Research Article

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Wine metabolome and sensory analyses demonstrate the oenological potential of novel grapevine genotypes for sustainable viticulture in warm climates

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Abstract

BACKGROUND: Genetic breeding is essential to develop grapevine genotypes adapted to warm climates and resistant to pathogens. Traditionally cultivated *Vitis vinifera* is susceptible to biotic and abiotic stresses. Winemakers and consumers, however, perceive wines from non-*vinifera* or hybrid cultivars as inferior. In this study, sensory analyses and comprehensive metabolite profiling by targeted and untargeted approaches were used to investigate the oenological potential of wines from grapes of genotypes developed throughout four breeding cycles to improve climate adaptation, sugar contents and berry color.

RESULTS: Novel genotypes had higher yields and the wines exhibited increased contents of polyphenols, including anthocyanins. Volatile monoterpenes in the wines decreased throughout breeding cycles in the absence of selective pressure. Polyphenol contents were higher in intermediate wines, with hydroxytyrosol contents reaching up to three times reported values. Mouthfeel attributes astringency, leafy taste, flavor and body, and persistency showed significant correlation with untargeted features. Supervised model-based analyses of the metabolome effectively discriminate wines from distinct genetic origins.

CONCLUSION: Taken together, the results demonstrate the potential of novel grapevine genotypes to a more sustainable viticulture and quality wine production in warm climates. Comprehensive metabolite profiling of the wines reveals that genotype clustering is dependent on the chemical class and that traits not submitted to selective pressure are also altered by breeding. Supervised multivariate models were effective to predict the genetic origin of the wines based on the metabolic profile, indicating the potential of the technique to identify biomarkers for wines from sustainable genotypes.

Supporting information may be found in the online version of this article.

Keywords: GC–MS; genetic breeding; grapevine; polyphenol; UHPLC; volatiles

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ABBREVIATIONS

ANOVA	analyses of variance
ANT	total anthocyanins
CIELab	Commission Internationale de l'Éclairage *L*a*b* color space
DAD	diode array detector
ESI	electrospray ionization
EM	expectation–maximization
GC–MS	gas chromatography–mass spectrometry
LTPRI	linear-temperature-programmed retention index
MFA	multiple factor analysis
MS	mass spectrometry
PC	principal component
PCA	principal component analysis
pH	potential of hydrogen
PLS	partial least square
PTFE	polytetrafluoroethylene
SPME	solid-phase microextraction
sPLS-DA	sparse partial least square discriminant analysis
TSS	total soluble solids
TA	titratable acidity
TPI	total polyphenols
TQD	triple quadrupole
UHPLC	ultrahigh-performance liquid chromatography

INTRODUCTION

Viticulture worldwide is challenged with requirements for increased sustainability.[1,2] Adaptation to biotic and abiotic conditions is crucial to crop resilience.[1,2] Agrochemical use is extensive in grape production, due to *Vitis vinifera* susceptibility to phytopathogens, berry color, and size exigences.[2,3] High temperatures during the winter negatively affect differentiation of uncommitted meristems and reduce grape yield.[4] They also reduce anthocyanin accumulation during ripening and hinder flower bud development in non-adapted genotypes.[3,5] Although these effects are drastic in warm climates, extreme conditions caused by climate changes also affect grapevine development in temperate regions, reinforcing the need for adapted cultivars worldwide.[1,2,5]

Genetic breeding has contributed to grapevine adaptation and disease tolerance for many years, and controlled crosses and selection in different environments allowed the development of improved genotypes.[2,5,6] Wines from recent hybrid genotypes are indistinguishable from those of traditional *Vitis vinifera* cultivars,[6-8] however preconceptions on wine quality remain.[2,8] Metabolite profiling, coupled with physicochemical properties, chromatic characterization, and sensory analyses, were used to discriminate wines by geographic location and vintage,[9-11] winemaking processes[12,13] and agronomical condition.[14,15] Association between high-throughput metabolic fingerprinting and genetics also contributes to elucidate the heritability of compounds underlying complex traits, as mouthfeel or 'bouquet'.[16-18]

This study aimed at determining the oenological potential of novel grapevine genotypes, developed for sustainable viticulture, by integrating physicochemical characterization, metabolite profiling, and sensory analyses of young red wines. Genotypes were developed by mutant selection and conventional breeding. Profiles of polyphenols and volatiles were determined by ultrahigh-performance liquid chromatography (UHPLC) and gas chromatography–mass spectrometry (GC–MS), respectively, and associated with UHPLC–MS fingerprinting. Genetic origin and oenological features were investigated by multivariate models and hierarchical clustering. Correlation analyses were used to associate metabolite contents with sensory grades. Complete metabolomes were used to construct sparse partial least square discriminant analysis (sPLS-DA) models and their performance to classify the wines according to genetic origin were assessed. The results may contribute to identifying biomarkers of sustainable grapevine genotypes.

MATERIAL AND METHODS

Plant material

Winemaking grapes were harvested from experimental vineyards in Bento Gonçalves (29.1650° S, 51.5264° W), Brazil. Scion genotypes were grafted onto Paulsen 1103, trained in a pergola system, using 2.5 m × 1.5 m spacing, on east–west orientation on slopes inferior to 5%. Local climate and soil are humid mesothermic (Cfb, Köppen-Geiger) (Supporting Information, Tables S1 and S2), and haplic cambisol. Vineyards were not irrigated and received the recommended phytosanitary and soil management practices.[19] Yield was determined by mass weighting and represents 10-year averages from vineyards older than 5 years. Genotype information is summarized in Supporting Information, Tables S3, S4, and Fig. S1. 'Bordo' wines were used as standard for color, phenolics and untargeted metabolomic analyses. Selected traits were adaptation to warm climates, fruitfulness, soluble solids contents, and berry color.[5]

Wine making

Small-scale vinifications were conducted at Embrapa Uva e Vinho, from 2016 to 2020. Berries were manually harvested and selected to eliminate the unripe, rotten, and fungus infected. Thus, 50 kg of berries from each genotype were mechanically destemmed and crushed. Musts were incubated in a tank at 25 ± 2 °C where they received potassium metabisulfite ($K_2S_2O_5$) to a final concentration of 50 mg L^{-1} and were inoculated with 0.2 g L^{-1} of yeast, *Saccharomyces cerevisiae* (Maurivin PMD). Musts were macerated for 4 days and then liquid was separated from the solid phase. Alcohol contents of 11.5% (*v/v*) were reached by correcting sugar levels up to 3% (*w/v*) (Table S5). Samples underwent malolactic fermentation for 60 days and were stabilized at 0 °C for 20 days, with $K_2S_2O_5$ correction until 30 mg L^{-1} of free sulfur dioxide (SO_2). Finished wines were bottled and analyzed within a 2-month period. Metabolic profiling was carried out using three replicates from independent bottles.

Sensory evaluations

Wines from two vintages (2017 and 2018) were submitted to quantitative descriptive analyses by a panel of eight trained members, in three independent tastings. Attributes in classes of mouthfeel (acidity, astringency, bitterness, sweetness, leafy, persistency, sharpness and frankness, body of the flavor, pungency and alcoholic power, tannins and structure, unpleasant taste, and jam or jelly characteristics), odor (fruity, leafy, unpleasant, and foxy), and color (intensity and violet hue) were evaluated from the lowest (0) to the highest perceivable intensity (5). Global appreciation was scored using a 0 to 100 scale. Analyses were conducted in a dedicated laboratory, with temperature at 20 ± 2 °C, in individual booths with white lights. Samples were prepared in

a separate area. The study purpose and treatments were not disclosed, and panelists were anonymized. Participants were presented with 30 mL of each wine identified by numeric codes. Panelists were instructed to evaluate samples independently and analyses were performed according to ABNT NBR ISO 1113.[20] Experiments were approved by the local ethical committee (CAAE number 79807924.4.0000.5305).

