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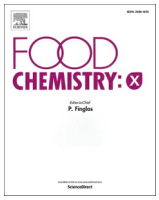

Inter- and intra-varietal genetic variations co-shape the polyphenol profiles of *Vitis vinifera* L. grapes and wines

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ABSTRACT

Inheritance and mutations are important factors affecting grape phenolic composition. To investigate the inter- and intra-varietal differences in polyphenolic compounds among grapes and wines, 27 clones belonging to eight varieties of *Vitis vinifera* L. were studied over two consecutive years. A total of 24 polyphenols (nine anthocyanins, three flavanols, five flavonols, and seven phenolic acids) were analyzed, and the physicochemical parameters of the grapes and wines were determined. Polyphenol profiles showed significant varietal and clonal polymorphisms, and malvidin-3-*O*-glucoside, peonidin-3-*O*- glucoside, and epicatechin were identified as key biomarkers distinguishing different grapes and wines when using an orthogonal partial least squares discriminant analysis. Further multivariate analysis classified these genotypes into three subclasses, and a somatic variant of 'Malbec', MBVCR6, had the most abundant polyphenolic compounds that were related to the titratable acid content. The current results reveal that varietal and clonal variations are important for obtaining wines with high polyphenol content.

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the most widely cultivated crops worldwide, and its high levels of phenolic compounds make it an important component of the human diet and wine industry. Phenolic compounds are secondary metabolites in grapes and wines, which are used to determine wine quality parameters and organoleptic properties, in addition to their distinct functions in plant biotic and abiotic environmental stress responses (Pantelić et al., 2016). Phenolic compounds in grapes are divided into two groups according to their carbon skeleton: flavonoids and non-flavonoids. Flavonoids mainly consist of anthocyanins, flavonols, and flavanols, which are involved in wine color, astringency, and bitterness. Non-flavonoids include stilbenes (resveratrol) and phenolic acids, and the latter include hydroxybenzoic and hydroxycinnamic acids, which have potential roles in stabilizing the color of red wine (Garrido and Borges, 2011). Studies on grape and wine polyphenols are increasing, particularly because of their importance when evaluating grape varieties and obtaining wines with distinctive or improved characteristics and antioxidant and free radical-scavenging properties, which can have positive effects on cardiovascular diseases, cancer, and diabetes (Pereira et al., 2013).

Phenolic composition and content depend on genetic (variety and clone) and environmental (light exposure, temperature, and soil) factors, as well as viticultural practices (canopy management, irrigation,

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nitrogen availability, and covering). For instance, Tian et al. (2023) applied gravel mulching within vineyard rows to promote anthocyanin and flavonol accumulation by regulating the cluster- and root-zone microclimates. In recent years, unstable weather conditions throughout the season due to climate change have had an increasing influence on berry composition and consequently, on the composition of wine. For example, a tendency toward reduced freshness and modifications in the ruby color of some Bordeaux wines has been observed (Drappier et al., 2017). A series of management methods has consequently been employed, including late winter pruning, apical leaf removal, and minimal pruning, to improve wine production (Gutiérrez Gamboa et al., 2020). Regardless of the effects of cultivation and environmental factors, some studies have shown that grape phenolic profiles, especially anthocyanins, are under strict genetic control and that their distribution varies considerably among different grape varieties (Revilla et al., 2001). The anthocyanin fingerprints of varietal wines have been proposed as analytical tools for authenticity certification (Kontoudakis et al., 2011).

Grape varieties have high genetic complexity and can be further subdivided into clones (Zombardo et al., 2022). Clones generally derive from spontaneous somatic mutations after reproduction by vegetative propagation, which generates some intra-varietal phenotypic variations, such as morphological (canopy thickness, bunch compactness, and fruit color in the grey and white variants of 'Pinot noir') and chemical traits (sugar, acidity, and polyphenols content) (Lemos et al., 2020; Vezzulli et al., 2012). The significant differences in the total anthocyanin and individual polyphenol contents are similar to those observed between varieties, as they are up to 0.4–2.4-fold among the berry skins of 'Barbera' and 'Kalecik Karası' clones (Ferrandino & Guidoni, 2010; Keskin et al., 2022), and there are reportedly threefold differences in the hydroxybenzoic acid content in 'Tempranillo' VN21 and RJ43 clone seeds (Royo et al., 2021). These genotype effects of the varieties and clones are critical determinants of grape and wine phenolic profiles and are thus important in determining grape and wine flavors in varieties with high levels of genetic diversity. Furthermore, it has been ascertained recently that intra-varietal variations in other traits that improve wine quality can arise as a plant responds to environmental stress, such as adaptations to climate change, through clone-dependent DNA methylation patterns (Xie et al., 2017). Therefore, clonal selection based on intra-varietal genetic polymorphisms has received considerable attention for the production of red wines with high polyphenol content, as they are responsible for key qualities, including sensory and health benefits (Royo et al., 2021). However, to the best of our knowledge, no detailed qualitative or quantitative studies have been conducted on the genetic effects of inter- and intra-varietal differences in polyphenol components in grapes and their corresponding wines.

In this investigation, we comprehensively examined the phenolic profiles of 27 clones belonging to eight different grape varieties to determine the influence of genetic diversity on grape and wine quality over two consecutive vintages (2019 and 2020). A total of 24 polyphenols (9 individual anthocyanins and 15 individual phenols) were quantified in all grapes and wines, and the possible correlation between the grape polyphenol profile and their physicochemical parameters and polyphenol content between grapes and wines were studied. Polyphenol profiles showed significant varietal and clonal variability, and orthogonal partial least squares discriminant analysis (OPLS-DA) identified malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, and epicatechin as key biomarkers that distinguished different grapes and wines into three subclasses by multivariate analysis. Furthermore, a somatic variant of 'Malbec', MBVCR6, had the most polyphenolic compounds, both in its grape and wine, which was attributable to the titratable acid content. The present results will aid in the future selection of suitable genotypes to obtain polyphenol-enriched wines with high antioxidant levels.

2. Materials and methods

2.1. Experimental site and plant materials

This study was conducted in a commercial vineyard located in Minqin County (38°3′45″–39°27′37″, elevation 1400 m), Gansu Province, China, during 2019 and 2020. The area is defined as being in a temperate continental desert climate. All self-rooted vines were planted in 2015, and the vineyard was oriented south to north, with 2.5 m × 0.8 m spacing and grass growing between the grape rows. The canopy was trained using a vertical shooting positioning trellis system. An integrated water and fertilizer dropper system was applied, and field management was carried out according to standard viticultural techniques.

A total of 27 clones from eight varieties, namely, 'Gamay', 'Pinot Noir', 'Grenache', 'Mourvedre', 'Cabernet Sauvignon', 'Malbec', 'Merlot', and 'Cabernet Franc', were studied (Supplementary Table S1). Most climate indices were similar over the two years (Supplementary Table S2), and the cumulative rainfall was <125 mm, which was consistent with the arid climate of the experimental site (Li et al., 2011). The cumulative temperature was sufficient to ensure grape metabolite synthesis; however, there was a 44 % difference in cumulative rainfall over the two years of this investigation, which may have had significant effects on berry metabolism. Most phenology time points showed similar patterns among the different varieties, except for maturity days, which varied significantly between the 'Mourvedre' (the longest) and 'Malbec' (the shortest) clones, with a difference of approximately 1–2 weeks (Supplementary Table S3).

At harvest, 10 grape clusters were sampled from each clone using an "S" sampling method and transported to the laboratory with ice packs for physicochemical analysis. For the winemaking test, 30 kg of grapes were picked from each clone for three replicates.

2.2. Winemaking protocol

At harvest, the grapes were manually destemmed and crushed, with three technical replicates for each sample. Must was fermented in 10 L vessels containing 60 mg/L SO_2 (0.12 g/L potassium metabisulfite), 30 mg/L pectinase (Lallzyme Ex, Lallemand, France), and 0.20 g/L *Saccharomyces cerevisiae* strain (CECA, Angel, China). Alcoholic fermentation was performed at 20–25 °C in a temperature-controlled workshop. Caps were punched down manually, and specific gravity was determined twice a day. When the specific gravity was <0.997, the skins and seeds were removed, and alcohol fermentation was allowed to proceed. When the reducing sugar level was <4 g/L, the wine samples were bottled in 750 mL bottles with 80 mg/L SO_2 and then stored at −4 °C for subsequent chemical analysis after three months.

