

Exploring Intra-varietal Variation for Complex Traits in Grapevine (*Vitis vinifera* L.)

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Abstract

Climate change poses significant challenges to global grapevine (*Vitis vinifera* L.) production, highlighting the urgent need for adaptive breeding strategies to accelerate genetic improvement. While clonal propagation preserves varietal identity and heterozygosity, it also limits the rate of genetic gain due to prolonged breeding cycles. This study assessed phenotypic and genetic variation within five large clonal populations of key grapevine varieties (the Pinot family, Riesling, Müller-Thurgau, Auxerrois, and Savagnin) using 14 years of data collected in Germany across six agronomic, quality, and disease-related traits. Estimates of broad-sense heritability, genetic correlations, and key variance components were derived using linear mixed models. Substantial intra-varietal phenotypic variation was observed across all traits, with moderate to high heritability estimates, confirming that a meaningful proportion of the phenotypic variation can be attributed to the genetic differences among clones. Substantial year and year-by-field variance components were found to contribute to the total phenotypic variance for most traits, aligning with previous reports of substantial genotype-by-environment interaction in clonal grapevine populations. Genetic correlations revealed both strong positive and strong negative trait relationships, emphasising the importance of informed multi-trait selection strategies. The results highlight considerable potential to enhance clonal selection by integrating predictive breeding tools such as genomic and phenomic selection. Optimization-based multi-trait selection approaches also offer promising alternatives to traditional index methods, particularly in the context of negative trait correlations. Ultimately, the high intra-varietal genetic variation uncovered in this study represents a valuable resource for improving adaptation to future environments while maintaining varietal integrity in grapevine.

Key Message

Centuries of clonal propagation have shaped remarkable intra-varietal genetic diversity in grapevine, offering valuable opportunities to dissect complex traits and accelerate genetic improvement while safeguarding varietal integrity.

Introduction

Grapevine (*Vitis vinifera* L.) stands apart in agriculture due to the great importance of traditional cultivars, like Pinot noir and White Riesling, representing living genetic heritage propagated continuously for centuries. In contrast to many agricultural sectors that readily adopt newly bred varieties, the global wine industry is uniquely anchored to these ancient cultivars. Market identity, regional typicity, and consumer recognition are inextricably linked to these traditional varieties, creating significant barriers to the adoption of new cross-bred cultivars (Töpfer & Trapp, 2022).

Despite this, the need for genetic improvement persists and is driven largely by the urgent challenges posed by climate change. Rising temperatures, in particular, are accelerating phenology and increasing vulnerability to spring frosts, while also increasing the speed of sugar accumulation in grapes, resulting in high alcohol content and altered flavour profiles in wines (van Leeuwen et al., 2024; Baltazar et al., 2025). In addition, altered precipitation patterns and increased humidity favour the spread of fungal diseases such as downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*), and botrytis bunch rot (*Botrytis cinerea*), leading to significant impacts on yield and fruit quality (van Leeuwen et al., 2024). Without targeted intervention it is very apparent that global grapevine production is on an unsustainable trajectory.

Faced with the limited market acceptance of new varieties, grapevine breeders have pivoted towards clonal selection as a key avenue to identify and harness complex trait variation for the genetic improvement of traditional cultivars (Schmidt et al., 2025). This approach contrasts sharply with clonal selection programs in many other clonally propagated species (e.g., potato, strawberry), where the objective is typically to generate new varieties through hybridization, followed by selection and multiplication of the best resulting individual clone. In grapevine, however, clonal selection focuses on identifying, evaluating, and propagating superior clones that have emerged *within* the existing populations of ancient cultivars (Callipo et al., 2025). This intra-varietal diversity originates from the gradual accumulation of somatic mutations and epigenetic modifications over centuries of vegetative propagation (Duhovnikoff and Dodd, 2015; Vondras et al., 2019), leading to phenotypic differences among clones classified under the same variety name (Pelsy, 2010). Somatic mutation is a genetic alteration occurring in non-reproductive cells, frequently accumulating over time through repeated cycles of clonal propagation and cell division. While epigenetic modifications such as DNA methylation and histone modification can induce phenotypic variation among genetically identical clones without altering the DNA sequence, typically arising in response to environmental stresses and potentially being heritable through vegetative propagation (Berger et al., 2023). Practices like polyclonal selection further leverage this existing diversity by propagation of a group of genetically distinct but complementary clones within a variety to enhance vineyard resilience while maintaining varietal identity (Martins and Gonçalves, 2015).