Physicochemical and color analyses

Physicochemical parameters were determined for berries and wines from five vintages (2016–2020), and consisted of total soluble solids (TSS, °Brix) determined by refractometry (Atago RX5000; Atago Co. Ltd, Tokyo, Japan), titratable acidity (TA) by sodium hydroxide titration, and total phenolics (TPs) by the Folin– Ciocalteau assay, all according to OIV guidelines.[21] Total monomeric anthocyanins contents were determined by differential pH,[22] using buffer solutions at pH 1.0 (potassium chloride) and pH 4.5 (sodium chloride) and spectrophotometric absorbance at 510 nm and 700 nm (Ultrospec-2000; Amersham-Pharmacia Biotech, Amersham, UK), shown as mg L^{-1} of malvidin-3,-5-diglucoside. The CIELab parameters lightness (L^*), red-green (a^*), yellow-blue (b^*), chroma (C^*), and tonality angle or hue ($h°$) at 450, 520, 570 and 630 nm were employed to determine wine color, with a colorimeter (CR-400; Minolta, Tokyo, Japan), as described in the literature.[23]

Target and non-target metabolome analyses

Phenolic compounds

Wine phenolic profiling was done by UHPLC,[24] using an Ultimate 3000 BioRS Dionex (Thermo Fisher Scientific Inc., Waltham, MA, USA) system, with columns Acclaim TM RSLC 120 C18 (Thermo Fisher Scientific Inc.) (2.2 µm, 2.1 mm × 50 mm) and a diode array detector (DAD), operating at 280 nm, 320 nm, 360 nm, and 520 nm. Prior to injection, samples were filtered through polytetrafluoroethylene (PTFE) 0.45 µm (Millipore, Bedford, MA, USA) and 20 µL were used. Ultrapure water with phosphoric acid 0.85% (A) and acetonitrile (B) were used as mobile phase. Compounds were eluted by a gradient of solvents A and B, starting at 100% of A, decreasing from 96% in 2.5 min, 92% 7.5 min, 88% in 15 min, 85% in 18 min, 80% in 20 min, 35% in 24 min, up to 25 min, with a final increase to 100% of A at 28 min, with a flow rate of 0.8 mL min^{-1}. Metabolite identification and quantification were performed by comparison with commercial standards and external standard method using calibration curves. Standards were from Sigma-Aldrich (St Louis, MO, USA) and consisted of gallic acid, catechin, *trans*-cinnamic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, *trans*-ferulic acid, *trans*-resveratrol, rutin, quercetin, luteolin, hydroxytyrosol, 3-*O*-methyl-quercetin, kaempferol, cyanidin-3,5-diglucoside, delfinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, malvidin-3,5-diglucoside, peonidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside.

Volatile organic compounds

Solid-phase microextraction (SPME) was used to capture wine volatiles that were analyzed with a QP-5000 (Shimadzu Corporation, Kyoto, Japan) gas chromatograph/mass spectrometer with electron impact (EI) ionization (70 eV).

An aliquot of 10 mL received 3 g of sodium chloride in a 35 mL glass flask with screw top and septum and the mixture was incubated under constant agitation at 30 °C for 30 min. The fiber [carboxen/divinylbenzene/polydimethylsiloxane CAR/DVB/PDMS, Supelco, Inc., Bellefonte, PA, USA] was exposed inside the vial (headspace) for 8 min, retracted and exposed inside the injector. Injector and detector temperatures were at 220 °C and 240 °C, respectively, using helium as carrier, at 1 mL min^{-1} flow. Samples were injected in the split 1/20 mode, and compounds separated using a DB-5 column (30 m × 0.25 mm × 0.25 µm) with a temperature program from 35 °C to 240 °C, with 3 °C min^{-1} stepwise increases. Retention indices were calculated by Van den Dool and Kratz equation[25] using a homologous series of C_8–C_{20} n-alkanes run under the same chromatographic conditions. Area normalization was employed for relative quantification and volatiles were identified by mass spectra and retention indices comparisons against the equipment library (Wiley 139, NIST 62) and literature data.[26]

Untargeted metabolite profiling

Untargeted metabolome was carried out by UHPLC (Acquity Chromatograph, Waters Corporation, Milford, MA, USA) with triple quadrupole (TQD) MS (Acquity; Waters Corporation). Wine bottles were opened under controlled conditions and 1.5 mL samples, filtered through 0.45 µm PTFE into MS-certified amber vials, before injection of 5 µL. Detection was done by electrospray ionization in negative ion (ESI−) and positive ion (ESI+) modes with capillary at ±3.5 kV, ±30 V cone, capillary temperature of 150 °C and desolvation temperature of 300 °C. Gradient elution was conducted using purified water with 0.1% formic acid as solvent A and acetonitrile as solvent B, with C18 BEH Acquity Waters (column 1,7 µm × 2,1 mm × 50 mm; Waters Corporation), at a flow rate of 0.2 mL min^{-1} and an oven temperature of 40 °C. Gradient program was initiated with 5% of solvent B, ramped to 50% in 8 min, and reached 95% of B in 9 min, with conditions held for 10 min, and returned to the initial conditions at 10.1 min. Column was kept equilibrated until 12 min. Raw data from ESI− and ESI+ were converted to mzXML, using Proteowizard[27] and pre-processed with XCMS.[28] Package IPO[27] was used to optimize XCMS parameter data and mass features were analyzed with 'MetaboAnalyst'.[29]

Data analysis

Extractions, metabolite profiling, and sensory analyses were performed in triplicate and are represented as mean ± standard deviation. Physicochemical and yield parameters were obtained from 5-year evaluations. Statistical analyses used R version 4.2.0.[30] Data normality was tested by Shapiro–Wilk´s before analysis of variance (ANOVA) and multiple mean comparisons, by Tukey Honestly Significant Difference (HSD) *t*-test at $P < 0.05$. Data were scaled, centered, and normalized. Heatmaps were built using *z*-score normalized data, hierarchically clustered and plotted with 'pheatmap'.[31] Correlation analyses were done with 'Hmisc'[32] and 'corrplot'.[33] Principal component analysis (PCA), multiple factor analysis (MFA), and sPLS-DA were done using 'FactoMineR',[34] 'factoextra'[35] and 'mixOmics'.[36] Breeding cycle was used as discriminant in sPLS-DA and relevance networks were constructed using similarity matrices from the sum of correlations between the original variables and their corresponding latent component in the model. Tuning threshold for relevant associations in the network corresponds to cut-off values. Optimal models were determined by overall and balanced classification error rates, using maximum, centroids and Mahalanobis distances.

RESULTS

Agronomical performance of the cultivars

Berry yield increased more than 85% with breeding (Fig. 1(A)), whereas contents of soluble solids was 1.2 times higher in grapes

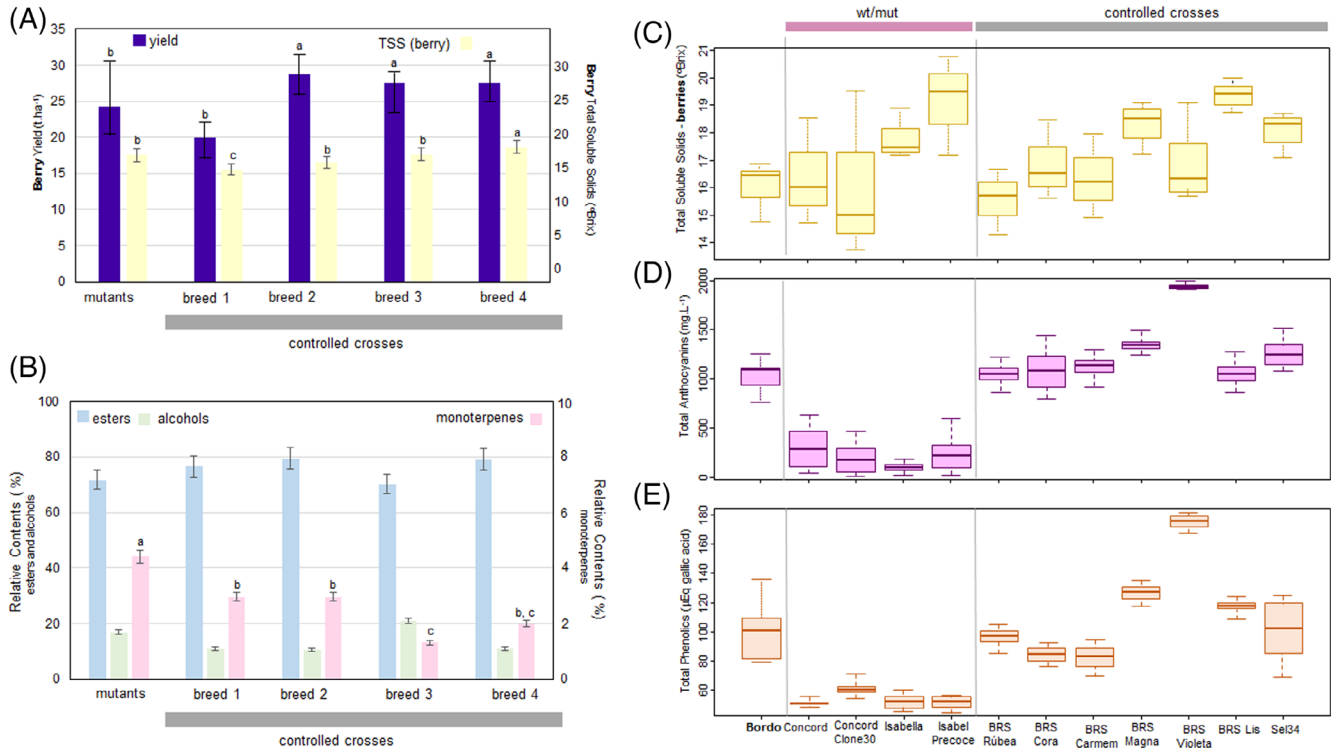

Figure 1. Genetic breeding effects on agricultural and oenological properties. Berry yield and total soluble solids (A), contents of wine volatile classes (B) per cycle. Boxplots of berry total soluble solids (C), wine total anthocyanins (D) and total phenolics (E) per genotype. Error bars represent standard deviation. Significant differences at $P < 0.05$ are shown by distinct letters.