2.3. Physiochemical parameters of grape and wine

Cluster weight and cluster tightness were measured using 10 grape clusters for each clone, according to the method of Aćimović et al. (2016). Then, 100 intact berries were randomly cut from the clusters according to the 5-point sampling method. The berries were weighed, peeled, and seeded to weigh the skin, seeds, and flesh using an electronic scale in each replicate. The reducing sugar and titratable acidity contents of the grapes and wines were determined according to Fehling's reagent reduction method and acid-base titration, respectively, and titratable acidity was expressed as g/L tartaric acid (Shi et al., 2018). Berry maturity was expressed as the ratio of reducing sugars to titratable acids. The wine alcohol content, volatile acidity, and dry extract content were determined according to the National Standard of the People's Republic of China (GB/T15038-2006). Three technical replicates were performed for each sample.

2.4. Analysis of the total phenolic compounds in grapes and wines

Hundred berries were peeled on ice for phenolic compound analysis per replicate, and three replicates were conducted. The berry skin was homogenized in liquid nitrogen and freeze-dried using an FD5-series vacuum freeze dryer (GOLD SIM, Newark, NJ, USA). To extract phenolics, 0.50 g of dried skin powder per sample was added to 10 mL of extract solution consisting of 60 % methanol and 0.1 % hydrochloric acid. The mixtures were then extracted ultrasonically in dark conditions at 28 ℃ for 30 min, and then centrifuged at 4 ℃ and 8000 × *g* for 10 min, and the supernatants were collected into clear 50 mL centrifuge tubes. After the extraction steps were repeated twice, all three supernatants were combined and stored at –80 ℃ for further analysis.

A spectrophotometer was used to measure the total phenol (TP), total tannin (TTA), total flavonoid (TFO), total flavanol (TFA), and total anthocyanin (TAN) contents, according to the Folin-Ciocalteu, methyl cellulose precipitation, aluminum chloride colorimetric, *p*-dimethylaminocinnamaldehyde-HCl (*p*-DMACA), and pH differential methods, respectively (Meng et al., 2018). The results were calculated using the standard curves of gallic acid for TP, catechin for TTA and TFA, and rutin for TFO, and were expressed as milligram per gram of dry skin weight (mg g^{-1} DW) (Supplementary Table S4). To analyze the total phenolic parameters of the wine, the solutions were mixed directly with wine, and their absorbances (mg/L) were determined after reaction.

2.5. Analysis of the polyphenol components of grapes and wines

Sample pre-treatment of the polyphenol components was performed according to the method described in Section 2.4.

2.5.1. Analysis of the anthocyanin contents

To extract individual anthocyanins, 0.50 g skin powder was dissolved ultrasonically in 10 mL of extraction buffer (formic acid/methanol, 2:98, v/v) for 10 min and then shaken in darkness for 30 min at 130 rpm and 25 ℃. The mixture was then centrifuged at 4 ℃ and 8000*g* for 10 min, and the supernatant was collected. The residues were re-extracted twice, and 30 mL of the supernatant was pooled and concentrated to dryness. The precipitates were redissolved in 10 mL of mobile-phase A (formic acid: acetonitrile: water, 2:6:92, v/v/v) and then filtered through a 0.45 μm polypropylene syringe filter (Jinteng, Tianjing, China) for quantitative analysis of anthocyanins. Samples were analyzed using high-performance liquid chromatography-tandem mass spectrometry according to the protocols described by Li et al. (2011). Individual anthocyanin contents were quantified using the Mv-glu standard curve and expressed as mg kg^{-1} skin dry weight (DW) for grapes and mg/L for wine.

2.5.2. Analysis of the non-colored polyphenol compound contents

The extraction of individual non-colored polyphenols was similar to that of individual anthocyanins, where 0.40 g of skin powder was extracted ultrasonically with 10 mL of extraction buffer (water/ethyl acetate, 1:9, v/v), and the final precipitates were redissolved in 1 mL of methanol. Samples were analyzed according to the protocol described by Li et al. (2011). Quercetin-3-*O*-glucoside (Qu-glu), catechin, and gallic acid standard curves were used to calculate flavonol, flavanol, and phenolic acid contents, respectively, and were expressed as mg kg^{-1} skin DW for grapes and mg/L for wine.

2.6. Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS version 22.0 (Tukey's test, $P < 0.05$). OPLS-DA was performed using Simca version 14.1. Principal component and hierarchical clustering analyses were performed using XLSTAT 2019 software. All figures were drawn using GraphPad Prism 8.0.2 and XLSTAT 2019.

3. Results and discussion

3.1. Morphological parameters of different grape varieties and their clones

Grape morphological parameters varied significantly among different varieties and clones. The 100-berry skin weights, 100-berry weights, and cluster weights for 'Mourvedre' and 'Grenache' were significantly higher than those of the other varieties, whereas those of 'Cabernet Sauvignon' and 'Cabernet Franc' were lower (Supplementary Fig. S1). 'Merlot' showed greater clonal polymorphism in cluster weight, 100-berry skin weight, and skin-fruit ratio when compared with the other varieties. The cluster weight was significantly higher in ML343 than in the other 'Merlot' clones, with a 58 %–101 % difference, which was also observed among 'Pinot Noir' clones in a previous study (Castagnoli & Vasconcelos, 2006). Similarly, there was a 39 %–79 % difference in 100-berry skin weights and skin-fruit ratios among 'Merlot' clones. Environmental factors seem to differentially affect grape morphological parameters, as there were no similar changes in trends for cluster tightness and skin-fruit ratio among different varieties and their clones over the two years. Cluster weight and cluster tightness were significantly lower in 2020 than in 2019, except in 'Grenache' and 'Mourvedre', which may be explained by the different rainfall patterns between the two years (Tian et al., 2023). These results were further supported by two-factor analysis, where the year factor significantly affected grape morphological parameters at $P < 0.001$ level (Supplementary Fig.S1).

3.2. Chemical parameters for different grape and wine varieties and their clones

The reducing sugar content, titratable acid content, and maturity of berries are shown in Supplementary Fig.S2. Titratable acid content and maturity varied significantly among different varieties and their clones, but a minor difference was found in the reducing sugar content. Notably, 'Malbec', 'Cabernet Sauvignon', and 'Pinot Noir' berries had higher titratable acid contents when compared with the other varieties, whereas they were lower in 'Mourvedre', 'Grenache', and 'Merlot' berries (Supplementary Fig. S2B). In contrast, 'Merlot', 'Mourvedre', and 'Grenache' berries had higher maturity than 'Malbec' berries. In terms of clones, berry maturity showed a large difference of 33 % among 'Mourvedre' clones and 43 % among 'Grenache' clones. These results suggest that berry titratable acid content and maturity are affected significantly by inter- and intra-varietal genetic variations (Lemos et al., 2020).

To study the effects of genetics and mutations on wine flavor in detail, 27 individual wines were vinified under the same conditions in 2020. Wine reducing sugar ranged from 2.17 to 3.39 g/L, volatile acids ranged from 0.35 to 0.77 g/L, and dry extracts ranged from 19.91 to 27.99 g/L (Supplementary Fig.S3). Among all varieties, 'Cabernet Franc' wine had the highest alcohol content, with an average of 14.08 % v/v, within its clones. However, titratable acid content varied significantly among varieties and their clones. 'Malbec' contained the highest and 'Merlot' contained the lowest amount of titratable acid (Supplementary Fig. S3). In addition, the intra-varietal difference of titratable acid reached 35 % between PNVCR18 and PNVCR20, with no similar differences found in previous studies (Cuadros-Inostroza et al., 2020). Wine acids are derived from their grapes, and in this study, the high titratable acid content in 'Malbec' wines was found to coincide with that of its grapes.

3.3. Berry polyphenol components in different varieties and their clones

3.3.1. Total phenolic parameters of berries

Polyphenolic compounds in grapes have been extensively studied. They possess excellent antioxidant properties, which are related to their

ability to interfere with free radical formation and propagation, chelate transition metals, and inhibit certain enzymatic reactions (Baiano & Terracone, 2011). In this study, 'Malbec' contained the highest amount of total anthocyanins (Fig. 1C) (12.44–22.99 mg g^{-1}), followed by 'Cabernet Sauvignon' (9.69–18.85 mg g^{-1}), 'Cabernet Franc' (7.26–15.75 mg g^{-1}), 'Merlot' (8.49–13.56 mg g^{-1}), 'Pinot Noir' (5.69–10.12 mg g^{-1}), 'Gamay' (6.91–9.01 mg g^{-1}), 'Mourvedre' (6.58–8.27 mg g^{-1}), and 'Grenache' (4.11–6.88 mg g^{-1}), which were mostly consistent with previous study results (Zhang et al., 2020). Among all clones, MBVCR6 had the highest total anthocyanin content (22.99–20.77 mg g^{-1}), whereas it was low in PNVCR9 (5.69 mg g^{-1}) and GN224 (4.11 mg g^{-1}). Furthermore, the largest intra-varietal differences were between MBVCR6 and MBVCR598 (85 %).