Traditional clonal selection is exceedingly slow, where the average grapevine breeding cycle can span up to, or even exceed, 25 years, severely limiting the rate of genetic gain. This is largely due to the use of clonal propagation to preserve high heterozygosity, coupled with a prolonged juvenile phase of up to five years, which delays early-stage phenotypic selection for key yield and quality traits. In phylloxera-prone regions, the need for grafted vines can prolong this process by one to two years. In addition, the perennial nature of grapevine makes field evaluations more resource-intensive, while prolonged exposure to varying environmental conditions underscores the importance of extensive multi-year assessments. These constraints are further compounded by the inherent inefficiencies of clonal propagation, requiring labour-intensive cutting, grafting, phytosanitary testing and nursery management, and when combined with limited resources typical of public breeding programs, restrict the scalability and pace of multi-environment field evaluations.

Prior research consistently demonstrates substantial phenotypic variation within clonal populations of diverse grapevine varieties, highlighting potential for clonal selection. Studies investigating cultivars like Cabernet Franc (van Leeuwen et al., 2012), Grenache (Buesa et al., 2021), Malbec (van Houten et al., 2020), and Tempranillo (Arrizabalaga et al., 2017; Portu et al., 2024) report significant intra-varietal diversity for yield and quality traits, often even within relatively modest population sizes (e.g., 9–33 clones). This finding is echoed in large-scale surveys across multiple French regions (Neethling et al., 2023) and among numerous ancient Portuguese varieties, where considerable diversity, particularly for yield (e.g. genotypic coefficient of variation up to 59%), was evident (Gonçalves and Martins, 2022). However, investigations into phenotypic correlations remain limited with the exception of one study in Tempranillo where

a negative yield-malic acid relationship was observed alongside expected positive correlations among acidity component traits (Portu et al., 2024). Crucially, despite documenting phenotypic variation, the majority of previous work has generally not partitioned this variance to quantify the specific contribution of the clonal genetic effect. Addressing this gap, a study by Laidig et al. (2009) analysed 30 White Riesling clones across a large scale, encompassing 16 locations over 36 years. This research is significant for explicitly partitioning variance using mixed models on extensive, unbalanced multi-environment trial data, thereby estimating the specific contribution of the clonal genetic effect. Their analysis quantified this clonal effect, finding it accounted for a small proportion (less than 1%) of the total phenotypic variation for key traits like yield, sugar content (total soluble solids), and acidity, with environmental factors (location, year, and their interactions) explaining the vast majority (around 95%). Accurately partitioning this variance is thus a critical first step towards leveraging clonal diversity through selection technologies.

Integrating modern predictive breeding approaches, such as genomic selection (GS) with the rich genetic resources found in varietal clonal populations presents a powerful strategy for accelerating genetic gain specifically tailored to the grapevine industry's constraints. For example, GS can potentially shorten the evaluation cycle by predicting clonal genetic merit early using genome-wide markers (Meuwissen et al., 2001). However, the success of such predictive models rests on accurately characterising the genetic architecture of traits within large, accurately phenotyped training populations. Addressing the limitations of previous research, namely the need for larger population sizes, multi-year trait evaluation, and robust variance component analysis, is crucial for building effective predictive models for clonal selection in grapevine.

This study aims to quantify the extent of phenotypic variation within five commercially-important varietal clonal populations by assessing multi-year trait data for yield, sugar, and main organic acids, as well as disease susceptibility. Using a linear mixed model framework, the study estimates the contribution of genetic effects to trait variation and evaluates broad-sense heritability across traits and populations. Additionally, trait genetic correlations are examined to identify key relationships influencing selection strategies, providing insights into opportunities for optimizing clonal selection and accelerating genetic improvement in grapevine.

