

Research Paper

Seasonal fluctuation of fruit quality is differentially affected by mediating mineral nutrients and targeted major metabolites in greenhouse grown bell pepper (*Capsicum annuum* L.) cultivars

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ABSTRACT

As bell peppers are produced year-round via greenhouse-based technology, the fruit quality of bell peppers fluctuates greatly depending on the bell pepper cultivar during winter. Thus, this study aimed to evaluate the seasonal fluctuation effects on fruit quality attributes, mineral nutrients, and targeted major metabolites in the 'Scirocco', 'DSP7054', and 'Volante' bell pepper cultivars grown in greenhouse cultivation system from September to June. Overall, fruit physiological and biochemical performance clearly diverged depending not only on bell pepper cultivar, but also on harvest time. The results of the partial least square-discriminant analysis (PLS-DA) loading plot indicated that amino acids were closely associated with fatty acids, however less associated with most mineral nutrients, chlorophylls, and physiological fruit quality attributes. According to the results of the variable importance in projection (VIP) score plot, 12 variables were highly important during winter season, regardless of bell pepper cultivars. While pericarp colour variables differentially responded to the pericarp colour of bell pepper cultivars, fruit quality attributes, soluble carbohydrates, citric acid, malic acid, glutamine, K, Mg, and total antioxidant scavenging activities were highly variable depending on bell pepper cultivar and sampling time during the winter season. The results of the correlation coefficient networks indicated that the overall fruit performance based on the results of physiological fruit quality attributes, mineral nutrients, and targeted major metabolites was highly different depending on the bell pepper cultivar. Thus, the overall pattern of correlation coefficient network was much more distinctive in 'Volante' cultivar than in 'Scirocco' and 'DSP7054' cultivars during the winter season. Therefore, the results indicate that fruit quality and performance derived from mineral nutrients and targeted major metabolites fluctuated depending on the bell pepper cultivar during the winter season.

1. Introduction

Bell peppers, also known as paprika, are widely cultivated, globally produced and characterised by their blocky shape, pericarp colour, and moderate taste (Kim et al., 2016; Kye et al., 2022). The bell pepper fruit contains a wide range of nutrients and bioactive compounds with beneficial health effects (Kim et al., 2011; Matsufuji et al., 1998; Rosa-Martínez et al., 2021; Saini and Keum, 2016). As annual or perennial crops, bell peppers can be cultivated in open fields (Juroszek and Tsai, 2009; Kabir et al., 2022) or greenhouses (Kim et al., 2023a, 2023b). Furthermore, protected cultivation technology derived from

greenhouse-based cultivation systems has been actively applied to the cultivation and production of bell peppers, along with state-of-the-art management control systems (Jung et al., 2021; Kim et al., 2023a; Nam et al., 2019). Under a highly advanced management system for greenhouse facilities, nutrition, irrigation, temperature, and irradiation management are sustainably under the control of better fruit quality and higher bell pepper yield (Jung et al., 2021; Shin and Son, 2015). Nonetheless, an irregular pattern of bell pepper production based on an imbalanced relationship between fruit set, plant sink, and source strength has occurred in a greenhouse-based cultivation system during the winter season (Marcelis et al., 2004). Thus, fluctuations in bell

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pepper fruit yield contribute to reducing the fruit quality of bell pepper cultivars (Heuvelink et al., 2004).

Fruit quality attributes highly fluctuate not only by growing season but also by bell pepper cultivar (Byeon et al., 2023). In addition, the incidence of fruit physiological disorders differs depending on the growing season, and the disorders are much more severe in blocky bell pepper cultivars than in conical cultivars (Byeon et al., 2023). The mean fruit size, marketable fruit ratio, and marketable yield fluctuated for three-year cultivation period, depending on the coloured bell pepper cultivars in the high tunnel cultivation system (Sideman, 2020). In addition, the marketable fruit number per plant is highly diverse among bell pepper cultivars, whereas marketable fruit weight per plant and total fruit weight per plant are not affected differently (Lang et al., 2020). However, with respect to yield traits and fruit quality parameters, sweet pepper cultivars not only affect fruit number per plant and total fruit yield, but also influence fruit length, fruit diameter, fruit thickness, and fruit fresh weight. With regard to fruit nutritional quality attributes, vitamin C content, titratable acidity (TA), soluble solids content (SSC), and total sugar ratio are clearly different among sweet pepper cultivars (Alsadon et al., 2013; Ibrahim et al., 2019). The incidence of bacterial soft rot is highly dependent on the ripe fruit of bell pepper cultivars and diversity of the skin colour (Díaz-Pérez, 2014). Thus, at harvest, fruit quality and marketable yield could be significantly affected by bell pepper cultivars.

The levels of mineral nutrients respond diversely to skin colour pigmentation during fruit ripening and maturation, depending on the bell pepper cultivar at harvest (Simonne et al., 1997). The content of individual mineral nutrients present in the bell pepper is diversely affected by numerous pre-harvest factors, including cultivar, cultivation location, and growing year in red pepper fruit (Kim et al., 2019), irrigation level and calcium fertilisation in bell pepper fruit (Díaz-Pérez and Hook, 2017), and fruit maturity and agricultural practices in sweet pepper fruit (Pérez-López et al., 2007). Although the contents of Ca and Sr do not differ between cultivars, the levels of Fe, S, and Zn are highly affected by the bell pepper cultivar (Abbasi et al., 2022). Moreover, the levels of mineral nutrients in bell pepper fruit are affected not only by solar radiation-based irrigation frequency but also by soil media composition (Jovicich et al., 2007). Salinity in nutrient media influences the relative level and composition of mineral nutrients in sweet pepper fruit, wherein major nutrients are not affected; however, minor nutrients including B, Mn, Fe, Na, and Cu, are highly accumulated (Piñero et al., 2019). The Ca^{2+} amount per fresh weight does not consistently respond to the increase in fruit diameter; however, Ca^{2+} amount per fruit gradually increases with increase in fruit diameter during the fruit development of bell peppers (Mayorga-Gómez et al., 2020). The application of plant growth regulators, such as indole-3-acetic acid (IAA), gibberellic acid (GA_3), and abscisic acid (ABA) does not significantly affect the mineral nutrient content of sweet pepper fruits (Pérez-Jiménez et al., 2015).

Glucose, fructose, and sucrose play major roles in soluble carbohydrate metabolism during fruit development in sweet pepper. Moreover, with advances in fruit growth and development, the glucose and fructose contents are similarly increased; however, sucrose was inversely correlated with starch response (Nielsen et al., 1991). Thus, glucose and fructose are the major soluble carbohydrates in the fruits of pepper cultivars (Bernardo et al., 2008; Matsufuji et al., 2007; Rosa-Martínez et al., 2021). Soluble carbohydrates are highly affected by greenhouse climate and irrigation frequency, along with the activity of soluble carbohydrate-metabolising enzymes (Turhan et al., 2006). Foliar application of GA_3 reduces the contents of glucose and fructose; however, contents of sucrose increase. Moreover, IAA and ABA do not affect the levels of glucose and fructose; however, significantly reduce sucrose levels in sweet pepper fruit (Pérez-Jiménez et al., 2015). Citric and malic acids play major roles in organic acid metabolism in pepper (Osorio et al., 2012); but, their relationship is inversely related to fruit ripening (Flores et al., 2012). The contents of citric and malic acids gradually

increase during the development of pericarp colour in pepper fruits (Manikharda et al., 2018; Matsufuji et al., 2007; Serrano et al., 2010). The levels of major organic acids in harvested bell peppers are cultivar-dependent (Lwin et al., 2022). The content and response of amino acids differ greatly depending on fruit maturity and the type of amino acids in the fruit of pepper (Jang et al., 2015; Kim et al., 2019; Osorio et al., 2012) and bell pepper cultivars (Aizat et al., 2014; Lwin et al., 2022). In addition, the levels of amino acids highly fluctuate not only due to the growing region and season (Kim et al., 2019), but also due to the foliar Ca application and saline irrigation in sweet pepper (Piñero et al., 2018). Tyrosine content is only enhanced by GA_3 application in sweet pepper fruits compared to ABA and IAA (Pérez-Jiménez et al., 2015). Moreover, bell pepper cultivars significantly affect the content of individual fatty acids, thereby altering the ratio of unsaturated fatty acids to saturated/total fatty acids at harvest (Lwin et al., 2022). The components and profiles of fatty acids are highly affected not only due to the growing region, but also due to the growing season in the fruit of pepper cultivars (Kim et al., 2019), along with pericarp pigmentation differences in sweet pepper cultivars (Saini and Keum, 2016). Furthermore, harvest time influences the content and profile of fatty acids in open-field-grown, fully mature red pepper fruits (Bhandari et al., 2016).

However, there is a lack of information on the seasonal responses of fruit quality attributes associated with mineral nutrients and targeted major metabolites, such as soluble carbohydrates, organic acids, amino acids, fatty acids, chlorophyll, and carotenoid, along with the total antioxidant scavenging activity in greenhouse-grown fruits of diverse pericarp-coloured sweet pepper cultivars during the winter season. Therefore, we tested the hypothesis that the responses of fruit quality attributes are highly associated with seasonal fluctuations in mineral nutrients and targeted major metabolites in greenhouse-grown fruit of bell pepper cultivars during the winter season. This study aimed to determine the physiological and biochemical responses of fruit quality attributes, mineral nutrients, and targeted major metabolites, and their association to matured fruits of the ‘Scirocco’, ‘DSP7054’, and ‘Volante’ bell pepper cultivars harvested during the winter season from December to June.

2. Materials and methods

2.1. Fruit materials

Three cultivars of bell pepper (*Capsicum annuum* L.) fruit, the red ‘Scirocco’, orange ‘DSP7054’, and yellow ‘Volante’ cultivars, were harvested four times during the winter season with two months intervals: on 14 December 2021, 18 February 2022, 21 April 2022, and 9 June 2022 from commercial orchard in Yeongam, Jeollanam-do, Republic of Korea (34°44'19.35"N 126°28'59.20"E). The daily average air temperature and relative humidity inside the greenhouse during the winter season were 23.5 °C and 20.9 %, respectively. Bell pepper fruits were cultivated according to standard agronomic practices and harvested at commercial maturity (RDA, 2023). After that, the harvested fruit were promptly transported to Chung-Ang University in Anseong, Republic of Korea (37°00'13.60"N 127°13'55.20"E). The fruit quality attributes were evaluated using 30 fruits as three replicates for each cultivar on individual harvest dates. Subsequently, the uniform cuts of pericarp samples were separated into two groups: one group was oven-dried at 70 °C for mineral nutrient analysis and the other was frozen in liquid nitrogen and stored at -80 °C for further analysis.