Similarly, the total flavonoid and total flavanol contents changed significantly among varieties and clones at $P < 0.05$. These contents were high in 'Malbec' and low in 'Gamay' clones (Fig. 1D and E). In contrast, 'Mourvedre' had high and 'Gamay' had low total phenol and total tannin contents (Fig. 1A and B). Additionally, 0.15–1.7-fold differences for these two parameters were found within 'Pinot Noir' clones. Baiano and Terracone (2011) found that the polyphenol components of different grapes depend mainly on varietal differences, whereas inter- and intra-varietal variation jointly affected berry polyphenol content in this study.

3.3.2. *Individual phenol components of berries*

3.3.2.1. Anthocyanins. Anthocyanins, which are responsible for the red color in grapes and wine, are mostly synthesized in the skin, and they begin to accumulate at veraison, which is defined as the onset of ripening (Tian et al., 2023). Therefore, the evaluation and identification of the anthocyanin composition of a variety is important for estimating its enological potential. Nine individual anthocyanins were studied (Supplementary Table S5), and the results of total individual anthocyanin analysis were grouped by variety and clone type (Fig. 2A, C, D, and F). All 27 clones of the eight varieties together showed that Mvs (mostly in Mv-glu) was the most abundant anthocyanin in grapes, and its contents ranged from 2477.83 to 10063.31 mg kg^{-1}, and the others accounted for a low proportion (Fig. 2B and E), which is in agreement with previous studies (Shi et al., 2018). It is well known that delphinidin-3-*O*-glucoside (Dp-glu) and cyanidin-3-*O*-glucoside (Cy-glu) are the precursors of Mv-glu, petunidin-3-*O*-glucoside (Pt-glu), and peonidin-3-*O*-glucoside (Pn-glu), and their decrease was accompanied by an increase in Pn-glu and Mv-glu during grape ripening (Kong et al., 2021). Thus, the accumulated malvidin derivatives may result from the continuous methylation of their precursors, as evidenced by the positive correlation between *O*-methyltransferase (OMT) expression and anthocyanin methylation levels during ripening (Muñoz et al., 2014).

By comparing the anthocyanin components of the different varieties and their clones, the results showed that the Mv-glu, malvidin-3-*O*-(*trans*-6-*O*-coumaryl)-glucoside (Mv-cmglu), and Pt-glu contents were the highest in 'Malbec' clones, especially in MBVCR6, and the maximum differences were 2.5–3.5-fold (vs. CS170 or GN224), 154.6–223.2-fold (vs. PNVCR20 or PN375), and 9.2–14.7-fold (vs. GA509 or GN224), respectively (Supplementary Table S5). However, most anthocyanin contents were low in the 'Grenache', 'Gamay', and 'Pinot Noir' clones (Fig. 2B and E). Previous studies have found that Mv-glu is responsible for the anthocyanin content and color intensity variations of 'Malbec' clones, due to its extremely high proportions in malvidin derivatives (Muñoz et al., 2014). In this study, 0.3–1.1-fold differences of malvidin derivatives were observed between MBVCR6 and MBVCR598. These changes may be related to the distinct gene expression levels of anthocyanin hydroxylase (F3′5′H), OMT1, and anthocyanin transporter (AM2)

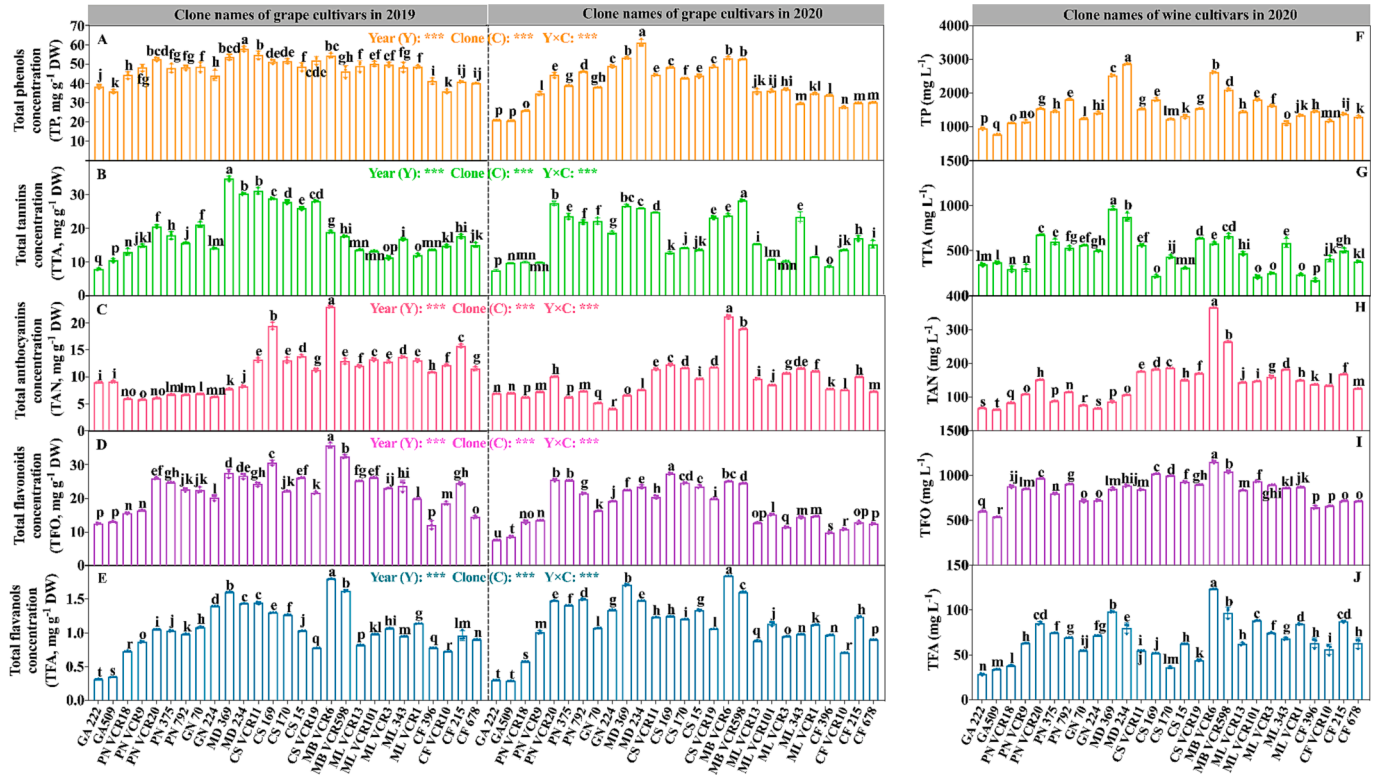

Fig. 1. Grape and wine total phenolic parameters among different varieties and their clones. Total phenols in 2019 and 2020 grape (A) and 2020 wine (F); total tannins in 2019 and 2020 grape (B) and 2020 wine (G); total anthocyanins in 2019 and 2020 grape (C) and 2020 wine (H); total flavonoids in 2019 and 2020 grape (D) and 2020 wine (I); total flavanols in 2019 and 2020 grape (E) and 2020 wine (J). GA, Gamay; PN, Pinot noir; GN, Grenache; MD, Mourvèdre; CS, Cabernet Sauvignon; MB, Malbec; ML, Merlot; CF, Cabernet Franc. Different lowercase letters indicate significant differences among all clones (Tukey's test, $P < 0.05$). Y and C indicate year and clone, respectively. ***, significant difference at $P < 0.001$.

