected to chemical similarity enrichment analysis using ChemRICH (Barupal and Fiehn, 2017) to elucidate significantly affected metabolite clusters during cold storage.

3. Results

3.1. Maintenance of postharvest quality in zucchini fruits across accessions

Cold storage induced notable differences among cultivars, particularly affecting the visual appearance of zucchini fruit (Fig. 1). A phenotypic assessment of harvested fruits from each cultivar was conducted by evaluating weight loss (WL) and chilling injury (CI) index after 3, and 14 days of cold storage (Table 1). Both parameters increased significantly after 3 days, with WL exhibiting a substantial increase of 200–300 % in S-cultivars compared to T-ones (Table 1). Substantial variability in the CI was also observed among cultivars at this time point. Notably, the T-cultivar PI 174,187 exhibited the lowest chilling injury (CI = 0.4), whereas the S-cultivar PI 172860 showed the highest susceptibility to cold-induced damage (CI = 1.8). These trends were accentuated further after 14 days of cold storage, with all S-cultivars displaying significantly greater WL than T-cultivars. Specifically, PI 525176 and PI 525166 reached weight loss values of 15.2 % and 13.8 %, respectively, both with a CI of 3.0. In contrast, PI 174187 and PI 174188 exhibited lower weight losses of 5.7 % and 4.5 %, respectively, with a CI of 2.1 and 2.2 (Table 1).

3.2. Changes in antioxidant properties and lipid peroxidation

Since preventing short-term oxidative damage is crucial for reducing

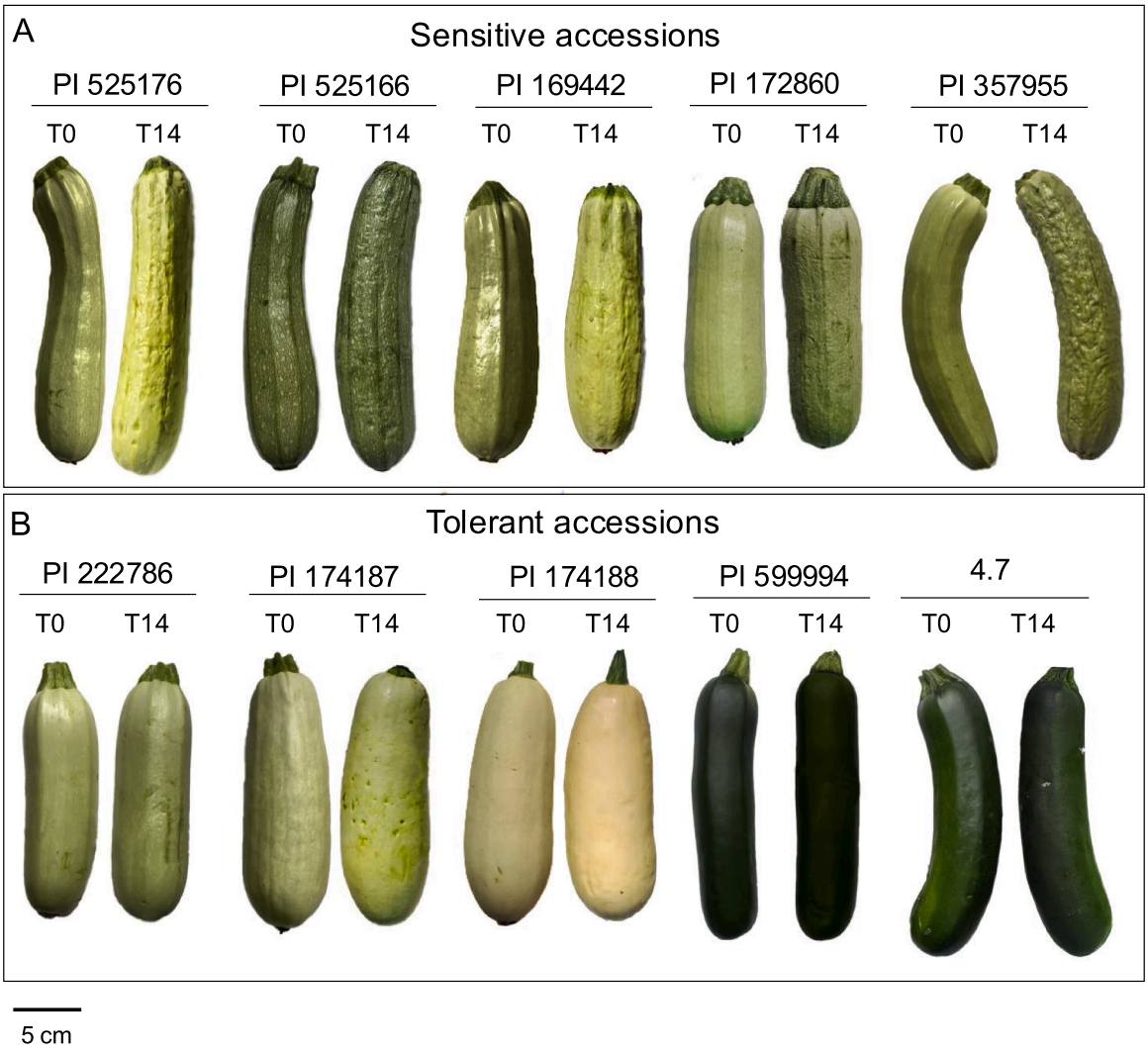

Fig. 1. Phenotypic variability of accessions evaluated for postharvest cold tolerance. T0: fruits at harvest, T14: fruits after 14 days of cold storage at 4°C. A) Accessions classified as sensitive (525176, 525166, 169442, 172860, and 357955) based on weight loss (% WL). B) Accessions classified as tolerant (222786, 174187, 174188, 599994, and 4.7).

Table 1
Postharvest quality parameters in zucchini fruits from different cultivars, showing changes in weight loss (WL) and chilling injury index (CI) after 3 and 14 days of cold storage at 4 °C.