2.2. Fruit quality attributes

The fresh weight of each fruit was measured using an analytical balance (WZ-3A, CAS Corp., Yangju, Republic of Korea). Fruit size (length and diameter) and pericarp thickness were determined using digital Vernier calipers (CD-15AX; Mitutoyo Co., Kanagawa, Japan). The

longest part of the fruit, from the shoulder to the blossom-end point, was measured as fruit length, and the average of the two orthogonal parts in the equatorial region was measured as the fruit diameter. The ratio of fruit length to diameter was calculated and the number of locules was counted after cutting the centre of the fruit horizontally. Peel colour coverage (%), peel colour variables [peel lightness (L^*), chroma (C^*), hue angle (h°), redness (a^*), and yellowness (b^*)], and fruit firmness (N) were measured as previously described (Byeon et al., 2023). The fruit respiration rate ($\mu\text{L CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$) was measured using six individual fruit sets in three replicates of two fruits for each cultivar using a previously established method (Byeon et al., 2023). Juice-squeezed pericarp samples of ten fruits per replicate were obtained using a household juicer to determine soluble solids content (SSC) and titratable acidity (TA). The SSC was measured using a digital handheld pocket reflectometer (PAL-1, Atago Co., Ltd., Tokyo, Japan) and the results were expressed as percentage (%). TA was measured using a bottle-top burette (Titrette® 50 mL, Brand GMBH + CO KG, Wertheim, Germany) to titrate the 5 mL juice with 0.1 N NaOH solution to an endpoint of pH 8.2 using the bench pH meter (ST3100, Ohaus Corporation, Parsippany, NJ, USA). TA was expressed as a percentage (%) of citric acid (Sadler and Murphy, 2010).

2.3. The contents of mineral nutrients

The oven-dried samples were finely ground using a mixer. The powder sample (0.1 g) was digested with 4 mL HNO_3 in a 50 mL glass beaker and incubated at 22 °C for 24 h, post which it was boiled at 200 °C on a hot plate until the solution cleared after treatment with 3 mL H_2O_2 . The solution was brought to a 10 mL with 1 N HCl containing 1000 ppm La^+ after filtration using a 0.45 μm hydrophilic PVDF filter and diluted 50-fold with deionised water. The inductively coupled plasma-optical emission spectrometer (Optima 5300 DV, PerkinElmer, Wellesley, MA, USA) was set to an operating power of 1300 Watts, plasma flow rate of 15.0 L min^{-1} , auxiliary flow rate of 0.2 L min^{-1} , and nebulizer flow rate of 0.8 L min^{-1} for mineral nutrients measurement. The results were expressed as g kg^{-1} dry weight (Lwin et al., 2023b).

2.4. Soluble carbohydrates, organic acids, and free amino acids

Fruit extraction for the analyses of soluble carbohydrates, organic acids, and free amino acids was performed as described by Latt et al. (2023). Briefly, ground frozen samples (5 g) were homogenised with 30 mL deionised water using a Polytron® homogeniser (PT 2500 E, Kinematic AG, Littau/Luzern, Switzerland), sonicated in ice-cold water for 30 min, then centrifuged using a LaboGene centrifuge (1580 R, Gyrozen Co., Ltd., Gimpo, Korea) at 3000 rpm for 10 min at 4 °C. The supernatant of the extract was filtered through a 0.45 μm hydrophilic PVDF membrane filter for high-performance liquid chromatography (HPLC). For soluble carbohydrates, 10 μL aliquot of the filtrate was injected into a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific, Sunnyvale, CA, USA) with an oven temperature set at 70 °C and equipped with a 300 $\times$ 6.5 mm Sugar-pak column (Waters Co., Milford, MA, USA) with a Shodex RI-101 detector (Showa Denko K.K., Kanagawa, Japan). The flow rate of the mobile phase (HPLC-grade water) was 0.5 mL min^{-1} . The collected data were organized using the Chromeleon version 6.0 Chromatography data system (CDS) software (Dionex, Sunnyvale, CA, USA). The results were expressed as g kg^{-1} fresh weight (Lwin and Lee, 2022). Similarly, for organic acids, 10 μL aliquot of filtrate was injected into the Dionex UltiMate 3000 HPLC system with oven temperature set at 40 °C and equipped with an Aminex 87H column (300 $\times$ 10 mm, Bio-Rad Co., Hercules, CA, USA) and an RI detector (RefractoMAX520, ERC Inc., Saitama, Japan) at 210 nm. The flow rate of the mobile phase, 0.01 N sulfuric acid, was 0.5 mL min^{-1} . Results were expressed as g kg^{-1} fresh weight (Lwin and Lee, 2022). Free amino acids were analysed using the Dionex UltiMate 3000 HPLC system equipped with Inno C₁₈ column (4.6 mm $\times$ 150 mm, 5 μm , Youngjin Biochrom Co., Seongnam,

Korea), Agilent 1260 Infinity fluorescence detector (Agilent Technologies Inc., Santa Clara, CA, USA) and an UV detector set at 338 nm. The oven was maintained at 40 °C with a flow rate of 1.5 mL min^{-1} . A 0.5 μL aliquot of filtrate was used with gradient elution that was conducted with 40 mM sodium phosphate (pH 7.0) as solvent A and a mixture of distilled water, acetonitrile, and methanol (10:45:45, v/v %) as solvent B. Results were expressed in mg kg^{-1} on a fresh weight basis (Lwin and Lee, 2020).

2.5. Chlorophyll and carotenoid contents

Ground frozen samples (1 g) were homogenised for 30 s using the PT 2500 E Polytron® homogeniser with 10 mL of 80 % cold acetone, and then incubated for 30 min in dark at 4 °C. The extract was centrifuged using the 1580 R LaboGene centrifuge at 3000 rpm for 10 min at 4 °C. Absorbance of the extract was measured using a microplate reader (Epoch™ 2, BioTek instruments, Inc., Winooski, VT, USA) using the Gen5™ analysis software (BioTek instruments, Inc., Winooski, VT, USA) at wavelengths of 663, 647, and 470 nm. The contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and total carotenoid were calculated using equations reported by Lichtenthaler and Buschmann (2001) with some modifications. The results were expressed in mg kg^{-1} on a fresh weight basis. The chlorophyll content was measured as follows:

$$\text{Chlorophyll } a = 12.25 A_{663} - 2.79 A_{647}$$

$$\text{Chlorophyll } b = 21.50 A_{647} - 5.10 A_{663}$$

$$\text{Total chlorophyll} = 7.15 A_{663} + 18.71 A_{647}$$

Total carotenoid = (1000 A_{470} - 1.82 chlorophyll *a* - 85.02 chlorophyll *b*)/198 where A_{663} , A_{647} , and A_{470} are maximum absorbances of the samples at 663, 647, and 470 nm, respectively.

2.6. Total phenolic compounds and total antioxidant activities

To determine the total phenolic compounds and 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities, the extraction methods followed the protocol described by Lwin et al. (2023a). Briefly, frozen ground samples (1 g) were homogenised in 10 mL of 80 % methanol, followed by extraction through sonication for 15 min. The mixture was centrifuged using a LaboGene centrifuge (1730 R, Gyrozen Co., Ltd., Daejeon, Korea) at 12,000 $\times$ g for 15 min at 4 °C and the supernatant was collected. The total phenolic compounds were measured using the Folin-Ciocalteu method (Ainsworth and Gillespie, 2007), where 0.2 mL of extract was mixed with 0.2 mL of Folin-Ciocalteu reagent and 2.6 mL of distilled water, post which it was incubated at 22 °C for 6 min. Then, a 2 mL of 7 % sodium carbonate solution was mixed and it was incubated in dark for 90 min. Absorbance at 750 nm was recorded using a BioTek microplate reader with Gen5 software. The content of total phenolic compounds was expressed as grams of gallic acid equivalent (GAE) per kilogram of fresh weight using a standard curve of gallic acid (0–500 mg mL^{-1}). ABTS and DPPH radical scavenging activities were measured using the method described by Re (1999), with some modifications. A 10 μL of extract was gently mixed with 190 μL of each working solution on a 96-well microplate. The absorbance of the ABTS and DPPH radical scavenging activities was read at 734 nm and 517 nm, respectively, using a BioTek microplate reader with Gen5 software. ABTS and DPPH radical scavenging activities were calculated as previously described (Latt et al., 2024).

2.7. Ascorbic acid

The ground frozen sample (1 g) was homogenised for 1 min in 5 mL of 5 % metaphosphoric acid and centrifuged at 3000 rpm for 15 min at 4 °C using the 1580 R LaboGene centrifuge. The supernatant was filtered with a PVDF membrane filter (0.45 μL) and transferred into the amber colour vial. A 10 μL aliquots of the filtrate were injected into the Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific Co., Germering,

Germany) equipped with a Shodex Asahipak NH2P-50 4E column (4.6 mm × 250 mm, Osaka Soda Co., Ltd., Osaka, Japan) and diode array detector (DAD-3000, Thermo Fisher Scientific Co., Germering, Germany) set at 254 nm. The oven temperature was 35 °C and the flow rate was 0.8 mL min⁻¹. The mobile phase used was aqueous solvent A containing 20 mM sodium monobasic phosphate and 30 mM phosphoric acid, and acetonitrile was used as solvent B. The gradient elution was changed from 40 % to 20 % solvent B for 10 min, held at 20 % for 2 min, and then changed from 20 % to 40 % for 3 min. The ascorbic acid content was expressed as mg kg⁻¹ fresh weight and calculated using a standard curve of ascorbic acid (0–1000 mg L⁻¹, $y = 0.8673x + 7.0985$, $R^2 = 0.9967$).