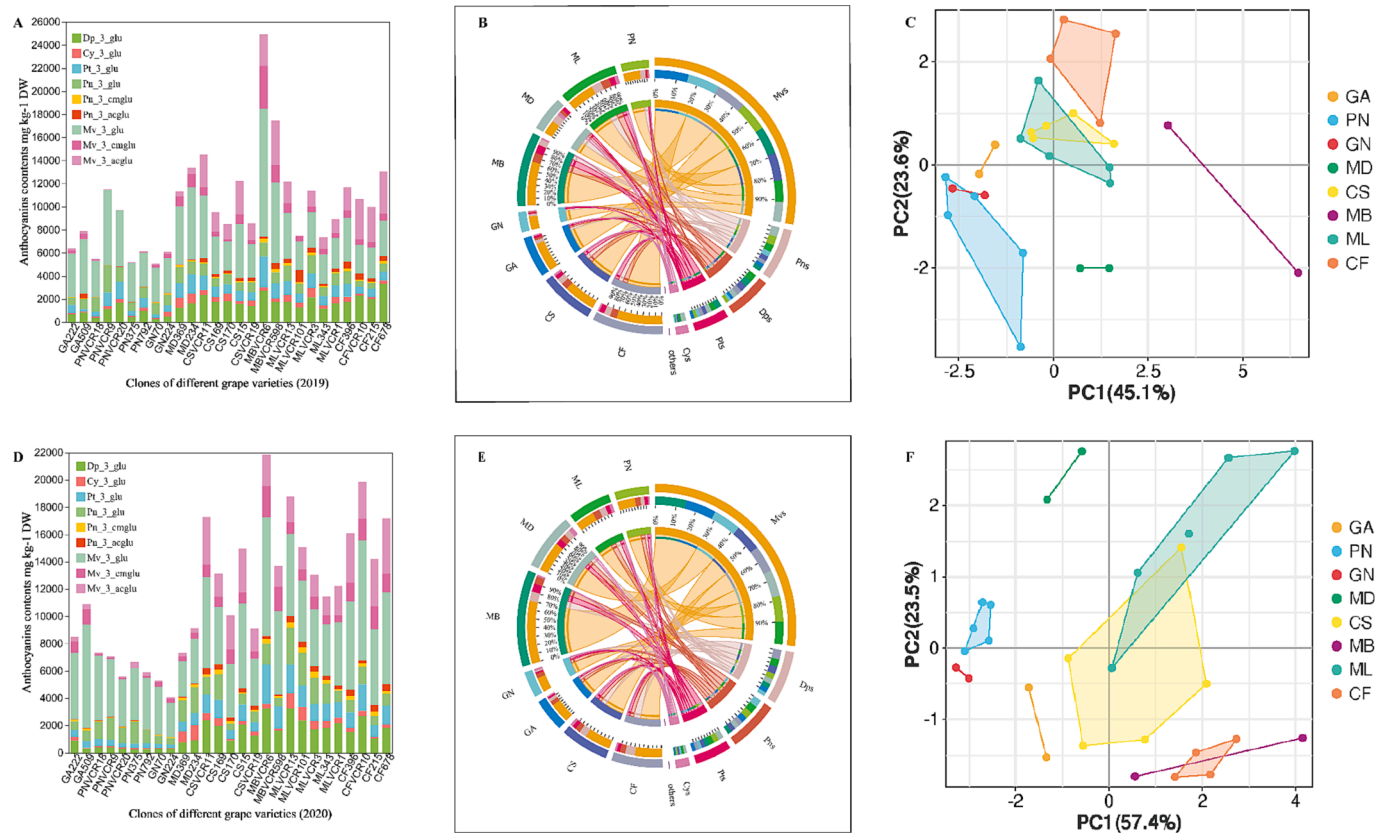

Fig. 2. The anthocyanin component distribution among different grape varieties and their clones. Individual anthocyanin contents in 2019 (A) and 2020 (D). Circos plot of anthocyanin distribution among different grape varieties in 2019 (B) and 2020 (E). Principal component analysis (PCA) based on grape individual anthocyanins in 2019 (C) and in 2020 (F). Mvs (Malvidin-3-*O*-glucoside, Malvidin-3-*O*-(6-*O*-acetyl)-glucoside, Malvidin-3-*O*-(6-*O*-coumaryl)-glucoside); Dps (Delphinidin-3-*O*-glucoside); Pns (Peonidin-3-*O*-glucoside, Peonidin-3-*O*-(6-*O*-acetyl)-glucoside, Peonidin-3-*O*-(6-*O*-coumaryl)-glucoside); Pts (Petunidin-3-*O*-glucoside); Cys (Cyanidin-3-*O*-glucoside). GA, Gamay; PN, Pinot noir; GN, Grenache; MD, Mourvèdre; CS, Cabernet Sauvignon; MB, Malbec; ML, Merlot; CF, Cabernet Franc.

in the anthocyanin biosynthesis pathway (Muñoz et al., 2014).

3.3.2.2. Flavonols. Flavonols were previously found to be the most abundant non-colored phenols in the 'Tempranillo' grape (Royo et al., 2021), and similar results were found in the current study (Fig. 3A and D). 'Malbec' contained the highest (570.57–624.66 mg kg^{-1}) and 'Grenache' contained the lowest (213.96–283.28 mg kg^{-1}) content of total individual flavonols, and the differences ranged from 1.2- to 3.1-fold (Fig. 3B and E, Supplementary Table S6). These results were consistent with the individual anthocyanin results (see Section 3.3.2.1). The significant intra-varietal difference between CSVCR11 and CS169 reached 1.9-fold, and that between PNVCR20 and PNVCR18 reached 2.3-fold (Supplementary Table S6). In terms of all clones, CSVCR11 (2019) and MBVCR6 (2020) had high and GN70 had low total individual flavonol contents. Qu-glu was the most abundant of the five individual flavonols studied, with an average content of 206.27 mg kg^{-1}, followed by rutin (80.4 mg kg^{-1}). It is worth noting that the rutin content varied between 268.02 and 285.33 mg kg^{-1} for 'Malbec' and between 9.73 and 10.69 mg kg^{-1} for 'Grenache', which may explain the inter-varietal variation of the total individual flavonols. Grape polyphenolic compounds are also affected by environmental factors, including sunlight exposure, temperature, and rainfall (Đorđević et al., 2017). According to the meteorological information at the experimental site, greater rainfall in 2019 than in 2020 may have caused the distinct distributions of the individual flavonols among the clones between the two years, which was consistent with the results of the two-factor analysis in Supplementary Table S6.

3.3.2.3. Flavanols and phenolic acids. In contrast to flavonols, low levels of flavanols and phenolic acids were found in the grapes (Fig. 3B and E). Total individual flavanol contents varied from 163.43 to 319.27 mg kg^{-1} for 'Cabernet Franc' to 46.42–72.35 mg kg^{-1} for 'Gamay', with a difference ranging from 2.5- to 3.4-fold. Moreover, CFVCR10 had high and GA509 had low content among all clones (Supplementary Table S6). The significant intra-varietal difference reached 0.8-fold between CS15 and CS169 and 1.0-fold between CF396 and CFVCR10, which was similar to that observed in other varieties (Mattivi et al., 2008).

In line with previous studies (Pantelić et al., 2016), the three major monomers of the five detected flavanol contents decreased in the following order: catechin (21.42–214.08 mg kg^{-1}) > epicatechin (0–109.04 mg kg^{-1}) > proanthocyanidinB2 (PB2) (6.39–56.65 mg kg^{-1}) (Supplementary Table S5). The inter-varietal variations of the epicatechin content represented that of the total individual flavanols, varying significantly from 'Cabernet Franc' (75.95–109.04 mg kg^{-1}) to 'Gamay' (undetected), which may play an important role in the classification of grape samples (Mattivi et al., 2008).

Seven phenolic acids were detected in all varieties, except for the absence of caffeic acid in 'Gamay' and 'Grenache' (Supplementary Table S6). 'Malbec' had the highest and 'Gamay' had the lowest phenolic acid contents, and the maximum difference ranged from 1.5- to 1.7-fold. However, the intra-varietal difference was 1.4-fold between ML343 and MLVCR13, and 1.5-fold between CSVCR11 and CS169. Component comparisons showed that *trans*-ferulic acid was the major compound, at 2.17–52.53 mg kg^{-1}, and this was followed by chlorogenic acid (3.29–32.17 mg kg^{-1}), *trans-p*-coumaric acid (1.4–20.05 mg kg^{-1}), and caffeic acid (0–15.87 mg kg^{-1}), but the other compounds had low levels (<10 mg kg^{-1}) in grape berries. These non-colored polyphenols significantly distinguished different varieties and clones (Fig. 3C and F).