		3 days at 4 °C		14 days at 4 °C	
		WL	CI	WL	CI
T-cultivars	PI 222786	1.6 ± 0.2^{b}	1.5 ± 0.2^{ab}	7.6 ± 0.8^{c}	2.8 ± 0.2^{a}
	PI 174187	1.1 ± 0.1^{b}	0.4 ± 0.2^{c}	5.7 ± 0.4^{cd}	2.1 ± 0.4^{b}
	PI 174188	1.0 ± 0.1^{b}	0.9 ± 0.1^{bc}	4.5 ± 0.4^{d}	2.2 ± 0.2^{b}
	PI 599994	1.6 ± 0.1^{b}	0.6 ± 0.2^{c}	6.8 ± 0.3^{cd}	2.3 ± 0.1^{b}
	4.7	1.5 ± 0.1^{b}	0.7 ± 0.2^{c}	6.5 ± 0.5^{cd}	2.9 ± 0.1^{a}
S-cultivars	PI 525176	3.3 ± 0.2^{a}	1.6 ± 0.2^{ab}	15.2 ± 0.8^{a}	3.0 ± 0^{a}
	PI 525166	3.4 ± 0.2^{a}	1.2 ± 0.2^{abc}	13.8 ± 0.6^{ab}	3.0 ± 0^{a}
	PI 169442	3.0 ± 0.3^{a}	1.2 ± 0.4^{abc}	12.6 ± 1.0^{b}	2.8 ± 0.2^{a}
	PI 172860	3.3 ± 0.1^{a}	1.8 ± 0.3^{a}	12.9 ± 0.8^{ab}	3.0 ± 0^{a}
	PI 357955	2.9 ± 0.2^{a}	1.6 ± 0.3^{ab}	12.1 ± 0.7^{b}	3.0 ± 0^{a}

Data are presented as the mean ± standard deviation based on a minimum of 6 fruits per cultivar for weight loss and chilling injury index. Different letters within each column indicate statistically significant differences between cultivars according to Duncan's test ($p < 0.05$).

susceptibility to cold stress, the antioxidant capacity and lipid peroxidation of cold-stored fruit from the different cultivars were evaluated at T0, T3, and T14. Values at T3 and T14 were expressed as percentage changes from T0 to compare cultivar responses over time.

In terms of antioxidant capacity, general upregulation was observed in T-cultivars at T3, with the highest increases exhibited by PI 174187, PI 174188, and PI 599994 exhibiting the highest increases (18.9 %, 31.8 %, and 22.9 %, respectively). Conversely, a marked decline in antioxidant capacity was detected in S-cultivars, with PI 169442 and PI 352955 showing reductions of −27.3 % and −23.7 %, respectively (Fig. 2A).

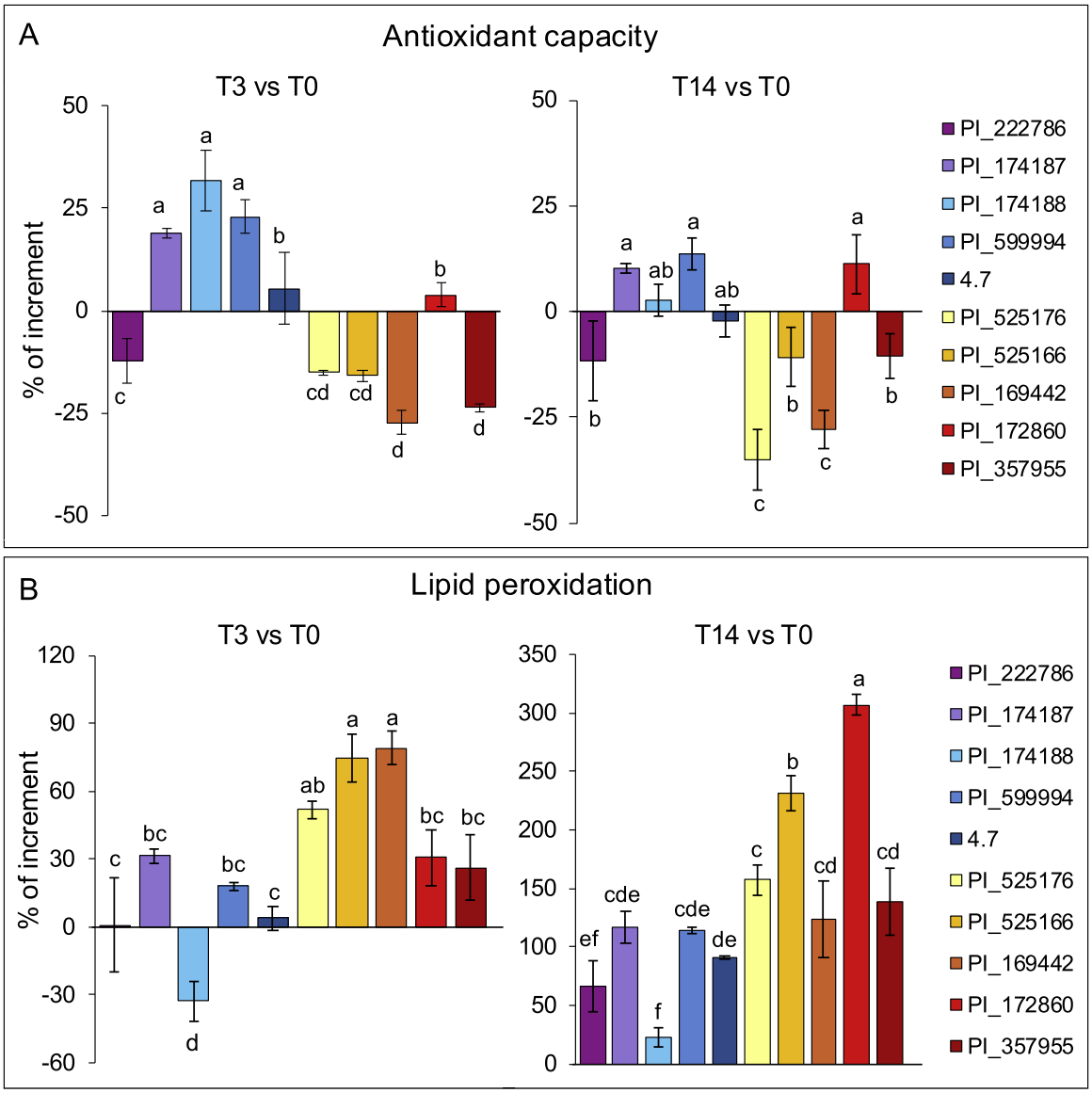

Fig. 2. Evaluation of fruit exocarp response to cold storage from each cultivar based on (A) antioxidant capacity measured by FRAP assay and (B) lipid peroxidation assessed by MDA content, after 3 and 14 days of storage relative to T0. Values are expressed as percentage change relative to T0 to account for baseline variability among cultivars. Bluish indicate cold-tolerant cultivars (222786, 174187, 174188, 599994, and 4.7), while reddish indicate cold-sensitive cultivars (525176, 525166, 169442, 172860, and 357955). Errors bars represent the mean ± standard error (SE). Different letters denote statistically significant differences between samples, as determined by post hoc comparison ($p < 0.05$).

These pronounced differences observed at T3 were slightly attenuated at the end of cold storage. However, the antioxidant capacity of fruits from T-cultivars was maintained at T14, whereas a significant diminution was again observed in S-cultivars, particularly in PI 525176 (−35 %) and PI 169442 (−28 %) (Fig. 2A).