2.8. Fatty acids

Fatty acid analysis was performed as previously described by Garces and Mancha (1993) with some modifications. The frozen samples were freeze-dried using the HyperCool™ freeze dryer (HC3110, Gyrozen Co., Ltd., Gimpo, Korea) at -110 °C for 72 h and then finely ground. The lyophilised powder sample (0.1 g) and 20 µL pentadecanoic acid (5 g L⁻¹, Sigma-Aldrich Co., St. Louis, MO, USA), used as an internal standard were placed in a tube with a Teflon cap. To this, a methylation mixture (2 mL) consisted of methanol, benzene, 2,2-dimethoxypropane, and sulfuric acid (39:20:5:2, v/v %) and heptane (1 mL) were added, which were followed by gentle shaking. The mixture was extracted at 80 °C for 2 h and then cooled to 22 °C. The supernatant was then transferred to a vial for fatty acids analysis using a gas chromatograph (Agilent 7890A, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with DB-23 columns (0.25 mm × 60 mm, 0.25 µm, Agilent Technologies, Inc., Santa Clara, CA, USA). The injected sample volume was 1 µL with a split ratio of 1:10. The injector temperature was set at 250 °C with helium flowing at 4 mL min⁻¹ as the carrier gas. The flame ionisation detector (FID) was maintained at 280 °C with a flow rate of 35 mL min⁻¹ for hydrogen, 350 mL min⁻¹ for air, and 35 mL min⁻¹ for helium. The column oven temperature was programmed to start at 50 °C for 1 min hold, then it was gradually increased to 130 °C at a rate of 25 °C min⁻¹, to 170 °C at a rate of 8 °C min⁻¹, to 215 °C at a rate of 2 °C min⁻¹ with 10 min hold, and finally to 250 °C at a rate of 10 °C min⁻¹. The fatty acids were identified using the internal standard and a fatty acid methyl ester mixture (Supelco™ 37 Component FAME Mix, Supelco Inc., Bellefonte, PA, USA) as a standard. The results were expressed as mg kg⁻¹ on a dry weight basis.

2.9. Statistical analyses

A completely randomised design was used to evaluate the fruit quality attributes, mineral nutrients, and target major metabolites between different cultivars and harvest times. The experiment included three replicates ($n = 3$) with ten fruits per replicate, and the results were presented as mean ± standard error (SE). Statistical analysis was performed using ANOVA and the least significant difference (LSD) test ($p < 0.05$) using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Normalised heatmap responses, principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), volcano plots, and Pearson's correlation coefficient heatmap matrix were used to evaluate the treatment effects on fruit quality attributes, mineral nutrients, and targeted major metabolites using MetaboAnalyst 6.0 (<https://www.metaboanalyst.ca/>) (Latt et al., 2024). The correlation coefficient-based networks of all variables were visualised using the MetScape (version 3.1.3) plugin in Cytoscape (version 3.10.1) (Latt et al., 2024).

3. Results

3.1. Overall responses of fruit quality attributes and targeted major metabolites

To evaluate the overall responses of fruit quality attributes, mineral nutrients, and targeted major metabolites during the winter season, a normalised heatmap matrix system was generated for the fruit of the three bell pepper cultivars, along with a hierarchical cluster analysis based on bell pepper cultivar and harvest time (Fig. 1). Based on the criteria of bell pepper cultivars and harvest season, two major clusters were detected at the top of the normalised heatmap matrix, in which the December and June seasons were mostly segregated from the February and April seasons, although some of the April season was included in the December and June clusters. While the results of the February and April season were inconsistently clustered, the results of the December and June season were clearly grouped together. On the other hand, in the aspect of individual variables in terms of fruit quality attributes, mineral nutrients, and targeted major metabolites, two major clusters were detected on the left side of normalized heatmap matrix. Two minor clusters were observed within each major cluster. In the upper major cluster, soluble carbohydrates, organic acids, and amino acids were mainly included; however, in the lower major cluster, mineral nutrients, chlorophyll and carotenoid, fruit peel colour variables, and so on were mostly included (Fig. 1).

3.2. Overall responses to bell pepper cultivars and harvest times

To evaluate the overall responses of fruit quality attributes, mineral nutrients, and targeted major metabolites to three different pericarp coloured bell pepper cultivars and harvest times, principal component analysis (PCA) score plots were applied (Fig. 2A). The PCA scores plot was used to determine the divergence and association of predictor variables during the winter season. The PC-1 and PC-2 values accounted for 39.1 % and 18.4 % of the total X and Y variables in the PCA scores plot, respectively, indicating that the overall divergence and segregation could be derived from cultivar differences and sampling times (Fig. 2A).

On the contrary, to evaluate seasonal difference effects on fruit quality attributes, mineral nutrients, and targeted major metabolites, PLS-DA scores plot model and loading plot system were used to determine the associations among harvest times and bell pepper cultivars (Fig. 2B and 2C). The PLS-DA scores plot showed variable segregation of harvest times, indicating that fruit quality attributes, mineral nutrients, and targeted major metabolites could be differentially affected by harvest time. LV-1 and LV-2 accounted for 19.2 % and 30.9 % of total variance, respectively. Within each harvest time, all variables were segregated by bell pepper cultivars. The results of the PLS-DA score plot progressed from December to June; however, the results of each harvest time overlapped with themselves (Fig. 2B).

Moreover, the PLS-DA loading plot model was adopted to evaluate variable associations among fruit quality attributes, mineral nutrients, and targeted major metabolites, irrespective of the harvest time and bell pepper cultivar. The latent variable (LV)-1 and LV-2 values in the PLS-DA loading plot accounted for a total variance of 18.3 % and 14.5 %, respectively. Most of the variables were widely distributed in the PLS-DA loading plot. However, the amino acids were closely associated with each other. At the same time, fatty acids were also closely associated with themselves and most amino acids. However, mineral nutrients were least associated with most fatty and amino acids. Fruit physiological quality variables were the most widely linked. Chlorophyll variables were closely associated with peel h° and peel L^* ; however, they were least associated with the total carotenoid content (Fig. 2C).

The variable importance in projection (VIP) score plot system was adopted to evaluate the variables that were significantly important during the winter season and depended on bell pepper cultivars based on more than one cut-off value (Fig. 2D). Twelve variables that scored high

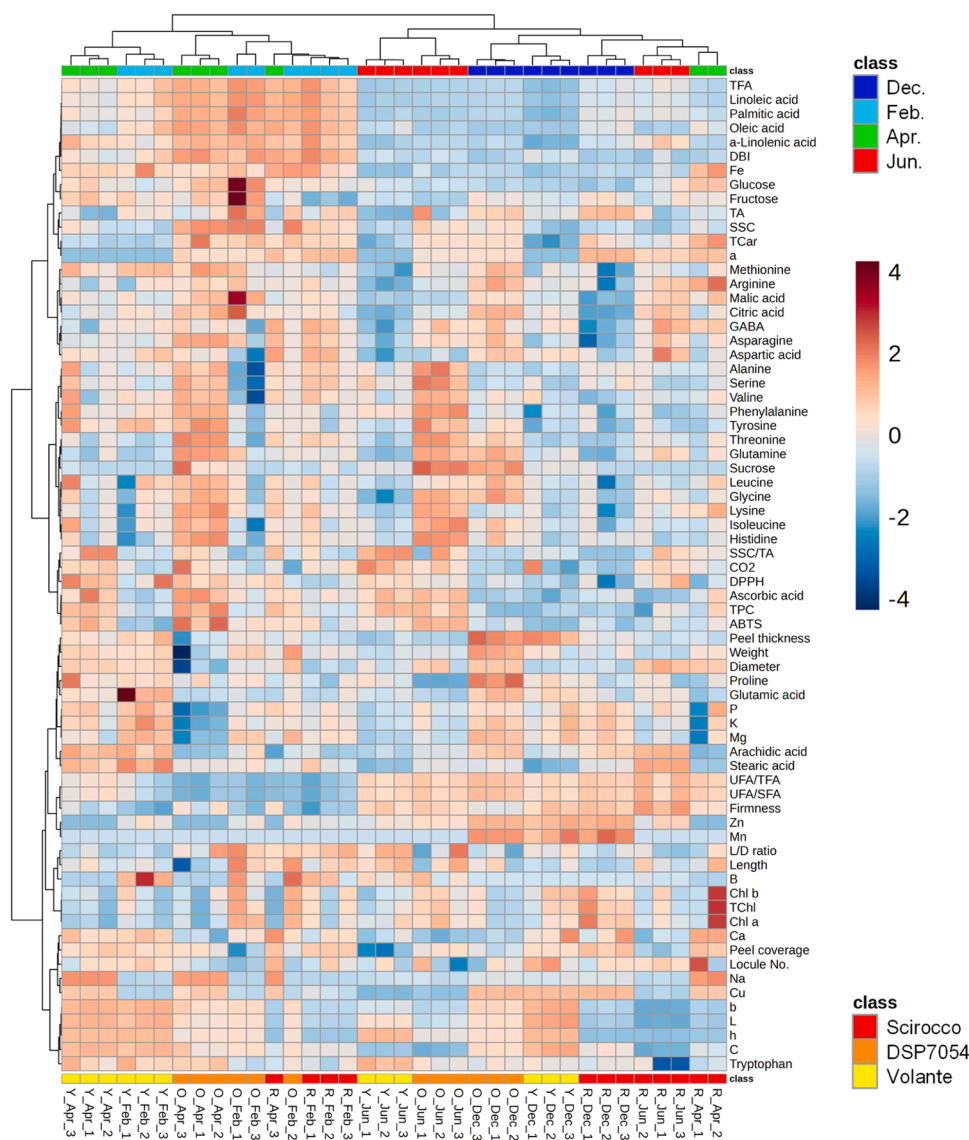


Fig. 1. Normalized heatmap matrix representing a hierarchical cluster analysis of physiological fruit quality attributes, soluble carbohydrates, organic acids, amino acids, fatty acids, mineral nutrients, chlorophylls, carotenoids, and total antioxidant activities in fruit of 'Scirocco' (red), 'DSP7054' (orange), and 'Volante' (yellow) bell pepper cultivars at harvest during winter season. Abbreviations are provided in Supplementary Table 1.

on the VIP score plot were listed from the PLS-DA model. Out of the twelve variables, peel C^* , peel b^* , K, pericarp thickness, and peel L^* were highest in December, compared with the other variables. Fruit fresh weight and Mg were highest in February. DPPH and ABTS radical scavenging activities and pericarp pigmentation (peel colour coverage) were highest in April. SSC/TA and fruit respiration were highest in June (Fig. 2D).