from later cultivars (Fig. 1(A)). Monoterpene contents were reduced, decreasing more than three times (Fig. 1(B)), although olfactory berry and wine characteristics were not selective criteria. Ester and alcohol levels were not significantly modified (Fig. 1(B)). Mutants exhibited uneven maturation, in comparison with later genotypes, and low contents of polyphenols (Fig. 1(C)–(E)). Breeding promoted a four-fold increase in total anthocyanins (Fig. 1(D)) and doubled polyphenols (Figs 1(E) and S3 (B)). Wine tonality also improved (Fig. S3 (C)), although not reaching 'Bordo' standard. Phenotypes under selection, as yield, berry TSS, and TPs, were the most affected (Fig. 1).

Sensory analyses

Most sensory variation of the wines was explained by the first two components (Fig. 2(A)). Color attributes and mouthfeel characteristics had good representation on the principal dimension (Fig. 2(B)). 'BRS Cora', 'BRS Carmem', and 'BRS Rúbea' wines associated with higher overall grades and positive features (Fig. 2(C)), whereas those from 'BRS Carmem' were associated with 'leafy' and 'bitter' mouthfeel (Fig. 2(B)). Wines from mutant genotypes were considered with 'foxy odor', contrasting the 'fruity odor' of wines from cultivars with greater *Vitis vinifera* representation (Figs 2(D) and S4). 'BRS Lis' wines associated with 'leafy odor' and 'tannins/structure' (Fig. 2(D)).

Wines chemical profile

Phenolic compounds

Twenty-one phenolic compounds were analyzed in wines from eight genotypes and one control (Table 1). Polyphenol total contents ranged from 430 to 450 mg L^{-1} in 'Isabel Precoce' and 'BRS Carmem' to 1080–1180 mg L^{-1} in 'BRS Lis' and 'BRS Violeta' wines, representing up to 13% higher polyphenol levels as 'Bordo' (Table 1). Wines from 'BRS Violeta' had approximately twice (1.78) the contents of anthocyanins as 'Bordo' (Table 1). Anthocyanin contents in wines from 'BRS Lis' were equivalent to 'Bordo' (Table 1). Contents of flavan-3-ols (cathechin and hydroxytyrosol) were higher in wines from all cultivars in comparison to 'Bordo', except for 'BRS Carmem' (Table 1).

'Bordo' wines had the most divergent polyphenol profile (Figs 3 (A) and S5 (A)). 'Isabel Precoce' and 'Concord Clone 30' wines had higher contents of 3,5-anthocyanidin diglucosides (Fig. 3). Wines from the second breeding cycle onward did not associate with anthocyanin 3,5-diglucosides (Fig. 3). Hydroxytyrosol contents were significant in 'BRS Rúbea', 'BRS Violeta', and especially 'BRS Magna' wines (Figs 3(A) and S5 (A)). Contents of monomeric anthocyanins correlated with color intensity and violet hue (Fig. 3(B)). Total and monomeric anthocyanins, and flavonoids were negatively correlated with b^* (Fig. 3(B)).

Volatile organic compounds

Twenty-two volatiles were identified by GC–MS (Table 2). Profiles changed throughout breeding cycles (Fig. 1(E) and Table 2). Most variation in the wines' volatiles (56.5%) was explained by the first two components (Fig. 3(C)). Volatiles from controlled cross wines were distinct from those of *labrusca* mutants (Fig. 3(C),(D)). 'BRS Carmem', 'BRS Violeta', and 'BRS Magna' volatile profiles were similar, whereas wines from earlier and later genotypes resembled those from *Vitis labrusca* mutants (Fig. 3(C),(D)). Distinct wine groups were formed based on polyphenol and volatile profiles (Fig. 3(A),(C)). Wines from recent genotypes were significantly associated with a hydroxylated derivate of ethyl propanoate, whereas those from 'Concord Clone 30', 'BRS Rúbea', 'BRS Cora',

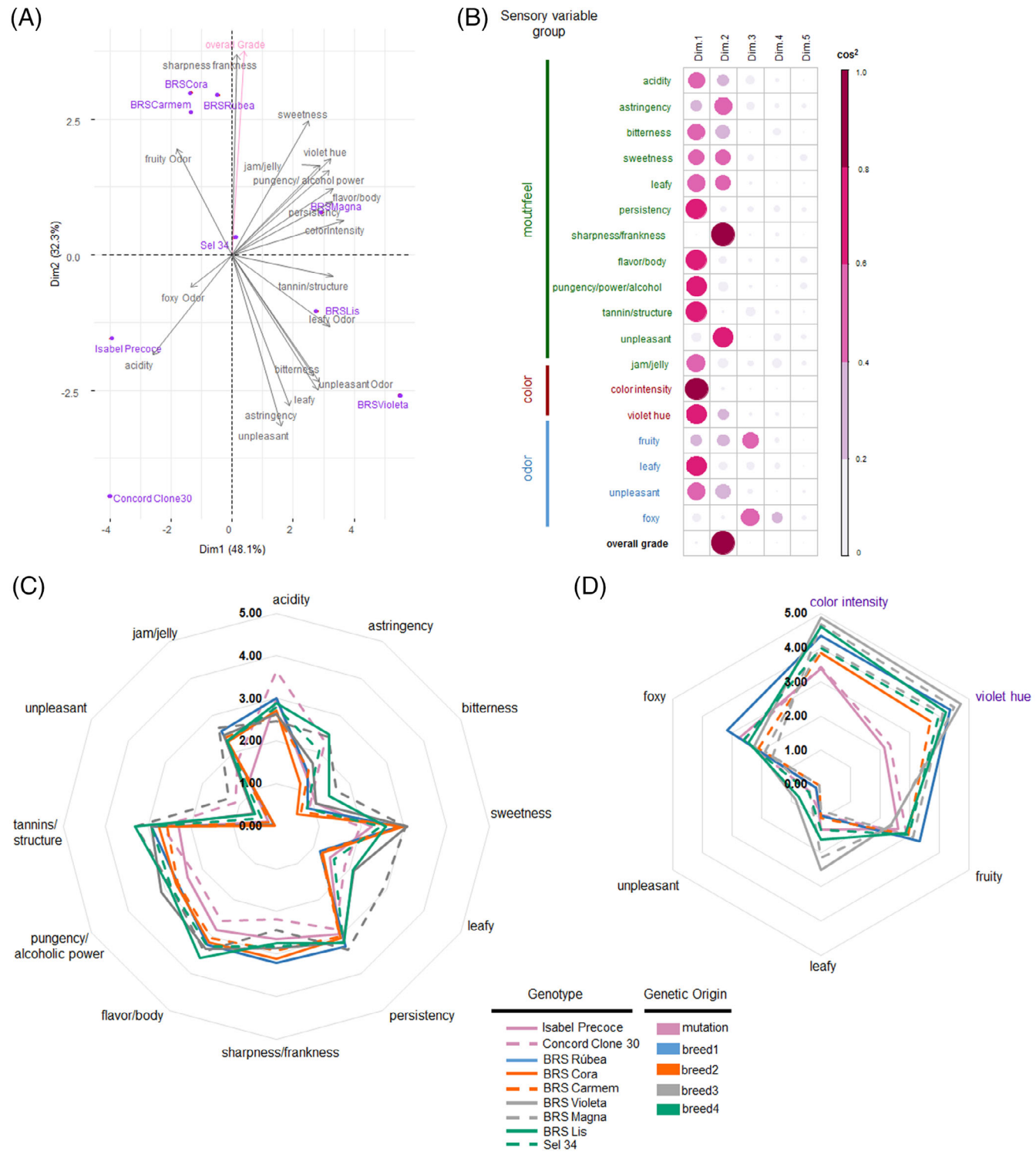

Figure 2. Multivariate analyses of sensory properties. MFA of mouthfeel, odor, and color groups of attributes, using the overall appreciation grade as supplementary quantitative variable (A). Variable representation (cos^2) from PCA of the sensory grades (B). Mouthfeel and taste attributes (C), odor and color attributes (D), color is shown in purple.

and 'BRS Violeta' were correlated with ethyl-hexanoate (Fig. 3(C), (D)). A position derivative of ethyl-2-hexanoate was also relevant in 'BRS Rúbea' wines (Fig. 3(C),(D)), considered to have 'fruity' odor (Fig. S5 (B)).