A rapid response in maintaining membrane integrity was also observed, as indicated by lipid peroxidation levels at T3. T-cultivars maintained MDA levels comparable to those at T0, with PI 174188 even showing a significant reduction of −32.8 %. In contrast, S-cultivars exhibited a marked increase in MDA content, with PI 525166 and PI 169442 almost doubling their initial levels (Fig. 2B). This trend persisted until T14, with PI 174188 continuing to exhibit the lowest lipid peroxidation levels. Meanwhile, PI 525166 and PI 172860 displayed substantial increases in MDA content, rising by 231 % and 307 %, respectively (Fig. 2B).

3.3. Multivariate statistical analysis reveals the hierarchical impact of cold storage on cultivar metabolic profiles

Exocarp samples from the evaluated cultivars were collected at T0, T3, and T14 and subjected to an untargeted metabolomic approach to investigate the effects of cold stress in each cultivar at a metabolome-wide level. Ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight high-resolution mass spectrometry (UHPLC/QTOF-HRMS) enabled the detection of 10,308 entities across the analyzed extracts. Of these, 1694 were putatively annotated against the FooDB database: 137 entities were annotated with MS^2 fidelity, 47 entities with MS^2 (in-silico) fidelity, and 1509 entities were reported at the MS-only level (Table S1). To explore the metabolic variability between cultivars and the impact of fruit cold storage, an unsupervised hierarchical cluster analysis (HCA) was conducted (Fig. 3). The clustering patterns indicated that cultivar-specific cold tolerance exerted the strongest influence on the exocarp metabolome. Specifically, T-cultivars predominantly clustered on the right side of the dendrogram, while S-

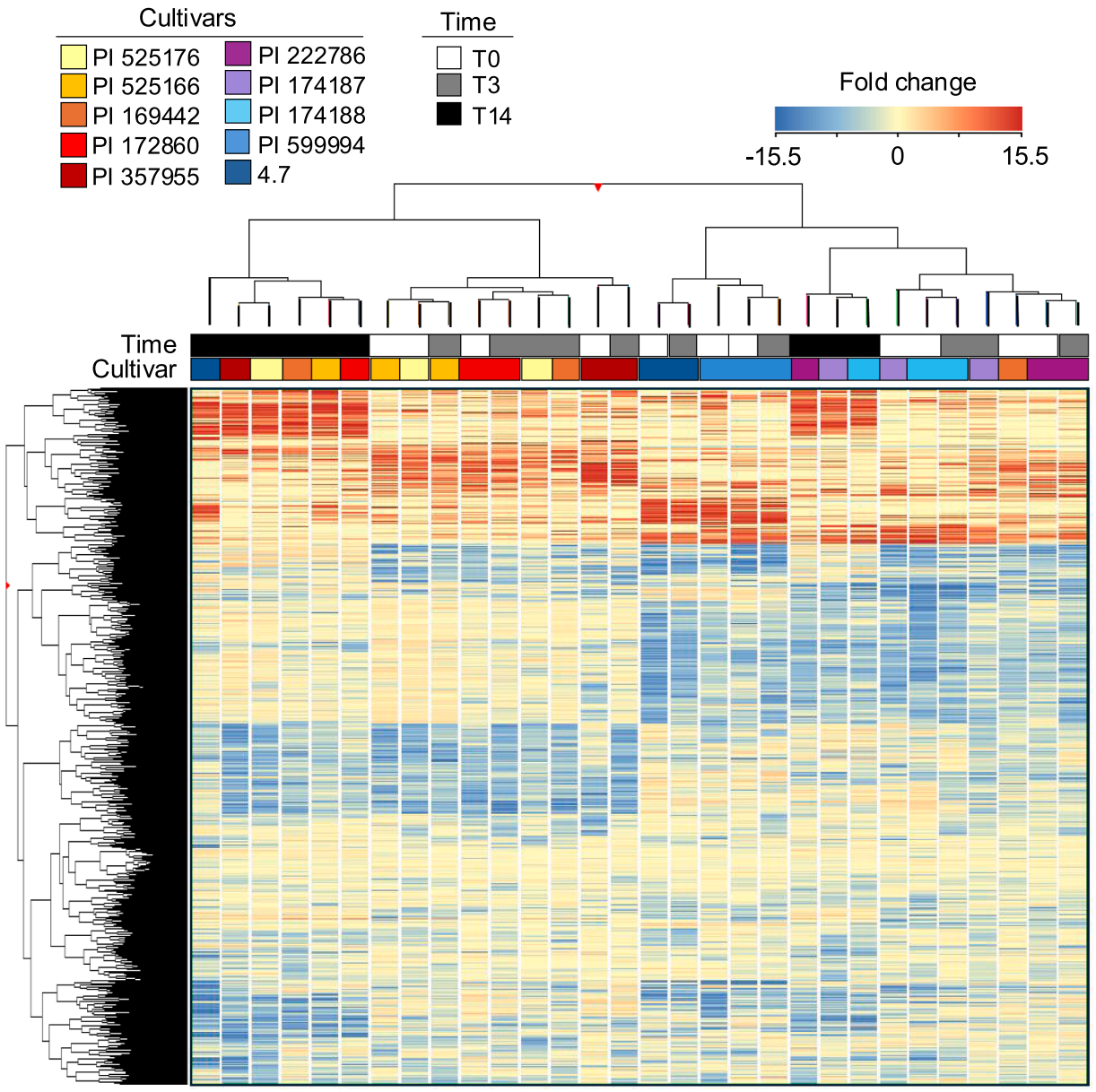

Fig. 3. Hierarchical cluster analysis (HCA) of the untargeted metabolic profile of zucchini fruit exocarp under cold storage. Greyish colors identify each cold storage period with light, medium and dark for T0, T3, and T14, respectively. Bluish colors correspond to tolerant cultivars (222786, 174187, 174188, 599994, and 4.7), whereas reddish colors correspond to sensitive cultivars (525176, 525166, 169442, 172860, and 357955). Clustering was performed according to the fold change-based heatmap, which was baselined to the median values of all samples (Euclidean distance, Ward's linkage algorithm).

cultivars grouped on the left (Fig. 3). Only the T0 samples of PI 169442 (S-cultivar) clustered with the tolerant group, suggesting that its baseline metabolome exhibited characteristics associated with cold tolerance. However, the progression of cold storage led to pronounced metabolomic alterations that likely contributed to increased susceptibility to cold stress in this cultivar. A similar trend was observed in the inbred line 4.7, which initially clustered with tolerant samples at T0 and T3. Nevertheless, at T14, its metabolomic profile shifted significantly, resulting in its clustering with the sensitive cultivars. These findings highlight the dynamic nature of the metabolomic response to cold stress and underscore the importance of temporal modulation in determining cultivar-specific tolerance.