3.3. Differential responses of variables to bell pepper cultivars

To determine the variable differences during the winter season, a PLS-DA scores plot model was adopted to evaluate seasonal differences in the effects of bell pepper cultivars on fruit quality attributes, mineral nutrients, and targeted major metabolites from December to June (Fig. 3). In the 'Scirocco' red bell pepper cultivar, LV-1 and LV-2 variables accounted for 41.7 % and 16.3 % of total variance of X-axis and Y-axis, respectively. All the variables progressed sequentially from December to June. The results of the response variable from February were more closely associated with themselves compared to the other sampling times (Fig. 3A). Based on the results of the VIP score plot, peel

C^* , a^* , and K were highest in December. Peels b^* and L^* were highest in February. SSC/TA, peel colour coverage rate, glucose, and fruit fresh weight were highest in April. DPPH activity, fruit diameter, citric acid, fruit respiration rate, malic acid, and glutamine levels were highest in June (Fig. 3B). In the 'DSP7054' orange bell pepper cultivar, LV-1 and LV-2 variables accounted for 37.5 % and 17.5 % of total variance of X- and Y-axis, respectively. The responses of all variables progressively clustered from December to June, depending on the sampling time were shown in Fig. 3A. However, the response variables from December were most tightly clustered compared to other sampling times (Fig. 3C). According to the results of the VIP score plot, peel b^* , fruit fresh weight, peel C^* , peel K, peel L^* , peel h^* , pericarp thickness, and Mg were highest in December. Fructose was highest in February. SSC/TA, ABTS, fruit respiration, peel colour coverage, and DPPH were highest in April. Moreover, sucrose level was highest in April (Fig. 3D). In the 'Volante' yellow bell pepper cultivar, LV-1 and LV-2 variables accounted for 45.8 % and 22.3 % of total variance of X-axis and Y-axis, respectively. Results of the response variables from February were most tightly clustered within 95 %; however, the results were least clustered from April (Fig. 3E). Based on the results of the VIP score plot, peel b^* , peel C^* , peel

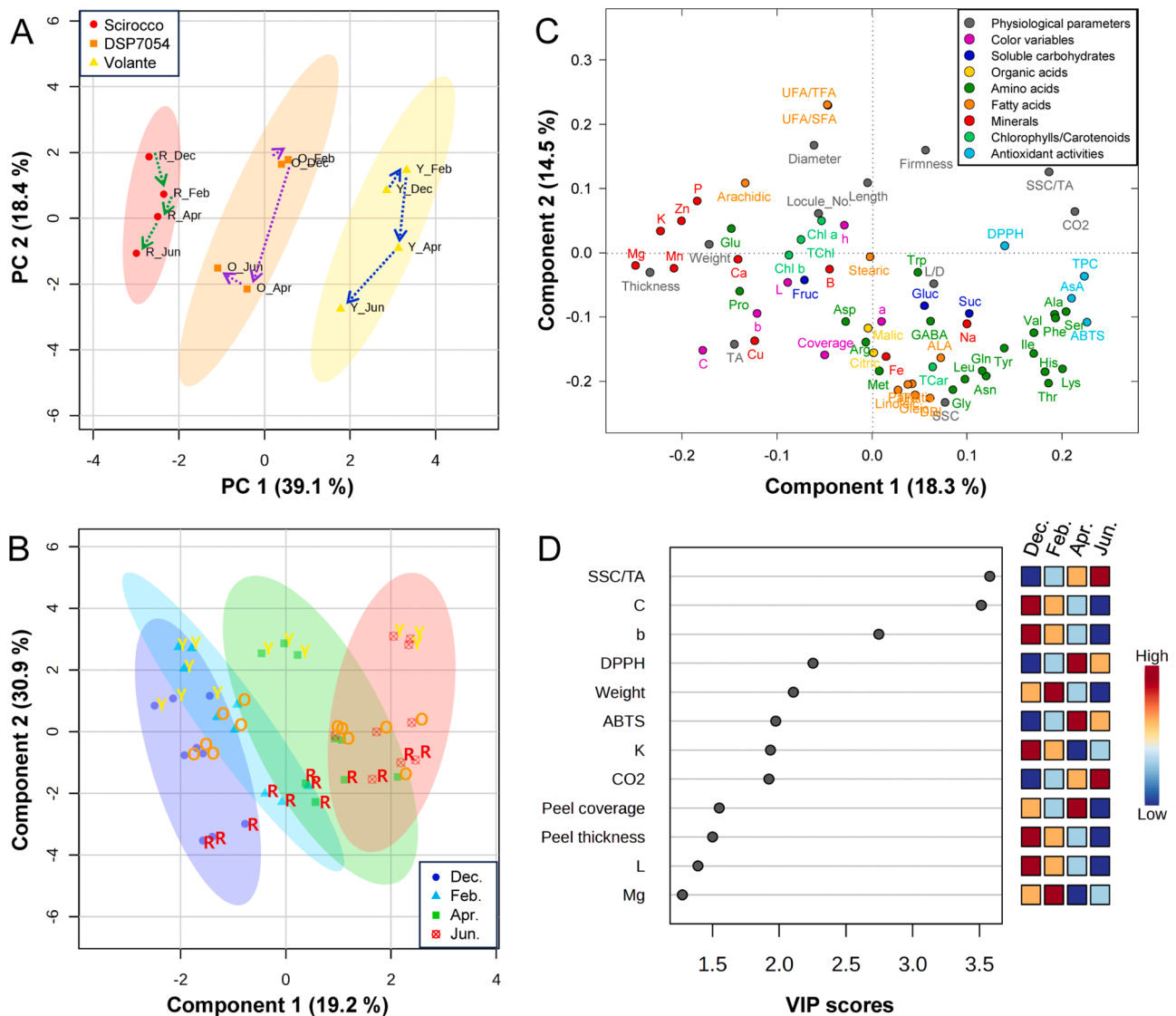


Fig. 2. Principal component analysis (PCA) scores plot (A), partial least squares-discriminant analysis (PLS-DA) scores (B) and loading (C) plots, and variable importance in projection (VIP) scores plot (D) in fruit of 'Scirocco' (red), 'DSP7054' (orange), and 'Volante' (yellow) bell pepper cultivars at harvest during winter season. (A) and (B) are for cultivar group and (C), and (D) are for monthly harvest time group. Abbreviations are provided in Supplementary Table 1.

L^* , and pericarp thickness were highest in December. K, citric acid, and Mg were highest in February. Peel colour coverage, ABTS, DPPH, and glucose were highest in April. Finally, SSC/TA, fruit respiration rate, and fruit length were highest in June (Fig. 3E).

3.4. Responses of individual variables to cultivars and season

Based on the results of the VIP score plots (Figs. 2D, 3B, 3D, and 3F), certain variables were selected and categorised into important variables in which the threshold level of the cut-off value was more than one value of the VIP scores. In terms of physiological fruit quality attributes, fruit fresh weight, fruit length, fruit diameter, pericarp thickness, fruit respiration rate, and SSC/TA ratio were determined, as shown in Fig. 4. Fruit fresh weight was highest in the 'DSP7054' bell pepper cultivar in December; however, it was lowest in April. Fruit length was highest in the 'DSP7054' bell pepper cultivar in February; however, it was lowest in April. Fruit diameter was highest in the 'Scirocco' bell pepper cultivar in April; however, it was lowest in the 'DSP7054' bell pepper cultivar in April. Pericarp thickness was gradually decreased from December to June, except for the 'Scirocco' bell pepper cultivar, in which pericarp thickness remained unchanged. In contrast, the fruit respiration rate and

SSC/TA gradually increased from December to June, irrespective of the bell pepper cultivar. Fruit respiration rate was highest in the 'Volante' cultivar in June but lowest in December. However, SSC/TA was highest in the 'Volante' cultivar in June but lowest in the 'Scirocco' cultivar in December.

All peel colour variables responded differently to bell pepper cultivar and sampling time. That is, peel L^* , C^* , h° , and b^* were highest in 'Volante' yellow bell pepper cultivar; however, it was lowest in the 'Scirocco' red bell pepper cultivar, compared with the others, regardless of sampling time. However, peel a^* was highest in the 'Scirocco' red bell pepper cultivar and gradually declined from December to June. Furthermore, peel a^* values from the 'DSP7054' and 'Volante' cultivars remained unchanged. Peel colour coverage was highest in the 'Scirocco' red bell pepper cultivar; however, it was lowest in the 'DSP7054' orange bell pepper cultivar from December to April, compared with the others. However, peel colour coverage was reduced in the 'Scirocco' and 'Volante' cultivars but not in the 'DSP7054' cultivar in June.

Among the major metabolites, sucrose, glucose, fructose, citric acid, malic acid, and glutamine were considered important variables. With respect to soluble carbohydrates, sucrose was only highly produced in the 'DSP7054' orange bell pepper cultivar except for in February,