Untargeted metabolome

Ninety-six UHPLC–MS features, 63 from negative (ESI−) and 33 in positive ionization modes (ESI+), were detected in the wines (Figs 4(A) and S6). ESI− produced approximately twice (1.91) the

Table 1. Profile of phenolic compounds from the red wines determined by ultrahigh-performance liquid chromatography (UHPLC) analysis

Metabolite (Class)	Molecular formula	Retention time	Bordo	Isabel Precoce	Concord Clone 30	BRS Rúbea	BRS Cora	BRS Carmem	BRS Violeta	BRS Magna	BRS Lis	Sel34
Anthocyanins												
Cyanidin-3,5-diglucoside	$C_{27}H_{31}O_{16}$	7.32	50.97 ± 0.46^{de}	—	55.14 ± 2.21^{de}	164.95 ± 17.57^{c}	213.81 ± 8.83^{bc}	39.84 ± 1.53^{ef}	544.01 ± 49.12^{a}	76.35 ± 9.15^{de}	100.80 ± 1.43^{d}	227.82 ± 15.46^{b}
Delphinidin-3-*O*-glucoside	$C_{21}H_{21}O_{11}$	7.55	26.68 ± 0.28^{d}	25.95 ± 1.40^{d}	36.60 ± 1.30^{c}	36.58 ± 1.33^{c}	48.77 ± 1.58^{a}	23.38 ± 0.27^{d}	51.18 ± 3.69^{a}	25.12 ± 0.52^{d}	42.37 ± 2.67^{a}	37.67 ± 1.67^{ac}
Cyanidin-3-*O*-glucoside	$C_{21}H_{21}O_{10}$	9.49	28.46 ± 1.16^{a}	0.44 ± 0.28^{c}	6.38 ± 0.44^{b}	1.29 ± 0.21^{c}	1.44 ± 0.07^{c}	—	—	—	8.93 ± 3.03^{b}	—
Pelargonidin-3-*O*-glucoside	$C_{29}H_{35}O_{17}$	9.64	1.44 ± 0.03^{b}	—	0.32 ± 0.04^{b}	0.90 ± 0.09^{b}	0.84 ± 0.14^{b}	—	—	—	—	5.93 ± 1.16^{a}
Malvidin-3,5-diglucoside	$C_{21}H_{21}O_{12}$	10.10	382.11 ± 17.0^{a}	41.50 ± 3.73^{d}	8.72 ± 0.74^{d}	6.19 ± 1.41^{d}	7.89 ± 1.20^{d}	201.67 ± 9.39^{b}	285.12 ± 24.55^{a}	115.03 ± 29.71^{c}	310.79 ± 22.68^{a}	42.09 ± 6.78^{d}
Peonidin-3-*O*-glucoside	$C_{22}H_{23}O_{11}$	11.09	0.96 ± 0.06^{c}	2.79 ± 0.30^{b}	0.33 ± 0.04^{c}	0.77 ± 0.19^{c}	0.95 ± 0.40^{c}	0.49 ± 0.33^{c}	0.64 ± 0.34^{c}	0.32 ± 0.07^{c}	8.47 ± 0.11^{a}	0.81 ± 0.32^{c}
Malvidin-3-*O*-glucoside	$C_{23}H_{25}O_{12}$	13.03	6.77 ± 0.25^{c}	28.41 ± 1.98^{a}	3.96 ± 0.11^{d}	3.41 ± 0.03^{d}	3.61 ± 0.04^{d}	6.55 ± 0.28^{c}	3.77 ± 0.08^{d}	3.72 ± 0.07^{d}	22.94 ± 0.70^{b}	4.78 ± 0.09^{cd}
Flavonoids												
Rutin	$C_{27}H_{30}O_{16}$	13.65	16.53 ± 0.00^{c}	9.33 ± 0.14^{e}	12.43 ± 0.31^{d}	15.71 ± 0.16^{c}	22.67 ± 1.86^{ab}	10.10 ± 0.20^{de}	16.62 ± 0.65^{c}	12.17 ± 0.21^{d}	25.25 ± 1.63^{a}	22.31 ± 1.15^{b}
Quercetin	$C_{15}H_{10}O_{7}$	19.62	9.34 ± 0.04^{a}	2.31 ± 0.14^{f}	2.41 ± 0.02^{ef}	2.85 ± 0.11^{ef}	3.44 ± 0.10^{de}	6.11 ± 0.57^{b}	5.79 ± 0.59^{b}	5.12 ± 0.63^{bc}	4.10 ± 0.20^{cd}	2.67 ± 0.30^{ef}
3-*O*-Methyl quercetin	$C_{16}H_{12}O_{7}$	21.20	1.52 ± 0.03^{a}	0.46 ± 0.12^{bc}	0.23 ± 0.04^{c}	0.13 ± 0.02^{c}	0.47 ± 0.36^{bc}	0.52 ± 0.19^{bc}	0.52 ± 0.11^{bc}	0.14 ± 0.01^{c}	1.87 ± 0.28^{a}	0.79 ± 0.22^{b}
Kaempferol	$C_{15}H_{10}O_{6}$	22.21	4.15 ± 0.16^{ab}	1.75 ± 0.55^{cd}	4.61 ± 1.13^{ab}	0.67 ± 0.05^{d}	2.88 ± 0.25^{bc}	4.53 ± 1.53^{ab}	3.03 ± 0.89^{bc}	0.34 ± 0.02^{d}	5.25 ± 0.19^{a}	3.25 ± 0.50^{abc}
Phenolic acids												
Gallic acid	$C_{7}H_{6}O_{5}$	0.74	180.81 ± 5.51^{bc}	50.01 ± 2.11^{e}	149.22 ± 12.28^{c}	102.49 ± 7.12^{d}	169.41 ± 18.01^{c}	18.85 ± 0.68^{f}	68.15 ± 3.39^{e}	20.66 ± 0.54^{f}	198.58 ± 7.87^{b}	242.63 ± 15.86^{a}
Caffeic acid	$C_{9}H_{8}O_{4}$	4.73	24.13 ± 0.96^{a}	14.09 ± 0.75^{cd}	12.72 ± 1.64^{de}	20.01 ± 0.75^{b}	8.11 ± 0.11^{f}	3.89 ± 0.85^{g}	3.87 ± 0.78^{g}	15.40 ± 0.54^{c}	24.56 ± 0.64^{a}	10.52 ± 0.69^{e}
Chlorogenic acid	$C_{16}H_{18}O_{9}$	5.48	117.52 ± 3.7^{a}	34.90 ± 0.51^{f}	46.69 ± 1.34^{e}	55.59 ± 3.33^{cd}	58.86 ± 1.79^{bc}	39.42 ± 0.35^{f}	47.82 ± 3.63^{e}	54.62 ± 1.08^{cd}	51.32 ± 2.05^{de}	62.24 ± 1.43^{b}
p-Coumaric acid	$C_{9}H_{8}O_{3}$	6.19	66.66 ± 2.94^{a}	15.98 ± 0.96^{de}	7.69 ± 0.63^{gh}	31.40 ± 4.84^{b}	23.12 ± 1.59^{c}	4.00 ± 0.41^{h}	10.84 ± 1.35^{efg}	18.60 ± 0.51^{cd}	10.52 ± 0.55^{fg}	14.18 ± 0.93^{def}
trans-Ferulic acid	$C_{10}H_{10}O_{4}$	7.94	4.54 ± 0.02^{b}	3.11 ± 0.03^{de}	3.20 ± 0.02^{d}	3.24 ± 0.03^{d}	4.26 ± 0.07^{b}	3.02 ± 0.00^{e}	3.05 ± 0.01^{de}	3.45 ± 0.09^{c}	3.48 ± 0.13^{c}	4.88 ± 0.07^{a}
trans-Cinnamic acid	$C_{9}H_{8}O_{2}$	13.94	0.65 ± 0.04^{de}	1.24 ± 0.08^{cd}	0.28 ± 0.03^{e}	6.24 ± 0.81^{a}	0.67 ± 0.02^{de}	0.82 ± 0.05^{de}	1.21 ± 0.08^{cd}	1.71 ± 0.37^{c}	0.95 ± 0.02^{cde}	3.00 ± 0.02^{b}
Stilbene												
trans-Resveratrol	$C_{14}H_{12}O_{3}$	24.96	0.06 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.01^{a}
Flavone												
Luteolin	$C_{15}H_{10}O_{6}$	20.58	3.60 ± 0.08^{bc}	3.18 ± 0.96^{bc}	1.33 ± 0.07^{d}	1.15 ± 0.06^{d}	1.74 ± 0.15^{d}	2.25 ± 0.17^{cd}	1.96 ± 0.40^{cd}	3.17 ± 0.47^{bc}	6.76 ± 0.80^{a}	4.46 ± 0.46^{b}
Flavan-3-ol												
Hydroxytyrosol	$C_{8}H_{10}O_{3}$	1.86	5.92 ± 0.01^{b}	2.07 ± 0.37^{b}	4.07 ± 0.81^{b}	12.99 ± 1.39^{a}	2.89 ± 0.30^{b}	2.23 ± 0.32^{b}	12.40 ± 2.57^{a}	13.14 ± 1.57^{a}	1.25 ± 0.41^{b}	2.27 ± 0.51^{b}
Catechin	$C_{15}H_{14}O_{6}$	4.47	107.14 ± 1.6^{d}	193.82 ± 15.95^{c}	204.39 ± 19.32^{bc}	236.34 ± 33.50^{abc}	287.54 ± 20.60^{a}	81.23 ± 0.50^{d}	119.96 ± 6.36^{d}	117.00 ± 7.12^{d}	252.56 ± 30.47^{ab}	203.57 ± 9.60^{bc}
Totals per class												
Anthocyanins			497.39 ± 25.59^{b}	99.10 ± 6.23^{e}	111.44 ± 4.13^{e}	214.08 ± 20.04^{d}	277.31 ± 11.21^{cd}	271.93 ± 11.74^{cd}	884.73 ± 73.53^{a}	220.54 ± 26.43^{d}	494.30 ± 26.36^{b}	319.10 ± 19.52^{c}
Flavonoids			31.54 ± 0.24^{b}	13.85 ± 0.61^{d}	19.68 ± 1.40^{c}	19.36 ± 0.11^{c}	29.46 ± 1.46^{b}	21.26 ± 1.41^{c}	25.96 ± 2.09^{b}	17.77 ± 0.85^{c}	36.46 ± 1.64^{a}	29.01 ± 0.79^{b}
Phenolic acids			394.31 ± 18.62^{a}	119.31 ± 2.65^{e}	219.79 ± 15.52^{d}	218.97 ± 15.70^{d}	264.43 ± 14.77^{c}	70.00 ± 0.80^{f}	134.94 ± 3.72^{e}	114.44 ± 1.26^{e}	289.41 ± 8.59^{c}	337.45 ± 13.62^{b}