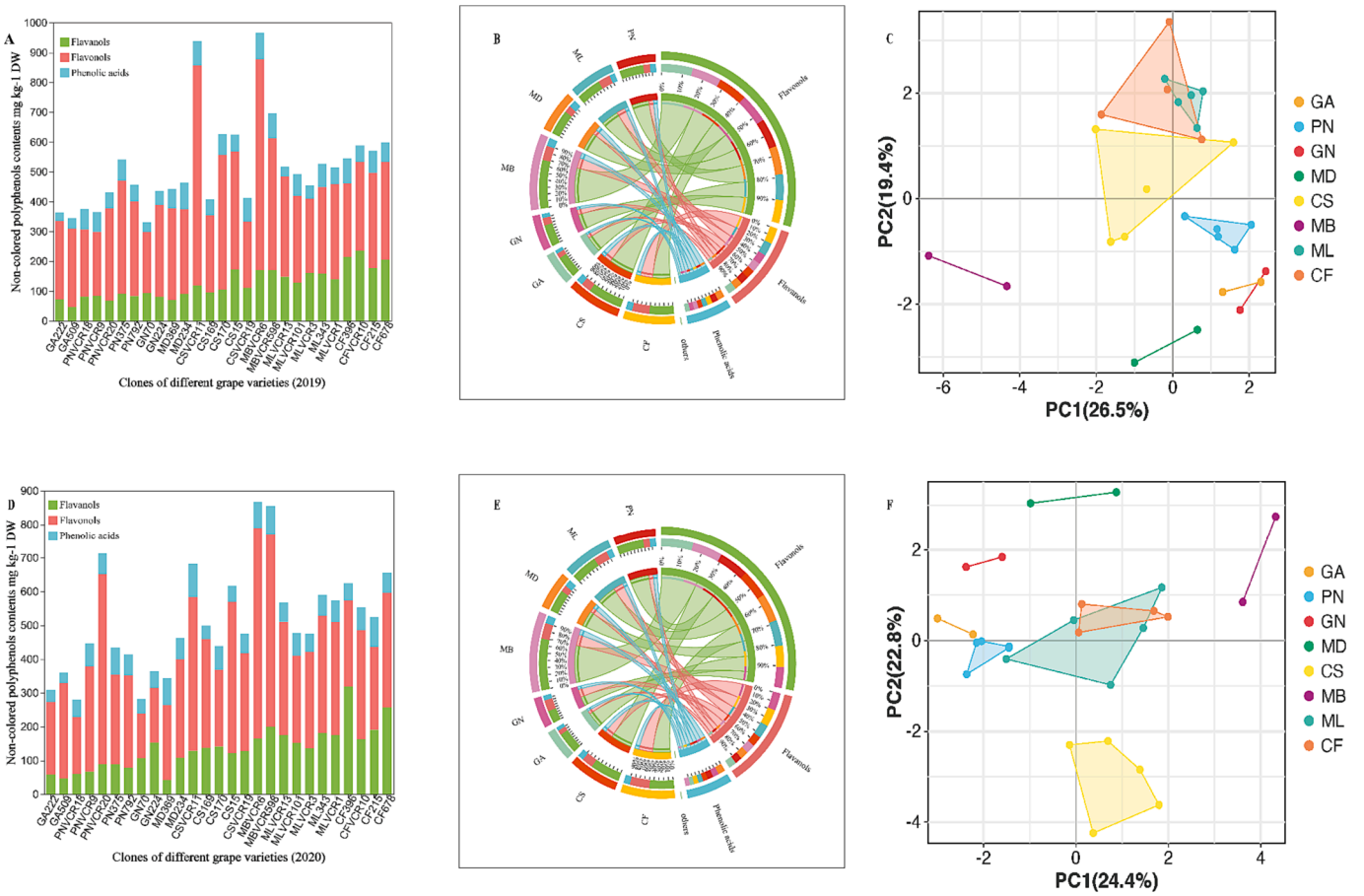

Fig. 3. The The non-colored polyphenol component distribution among different grape varieties and their clones. Non-colored polyphenol contents in 2019 (A) and 2020 (D). Circos plot of the non-colored polyphenol distribution among different grape varieties in 2019 (B) and 2020 (E). Principal component analysis (PCA) based on grape non-colored polyphenols in 2019 (C) and 2020 (F). GA, Gamay; PN, Pinot noir; GN, Grenache; MD, Mourvèdre; CS, Cabernet Sauvignon; MB, Malbec; ML, Merlot; CF, Cabernet Franc.

3.4. Wine polyphenol components of different varieties and their clones

3.4.1. Total phenolic parameters of wines

In this study, from the MBVCR6 clone of 'Malbec' to the 'Gamay' clones, wine total anthocyanin, total flavonoid, and total flavanol contents were 63.07–365.28 mg/L, 540.13–1165.52 mg/L, and 27.29–123.73 mg/L, respectively (Fig. 1H, I, and J). The significant inter-varietal differences for these three parameters were observed between 'Malbec' and 'Gamay' wines, and the intra-varietal differences ranged from 0.2- to 1.3-fold. Wine total phenol and total tannin contents were 774.19–2843.29 mg/L and 215.00–993.00 mg/L (Fig. 1F and G), respectively, corresponding with reported results of 160–3200 mg/L (Li et al., 2009). Similar to the results of grape, the total phenol and total tannin contents varied significantly among 'Mourvèdre', Gamay', and 'Merlot' wines. Wine polyphenols originate partly from skin impregnation, and other compounds are synthesized by complex physicochemical reactions during fermentation and aging processes (Zänglein et al., 2007), which may explain the different patterns of total phenolic parameters among varieties.

3.4.2. Individual phenol components of wines

3.4.2.1. Anthocyanins. To further compare the distribution of wine anthocyanins among different varieties and their clones, nine individual anthocyanins were analyzed, with the exception of malvidin-3-*O*-(6-*O*-acetyl)-glucoside (Mv-acglu) in PNVCR375; Cy-glu in GA222, GA509, and MD369; and peonidin-3-*O*-(6-*O*-acetyl)-glucoside (Pn-acglu) in PNVCR375 and MD369 (Supplementary Table S7). Mv-glu and Mv-acglu were the top two anthocyanins in all wines, at 29.12–188.33 mg/L and 0–40.57 mg/L, respectively. These were followed by Pt-glu (2.63–36.19 mg/L), Pn-glu (1.23–31.76 mg/L), Dp-glu (1.11–26.81 mg/L), Cy-glu (0–18.14 mg/L), Mv-cmglu (0.51–11.34 mg/L), Pn-acglu, and peonidin-3-*O*-(*trans*-6-*O*-coumaryl)-glucoside (Pn-cmglu) (0–8.31 mg/L) (Rutan et al., 2018). Anthocyanin content was observed to be significantly lower in wines than in grapes, and a similar decreasing trend has been observed in other red wines (Burin et al., 2011). Previous studies have reported that anthocyanins change constantly during red wine aging and storage due to their degradation and condensation with other phenolic compounds to generate more stable polymeric pigments or proanthocyanins (Zhao et al., 2021). The reaction between anthocyanins and hydroxycinnamic acids reportedly decreases wine anthocyanin content (Zänglein et al., 2007). These factors may explain the differences in anthocyanin content between the grapes and wines in this study.