Samples within S-cultivars exhibited a clear time-dependent clustering pattern, with T14 forming a distinct subcluster, separated from T0 and T3 (Fig. 3). This temporal segregation suggests that cold storage had a pronounced impact on the metabolomic profiles of these cultivars, leading to a convergent response at T14 regardless of genetic background. In contrast, such a strong time-dependent clustering effect was observed in only three T-cultivars. Notably, all samples of PI 599994 formed a separate cluster, as did the T0 and T3 samples of the inbred line 4.7, implying a cultivar-specific modulation of defense mechanisms against cold stress. These findings suggest that chilling injury had a comparatively lower impact on the metabolomic profile of the tolerant group, suggesting a more stable or adaptive metabolic response to prolonged cold exposure.

3.4. Identification of metabolomic markers in response to the cold storage of zucchini

To gain further insight into the impact of cold storage on the metabolome of the fruit of each cultivar, a supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) was conducted. Regarding T-cultivars, all OPLS-DA models exhibited high-quality parameters in terms of goodness-of-fit ($R^2Y > 0.997$) and

goodness-of-prediction ($Q^2 = 0.808 – 0.969$) (Table S2). However, cross-validated ANOVA (CV-ANOVA) results indicated that significant metabolomic alterations ($p < 0.05$) were only evident after prolonged cold exposure, suggesting that the metabolome of T-cultivars remained relatively stable under short-term cold stress. In contrast, S-cultivars exhibited stronger model performance ($R^2Y > 0.998$; $Q^2 = 0.904 – 0.971$), with significant metabolomic changes detected not only at T14, but also at short-term. Specifically, cultivars PI 525166, PI 172860, and PI 357955 showed significant alterations at T3, underscoring a heightened sensitivity to cold stress (Table S2). Collectively, these findings support the notion that cold tolerance mechanisms in T-cultivars confer short-term protection to fruit metabolism, whereas such mechanisms appear weak in the S-cultivars under study.

To identify the specific metabolites driving group separation in each OPLS-DA model, a Variable Importance in Projection (VIP) analysis was performed. The comprehensive list of compounds for each model, together with their VIP score and fold-change, is included in Supplementary Table S2. Metabolites with VIP scores > 1.4 were considered key discriminant features (VIP markers). Due to the pronounced metabolic diversity among cultivars, a wide range of differentially accumulated metabolites was identified during cold storage, with each cultivar exhibiting a distinct metabolic response. To discern common metabolic responses associated with cold tolerance, VIP markers were further analyzed using Venn diagrams, comparing tolerant and sensitive cultivars separately after 3 and 14 days of cold storage (Fig. 4). As shown in Fig. 4, each cultivar modulated its response to cold stress uniquely, with only a limited number of metabolites being commonly accumulated. At T3, tolerant cultivars shared a greater number of VIP markers (10) compared to sensitive cultivars (5). This trend was reversed at T14 days of cold exposure, when 4 and 12 common VIP markers were found in the fruit T- and S-cultivars, respectively. Interestingly, some of these metabolites were similarly accumulated in T- and S-cultivars at T3; however, by T14, the profiles diverged completely. In fact, three metabolites (N6,N6,N6-Trimethyl-L-lysine, 6-hydroxymethyl-7,8-dihydropterin and 2,12–2,12-Tetradecadiene-4,6,8,10-tetrayne) were consistently up-accumulated in all cultivars at T3, maintaining induction until T14 in T-cultivars. This pattern highlights their potential role as core metabolic indicators of long-term cold tolerance in zucchini fruit. Conversely, the metabolites uniquely induced in S-cultivars at T14 may serve as candidate biomarkers of chilling injury (Fig. 4), offering potential targets for future research on stress mitigation strategies.

3.5. *Chemical enrichment analysis identifies cultivar-dependent metabolic functions during cold storage*

To gain deeper insights into the metabolome-wide shifts for each cultivar by cold storage, a chemical enrichment analysis (ChemRICH) was performed, considering the differentially accumulated metabolites (DAMs) provided by Volcano analysis ($p < 0.01$ and FC cut-off = ±3) (Figure S1). To provide a comprehensive overview, the increase ratio (IR, defined as the proportion of significantly accumulated metabolites within each cluster due to each cold storage timepoint, T3 and T14 versus T0) of each metabolic class was visualized through a heatmap, summarizing the outcomes of all ChemRICH analyses across cultivars and time points (Fig. 5). The full list of DAMs is reported in Table S3, together with the corresponding clusters and their key metabolites.

Regarding the metabolic classes associated with lipid metabolism, a general up-accumulation was reported during cold storage, particularly among acylglycerides (monoacylglycerols (MAGs), diacylglycerols (DAGs), and triacylglycerols (TAGs)) as well as saturated fatty acids (SFAs) (Fig. 5). In contrast, terpenoid induction exhibited broad down-accumulation across all cultivars in response to cold stress. An exception was observed in the case of triterpenoids, which consistently accumulated in all S-cultivars at both T3 and T14. In T-cultivars, however, triterpenoid accumulation was induced later, becoming evident only at the final stage of cold storage.

Cold storage also affected the regulation of nitrogen-containing compounds (NCCs), particularly through the induction of alkaloid accumulation. In this regard, alkaloid accumulation was observed at T3 across all cultivars (IR= 0.5 – 1) and was maintained in T-cultivars until T14 (IR= 0.6 – 0.8) (Fig. 5). Conversely, a pronounced down-accumulation of nucleotides was noted in S-cultivars at T14 (IR= 0 – 0.2). Among the most affected compounds were: biotinyl-5′-AMP and FADH (Table S3). In contrast, the T-cultivar PI 174188 showed an IR=0.8, accumulating FADH and enhancing the ATP signaling, pointing to diadenosine triphosphate and ADP. This response was maintained through to T14, with further accumulation of key nucleotides including dGTP and diadenosine triphosphate.

A notable down-accumulation of phenolic compounds was observed in S-cultivars, with only isolated cases of transient up-accumulation. In contrast, three T-cultivars (PI 174187, 174188, and 599994) showed an important accumulation of phenolic compounds in response to cold stress, likely inducing the phenylpropanoid pathway. These cultivars consistently up-accumulated flavonoid glucosides (IR= 0.5 – 1) and phenolic glucosides (IR= 0.8 – 1) at both T3 and T14. Moreover, they showed an important accumulation of additional phenolic subclasses, including phenylpropanoids, phenolic acids, flavonoids, alkylphenols, and lignans throughout the cold storage period (Fig. 5).

Regarding primary metabolism-related compounds, a general up-accumulation of amino acids and dipeptides was observed in response to cold storage, except for the S-cultivar PI 357955, which exhibited a marked down-accumulation of these compounds. In terms of phytohormonal regulation, a consistent down-accumulation of kinetin was detected across all cultivars by the end of the cold storage period. Other phytohormones displayed cultivar- and time-specific regulation. Notably, indole-3-acetyl-isoleucine (a conjugated auxin derivative) was significantly accumulated in T-cultivars PI 222786 and PI 174187 at both T3 and T14, whereas the S-cultivar PI 357955 exhibited a pronounced downregulation of this compound at T14. Moreover, a differential mobilization of salicylates was observed in T-cultivars, characterized by the down-accumulation of salicylic acid 2-β-D-glucoside in PI 222786 and in the inbred line 4.7 at T3 and T14. Concurrently, the salicylate derivative 2-(10-heptadecenyl)-6-hydroxybenzoic acid was up-accumulated in the T-cultivars PI 222786 and PI 174187, while it was down-accumulated in the S-cultivar PI 169442.