Table 1. Continued

Metabolite (Class)	Molecular formula	Retention time	Bordo	Isabel Precoce	Concord Clone 30	BRS Rúbea	BRS Cora	BRS Carmem	BRS Violeta	BRS Magna	BRS Lis	Sel34
Stilbene			0.06 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.01^{a}
Flavones			3.60 ± 0.08^{bc}	3.18 ± 0.96^{bc}	1.33 ± 0.07^{d}	1.15 ± 0.06^{d}	1.74 ± 0.15^{d}	2.25 ± 0.17^{cd}	1.96 ± 0.40^{cd}	3.17 ± 0.47^{bc}	6.76 ± 0.80^{a}	4.46 ± 0.46^{b}
Flavan-3-ol			107.14 ± 1.6^{d}	193.82 ± 15.95^{c}	204.39 ± 19.32^{bc}	236.34 ± 33.50^{abc}	287.54 ± 20.60^{a}	81.23 ± 0.50^{d}	119.96 ± 6.36^{d}	117.00 ± 7.12^{d}	252.56 ± 30.47^{ab}	203.57 ± 9.60^{bc}
General totals												
Polyphenols			1039.26^{b}	431.26 ± 23.76^{f}	560.70 ± 39.17^{e}	702.90 ± 66.30^{d}	863.38 ± 28.93^{c}	448.90 ± 12.72^{f}	1179.95 ± 77.60^{a}	486.07 ± 20.81^{f}	1080.74 ± 26.00^{b}	895.86 ± 26.22^{c}
Monomeric anthocyanins			181.13 ± 0.19^{d}	109.99 ± 11.19^{d}	89.73 ± 6.36^{d}	527.68 ± 40.50^{c}	498.46 ± 15.93^{c}	780.95 ± 63.71^{b}	1226.25 ± 66.59^{a}	835.97 ± 31.03^{b}	499.57 ± 34.67^{c}	753.95 ± 12.30^{b}

Note: Contents are expressed in mg L^{-1} and represent means from three replicates per genotype ± standard deviation (n = 24). Distinct lettering corresponds to statistically significant differences in the rows, Tukey HSD $P < 0.05$. —, not detected.

number of features as ESI+. Intra-group correlation analyses of the modes are shown (Fig. S7). Wines were grouped in two clusters, one consisting of mutants and early cultivars, and another with recent genotypes (Fig. 4(A)). Untargeted profiles of 'BRS Cora' and 'BRS Carmem' wines were divergent, the first similar to 'Isabel Precoce' and 'Concord Clone 30' and the later, to recent cultivars (Figs 4(A) and S6). The sPLS model, using genetic information as discriminant, provided a clear distinction of the groups and accurate representation of their genetic relationships (Fig. 4(C)). Relevance networks associated chemical features to genetic origins (Fig. S8 (C)). Mouthfeel grades for astringency, leafy taste, flavor and body, and persistency exhibited positive correlation with UHPLC–MS features (Fig. 4(B)).

Integrated metabolome and genetic origin prediction

Genotype clusters based on wine polyphenol and volatile profiles, and untargeted metabolome features were distinct (Figs 2 and 3). A comprehensive model, based on targeted (21 polyphenols, 22 volatiles) and untargeted (96 features) metabolomes, produced two large groups, one with mutant cultivars and early genotypes, and another with wines from recent materials (Figs 4(C) and S8 (A)). Wines from sibling cultivars 'BRS Cora' and 'BRS Carmem' did not cluster together, despite the shared parentage (Figs S8 (A) and S2 (D)). Optimized sPLS-DA model exhibited sensitivity greater than 95.0% and specificity of 99.5% to discriminate wines from breeding cycles (Fig. 4(C)). Error rates were lower than 0.1 for more than two components (Fig. S8 (B)), demonstrating the absence of overfitting and effective cross-validation. Relevance network analyses associated wines from recent breeding cycle with luteolin, gallic acid, and 3-*O*-methyl-quercetin (Fig. 4(D)).

DISCUSSION

Novel grapevine genotypes are crucial for viticulture sustainability in traditional and new wine-producing regions worldwide, although, acceptance of hybrid cultivars remains restricted due to preconceptions on wine quality.[1,2,5,6] This study takes advantage of a long running grapevine breeding program and high-throughput metabolite profiling to investigate the oenological characteristics of cultivars developed for sustainable viticulture.

Breeding affects selected and unintended oenological traits

The cultivars are genetically close, with distances ranging from 0.13 ('Bordo' to 'BRS Rúbea') to 0.45 ('Isabel Precoce' to 'BRS Magna').[37] However, wine chemical and sensory properties were distinct. Mutant genotypes were selected for their shorter berry ripening cycle, which expands the productive and processing cycles in sub-tropical conditions.[5] Yield and soluble solid contents were increased in recent cultivars. In an F_2 population from wild *Vitis riparia* and hybrid 'Seyval', soluble solids and acid contents were closely associated and controlled by a narrow region in chromosome 6.[17] These traits were similar in wines of genotypes from controlled crosses also suggesting co-localization. Physicochemical properties of wines from breeding genotypes were closer to those reported for *Vitis vinifera* than for hybrids.[8-10]

Color in young red wines depends on grape contents of anthocyanins and other co-pigment polyphenols.[38] Polyphenol biosynthesis and accumulation in berries is highly influenced by the environment,[3,38,39] as observed for wines. Juices from 'BRS Cora'