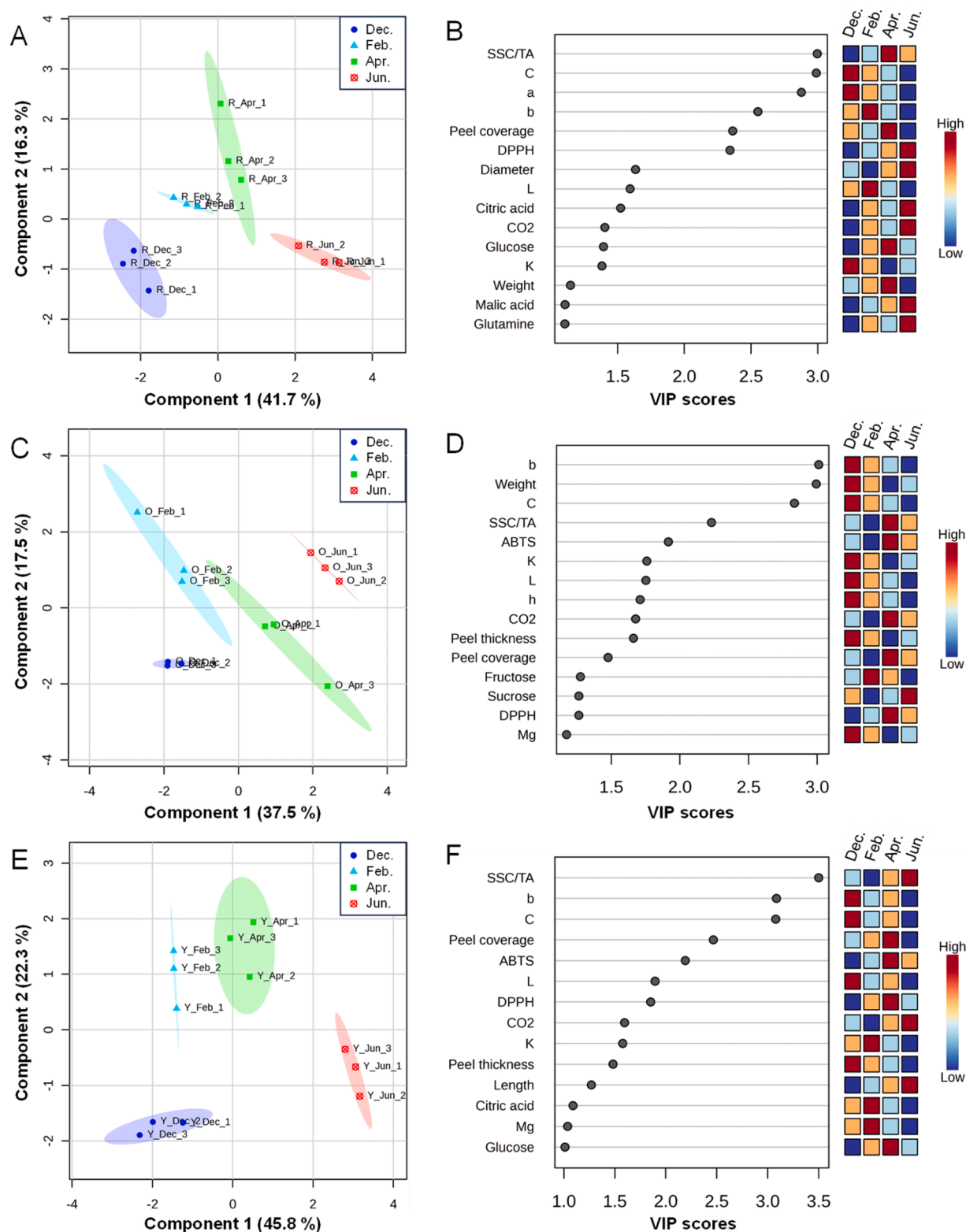


Fig. 3. Partial least squares-discriminant analysis (PLS-DA) scores plots (A, C, and E) and variable importance in projection (VIP) scores plots (B, D, and F) in fruit of 'Scirocco' (red; A and B), 'DSP7054' (orange; C and D), and 'Volante' (yellow; E and F) bell pepper cultivars at harvest during winter season.

compared with the other cultivars. Glucose and fructose were highest in the 'DSP7054' cultivar in February; however, glucose was lowest in the 'Volante' yellow bell pepper cultivar in December and fructose was lowest in the 'Scirocco' red bell pepper cultivar in February. Citric and malic acids were highest in the 'DSP7054' orange bell pepper cultivar in February, but lowest in the 'Scirocco' red bell pepper cultivar in

December. Moreover, glutamine was highest in the 'DSP7054' cultivar in December; however, it was lowest in 'Scirocco' cultivar in December.

Among the mineral nutrients, only K and Mg were selected as important variables. K and Mg were highest in 'Volante' cultivar in February, but lowest in the 'DSP7054' cultivar in April. The levels of K and Mg gradually declined from December to June, irrespective of the

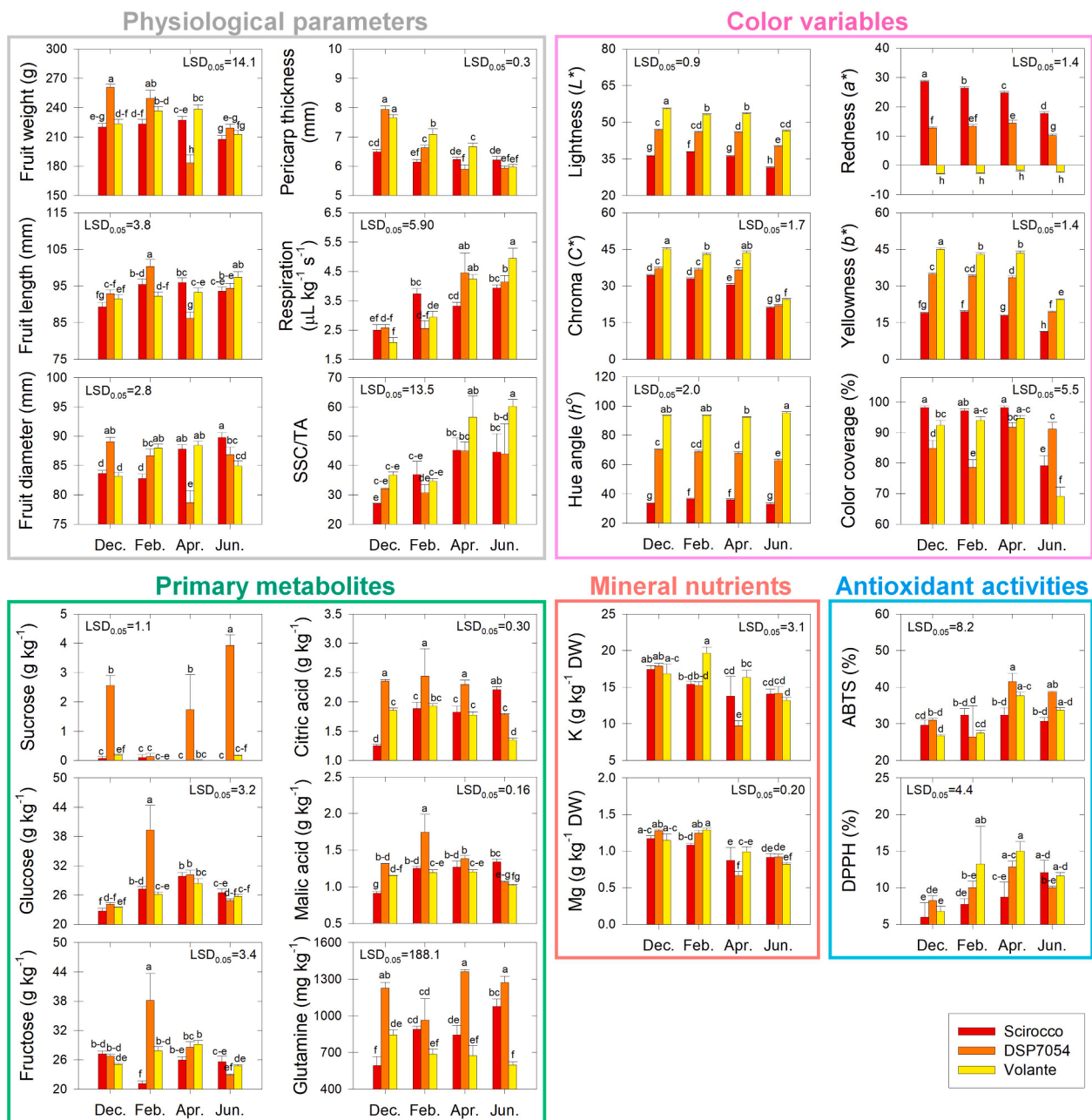


Fig. 4. The responses of individual variables in fruit of ‘Scirocco’ (red), ‘DSP7054’ (orange), and ‘Volante’ (yellow) bell pepper cultivars at harvest during winter season. Fresh weight, pericarp firmness, fruit length, fruit diameter, and peel colour variable data points are expressed as the mean of 30 bell pepper fruits as replicates ($n = 30$) $\pm$ standard error (SE). However, ethylene production and respiration rate data points indicate the mean of six bell pepper fruits as replicates ($n = 6$) $\pm$ SE. SSC, TA, SSC/TA, primary metabolites, mineral nutrients, and total antioxidant scavenging activities data points indicate the mean of three replicates ($n = 3$) $\pm$ SE. The mean difference analysis was significantly determined by least significant difference (LSD) test at $p = 0.05$.

bell pepper cultivar. On the other hand, ABTS was highest in the ‘DSP7054’ cultivar in April, but lowest in the ‘Volante’ cultivar in December. DPPH was highest in the ‘Volante’ cultivar in April, but lowest in the ‘Scirocco’ cultivar in December. As variables for total antioxidant scavenging activity, ABTS and DPPH antioxidant scavenging activities were differently responded to bell pepper cultivar and harvest time during season (Fig. 4).

3.5. Overall correlation among fruit quality attributes, mineral nutrients, and targeted major metabolites

To evaluate the relationships among all variables, an overall correlation coefficient heatmap matrix system was applied, irrespective of

bell pepper cultivar or harvest time (Fig. 5). Several positively correlated clusters were identified. From the lower left side of the correlation coefficient heatmap matrix, the total carotenoid content was positively correlated with peel a^* . Chlorophyll variables were positively correlated with each other. As one of the major positive correlation clusters, glucose, SSC, TFA, linoleic acid, palmitic acid, α -linolenic acid, DBI, oleic acid, and Fe were positively correlated with themselves. In turn, malic acid, citric acid, methionine, stearic acid, GABA, arginine, and aspartic acid were also positively correlated with themselves. Alanine, serine, valine, isoleucine, histidine, sucrose, leucine, asparagine, glycine, glutamine, lysine, threonine, phenylalanine, and tyrosine were positively correlated to each other. Moreover, UFA/TFA ratio, UFA/SFA ratio, and pericarp firmness were positively correlated with Zn and Mn.