Other potential contributors to cold tolerance are vitamins. This group was generally increased across all cultivars (IR= 0.7 – 1), pointing to the accumulation of alpha-tocopherol acetate and formiminotetrahydrofolic acid at both T3 and T14 in response to cold storage. Additionally, pyridoxal (Vitamin B6) exhibited cultivar-specific induction, being markedly accumulated in T-cultivars at T3 (PI 599994) and at T14 (PIs 222786, 174187, and 174188), with no significant induction detected in S-cultivars (Table S3), resulting also as a VIP marker for these cultivars (Table S2).

Finally, the induction of cucurbitacins was also cultivar dependent, with a pronounced up-accumulation of cucurbitacin C at both T3 and T14 in the S-cultivars PI 525176 and PI 525166. Remarkably, the inbred line 4.7 exhibited a unique response, significantly enhancing the accumulation of multiple cucurbitacin classes, including cucurbitacin I and cucurbitacin B at T14, being the only genotype with broad-spectrum induction (IR=1) within this metabolite family.

To further investigate the relationships between metabolomic responses and physiological performance during cold storage, a Pearson correlation analysis was conducted between ChemRICH metabolite classes (expressed as increase ratio, IR) and the physiological parameters CI, WL, MDA, and FRAP (Figure S2). As expected, CI, WL, and MDA showed strong positive correlations among themselves ($r = 0.75–0.92$), indicating a close association between chilling injury development, fruit dehydration, and oxidative damage. These parameters were negatively correlated with several classes of specialized metabolites, including flavonoids, phenylpropanoids, and phenolic glucosides ($r = −0.42$ to $−0.67$), as well as lignans ($r = −0.55$), steroids ($r = −0.74$), and amino

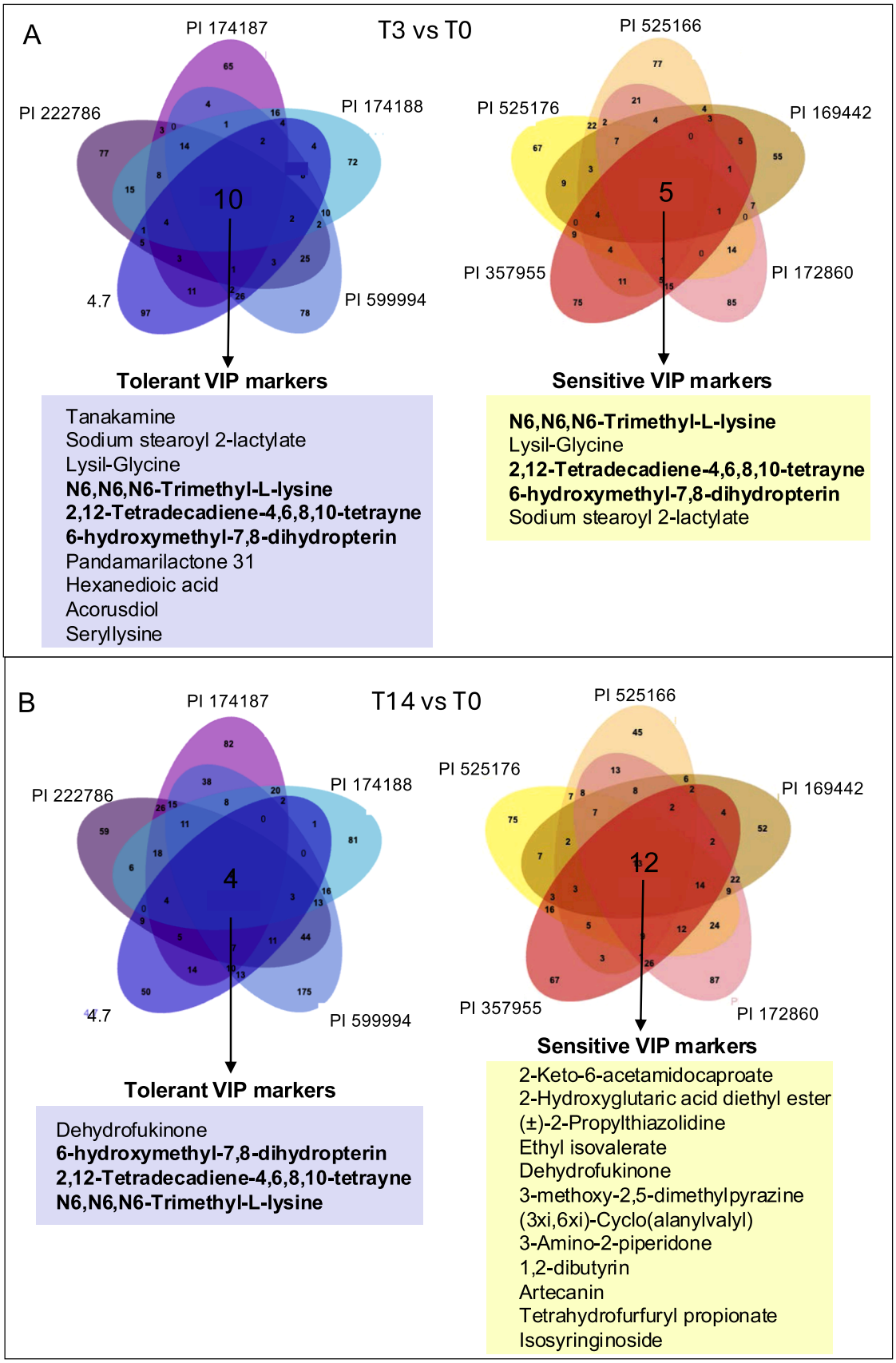

Fig. 4. Venn diagram showing Variable Importance in Projection (VIP) markers (VIP score > 1.4) identified as discriminant metabolites in the OPLS-DA models comparing tolerant (T-cultivars) and sensitive (S-cultivars) zucchini accessions. Comparisons were performed independently for T3 vs T0 (A) and T14 vs T0 (B). Bluish colors correspond to tolerant cultivars (222786, 174187, 174188, 599994, and 4.7), whereas reddish colors correspond to sensitive cultivars (525176, 525166, 169442, 172860, and 357955). Shared VIP markers between the two storage times are highlighted in bold.