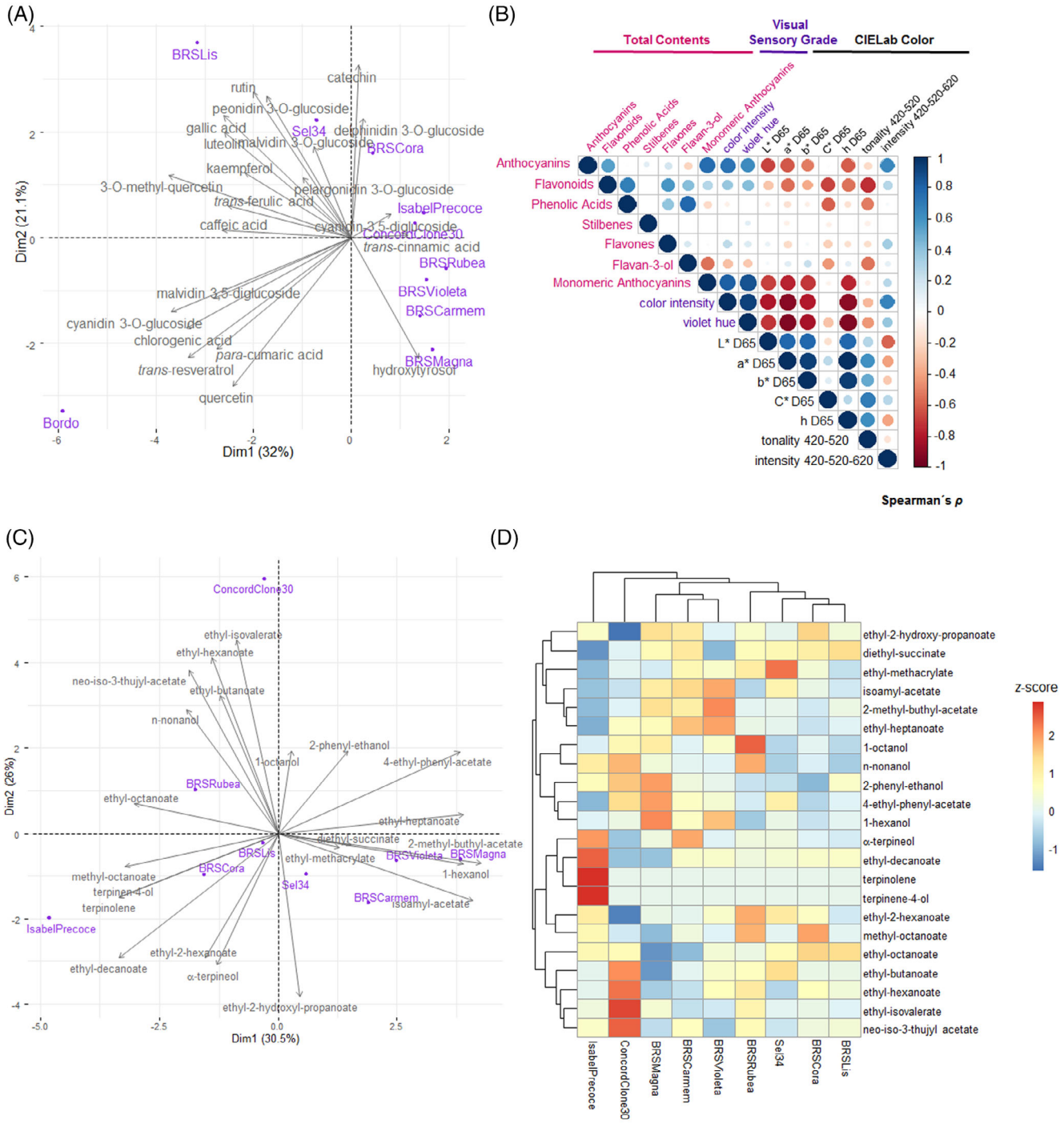

Figure 3. Phenolic and volatile profiles of the wines. PCA biplot of phenolic compounds (A) and correlation between class contents, visual sensory grades, and color parameters (B). PCA biplot (C) and hierarchical cluster (D) of volatile compounds.

and 'BRS Carmem' had high contents of polyphenols,[37,40] as their wines. These results support the contribution of genetics to increase polyphenol contents. Although not selected, monoterpene levels in the wines decreased linearly throughout breeding cycles. In grapes, monoterpenes are associated with muscat flavor,[41] and wines from mutant cultivars ranked as 'intensely flavored muscats' (4.4 mg L^{-1}) and from other genotypes, as 'aromatic, non-muscat' (2.96 to 1.31 mg L^{-1}). Contents of ester and alcohol were not significantly modified as these volatiles are predominantly of yeast origin.[42,43]

Chemical signatures of the wines from new genotypes

Phenolic compounds

Phenolics profile in grapes is extensively controlled by genetics.[3,14] In disease-resistant hybrids, genetic background determines the contents of most polyphenols, except for epicatechin and caftaric acid.[8,44] Young red wines are considered good representatives of grape contents of most phenolics, except for gallic acid.[8,44] 'Bordo' wines had the most divergent profile, reflecting its genealogy with extensive *Vitis labrusca* parentage. 'Isabel Precoce' and 'Concord Clone 30' gave rise to wines with higher

Table 2. Profile of volatile metabolites from the red wines determined by gas chromatography–mass spectrometry (GC–MS) analysis