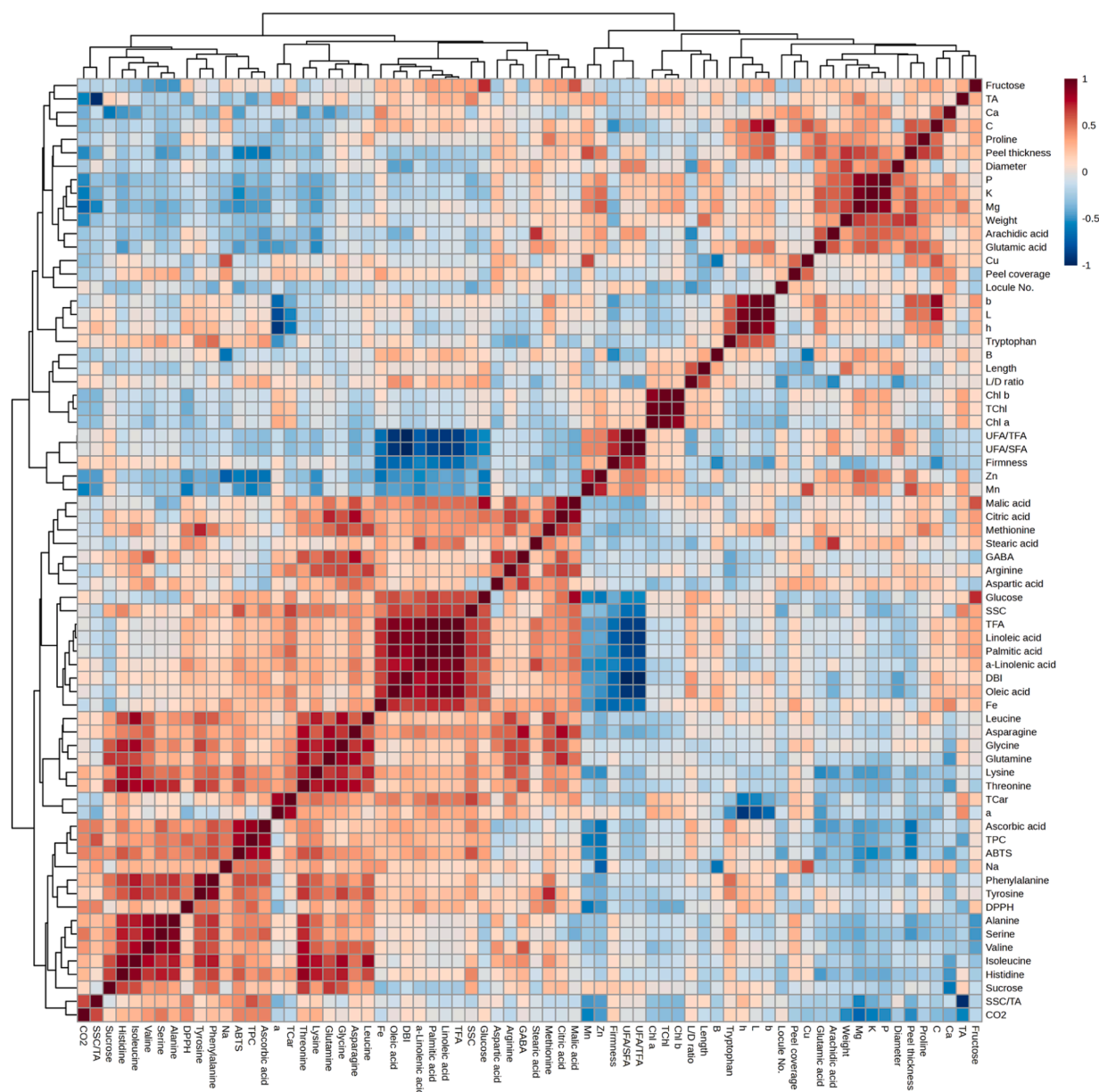


Fig. 5. Pearson correlation coefficient heatmap matrix with a hierarchical cluster analysis of physiological fruit quality attributes, soluble carbohydrates, organic acids, amino acids, fatty acids, mineral nutrients, chlorophylls, carotenoids, and total antioxidant activities in the fruit of ‘Scirocco’ (red), ‘DSP7054’ (orange), and ‘Volante’ (yellow) bell pepper cultivars at harvest during winter season. The figure legend indicates the results of the correlation coefficient (r) from -1 to 1 . The red and blue colors in boxes indicate positive and negative correlation coefficients between variables, respectively. Abbreviations are provided in Supplementary Table 1.

This positive correlation cluster was negatively correlated with glucose, SSC, TFA, linoleic acid, palmitic acid, α -linolenic acid, DBI, oleic acid, and Fe. Peel b^* , L^* , and h^* were positively correlated with tryptophan. As the last positive cluster was located at the upper right side of the correlation heatmap matrix, P, K, Mg, fruit fresh weight, arachidic acid, glutamic acid, proline, and pericarp thickness were positively correlated with themselves (Fig. 5). Results of the correlation coefficient heatmap matrix for each cultivar are shown in Fig. S9, irrespective of the sampling time.

3.6. Cultivar dependent correlation coefficient network

A Pearson correlation coefficient network system was generated to evaluate bell pepper cultivar-dependent effects on fruit quality attributes, mineral nutrients, and targeted major metabolites during the winter season, irrespective of harvest time (Fig. 6). In this case, the cut-

off value for the correlation coefficient was greater than $|0.75|$. In the ‘Scirocco’ red bell pepper fruit, fatty acids except for stearic and arachidic acids were tightly correlated with themselves, but positively correlated with certain amino acids, including alanine, serine, valine, threonine, and aspartic acid. Peel colour variables, except h^* , were positively correlated with each other as a cluster, but negatively correlated with stearic acid, citric acid, fruit diameter, and pericarp firmness. Lysine, arginine, leucine, phenylalanine, isoleucine, tyrosine, glycine, and methionine were positively correlated and formed clusters. At the same time, Mn was negatively correlated with lysine, methionine, glutamine, asparagine, GABA, citric acid, malic acid, fruit respiration, and glucose (Fig. 6A). In the ‘DSP7054’ orange bell pepper cultivar, most amino acids were positively correlated with themselves except for methionine, arginine, proline, and glutamic acid. Peel colour variables were closely and positively correlated with each other, except for peel colour coverage. Fatty acid variables, except for arachidic acid, were

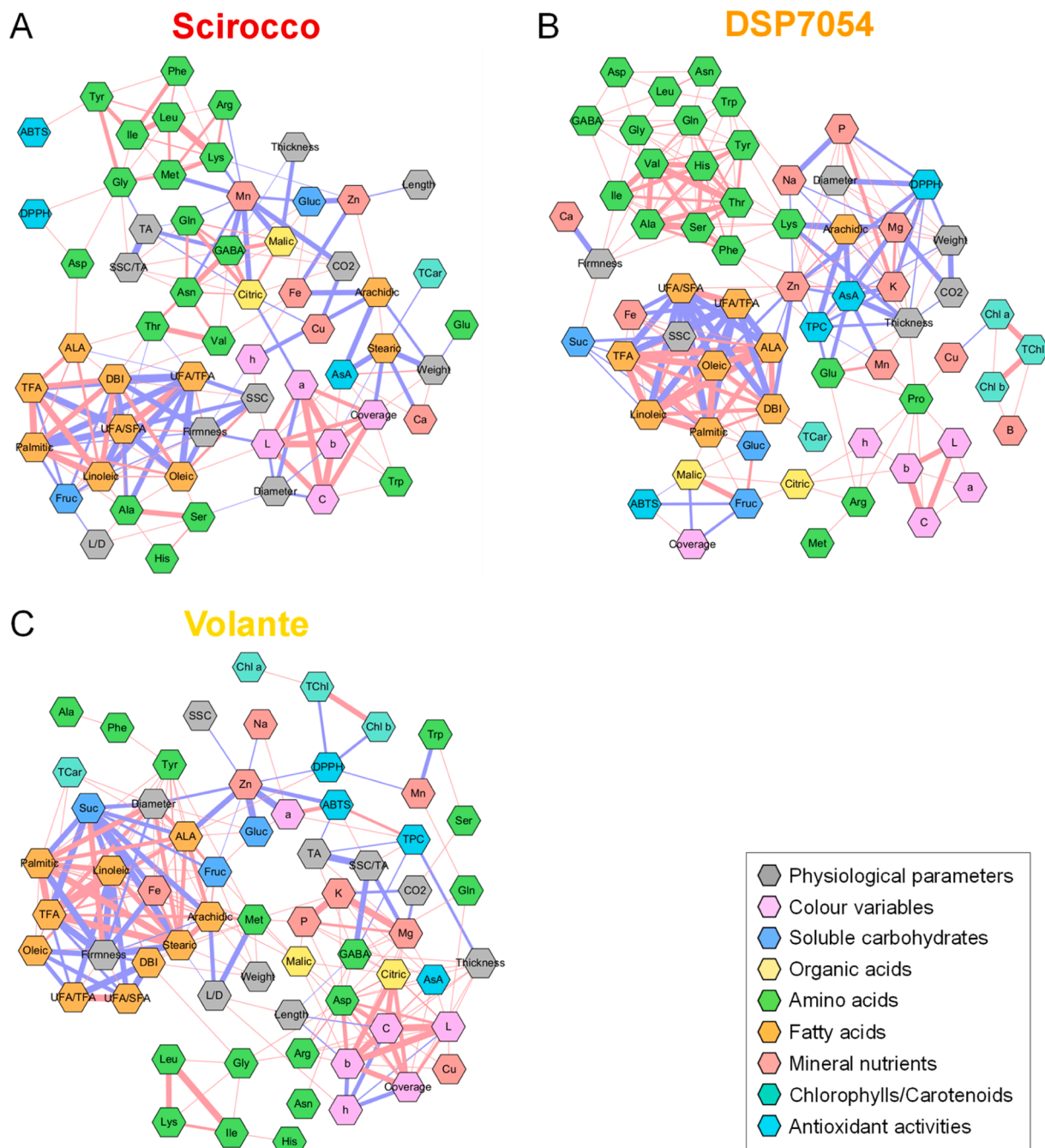


Fig. 6. Pearson correlation coefficient ($r \geq |0.75|$) network among physiological fruit quality attributes, soluble carbohydrates, organic acids, amino acids, fatty acids, mineral nutrients, chlorophylls, carotenoids, and total antioxidant activities in the fruit of ‘Scirocco’ (red; A), ‘DSP7054’ (orange; B), and ‘Volante’ (yellow; C) bell pepper cultivars at harvest during winter season. Red and blue lines indicate positive and negative correlations among variables, respectively. The thickness of the correlation coefficient network lines provides the significance of the correlation coefficient (r) among variables. The abbreviation of response variables is provided in Supplementary Table 1.

tightly correlated with each other, irrespective of positivity and negativity. Moreover, pericarp firmness was negatively correlated with Ca content. In addition, chlorophyll variables were positively correlated with each other, along with B, however negatively correlated with Cu. However, total carotenoid was positively correlated with DBI. Some mineral nutrients, including P, K, Mg, Zn, and Na, were negatively correlated with DPPH, TPC, and physiological fruit quality attributes, such as fruit diameter, fruit fresh weight, pericarp thickness, and fruit respiration (Fig. 6B). In the ‘Volante’ yellow bell pepper cultivar, most peel colour variables except for peel a^* were positively correlated with each other, but negatively correlated with peel h^* . At the same time, this

positive correlation cluster was positively correlated with Mg, K, P, Ca, Cu, citric acid, arginine, aspartic acid, asparagine, and ascorbic acid. However, fatty acid variables were strongly correlated with each other, regardless of positivity or negativity. Moreover, the fatty acid correlation cluster was negatively correlated with sucrose and pericarp firmness, but positively correlated with tyrosine, which was positively linked with phenylalanine and alanine. Zn was negatively correlated with SSC, Na, peel a^* , ABTS, glucose, fruit diameter, α -linolenic acid (ALA), and DPPH. DPPH was negatively correlated with the total chlorophyll, chlorophyll b, and Mn. Unlike previous bell pepper cultivars, amino acids were not tightly correlated with each other. The amino acids were

widely distributed and thus individually linked with other variables (Fig. 6C).