Materials and Methods

Collection and establishment of clonal populations

Five clonal populations evaluated in this study represent the varieties Auxerrois ($n = 87$), Müller-Thurgau ($n = 123$), the Pinot family ($n = 536$), Riesling ($n = 1,087$), and Savagnin ($n = 98$). Individual clones within each group were selected from historic vineyards across Germany, France, Austria, and Luxembourg (Supplementary Fig. 1), based on advantageous agronomic traits. The Pinot population includes clones classified as Pinot noir, Pinot gris, Pinot blanc, and Pinot précoce; and the Savagnin group comprises Savagnin blanc, Savagnin rose, and Gewürztraminer clones. While some of these are registered as separate varieties, they were treated as part of a single clonal population due to their close genetic relationships and shared breeding histories. Following virus testing and varietal confirmation, clones were propagated from single-bud cuttings and grafted onto phylloxera resistant rootstock varieties (Supplementary Table 1). The varietal clonal populations were planted in the experimental vineyards of Geisenheim University, across eight fields without replication (Supplementary Fig. 2A), and depending on the clone and population, clones were planted across years with the year of planting recorded. All vineyard fields were managed consistently according to standardized viticultural practices, i.e. uniform canopy, pruning level, soil, and cover crop management, identical plant protection regimes, and no yield-reducing interventions, and were laid out in a two-dimensional format, with rows in the horizontal direction and plots in the vertical direction (Supplementary Fig. 2B). Each vineyard plot consisted of three vines per clone (Supplementary Fig. 2C), allowing for within-plot replication to account for small-scale heterogeneity in soil conditions, plant spacing, or microclimate.

Phenotypic trait observations

The five clonal populations were evaluated for yield, juice quality parameters and botrytis susceptibility, across 14 seasons from 2009 until 2023. Due to breeding program resource constraints, not all clones were sampled for all traits in all 14 years. Supplementary Fig. 2B graphically depicts the distribution of clonal populations across the Geisenheim University experimental vineyard as well as the sampling frequency of each plot across the duration of the study. The size of the clonal populations varied, with the Auxerrois population consisting of 87 clones, the Müller-Thurgau 123 clones, Pinot 535 clones, Riesling 1,087 clones and the Savagnin consisting of 98 clones.

Six grapevine breeding priority traits were phenotyped as part of the study, including total yield (g/m^2), total soluble solids (brix °Brix), total acidity (g/L ; titratable acidity expressed as tartaric acid), malic (g/L) and tartaric acid content (g/L) and botrytis infection (%). To measure the juice quality parameters, clusters from each plot were pressed using a 20 L hydraulic basket press (Speidel Tank- und Behälterbau GmbH, Ofterdingen, Germany). Prior to coarse filtration ($16 \mu\text{m}$ Munktell 33/N, 90 g m^{-2} folded filter; Ahlstrom, Helsinki, Finland), 8 mL hL^{-1} of pectolytic enzymes (Trenolin 4000 DF; Erbslöh GmbH, Geisenheim, Germany) were added to the juice. A single bulk juice sample per plot was collected for analysis. brix, total acidity, malic acid, and tartaric acid concentrations were measured using Fourier-transform infrared (FTIR) spectroscopy with a Winescan FT2 spectrometer (FOSS, Hillerød, Denmark) and an in-house grape must calibration. Botrytis bunch rot was assessed through visual inspection of infected berries, using the seven-step EPPO guideline 1/031(3), with a scale from 0% (no symptoms) to 100% (complete infection). One overall severity score was recorded per plot.

Raw data curation

The raw data collect across 14 years was compiled individually for each of the five varietal clonal populations and herein all populations were analysed separately. All data analyses were performed using R (R Core Team, 2024). To remove outliers prior to calculating descriptive statistics and graphical representation of raw observations, a base linear mixed model was fit to each population using the following equation:

$$y = X\backslash\text{varvec}\tau + Zu + e$$

1

where X and Z are the design matrices associated with the fixed $\backslash\text{varvec}\tau$ and the random u effects, and e is the residual error term. In this base model, the intercept was fitted as a fixed effect, while clone, year, and field effects were modelled as random. A separate residual variance was estimated for each individual year, allowing for year-specific plot error. Following base model convergence, the model was updated to estimate the scaled residuals, and plot observations that were above or below a standardized residual threshold value of 4 were identified as outliers and removed. The base model was updated and the outlier detection process repeated until no observations exceeded the scaled residuals threshold. All standard assumptions of the linear mixed model, including the normality of residuals, homogeneity of variances, and independence of errors, were assessed through diagnostic plots and deemed to be satisfactorily met. The exception was the trait botrytis, which required a logarithmic transformation (i.e. $\log(1 + x)$) to reduce skewness and stabilize residual variance, thereby improving adherence to model assumptions. The base linear mixed model was fit in the R package 'ASReml-R' (Butler et al., 2009) and all graphical outputs created using 'ggplot2' (Wickham, 2016). Following outlier removal, descriptive statistics for each trait measured within each population were calculated, including mean, variance, standard deviation and the coefficient of variation % (CV%), calculated as standard deviation divided by the mean multiplied by 100 and reported Supplementary Table 2.