Metabolite (class)	Molecular formula	LTPRI	Isabel Precoce	Concord CI 30	BRS Rúbea	BRS Cora	BRS Carmem	BRS Violeta	BRS Magna	BRS Lis	Sel34
Esters											
Ethyl butanoate	$C_6H_{10}O_2$	800	0.75 ± 0.13^{bc}	1.07 ± 0.12^{a}	0.84 ± 0.04^{ab}	0.76 ± 0.04^{bc}	0.74 ± 0.06^{bc}	0.82 ± 0.15^{abc}	0.55 ± 0.04^{c}	0.76 ± 0.06^{bc}	0.96 ± 0.12^{ab}
Ethyl metacrylate	$C_6H_{10}O_2$	812	1.03 ± 0.81^{e}	2.27 ± 0.34^{cde}	3.88 ± 0.37^{b}	2.80 ± 0.22^{bcd}	3.47 ± 0.42^{bc}	3.01 ± 0.54^{bcd}	2.00 ± 0.18^{de}	1.78 ± 0.20^{de}	5.68 ± 0.70^{a}
Ethyl isovalerate	$C_7H_{14}O_2$	850	0.43 ± 0.28^{b}	0.98 ± 0.19^{a}	0.57 ± 0.05^{b}	0.40 ± 0.04^{b}	0.29 ± 0.07^{b}	0.32 ± 0.03^{b}	0.46 ± 0.08^{b}	0.35 ± 0.01^{b}	0.37 ± 0.04^{b}
Isopentyl acetate	$C_7H_{14}O_2$	875	2.14 ± 0.27^{c}	2.81 ± 0.34^{c}	2.54 ± 0.31^{c}	3.45 ± 0.14^{bc}	5.31 ± 0.46^{a}	5.98 ± 1.26^{a}	4.83 ± 0.40^{ab}	2.93 ± 0.15^{c}	4.53 ± 0.57^{ab}
Ethyl hexanoate	$C_8H_{16}O_2$	1001	13.51 ± 2.39^{b}	19.31 ± 0.11^{a}	16.26 ± 1.79^{ab}	15.14 ± 0.77^{ab}	12.57 ± 0.54^{b}	15.15 ± 2.12^{ab}	11.86 ± 2.21^{b}	14.28 ± 0.82^{b}	12.16 ± 1.70^{b}
Ethyl heptanoate	$C_9H_{18}O_2$	1099	—	0.68 ± 0.32^{ab}	0.48 ± 0.03^{ab}	0.29 ± 0.03^{ab}	1.13 ± 0.86^{a}	1.24 ± 0.34^{a}	0.73 ± 0.33^{ab}	0.40 ± 0.03^{ab}	0.44 ± 0.03^{ab}
Methyl octanoate	$C_9H_{18}O_2$	1125	0.43 ± 0.03^{b}	0.16 ± 0.05^{cd}	0.68 ± 0.09^{a}	0.72 ± 0.12^{a}	0.29 ± 0.03^{bc}	0.19 ± 0.11^{cd}	—	0.22 ± 0.01^{c}	0.22 ± 0.09^{c}
Diethyl succinate	$C_8H_{14}O_4$	1182	3.42 ± 2.98^{b}	6.24 ± 0.21^{ab}	8.05 ± 0.46^{a}	8.84 ± 0.54^{a}	9.13 ± 0.71^{a}	4.24 ± 1.28^{b}	8.22 ± 0.51^{b}	9.67 ± 1.14^{a}	8.24 ± 0.95^{a}
Ethyl octanoate	$C_{10}H_{20}O_2$	1199	34.50 ± 3.71^{ab}	34.32 ± 2.85^{ab}	31.15 ± 0.80^{bc}	38.14 ± 0.64^{a}	25.8 ± 60.69^{cd}	32.27 ± 1.82^{abc}	23.65 ± 1.14^{d}	37.74 ± 1.54^{a}	34.34 ± 3.94^{ab}
4-Ethylphenyl acetate	$C_{10}H_{12}O_2$	1244	—	0.92 ± 0.03^{ab}	0.30 ± 0.01^{de}	0.17 ± 0.02^{ef}	0.59 ± 0.04^{c}	0.53 ± 0.22^{cd}	1.16 ± 0.10^{a}	0.32 ± 0.06^{de}	0.75 ± 0.09^{bc}
Ethyl decanoate	$C_{12}H_{24}O_2$	1397	8.17 ± 7.62^{a}	1.89 ± 0.10^{b}	3.86 ± 0.72^{ab}	4.35 ± 1.48^{ab}	4.36 ± 0.30^{ab}	4.31 ± 0.63^{ab}	1.91 ± 0.35^{b}	4.42 ± 0.44^{ab}	4.15 ± 0.49^{ab}
Alcohols											
n-Hexanol	$C_6H_{14}O$	866	3.93 ± 0.57^{bcd}	4.69 ± 0.33^{bc}	2.08 ± 0.23^{d}	3.21 ± 0.10^{cd}	5.93 ± 0.46^{b}	9.05 ± 1.59^{a}	10.61 ± 0.67^{a}	4.16 ± 0.05^{bc}	4.50 ± 0.94^{bc}
n-Octanol	$C_8H_{18}O$	1073	0.47 ± 0.18^{cd}	0.84 ± 0.08^{b}	1.51 ± 0.12^{a}	0.51 ± 0.06^{cd}	0.50 ± 0.08^{cd}	0.71 ± 0.04^{bc}	0.95 ± 0.08^{b}	0.33 ± 0.02^{d}	0.32 ± 0.09^{d}
2-Phenylethanol	$C_8H_{10}O$	1111	8.19 ± 0.39^{bc}	11.22 ± 0.43^{ab}	4.66 ± 0.42^{de}	2.62 ± 0.26^{e}	6.68 ± 0.92^{cd}	6.01 ± 2.53^{cd}	12.51 ± 1.67^{a}	7.62 ± 0.94^{cd}	4.39 ± 0.98^{de}
n-Nonanol	$C_9H_{20}O$	1172	1.86 ± 0.34^{b}	2.49 ± 0.15^{a}	2.54 ± 0.36^{a}	0.73 ± 0.22^{cde}	0.86 ± 0.16^{cd}	0.81 ± 0.15^{cde}	1.14 ± 0.06^{c}	0.27 ± 0.05^{e}	0.28 ± 0.03^{ce}
Monoterpenes											
Terpinolene	$C_{10}H_{16}$	1087	0.44 ± 0.09^{a}	—	—	—	—	—	—	—	—
Terpinen-4-ol	$C_{10}H_{18}O$	1176	0.29 ± 0.09^{a}	—	—	—	—	—	—	—	—
α-Terpineol	$C_{10}H_{18}O$	1190	1.53 ± 0.09^{a}	—	0.55 ± 0.04^{b}	0.36 ± 0.21^{b}	1.47 ± 0.16^{a}	0.38 ± 0.01^{b}	0.54 ± 0.02^{b}	0.31 ± 0.19^{bc}	0.57 ± 0.12^{b}
neo-iso-3-Thujyl-acetate	$C_{12}H_{20}O_2$	1279	2.13 ± 0.28^{bc}	4.42 ± 0.14^{a}	2.41 ± 0.30^{b}	1.84 ± 0.02^{c}	2.25 ± 0.03^{bc}	0.65 ± 0.10^{e}	1.03 ± 0.08^{de}	1.93 ± 0.06^{c}	1.20 ± 0.14^{d}
Totals per class											
Esters			64.37 ± 9.34^{bc}	70.62 ± 2.20^{bc}	68.5 ± 2.20^{abc}	74.98 ± 1.89^{a}	63.61 ± 1.58^{bc}	67.94 ± 3.05^{bc}	55.26 ± 2.62^{c}	72.75 ± 2.15^{ab}	71.74 ± 4.52^{ab}
Alcohols			14.45 ± 0.79^{cd}	19.24 ± 0.57^{b}	10.79 ± 0.61^{ef}	7.07 ± 0.36^{g}	13.97 ± 1.04^{cd}	16.58 ± 2.99^{bc}	25.20 ± 1.80^{a}	12.38 ± 0.94^{de}	9.50 ± 1.36^{fg}
Monoterpenes			4.38 ± 0.32^{a}	4.42 ± 0.30^{a}	2.96 ± 0.30^{c}	2.20 ± 0.21^{d}	3.72 ± 0.16^{b}	1.04 ± 0.10^{f}	1.58 ± 0.08^{e}	2.23 ± 0.20^{d}	1.77 ± 0.18^{de}
Total identified			83.21 ± 9.38	94.26 ± 2.99	82.25 ± 2.30	84.25 ± 1.94	81.30 ± 1.90	85.55 ± 4.27	82.02 ± 3.18	87.37 ± 2.35	83 ± 4.72

Note: Relative contents are expressed in percentage (%). The values represent means from three replicates per genotype ± standard deviation ($n = 24$). Experimental linear-temperature-programmed retention indices (LTPRIs) are shown. Distinct lettering corresponds to statistically significant differences in the rows, Tukey HSD $P < 0.05$. —, not detected.

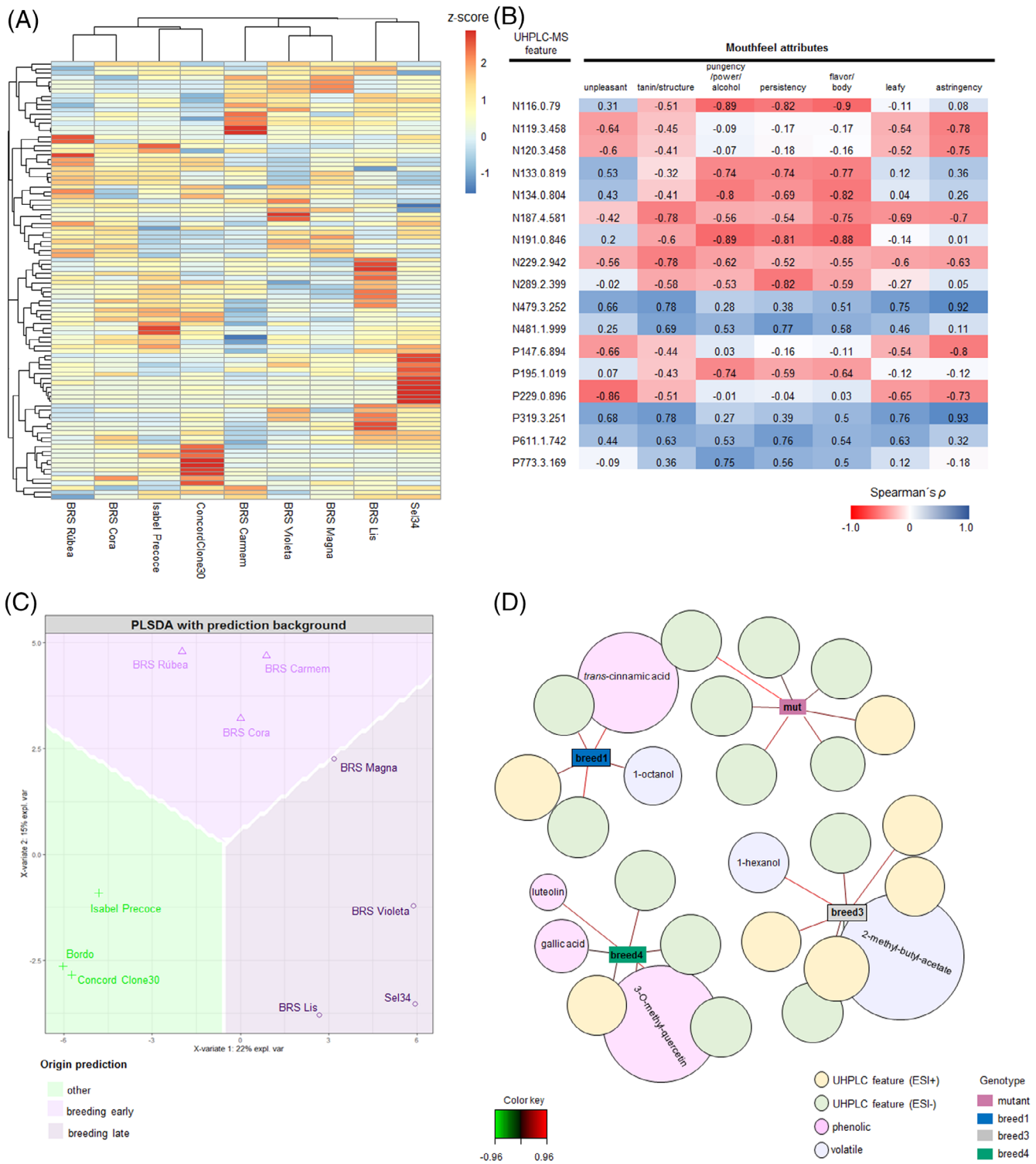

Figure 4. Untargeted metabolome by UHPLC–MS and integrated model. Hierarchical cluster of ESI+ and ESI− features (A). Correlation between features and mouthfeel sensory grades (B). Predictive performance of the integrated sPLS-DA model (UHPLC, GC–MS, and UHPLC–MS) (C). Background represents class boundaries calculated using maximum distance. Model tuning by repeated cross-validation (10 × 10-fold). Relevance network (D) of the associations between metabolites/features and wines genetic origins (cut-off = 0.75).

contents of 3,5-anthocyanidin diglucoside derivatives. Although diglucoside anthocyanins are considered markers of non-*vinifera* cultivars in winemaking,[8,44] recent hybrid cultivars exhibit levels of diglucoside anthocyanins like those in *Vitis vinifera* wines.[8,44] Wines from the second breeding cycle did not associate with anthocyanin 3,5-diglucosides, demonstrating their *vinifera*-like profile.