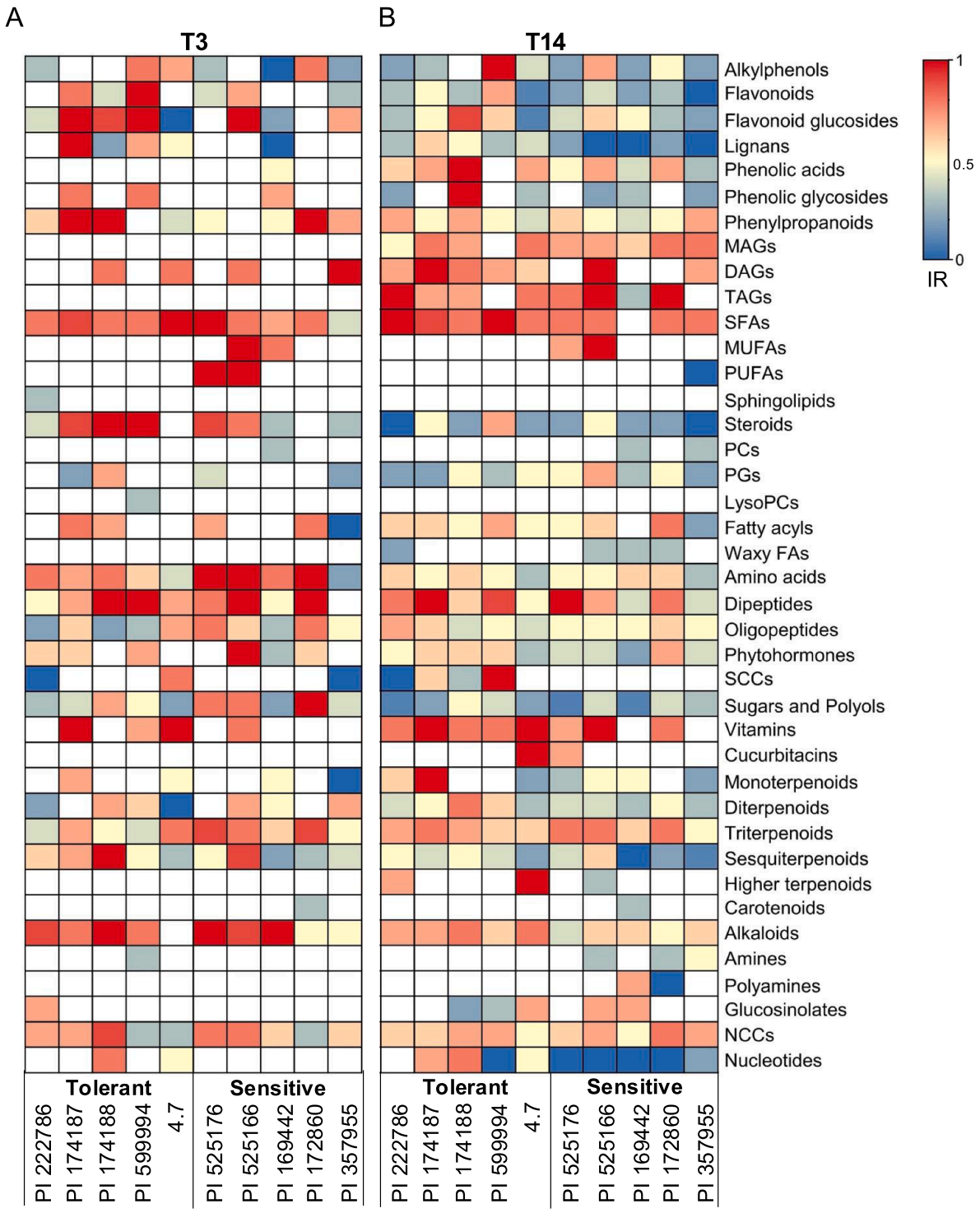

Fig. 5. Heatmap representing the impact ratio (IR) of differential metabolite classes identified through ChemRICH enrichment analysis across tolerant and sensitive zucchini cultivars during cold storage. Comparisons correspond to T3 vs T0 (A) and T14 vs T0 (B). Columns represent cultivars and rows represent metabolite classes. The IR value indicates the proportion of significantly altered metabolites within each class, where higher values (red) indicate a greater proportion of up-accumulated metabolites and lower values (blue) indicate a predominance of down-accumulated metabolites.

acids ($r = -0.46$). Conversely, FRAP showed positive correlations with several of these classes, particularly lignans ($r = 0.52$), nucleotides ($r = 0.40$), and flavonoids ($r = 0.43$), suggesting a contribution of these metabolites to the antioxidant capacity of zucchini fruit during cold storage. Additionally, strong positive correlations were observed among certain metabolite classes, including cucurbitacins with vitamins ($r = 0.91$), as well as with lipid-related classes such as MUFAs and waxy fatty acids ($r = 0.86$–0.94).

4. Discussion

4.1. Metabolic markers associated with cold storage

Exploring the natural variability conserved in germplasm banks is

essential for developing novel breeding strategies that address global agricultural challenges. Multi-omics analysis is being used in the search for key genes regulating metabolites associated with agronomic interest traits (Habibi et al., 2024). In recent years, metabolomic approaches have been applied for this purpose, contributing to the enhancement of nutritional quality and postharvest management in crops, such as palm fruit, cassava, banana, strawberry or sweet cherry (Karagiannis et al., 2018; Kim et al., 2023; Yin et al., 2022). In this context, the present study aims to uncover the metabolomic responses, common or independent, during cold storage behind the differences observed for quality maintenance and shelf life in tolerant and sensitive zucchini cultivars. The results revealed a differential induction of antioxidant defense among cultivars, which was accompanied by a distinct modulation of lipid peroxidation as a stress marker during the postharvest period. The metabolic variability of zucchini fruit was assessed across 5 cold-sensitive and 5 tolerant cultivars, identifying cold tolerance as the principal factor differentiating the samples. Notably, cold storage induced a distinct metabolic reprogramming in sensitive cultivars, leading to the clustering of all T14 samples into a separate subgroup. These results are consistent with other studies carried out in sensitive cultivars of zucchini fruit (Castro-Cegrí et al., 2024b, 2025a).