Individual anthocyanin contents showed significant inter- and intra-varietal variability among wines (Fig. 4A and B, Supplementary Table S7). Mv-glu, Mv-cmglu, Pt-glu, and total individual anthocyanin contents were high in 'Malbec' wines (mostly in MBVCR6) and low in 'Merlot', 'Pinot Noir', and 'Grenache' wines (Supplementary Table S7). With a similar qualitative composition detected in grapes, good maceration and extraction of skin anthocyanins explained the abundance of anthocyanins in 'Malbec' clones (Royo et al., 2021). Mv-acglu and Pn-acglu were the most abundant in 'Cabernet Franc' wines (Pn-acglu in CF678), and Dp-glu, Cy-glu, and Pn-glu in 'Pinot Noir' wines

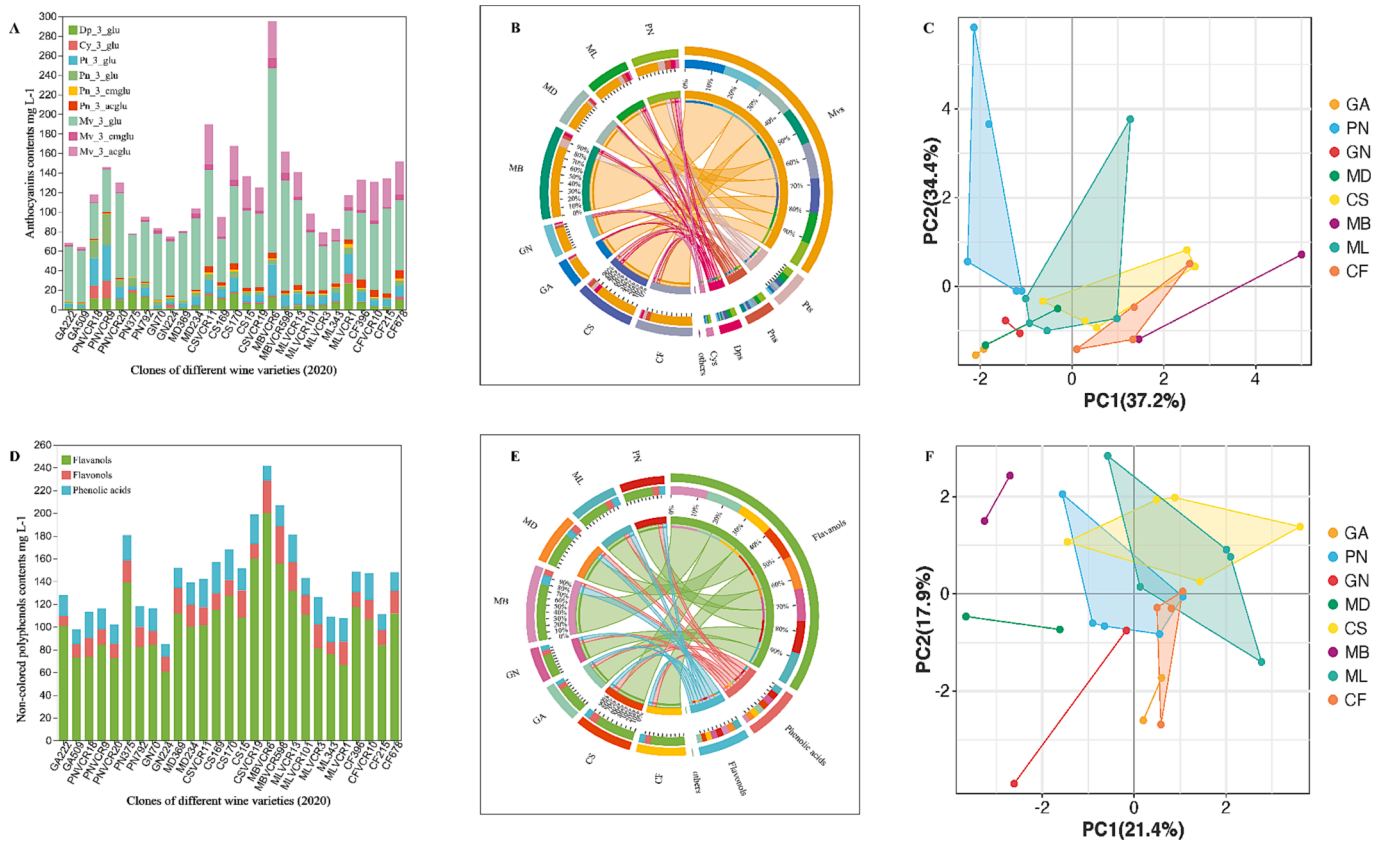

Fig. 4. The anthocyanin and non-colored polyphenol component distribution among different wine varieties and their clones. The contents of individual anthocyanin (A) and non-colored polyphenol (D) of wine in 2020. Circos plot of anthocyanin (B) and non-colored polyphenol (E) distribution among different wine varieties. Principal component analysis (PCA) based on wine individual anthocyanins (C) and non-colored polyphenols (F). GA, Gamay; PN, Pinot noir; GN, Grenache; MD, Mourvèdre; CS, Cabernet Sauvignon; MB, Malbec; ML, Merlot; CF, Cabernet Franc.

(mostly in PNVCR9), whereas they were low in 'Grenache' wines (Mv-acglu, Pt-glu, and Dp-glu) and 'Gamay' wines (Pns) (Supplementary Table S7). The intra-varietal differences varied significantly, from 1.0-fold for Pn-acglu within 'Cabernet Franc' wines to 25.3-fold for Cy-glu within 'Pinot Noir' wines. These specific anthocyanin profiles of different genotypes may contribute to wines' signature color intensity and tonality (Burin et al., 2011) and significantly differentiate between varieties and their clones (Fig. 4C).

3.4.2.2. Flavonols. Five individual flavonols were analyzed in wines, except for kaempferol-3-*O*-glucoside (Ka-glu), which was undetected in 'Mourvedre', and quercetin-3-*O*-galactoside (Qu-gal) in CSVCR19 and CF215 (Supplementary Table S8). Qu-glu was the most abundant flavonol in wines, and its content decreased in the order 'Malbec' > 'Mourvedre' > 'Merlot' > 'Cabernet Franc' > 'Pinot Noir' > 'Cabernet Sauvignon' > 'Grenache' > 'Gamay', ranging from 2.86 to 20.93 mg/L. The abundant Qu-glu also contributed mainly to wine total individual flavonol variations owing to a similar inter-varietal trend (Fig. 4D and E). Consistent with the study of Đorđević et al. (2017), significant intra-varietal difference was found in 'Merlot' clones, at up to 1.0-fold for Qu-glu and 1.3-fold for total individual flavonols.

Notably, the wine flavonol profiles differed from those of the corresponding grapes. We observed that total individual flavonols were the lowest in 'Gamay' wines and 'Grenache' grapes, and the highest in MBVCR598 wine and MVCR6 grapes (Supplementary Tables S6 and S8). These results are most likely attributable to the distinct extractability of berry polyphenols during maceration and grape maturity, as has been observed in 'Merlot' and 'Cabernet' grapes and wines (Pantelić et al., 2016). Furthermore, most phenolic compounds are more unstable in wine than in grapes because of complex oxidation, polymerization, and copigmentation reactions with other compounds during fermentation, which would presumably lead to polyphenol variation due to the changes in the redox balance and intensity and complexity of wines (Zhao et al., 2021). The limited number of individual flavonols detected in the present study may also be linked to these differences.

3.4.2.3. Flavanols and phenolic acids. Flavanols are important components of wine pigments and tannins that directly affect the degree of proanthocyanidin polymerization, wine astringency, and bitterness (Casassa et al., 2013). Consistent with the grape results, three individual flavanols were detected in wines (Supplementary Table S8). Epicatechin was the most abundant flavanol in the studied wines (19.60–114.22 mg/L), followed by catechin (6.11–76.34 mg/L) and PB2 (5.35–21.99 mg/L), which is consistent with previous studies (Garrido & Borges, 2011). Epicatechin, catechin, and total individual flavanol contents were the most abundant in 'Malbec' wines (mostly in MBVCR6), and PB2 in 'Merlot' wines, and the lowest in 'Grenache' and 'Pinot Noir' wines (Fig. 4D, Supplementary Table S7). Casassa et al. (2018) found that 'Pinot noir' wines typically contain a lower level of polyphenolic compounds than those of other red wines, which is why they are typically considered to have soft and delicate mouthfeel attributes (Parr et al., 2020). Flavonol variations thus tend to affect wine organoleptic perceptions (Cuadros-Inostroza et al., 2020). In addition, we found that CS169 contained the highest catechin content and the lowest epicatechin content, which was similar to the flavanol distribution results in the canes of different grape varieties (Loupit et al., 2020).

Seven phenolic acids were analyzed in wines, except for the absence of chlorogenic acid in GA509, PNVCR18, PN792, GN224, and CSVCR11 (Supplementary Table S8). In contrast to the flavanol results, the total phenolic acid contents were found to vary significantly from 'Cabernet

Sauvignon' wines (the highest in CS169) to 'Grenache' wines (Fig. 4D and E). Gallic acid and protocatechuic acid were the most abundant phenolic acids of wine, with other contents below 10 mg/L (Supplementary Table S8). Gallic acid serves as the precursor of hydrolyzable tannins and is involved in condensed tannin formation in wines (Garrido & Borges, 2011). At independent vineyard sites, CS169 has higher catechin and gallic acid contents, as well as other polyphenols, when compared with other 'Cabernet Sauvignon' clones (Burin et al., 2011), which was further supported by our results. Generally, total non-colored polyphenols showed greater genetic variability and were grouped by different varieties and their clones (Fig. 4D and F).