'BRS Rúbea', 'BRS Violeta', and especially 'BRS Magna' wines exhibited high contents of hydroxytyrosol, considered to have higher antioxidant activity than other polyphenols due to the

ortho-dihydroxy conformation of its aromatic ring.[45] Studies demonstrated strong anti-inflammatory activity of hydroxytyrosol by inhibiting cytokines, and neuroprotective role by modulating microglial cells activity, which reduces neuroinflammation and inhibits α-synuclein and β-amyloid fibrils formation.[45] However, the compound is only present in olives, virgin olive oils, and wine.[45] 'BRS Rúbea', 'BRS Violeta', and 'BRS Magna' wines had more than three times the average reported concentration of hydroxytyrosol.

Wine color intensity and violet hue positively correlated with levels of monomeric anthocyanins. Contents of all classes of phenolic compounds were intercorrelated due to their shared metabolic pathways.[46] Wine color is influenced by the flavylium cation of malvidin-3-glucoside and its interaction with quercetin 3-*O*-β-glucopyranoside.[47] 'BRS Violeta' and 'BRS Magna' wines received higher color grades than 'BRS Lis', with greater malvidin-3-glucoside and quercetin contents. Discrepancies of the CIE *L*a*b** color space were demonstrated for highly saturated red regions, where it does not faithfully represent eye perception.[48] Despite these divergences, *b** coordinate is the closest approximation to perceived hue in red wines,[48] agreeing with the negative correlation between anthocyanins, flavonoids, and *b** in our results.

Volatile compounds

Clustering analyses based on polyphenol and volatile profiles generated distinct groups, suggesting different effects on metabolic pathways submitted to or free of selective pressure by breeding. In highbush blueberry (*Vaccinium corymbosum*), flower volatiles were influenced by environment, genomic context, and metabolite.[18] Wines' polyphenol profile became more divergent, whereas the blend of volatiles turned more similar along breeding cycles. Despite the absence of selection, monoterpene contents decreased in wines from recent cultivars. Although volatiles of *vinifera* and hybrid genotype wines are generally considered different, intragroup variation is high.[9,10] Moreover, winemaking technologies and microorganisms exert great influence on volatiles.[49] Thus, no clear association between volatiles and hybrid grapes is known. Wines from French disease-resistance hybrids had ethyl propanoate, whereas ethyl-hexanoate was predominant in wines from *Vitis vinifera* cultivars.[9,10] In our study, a hydroxylated derivate of ethyl propanoate was detected in association with wines from recent genotypes and ethyl-hexanoate, in those from 'Concord Clone 30', 'BRS Rúbea', 'BRS Cora', and 'BRS Violeta'. In *vinifera* wines, the compound is associated with green apple, strawberry, and pineapple aromas,[10] agreeing with the 'fruity' odor of 'BRS Rúbea' and 'BRS Cora' wines. However, the association between volatile profiles and sensory analyses were inconclusive, as in other studies.[42,43]

Sensory ratings and volatile profiles of 'BRS Rúbea', 'BRS Cora', and 'BRS Carmem' wines were distinct from previously reported.[12,13] These differences are likely due to distinct vineyard environments and winemaking techniques. In contrast, the 'leafy'/'vegetal' trait of 'BRS Violeta' wines, observed previously[13] was replicated in our study. The 'leafy odor' of 'BRS Lis' wines is likely due to interaction between pulp polyphenols and volatiles.

Untargeted metabolome

Ninety-six features were detected in the wines, with a higher number from ESI−. Similar studies of red wines, using UHPLC and liquid chromatography/ion mobility with quadrupole time-of-flight mass spectrometry (LC-IM-QTOF-MS), detected 2145 and 2384 features in 'Cabernet Sauvignon' wines from three regions of China and in 114 commercial wines from different vintages and regions, respectively.[11,50] Both ionization modes exhibited similar chemical fingerprints.[11,50] Here the differences could be due to higher contents of phenolic acids, flavanols, and flavan-3-ols that generate stronger signals in ESI−, in comparison to anthocyanins, that tend to ionize in ESI+. These observations agree with HPLC results, where polyphenol/monomeric anthocyanin ratios were greater than or close to 1.0 for seven out nine genotypes.

Prediction of wine quality from chemical analyses is desirable for the industry[51,52] and breeding programs. In young red wines, sensory features originate from the berry (varietal or primary traits), winemaking processes (secondary traits), and alcoholic and malolactic fermentations (fermentation traits).[8,53] Wine ethanol and polyphenol contents and their interactions create oral-tactile stimulation during tasting, inducing salivary film modifications.[54] Studies demonstrated that integrative approaches, combining sensory and instrumental analyses, are required to investigate mouthfeel perception.[53,54] Our results show significant correlation between UHPLC–MS features and sensory traits.

Metabolite identification remains challenging in untargeted metabolomics due to requirements for accurate molecular weight, retention time, and ion fragments that are specially difficult for plant specialized metabolites with high structural variability.[55] Discriminant compounds in untargeted wine analyses include flavonoids, organic acids, amino acids, terpenes, and fatty alcohols.[8,11,50,53] We tentatively identified quercetin (N301/5.46), 3-*O*-methyl-quercetin (P3017/4.559), chlorogenic (N353/4.187), and *trans*-ferulic (N193/0718) acids but most compounds remain to be characterized.

Integrated metabolome and genetic origin prediction

Genotype clustering was different for each metabolite set, as in grape juice.[42] Groups formed from sensory data and chemical profiles were also distinct. An integrative model discriminated wines from early and later genotypes and predicted genetic origin. Minor discrepancies are likely due to yeast influence on wines chemical signature[53] and high heterozygosity of grapevine.[1,2,56]

CONCLUSIONS

Genetic breeding improved grapevine adaptation and yield. Wine phenolic contents almost doubled in four breeding cycles. Breeding reduced monoterpene levels without selection. Wines exhibit distinct sensory properties with great contribution of color and mouthfeel attributes. The profile of phenolic metabolites was divergent for wines from recent genotypes. Wines from 'BRS Rúbea', 'BRS Violeta', and especially 'BRS Magna' had elevated contents of hydroxytyrosol, a powerful natural antioxidant. Monomeric anthocyanin contents correlated with wine color intensity and violet hue, whereas phenolic acids and flavonoids contents were negatively associated with saturation and tonality. The profile of volatiles also allowed discriminating genotypes from different breeding cycles. Wines from recent genotypes had lower contents of monoterpenes. Untargeted metabolome was less effective to discriminate the wines, but unveiled significant correlation between features and sensory attributes. Integrative modeling of chemical profiles allowed accurate representation and prediction of genetic origin, suggesting that further studies may permit identification of wine biomarkers for sustainable viticulture.

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CONFLICT OF INTEREST STATEMENT

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 article. The sponsors had no role in study design; in the collection, analysis and interpretation of data; in the writing of the article; and in the decision to submit the article for publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in REDAPE at http://www.redape.dados.embrapa.br/, reference number http://doi.org/10.48432/RTVDLE.

AUTHOR CONTRIBUTIONS

H.A.G. Gomez: methodology and investigation; G. F. Niederauer: investigation; I. O. Minatel: investigation; E. R. M. Antunes: data curation and analyses; M.J. Carneiro: methodology, investigation, and original writing; A.C.H.F. Sawaya: data curation, supervision, and writing – reviewing and editing; M. C. Zanus: supervision; V. Quecini: conceptualization, data curation, and writing – reviewing and editing; P. S. Ritschel: conceptualization, data curation, supervision, and writing – reviewing and editing; G. P. P. Lima: supervision, and writing – reviewing and editing; M. O. M. Marques: data curation, supervision, and writing – reviewing and editing.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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