After investigating the metabolites commonly induced in both tolerant and sensitive cultivars after 3 and 14 days of cold storage using OPLS-DA models, several compounds were identified as potential markers during cold storage. Among these, the accumulation of N6,N6,N6-trimethyl-L-lysine, an essential intermediate in the carnitine biosynthesis pathway in plants (Rippa et al., 2012), was one of the most prominently induced in response to chilling stress, being up-accumulated after 14 days of cold storage just in T-cultivars. This finding may indicate an active carnitine biosynthetic process, a pathway associated with mitochondrial metabolism and fatty acid transport, and therefore potentially related to energy balance under stress conditions (Kerner and Hoppel, 2000). Recently, carnitine application was studied in tomato and strawberry fruit, enhancing vitamin C but non improving ripening (Henschel et al., 2025). In zucchini fruit, carnitine metabolism was also related to cold tolerance due to riboflavin treatment (Castro-Cegrí et al., 2025a). Therefore, further investigation of N6,N6,N6-trimethyl-L-lysine and its role in carnitine biosynthesis could help to elucidate its contribution to postharvest cold tolerance, particularly given the limited number of studies addressing this pathway under chilling stress conditions. In parallel, another metabolite identified as a marker for cold tolerance was 6-hydroxymethyl-7,8-dihydropterin, a key intermediate in folate biosynthesis derived from pterins. These compounds function as cofactors, with 5-methyltetrahydrofolate serving as the methyl donor for the conversion of homocysteine to methionine, a precursor of S-adenosylmethionine and ethylene biosynthesis (Wang et al., 2002). Moreover, ethylene has been associated with postharvest cold tolerance in zucchini fruit (García et al., 2020; 2024; Megías et al., 2016b). Previous studies revealed that folic acid and ethylene affects postharvest ripening of fruits as tomato or avocado (Garza-Aguilar et al., 2020), and interestingly, folic acid mitigated chilling injury, increasing antioxidant defense in tomato (Ghorbani et al., 2025). Therefore, the ability of cold-tolerant cultivars to maintain elevated levels of 6-hydroxymethyl-7,8-dihydropterin during prolonged cold storage could be exploited to develop strategies to enhance the shelf life of zucchini fruit through increased folate biosynthesis. Thus, the simultaneous accumulation of these two metabolites may reflect a coordinated metabolic adjustment to cold stress, integrating energy-related and redox-related processes, rather than the activation of two unrelated pathways. However, their precise functional contribution to postharvest cold tolerance remains to be experimentally validated. Finally, tetradecadiene-4,6,8,10-tetrayne also exhibited a similar pattern of accumulation. This unsaturated hydrocarbon, containing both alkene and alkyne groups, is likely involved in the composition of cuticular waxes, which form the first barrier against oxidative damage during postharvest storage in fruits such as citrus, litchi, and zucchini (Carvajal et al., 2021; Huang et al., 2022; Yang et al., 2022).

Future studies should aim to validate the identity of the key metabolites identified in this work using authentic reference standards and targeted metabolomics approaches, in order to further confirm their role as biomarkers of postharvest cold tolerance.

4.2. *Differential metabolic class induction during cold storage in zucchini cultivars*

Based on wide-scale metabolomic changes detected through ChemRICH analysis, several metabolite classes were identified as being significantly affected by cold storage during the postharvest period across all evaluated zucchini accessions. Among these, a general up-accumulation of acylglycerides and saturated fatty acids (SFAs) was observed. Membrane stability is highly dependent on the composition and regulation of membrane lipids, including phospholipids, diacylglycerols (DAGs), and triacylglycerols (TAGs). Lipid degradation, which compromises membrane integrity, is a major cause of fruit damage under low-temperature conditions, as previously reported in peach or mango (Hsiao et al., 2026; Song et al., 2022). Cold storage adversely affects membrane stability, leading to increased lipid peroxidation across all zucchini cultivars. However, T-cultivars exhibited reduced membrane damage and greater antioxidant capacity compared to S-cultivars. Furthermore, cold storage directly promoted the accumulation of SFAs in all cultivars analyzed. Since SFAs reduce membrane fluidity and stability, their accumulation negatively impacts fruit shelf life and storability, as has been observed in crops, such as banana and cucumber (Zhang et al., 2022; Zhang et al., 2022).

Regarding redox balance and energy metabolism, a general down-accumulation of $FADH_2$ was observed during the cold storage period in S-cultivars. This reduced coenzyme entering the electron transport chain serves to the generation of ATP (Pappas et al., 2023) and participates in key steps of the tricarboxylic acid cycle, catalyzing the synthesis of fumaric acid (Martínez-Reyes and Chandel, 2020). This metabolic adaptation of energy management has also been associated with improved fruit quality maintenance in mango (Huang et al., 2024) or zucchini (Palma et al., 2019).

Another key mechanism underlying the induction of chilling tolerance in fruit is the enhancement of the non-enzymatic antioxidant response through activation of the phenylpropanoid pathway, which drives the synthesis of phenolic compounds (Wang et al., 2021). With this aim, various postharvest treatments have been shown to preserve fruit quality by promoting polyphenol accumulation, such as a melatonin treatment in sweet cherry (Michailidis et al., 2021), or abscisic acid (ABA) and riboflavin treatments in chilling-sensitive zucchini varieties (Castro-Cegrí et al., 2024a, 2023). In the present study, several T-cultivars (PI 174187, 174188, and 599994) exhibited similar metabolic profiles, as revealed by cluster analysis, with a pronounced accumulation of polyphenols, pointing to an induction of phenolic compounds biosynthesis. These cultivars showed significant up-accumulation of flavonoid glucosides, phenolic glucosides, phenylpropanoids, and phenolic acids. Remarkably, these accessions also recorded the highest antioxidant capacity at T3, which likely contributed to delaying the appearance of chilling injury symptoms in zucchini fruit by increasing phenolic content, as was also proved in other fruits during the postharvest period (Liu et al., 2024). Consistently, the correlation analysis between metabolite classes and physiological traits revealed negative associations between phenolic-related classes (flavonoids, phenylpropanoids and phenolic glucosides) and chilling injury indicators such as CI, WL and MDA, while showing positive relationships with antioxidant capacity (FRAP), supporting their potential role in mitigating oxidative damage during cold storage.

The modulation of amino acids and dipeptides represents a key mechanism of tolerance during the postharvest period, as these compounds play critical roles in stress response pathways in fruits, such as strawberry and kiwi (Lv et al., 2022; Salzano et al., 2019). Our findings

further support the relevance of this mechanism in zucchini fruit, as it appeared to be consistently induced across all evaluated cultivars, regardless of their morphological and genomic differences.

In relation to phytohormonal regulation, a general down-accumulation of kinetin was detected across all cultivars in response to cold storage, which may impact the postharvest maintenance of zucchini fruit, as has been observed in banana or tomato (Chen et al., 2025; Ghimire et al., 2021). Nevertheless, phytohormone responses displayed cultivar- and time-specific patterns, since cold storage affected the accumulation of auxins and salicylate derivatives in the tolerant cultivars PI 222786 and PI 174187. Increased auxin levels have been associated with alleviating chilling injury and delaying tomato fruit softening by affecting phytohormonal regulation and cell wall metabolism (Liu et al., 2025). Additionally, the accumulation of salicylate derivatives in these T-cultivars (PI 222786 and PI 174187) may contribute to alleviating postharvest chilling injuries, as previously reported in tomato and cucumber (Aghdam et al., 2014; Zhang et al., 2015).