3.7. Comparative responses of all the variables to cultivars

To evaluate the overall performance of bell pepper cultivars based on the results of the response variables, a volcano plot was generated, as shown in Fig. 7. The volcano plot was determined based on a fold change (FC) cut-off value of 1.5 at a false discovery rate (FDR) adjusted p -value < 0.05 (Fig. 7A, 7C, and 7E). Based on the results from the corresponding volcano plots, the violin plot system was adopted to evaluate the overall distribution pattern of the corresponding variables in bell pepper cultivars (Fig. 7B, 7D, and 7F). Peel a^* was only up-regulated in the 'Scirocco' cultivar, compared with the 'DSP7054' cultivar, but peel h° , sucrose, and peel b^* were highly up-regulated in the 'DSP7054' cultivar (Fig. 7A). The results of violin plots between the 'Scirocco' and 'DSP7054' cultivars indicate that peel h° , sucrose, and peel b^* were highly distributed in the 'DSP7054' cultivar than in the 'Scirocco' cultivar, but peel a^* was only highly distributed in the 'Scirocco' cultivar (Fig. 7B). When the 'DSP7054' cultivar was compared with the 'Volante' cultivar, peel a^* , sucrose, total carotenoid, and glutamine were only highly up-regulated in the 'DSP7054' cultivar, however, no variables were up-regulated in the 'Volante' cultivar (Fig. 7C). According to the results of violin plots, the four variables (peel a^* , sucrose, total carotenoid, and glutamine) were only highly responded and distributed in the 'DSP7054' cultivar than in the 'Volante' cultivar (Fig. 7D). When the 'Volante' cultivar was compared with the 'Scirocco' cultivar, peel h° , peel b^* , and tryptophan were highly up-regulated in the 'Volante' cultivar; however, peel a^* and total carotenoid were only highly up-regulated in the 'Scirocco' cultivar (Fig. 7E). Thus, according to the results of violin plots, peel h° , peel b^* , and tryptophan were highly responded and distributed in the 'Volante' cultivar, but peel a^* and total carotenoid only were highly responded and distributed in the 'Scirocco' cultivar (Fig. 7F).

4. Discussion

Bell pepper fruits are cultivated throughout the year and are produced using greenhouse systems (Aloni and Karni, 2002). Subsequently, the fruits are collected into packinghouses by local growers, sorted into a relevant fruit quality in terms of fruit size, shape, and pericarp pigmentation, packed into a relevant packaging style depending on vendor demand, and then distributed via a distribution networking system (González and Tiznado, 1993; Meir et al., 1995; Rodov et al., 1995; Sargent et al., 1992). However, fruit quality fluctuates depending on numerous fruit quality-controlling factors, such as local climatic conditions, greenhouse cultivation systems, mineral nutrient management systems, integrated pest management systems, postharvest fruit distribution systems, and cultivar differences (Castañeda et al., 2023; Elizondo-Cabalceta and Monge-Pérez, 2017; Sherafati et al., 2010). In this study, the overall fruit quality of the harvested bell peppers was not only seasonally different but also cultivar-dependent throughout the growing period (Figs. 1–3). The results of the normalised heatmap matrix with a hierarchical cluster analysis indicated that the overall fruit quality of the bell peppers could be mainly clustered into two major clusters: fruit quality harvested in December and June was clearly distinct from that in February and April, irrespective of bell pepper cultivars (Fig. 1). However, the overall fruit quality of the bell peppers gradually improved from December to June (Figs. 2A, 3A, 3C, and 3E); although, fruit quality was slightly different depending on the bell pepper cultivars. Fruit quality attributes and physiological disorders respond differently to the harvest season (winter vs. summer) and bell pepper fruit type (blocky vs. conical) (Byeon et al., 2023). They also respond differently to bell pepper cultivars in terms of fruit shape and pericarp pigmentation at harvest (Kye et al., 2022). The yield and fruit quality of bell peppers fluctuate greatly during the growing season in

greenhouses (Joshi et al., 2019), and the marketable fruit and yield vary seasonally (Sideman, 2020). Additionally, agronomic fruit characteristics in terms of yield and fruit quality are clearly different between Bola-type paprika red pepper cultivars (García et al., 2007) and bell pepper genotypes (Elizondo-Cabalceta and Monge-Pérez, 2017). Thus, overall fruit quality could be highly affected by both bell pepper cultivar and seasonal cultivation factors.

Among the individual fruit quality parameters covered with fruit quality attributes, mineral nutrients, and targeted major metabolites, most amino acids, organic acids, glucose, and sucrose were highly segregated from fruit quality attributes and mineral nutrients; although, some amino acids, such as glutamic acid and proline were inconsistent. Additionally, individual fatty acids were closely associated with amino acids and total carotenoid but were less associated with chlorophyll variables (Fig. 2C). Based on the results of the VIP score plot, 12 variables were highly important, regardless of the bell pepper cultivar (Fig. 2D). However, with respect to bell pepper cultivar, peel a^* , fruit diameter, citric acid, glucose, malic acid, and glutamine were highly important only in the 'Scirocco' red bell pepper cultivar (Fig. 3B). Peel h° , fructose, and glucose were only highly important in the 'DSP7054' yellow bell pepper cultivar (Fig. 3D). Moreover, fruit length, citric acid, and glucose were only highly important in the 'Volante' yellow cultivar (Fig. 3F). The results of the VIP score plot indicated that certain fruit quality variables could respond differently to bell pepper cultivars. Most mineral nutrients in pepper fruit are clustered differently, with glucose, fructose, ascorbic acid, and certain secondary compounds (Rosa-Martínez et al., 2021). The contents of ascorbic acid, capsaicinoids, and flavonoids differentially respond to the growing season, pepper cultivar, and fruit maturity of greenhouse-grown pepper fruits (Bae et al., 2014). Additionally, the dry matter, ascorbic acid, carotenoid, and phenolic compound contents in bell pepper fruits are highly affected by the cultivation system and bell pepper cultivar (Hallmann and Rembiałkowska, 2012). However, it is difficult to evaluate the seasonal effect of fruit quality parameters on the relationship among fruit physiological quality attributes, mineral nutrients, and targeted major metabolites from harvested fruits of three different bell pepper cultivars during the entire winter season because of the lack of whole-season-based research results. However, the overall results indicated that it could determine the differential responses of certain targeted parameters, such as soluble carbohydrates, organic acids, amino acids, pericarp colour variables, and mineral nutrients, depending on the season or cultivar, as shown in Figs. 4 and 7. Thus, the results suggest that certain fruit quality parameters could inconsistently respond to either the growing season or bell pepper cultivar.

From the fruit quality variables, pericarp colour variables, such as peel L^* , peel C^* , peel h° , peel a^* , peel b^* , and peel colour coverage, responded distinctively to the bell pepper cultivar during the entire growing season (Figs. 2–4, 6, and 7). Thus, different responses of pericarp pigmentation were derived from the fruit pericarp pigmentation characteristics of each bell pepper cultivar, based on cultivar-dependent pericarp colour attributes. The different bell pepper cultivars for red and yellow pigments were distinctively separated from the total pigment content, thereby contributing to the segregation of the values of the colorimetric coordinates a^* and b^* (Gómez et al., 1998). Additionally, peel a^* and b^* values are highly associated with the total pigment concentration of paprika cultivars (Gómez et al., 1998). The results of colour attributes are cultivar-dependent in paprika peppers and are also distinctively different from the fruit ripening phases because the colour attributes indicate the total pigment concentration synthesized in the fruit and their relationship with carotenoids in the fruit of paprika cultivars (de Guevara et al., 1996). The pericarp colour attributes were strongly associated with the chlorophyll and carotenoid contents, but peel a^* was only positively correlated with total carotenoid in the overall correlation coefficient heatmap matrix (Fig. 5). Thus, the differential responses of pericarp colour attributes mainly resulted from the levels of chlorophyll and carotenoid, and thus were clearly segregated