Overall, wine anthocyanins and non-colored polyphenols were analyzed together, revealing that the contents of Mv-glu, Mv-cmglu, and epicatechin were the highest in MBVCR6; Mv-acglu in CSVCR11; Pn-acglu in CF678; Dp-glu and Pn-cmglu in MLVCR1; Pt-glu, Pn-glu, and Cy-glu in PNVCR9; Qu-glu in MBVCR598; PB2 in GA222; and catechins and phenolic acids in CS169. These results indicate that grape inter- and intra-varietal variation potentially co-shapes wine phenolic profiles, and that these putative biomarkers can sufficiently discriminate wine quality when associated with their abundance changes and wine nutritional and sensorial properties (Cuadros-Inostroza et al., 2020).

3.5. Principal component (PCA) and hierarchical clustering (HCA) analysis reveals polyphenol variation among grapes and wines

PCA was performed to visualize polyphenol differences among varieties and their clones, considering all 9 anthocyanins and 15 non-colored polyphenols. In 2019 grapes, PC1 explained 40.5 % of the total variance that significantly distinguished 'Malbec', but no variety was grouped thoroughly by PC2 that explained 17.5 % of the total variance (Fig. 5A). Three anthocyanins (Mv-glu, Mv-cmglu, and Pt-glu) and most phenolic acids were the highest in the 'Malbec' clones; Ka-glu in 'Mourvedre' clones; Pn-acglu and epicatechins in 'Cabernet Franc'; and Qu-gal and protocatechuic acid in 'Merlot'. In 2020 grapes, PC1 explained 42.7 % of the total variance that distinguished 'Malbec', 'Merlot', 'Cabernet Franc', and 'Cabernet Sauvignon' from the other varieties, and PC2 explained 16.0 % of the total variance that distinguished 'Mourvedre' from the other varieties (Fig. 5C). In 2020 wine, PC1 and PC2 explained 39.8 % and 17.5 % of the total variance, respectively, but only PC1 distinguished 'Malbec' from the other varieties (Fig. 5E). Mv-glu, Mv-cmglu, and epicatechin were the highest in 'Malbec' clones. In addition, a supervised analytical model (OPLS-DA) was applied, and 10, 12, and 6 differential metabolites (VIP $>$ 1) were screened in 2019 and 2020 grapes and 2020 wine, respectively, with Mv-glu, Pn-glu, and epicatechin as the key metabolites (Supplementary Fig.S4).

As expected, HCA further significantly distinguished different varieties and clones. All 27 genotypes were clustered into three subclasses in 2019 grapes and 2020 wines, and two subclasses in 2020 grapes, according to the distance relationships of 24 individual components (Fig. 5B, D, and F). It is worth highlighting that MBVCR6 was independent of other individual genotypes, and 'Pinot Noir', 'Gamay', and 'Grenache' clones were always grouped together, both for grapes and wines. The current results indicate that inter- and intra-varietal variations significantly affected grape and wine polyphenol profiles, and their combinations amplified genetic differences among varieties, which led to MBVCR6 being identified as an excellent genotype for winemaking.

3.6. Correlation analysis between grape physicochemical properties, grape and wine polyphenolic components

Wine quality and character are directly correlated with grape quality, owing to the direct chemical composition input (Cuadros-Inostroza et al., 2020). In this study, a correlation analysis was conducted to explore the relationship between grape physicochemical properties and grape polyphenols over two years, as well as the polyphenol relationship between grapes and wines in 2020 (Fig. 6). Interestingly, the correlation matrix in the dynamic heat map presented a significant negative correlation between the total anthocyanin content and maturity days, cluster weight, cluster tightness, berry weight, and maturity. However, the opposite results were observed among the total phenol, flavonoid, and tannin contents and maturity days, cluster weight, and skin weight (Fig. 6A). These results can be explained by the fact that anthocyanins and non-colored polyphenols accumulate differently in berries during ripening. Similarly, the network diagram showed that individual anthocyanin and polyphenol accumulation was significantly affected by most physicochemical parameters, except for residual sugar, skin-berry ratio, pulp weight, and berry weight. In contrast to previous studies showing that sugar accumulation positively regulates anthocyanin synthesis in many plants through its metabolic and signaling functions (Duran et al., 2020), grape anthocyanin content was positively correlated with titratable acid content in the current study (Fig. 6A). The results showed that anthocyanins accumulated highly in 'Malbec' clones with high titratable acid content, which was similar to the findings for the 'Gamay Fréaux' grape (Kong et al., 2021). This may be because the anthocyanin pathway also serves as a potential carbon sink, competing with sugar accumulation for carbon sources in fruits (Soubeyrand et al., 2018), thus leading to the uncoupling of anthocyanins and sugar accumulation in particular varieties in this study. However, the negative relationship between anthocyanin content and maturity can be explained by the fact that anthocyanin content decreases during later ripening owing to degradation (Xie et al., 2021). Wine anthocyanins usually exist in the flavyl form, which degrades rapidly as the pH rises, making them unstable in wines (West & Mauer, 2013). Therefore, our observations that 'Malbec' wines with abundant anthocyanins can be explained partly by the high titratable acid content increasing anthocyanin stability. These results strongly imply a more complex relationship between grape maturity and anthocyanin accumulation than was expected, and further research will be required to resolve this.

Intergroup correlation analysis revealed that most individual anthocyanins (especially Mvs and Pn-acglu) in grapes were significantly positively associated with those in wines, except for Pn-cmglu and Cy-glu (Fig. 6B). These results suggest that MVs migrated more stably from grapes to wines, possibly because the number of methylations on the B ring stabilized their carbon skeleton and protected them from degradation (Garrido & Borges, 2011). However, only six of the 15 non-color individual phenols studied showed a good correlation between grapes and wines, including PB2, rutin, and Qu-gal. This was partly due to the complexity and unpredictability of fermentation, considering the reaction of polyphenols with other substances. These results suggest that the variation in grape physicochemical parameters among different varieties and their clones directly affects grape and wine polyphenol components, especially the relationship between maturity days, titratable acid content, and anthocyanin content.

4. Conclusion

In this study, the polyphenol profiles of 27 clones from eight *Vitis vinifera* L. varieties were investigated. The results showed that Mvs was the major anthocyanin, Qu-glu was the major flavonol, and catechin and epicatechin were the major flavanols in grapes and their resulting wines. As expected, polyphenolic compounds showed significant inter- and intra-varietal genetic variability, with the maximum differences ranging from 1.2- to 223.2-fold between 'Malbec' and other varieties and clones both of grapes and wines. Furthermore, a combined principal component and hierarchical cluster analysis classed all clones into different subclasses according to the polyphenol profiles and revealed that a somatic variant of 'Malbec', MBVCR6, had the highest anthocyanin and non-colored polyphenol contents. Correlation analysis of the polyphenol components and physicochemical parameters indicated that grape and wine anthocyanin contents were affected positively by the titratable acid content. To the best of our knowledge, this is the most comprehensive