A widespread up-accumulation of vitamins was observed in response to cold storage, with folates, whose role has been previously characterized, and tocopherols emerging as the most strongly up-accumulated across cultivars. Tocopherols (Vitamin E), a class of plant-derived isoprenoids with potent antioxidant properties, have been associated with enhanced chilling tolerance during the postharvest period in fruits, such as mandarin and grapefruit (Rey et al., 2021b, 2021a). Notably, a recent study identified an avocado variety with elevated tocopherol levels, highlighting its potential role in cold storage performance across a diverse panel of genotypes (Vincent et al., 2020). Given the observed accumulation of tocopherols in response to cold storage, further research is needed to determine whether inherent or cold-induced tocopherol levels directly contribute to enhanced postharvest tolerance in zucchini cultivars. In addition, pyridoxal (Vitamin B6) was generally up-accumulated in the T-cultivars. This vitamin has been shown to improve postharvest quality and extend shelf life in apples by promoting cell wall biosynthesis, thus enhancing firmness, and by downregulating oxidative enzymes such as polyphenol oxidases, peroxidases, and lipoxygenases, ultimately reducing MDA levels (Ali et al., 2024). These findings suggest that pyridoxal may be involved in the maintenance of postharvest quality in cold-tolerant zucchini cultivars, potentially explaining the associated significant reductions observed in MDA content. Interestingly, vitamins also displayed strong correlations with several lipid-related classes, including MUFAs and waxy fatty acids, suggesting coordinated metabolic responses potentially linked to membrane stability and oxidative balance during cold storage.

In contrast to previous findings, the chilling tolerance observed in the inbred line 4.7 did not appear to be primarily driven by a wide range of metabolomic changes, since its overall metabolic profile after 14 days of cold storage was similar to that of sensitive cultivars. However, only this cultivar exhibited a notable increase in cucurbitacin accumulation, specifically in cucurbitacin I and B levels. These compounds are highly oxidized tetracyclic triterpenoids associated with the bitter taste characteristic of the Cucurbitaceae family (Shang et al., 2014), and have been described to be involved in herbivore defense and preventing plant diseases (Brzozowski et al., 2020; Zhong et al., 2022). In addition, the antioxidant capacity of these compounds also confers more resistance to abiotic stresses, as is the case with cold-induced stress. Although the physiological roles of these compounds in fruit remain poorly understood, cucurbitacin I has been positively correlated with improved leaf gas exchange and chlorophyll fluorescence, suggesting a potential role in drought tolerance mechanisms in *Lagenaria siceraria* plant (Mashilo et al., 2018). In fact, *Lagenaria siceraria* accessions with increased cucurbitacin accumulation were more resistant to drought stress (Mkhize et al., 2023). Additionally, the therapeutic properties of these compounds are being investigated, showing a potential effect of cucurbitacins in cancer treatments (Peña et al., 2024; Xu et al., 2022). Interestingly, cucurbitacins showed strong positive correlations with vitamins and lipid-related classes such as MUFAs and waxy fatty acids, suggesting a coordinated metabolic response during cold storage. Although the physiological role of cucurbitacins in postharvest stress responses remains poorly understood, these associations may indicate their participation in broader metabolic adjustments linked to cold stress. These novel findings position the inbred line 4.7 as a promising candidate for future studies aimed at uncovering the role of cucurbitacins I and B in modulating postharvest cold tolerance of zucchini fruit.

Overall, the metabolomic responses observed in this study are consistent with physiological mechanisms commonly associated with chilling injury in fruits, including membrane lipid remodeling, alterations in energy metabolism, and modulation of antioxidant defenses during cold stress. Within this framework, our results highlight several metabolite classes potentially associated with cultivar-dependent cold tolerance in zucchini fruit. These metabolites could represent promising candidates for the development of biochemical markers useful in high-throughput phenotyping or metabolite-assisted selection strategies aimed at improving postharvest cold tolerance. Moreover, integrating metabolomic markers with genomic approaches may facilitate the identification of candidate genes controlling cold stress responses in zucchini. However, it should be noted that the present study identifies statistical associations rather than direct causal relationships. Future work combining targeted metabolomics with transcriptomics approaches (RNA-seq), as well as functional validation experiments, such as exogenous metabolite application, gene expression analyses, or genetic manipulation, will be necessary to confirm the biological role of key metabolites identified here, including compounds such as N6-trimethyl-L-lysine or cucurbitacins.

5. Conclusions

This metabolomics-driven study reveals both shared and cultivar-specific biochemical mechanisms underlying postharvest cold tolerance in zucchini fruit. Across genotypes, we identified a conserved cold-storage metabolic signature characterized by the induction of N6,N6,N6-trimethyl-L-lysine and 6-hydroxymethyl-7,8-dihydropterin, a response that persisted until T14 only in cold-tolerant cultivars. These findings point to core protective modules associated with improved metabolic homeostasis under chilling conditions.

Cold-tolerant accessions also maintained higher antioxidant capacity and lower lipid peroxidation, consistent with their metabolomic profiles. In particular, tolerant cultivars exhibited coordinated adjustments in amino acids and vitamins, including folate and pyridoxal, supporting a more stable energy and redox balance during storage. Additionally, tolerant lines displayed a selective reinforcement of specialized metabolites, marked by the accumulation of flavonoid and phenolic glucosides and lignans, together with targeted modulation of phytohormonal pathways involving auxin conjugates and salicylates. Cucurbitacins emerged as genotype-dependent traits, with the inbred line 4.7 uniquely up-accumulating cucurbitacins B and I throughout storage.

Collectively, these metabolomic and physiological shifts are consistent with the reduced chilling injury and lower weight loss observed in tolerant cultivars, providing chemically actionable biomarkers to support rapid screening, breeding strategies, and the preservation of functional and nutritional quality. Future work should aim to establish causal roles for key metabolites and to validate the robustness of these biomarkers across seasons, environments, and postharvest supply chains.

CRediT authorship contribution statement

Alicia García: Writing – review & editing, Writing – original draft, Investigation. **Alejandro Castro-Cegrí:** Writing – review & editing, Writing – original draft, Methodology. **Pascual García-Pérez:** Software, Methodology. **Francisco Palma:** Validation, Supervision. **Cecilia Martínez:** Validation, Supervision. **Dolores Garrido:** Writing – review &

editing, Resources, Project administration. **Manuel Jamilena:** Writing – review & editing, Resources, Project administration. **Luigi Lucini:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

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

Acknowledgements

This work was supported by research grant PID2020-118080RB-C21 at UAL and PID2020-118080RB-C22 at UGR, funded by 'Ministerio de Ciencia, Innovación y Universidades' together with EU FEDER funds. Pascual García-Pérez thanks the financial support through the Ramón y Cajal program (reference: RYC2023-044123-I) by the Spanish Ministry of Science, Innovation and Universities, the National Research Agency (MCIU/AEI/10.13039/501100011033) and the European Social Fund Plus (FSE+).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2026.114766.

Data availability

Data will be made available on request.

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