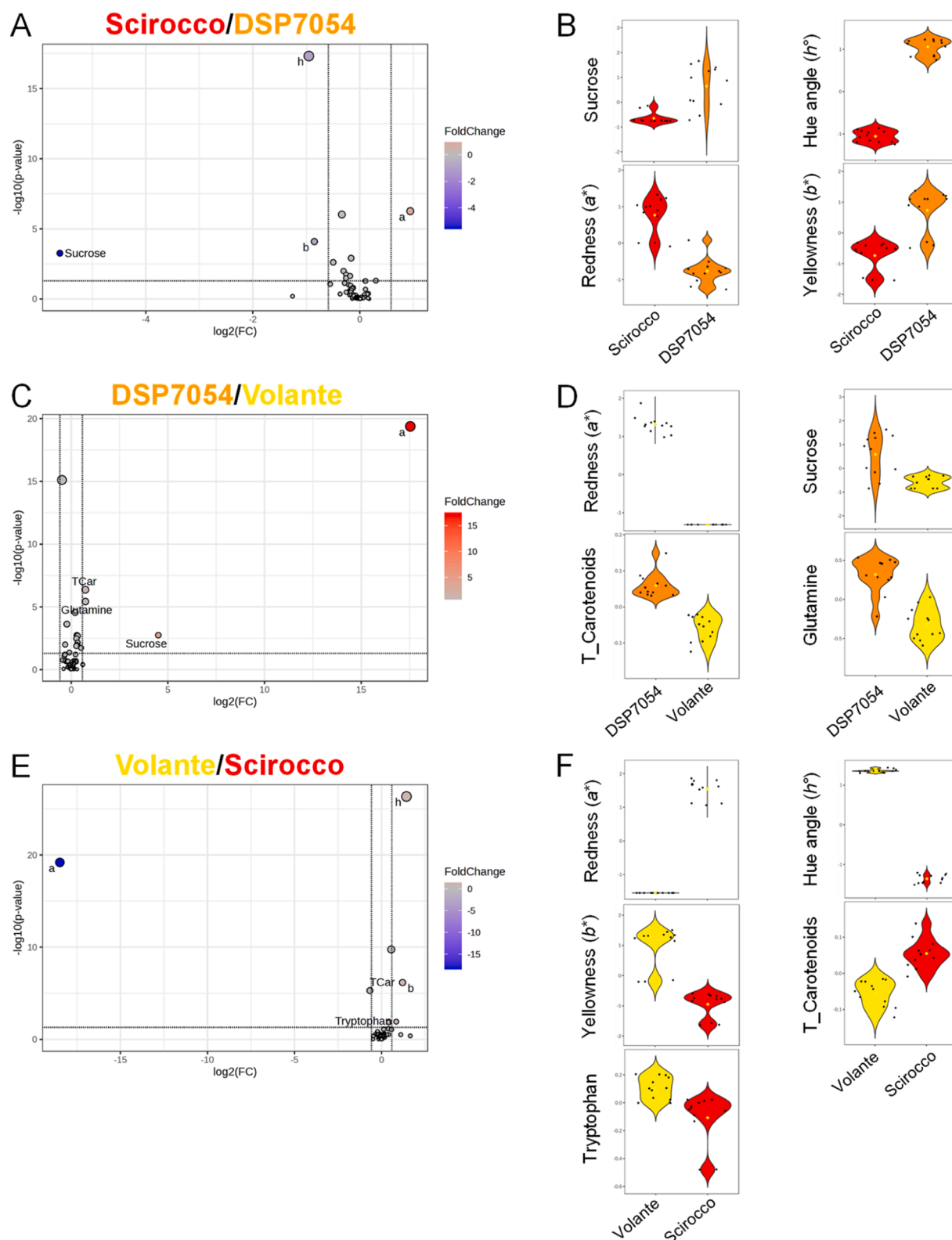


Fig. 7. Volcano plots (A, C, and E) and violin plots (B, D, and F) in fruit of ‘Scirocco’ vs ‘DSP7054’ (red vs orange; A and B), ‘DSP7054’ vs ‘Volante’ (orange vs yellow; C and D), and ‘Volante’ vs ‘Scirocco’ (yellow vs red; E and F) bell pepper cultivars at harvest during winter season. Determination of volcano plots is based on fold change (FC) cut-off value = 1.5 at false discovery rate (FDR) adjusted p -value < 0.05 with that red dots represent up-regulated variables, blue dots represent down-regulated variables, and gray dots represent variables with no significant differences. Violin plots represent the approximate frequency distribution of the corresponding data. Abbreviations are provided in Supplementary Table S1.

by the pericarp colour of bell pepper cultivars.

Of the mineral nutrients, only K and Mg were highly important, based on the results of the VIP score plot, irrespective of the cultivar (Fig. 2D). However, bell pepper cultivars responded differently to these two minerals (Fig. 3B, 3D, and 3F). Thus, mineral nutrients were relatively less correlated with other fruit quality parameters in the ‘Scirocco’ bell pepper cultivar, compared with those in two other cultivars (Fig. 6). Some mineral nutrients, such as N, P, K, Mg, and S, are strongly affected by irrigation levels in open-field-cultivated bell pepper fruit (Kabir et al., 2021). The levels of certain mineral nutrients, including Fe, Ca, Mg, and K, gradually decline with fruit maturity in greenhouse-grown sweet peppers (Pérez-López et al., 2007). Additionally, K, Ca, and Mg contents are highly affected by harvesting time in greenhouse-grown fully mature pepper fruits (Flores et al., 2009). The content and profiles of mineral nutrients in bell pepper fruits respond differently to different pericarp pigmentation-based bell pepper cultivars (Simonne et al., 1997). Therefore, the differential responses of mineral nutrients were strongly associated with harvest time and pericarp pigmentation in bell pepper cultivars.

Most amino acids were closely associated with glucose, sucrose, organic acids, and fatty acids in the PCA loading plots (Fig. 2C). However, among the targeted major metabolites, none were detected in the VIP score plot, regardless of the bell pepper cultivar (Fig. 2D). Of the soluble carbohydrates from the results of individual cultivar VIP score plots, glucose was only highly valuable in the red and yellow bell pepper cultivars, whereas fructose and sucrose were only distinctively important in the orange bell pepper cultivar (Fig. 3). Furthermore, based on the results of the volcano plot analysis, sucrose was only highly up-regulated in the ‘DSP7054’ bell pepper cultivar (Fig. 7). Sugar-related metabolism regulates sucrose hydrolysis, and starch, cellulose, and trehalose biosynthesis are specifically expressed as fruit maturity advances in paprika fruit (Nie et al., 2023). Of numerous soluble carbohydrates, glucose and fructose play a pivotal role in soluble carbohydrate metabolism, compared to the role of sucrose (Bernardo et al., 2008), as shown in this study (Fig. 4). Coloured sweet pepper fruits are covered with a major portion of soluble carbohydrates compared to sucrose, regardless of pericarp pigmentation in sweet pepper cultivars (Matsufuji et al., 2007). The relationship between glucose/fructose and sucrose is inversely related to the advancement of fruit ripening in sweet pepper (Nielsen et al., 1991). Although the absolute contents of glucose and fructose are mainly covered by the main portion of soluble carbohydrates during fruit maturity, the seasonal fluctuation of sucrose, which consists of a relatively small portion of the soluble carbohydrate pool, would critically respond to the cultivar during the growing season.

Of the targeted metabolites, most amino acids were closely associated with each other, except for some amino acids in the PLS-DA loading plot (Fig. 2C). Most amino acids are closely clustered in the PCA loading plot of 11 chili pepper cultivars, but are less associated with most soluble carbohydrates and organic acids (Florentino-Ramos et al., 2019). However, the overall responses of the amino acids were differentially correlated with other major metabolites depending on the bell pepper cultivar. Amino acids were much more tightly correlated with themselves in the ‘DSP7054’ cultivar than in the others, where amino acids were relatively spread out and less closely correlated with themselves (Fig. 6). The results indicate that the alteration of amino acids could be closely responded during the winter growing season compared to the other major metabolites. However, overall amino acid responses may be highly dependent on bell pepper cultivars during the winter growing season. Typically, most amino acids gradually increase with advancing fruit maturity in bell pepper (Aizat et al., 2014). Although the results do not cover the entire growing season, the content and profiles of amino acids are highly dependent on the bell pepper cultivar (Lwin et al., 2022), chili pepper cultivar (Florentino-Ramos et al., 2019), and habanero pepper cultivar (Ramírez-Meraz et al., 2024). In addition, no amino acids were identified as important variables in the VIP score plot

(Fig. 2D). Glutamine was only detected as an important variable from the VIP scores plot of the ‘Scirocco’ bell pepper cultivar (Fig. 3B), compared to the results from the other cultivars (Fig. 3D and 3F). However, glutamine was only up-regulated in the ‘DSP7054’ cultivar, compared to the ‘Volante’ cultivar (Fig. 7D) and tryptophan was only up-regulated in the ‘Volante’ cultivar, compared to the ‘Scirocco’ cultivar (Fig. 7F). Irrespective of the cultivar, tryptophan was only closely clustered with pericarp colour variables in the normalised heatmap matrix compared to the other amino acids (Fig. 1), thereby resulting in a positive correlation between colour variables and tryptophan (Fig. 5). Thus, tryptophan was only positively correlated with pericarp colour variables in the ‘Scirocco’ cultivar, compared to the others (Fig. 6). Moreover, based on the difference in the pericarp colour between the ‘Scirocco’ and ‘Volante’ cultivar (Fig. 7E and 7F), total carotenoid was highly up-regulated in the ‘Scirocco’ cultivar and thus resulted into higher up-regulation of pericarp redness (a^*), thereby contributing to altering tryptophan regulation as only amino acids, compared with the ‘Volante’ cultivar. It has been reported that degradation of chlorophyll and alteration of red and yellow carotenoids are highly regulated by the amino acid substitution of tryptophan (an aromatic amino acid) with arginine during pepper fruit ripening (Borovsky and Paran, 2008). In contrast, glutamine and total carotenoid were differentially regulated only between the orange and yellow bell pepper cultivars (Fig. 7D). Thus, these two parameters were positively correlated in the overall correlation coefficient heatmap matrix (Fig. 5). The up-regulation of glutamine levels is strongly involved in fruit ripening in cold-stored fig (Byeon and Lee, 2021). Thus, the unique regulation of tryptophan and glutamine could be derived from the alteration of carotenoid, based on the phenotypic responses of different pericarp pigmentations, depending on the bell pepper cultivar.

5. Conclusion

In this study, fruit quality attributes, mineral nutrients, and targeted major metabolites were determined throughout the winter season in three distinctive pericarp-coloured bell pepper cultivars. All response variables, including fruit quality attributes, mineral nutrients, and major targeted metabolites, were differentially clustered depending on the bell pepper cultivar or harvest time during the winter season. Additionally, the overall fruit quality was clearly segregated between February, April, December, and June. Most amino acids and fatty acids were tightly associated with each other, but least with most mineral nutrients, physiological fruit quality attributes, and chlorophyll variables. Moreover, comparing each cultivar, all colour variables were strongly associated with total carotenoid. Certain metabolites, including citric acid, glucose, malic acid, glutamine, fructose, and sucrose, were highly specific to certain bell pepper cultivars. Amino and fatty acids were closely correlated with each other in ‘DSP7054’ cultivar, and sucrose and glutamine were highly up-regulated in the ‘DSP7054’ cultivar, compared to the ‘Scirocco’ or ‘Volante’ cultivar. Tryptophan was up-regulated in the ‘Volante’ cultivar, compared to the ‘Scirocco’ cultivar. Therefore, the result indicated that fruit quality could be influenced by either the growing season or bell pepper cultivar, with both factors playing critical roles in winter season. Furthermore, the results of correlation coefficient tests could guide the potential research concepts of the metabolic pathways underlying this response, which provide a route to develop the predictive models for the evaluation of fruit quality under various growing conditions. Nonetheless, this study could be limited by focusing on a single winter growing season in a controlled greenhouse environment, which may be relatively less strong enough to guarantee fruit quality for other seasons or growing conditions. Although there are some limitations, our research stands as the platform of future research for supporting sustainable and efficient bell pepper cultivation and fruit quality under various seasonal conditions.

CRediT authorship contribution statement

Si-Eun Byeon: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Hyowon Park:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Theint Thandar Latt:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Sangyo Jeong:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Jinhee Lee:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Hnin Phyu Lwin:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Jinwook Lee:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

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.

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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.2024.113898](https://doi.org/10.1016/j.scienta.2024.113898).

Data availability

Data will be made available on request.

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