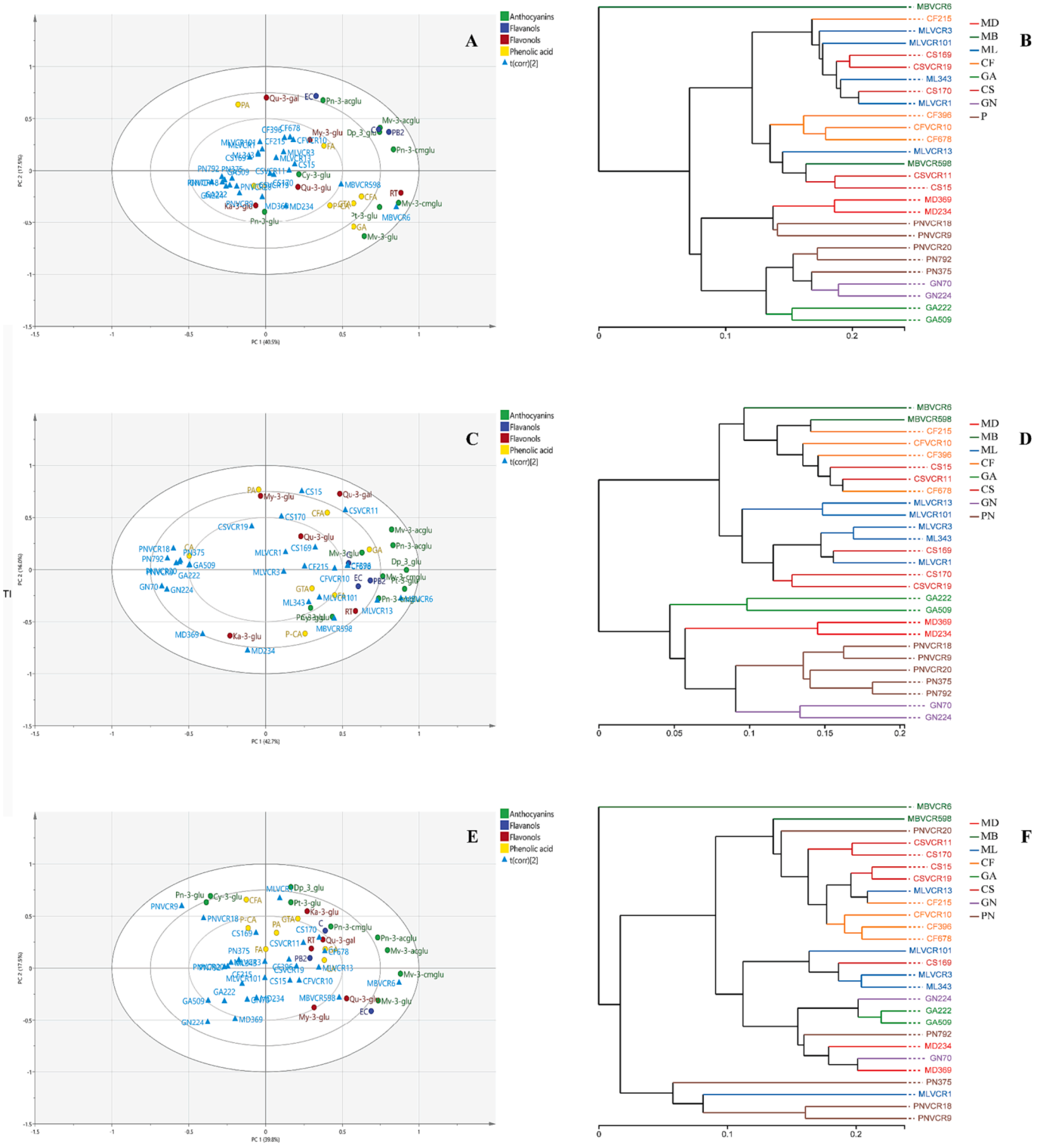

Fig. 5. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) of different varieties and their clones in grapes and wines. PCA based on the polyphenolic compounds of grape in 2019 (A) and 2020 (C) and wine in 2020 (E). HCA based on the polyphenolic compounds of grape in 2019 (B) and 2020 (D) and wine in 2020 (F). GA, Gamay; PN, Pinot noir; GN, Grenache; MD, Mourvèdre; CS, Cabernet Sauvignon; MB, Malbec; ML, Merlot; CF, Cabernet Franc. Cy: Cyanidin-3-*O*-glucoside; Dp: Delphinidin-3-*O*-glucoside; Mv: Malvidin-3-*O*-glucoside; Pn: Peonidin-3-*O*-glucoside; Pt: Petunidin-3-*O*-glucoside; Pn-acglu: Peonidin-3-*O*-(6-*O*-acetyl)-glucoside; Mv-acglu: Malvidin-3-*O*-(6-*O*-acetyl)-glucoside; Pn-cmglu: Peonidin-3-*O*-(6-*O*-coumaryl)-glucoside; Mv-cmglu: Malvidin-3-*O*-(6-*O*-coumaryl)-glucoside. C, Catechin; EC, Epicatechin; PB2, ProanthocyanidinB2; Qu-glu, Quercetin-3-*O*-glucoside; Ka-glu, Kaempferol-3-*O*-glucoside, Qu-gal, Quercetin-3-*O*-galactoside; My-glu, Myricetin-3-*O*-glucoside; RT, Rutin; GA, Galic acid; PA, Protocatechuic acid; CA, Chlorogenic acid; GTA, Gentitronic acid; CFA, Caffeic acid; P-CA, *trans*-*p*-coumaric acid; FA, *trans*-ferulic acid.

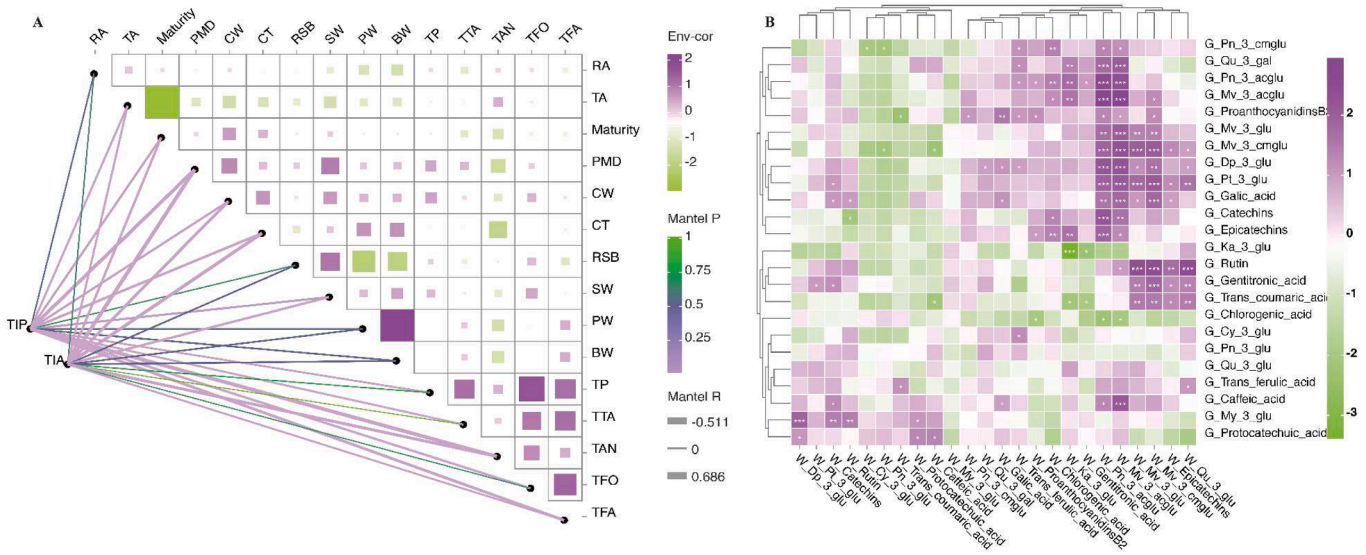

Fig. 6. Correlation analysis among physicochemical parameters and polyphenolic compounds of grapes and wine. Correlation analysis among grape physicochemical parameters, total phenolic parameters, and individual phenol parameters (unweighted UniFrac distances) including the total individual anthocyanins (TIA) and total individual non-colored polyphenols (TIP) determined using the Mantel test (A). The line and square color and represent the significance differences level (*P*-values). The line and square size represent the correlation coefficients (Mantel's r). RA, reducing sugar; TA, Titratable acid; PMD, maturity days; CW, cluster weight; CT, cluster tightness; RSB, the ratio of skin to berry weight; SW, skin weight; PW, pulp weight; BW, berry weight; TP, total phenols; TTA, total tannins; TAN, total anthocyanins; TFO, total flavonoids; TFA, total flavanols. Heat map of intergroup correlation analysis between grape polyphenols (G-) and wine polyphenols (W-) (B), * significant difference at $P < 0.05$; ** significant difference at $P < 0.01$; *** significant difference at $P < 0.001$ (Tukey's test).

study on polyphenol component variation in grapes and wines from different *Vitis Vinifera* L. varieties and their clones. The results highlight the importance of combining genetic breeding and clonal selection to improve polyphenol traits. Furthermore, MBVCR6 could be considered the most valuable genotype from which to obtain high-quality wines, considering its advantageous wine color and aging potential.

CRediT authorship contribution statement

Ruihua Ren: Writing – original draft, Writing – review & editing. **Jingjing Shi:** Data curation, Visualization. **Maoyu Zeng:** Data curation, Visualization. **Zizhu Tang:** Investigation, Resources. **Sha Xie:** Investigation, Resources. **Zhenwen Zhang:** Conceptualization, Funding acquisition, Supervision.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101030.

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