Univariate linear mixed model and associated variance estimates

Post raw data curation, a final linear mixed model, following the equation outlined in (1) was fit individually for each trait within each population. In the final model, for all populations, the fixed effects included an overall intercept and the random effects included effects for clone, year, field and a diagonal variance structure fit to the year-by-field interaction. The residuals were modelled as year-by-plot with a diagonal variance structure assigning a separate variance for each year. The diagonal structure accommodates heterogeneous variances across levels of a factor (e.g. year) while assuming zero covariances. For populations Auxerrois and Müller-Thurgau, planting year was also fitted as a continuous fixed effect, and was tested for significance at $p < 0.05$ using the Wald test statistic. For the Pinot, Riesling and Savagnin populations, the planting year was not found to be significant and was not included as a fixed effect in the model. The final model ASReml-R script is detailed in Supplementary Materials. Based on log-likelihood ratio tests and Akaike Information Criterion (AIC), the diagonal variance structure provided the best fit for both the random year-by-field interaction and the residual year-by-plot interaction. Although a correlated heterogeneous variance structure was considered for the year component of the year-by-field random effect, to account for potential heterogeneity and covariance between fields observed in different years, the model failed to converge, likely due to data sparsity in certain year-field combinations. Given the perennial nature of grapevine and the expectation of residual correlation across years within plots, a first-order autoregressive residual structure (ar1), was fit to the year-by-plot interaction. While a weak negative correlation was estimated, the model fit was not improved based on the REML log-likelihood and AIC. To better accommodate heterogeneity in residual variance across years, an ar1h structure was also tested; however, due to the sparsity and imbalance of the data and especially in respect to the variance in sampling the same plot each year, the model failed to converge. As such, a simpler diagonal residual structure was retained. Following model convergence, variance components and associated z-ratios were extracted. Multi-year best linear unbiased predictors (BLUPs) were obtained for each clone and the scaled average pairwise prediction error variance (A_{tt}). Broad-sense heritability (H^2) was calculated based on these components using the method described by Cullis et al., (2006), which accounts for unbalanced data and the prediction uncertainty:

$$\backslash\text{varvec}H^2 = 1 - \frac{\backslash\text{varvec}A_{\backslash\text{varvec}t\backslash\text{varvec}t}}{2\backslash\text{varvec}*\backslash\text{varvec}\sigma_{\backslash\text{varvec}g}^2}$$

2

where A_{tt} refers to the mean of average variance of a difference of the BLUPs and σ_g^2 the genetic variance. Genotypic coefficient of variation (CV_G%), as a percentage, was expressed as the amount of genetic variation for a trait relation to the means of the trait BLUPs.

Bi-variate linear mixed models to estimate genetic correlations

A series of bivariate linear mixed models were fitted to estimate genetic correlations between each pairwise trait combination within each clonal population. To ensure comparability across traits, all trait values were scaled prior to analysis. The fixed, random, and residual terms followed the univariate model structure described above. To model trait-specific variance and covariance across model components, 2×2 unstructured variance-covariance matrices were fitted to the following random effects: trait-by-clone, trait-by-year, and trait-by-year-by-field. For the trait-by-field and trait-by-unit residual effects, a diagonal structure was used to account for trait-specific variances while assuming no covariance between traits. These two terms were simplified due to convergence issues and data sparsity, which precluded fitting a full unstructured variance-covariance structure. This approach allows estimation of trait-specific genetic variances and covariances from the trait-by-clone effect, from which genetic correlations r_g between traits were derived using the standard formula:

$$\backslash\text{varvec}r_{\backslash\text{varvec}g} = \frac{\backslash\text{varvec}C\backslash\text{varvec}o\backslash\text{varvec}v_{\backslash\text{varvec}c\backslash\text{varvec}c\backslash\text{varvec}o\backslash\text{varvec}n\backslash\text{varvec}e}(\backslash\text{varvec}T1, \backslash\text{varvec}T2)}{\sqrt{\backslash\text{varvec}V\backslash\text{varvec}a\backslash\text{varvec}r_{\backslash\text{varvec}c\backslash\text{varvec}c\backslash\text{varvec}o\backslash\text{varvec}n\backslash\text{varvec}e}(\backslash\text{varvec}T1) \cdot \backslash\text{varvec}V\backslash\text{varvec}a\backslash\text{varvec}r_{\backslash\text{varvec}c\backslash\text{varvec}c\backslash\text{varvec}o\backslash\text{varvec}n\backslash\text{varvec}e}(\backslash\text{varvec}T2)}}$$

3

Where $Cov_{clone}(T1, T2)$ is the estimated genetic covariance between traits T1 and T2, and $Var_{clone}(T)$ is the genetic variance for trait T.

Results

Phenotypic variation across years and varietal clonal populations

Wide variation is evident for the raw observations of the six key grapevine traits (Fig. 1). The patterns of variation appear unique to each population for a given trait, highlighting the distinct phenotypic diversity within varietal clonal populations. For example, in the Müller-Thurgau population, yield distribution ranges from 478 to 3860 g/m², where as in the Auxerrois and Savagnin populations yield distribution is skewed to the right. The trait CV%, as a measure of scale-independent variability, ranged from 28.5–39.7% for yield, 6.0–9.1% for brix, 14.2–23.8% for tartaric Acid, 32.3–39.0% for malic acid, and 16.2–22.1% for total acidity (Supplementary Table 2). In a comparison of phenotypic variance across populations, the Pinot population had the highest CV% for all traits except brix, where it had the second highest following Auxerrois.

When raw trait observations were separated based on year, substantial phenotypic variation was observed for all traits, both within individual years and across all years measured in each varietal clonal population (Fig. 2). For example, in the Riesling population, yield distributions were right-skewed in 2010, 2013, and 2014, whereas a more normal distribution was observed from 2018 to 2022 (Fig. 2a). Some general year-to-year trends were evident across clonal populations, such as approximately normal yield distributions in 2015 and 2016. However, distinct population-specific trends also emerged within certain years, and similar trends can be observed for brix (Fig. 2b.) and the remaining traits (Supplementary Fig. 3A-F).

Clonal effect contributes to phenotypic variation for complex traits

Variance parameters were estimated from the multi-year linear mixed model fit individually for each trait within each clonal population, and the proportional breakdown of these specific sources of variation are presented in Fig. 3. Across populations, the contribution of clonal effect to total variance was generally largest for yield, followed by brix and total acid content, while botrytis susceptibility had the smallest effect. Within a trait, variance in the clonal contribution is evident, for instance, in the Pinot population the clonal contribution to yield is 16.1%, and similarly 13.5% in Riesling, while the contribution in Auxerrois is 6.0% (Supplementary Table 3). Similarly, for brix, the clonal contribution was highest in Müller-Thurgau at 14.0%, followed by Riesling at 8.4%, while Auxerrois had the lowest clonal contribution at 4.3%. The proportion of variance attributed to the year and year-by-field effects are substantial and predominate for most traits, where the year effect is dominate for malic (ranging from 22.4–82.9%) and total acid content (12.8–78.7%) for most populations. On its own, the contribution of field has a small effect on total variance with the exception of tartaric acid, and in particular for the Savagnin population with 51.0% of the variance explained by field term. The residual variance ranged from 4.3–40.0% across traits and populations, while the largest proportion of residual variance was observed for botrytis susceptibility in the Savagnin population, followed by yield for Müller-Thurgau at 35.6%.

Given the unbalanced nature of the multi-year dataset, variance component estimates for the clonal effect must be interpreted considering both cumulative replication across years and the pairwise prediction error variance, as the latter directly impacts the reliability of ranking clones for accurate selection. To account for this, broad-sense heritability was calculated using the method described by Cullis et al. (2016). Heritability estimates across populations ranged from 0.47–0.70 H² for yield, 0.50–0.72 for brix, 0.13–0.87 for tartaric Acid, 0.40–0.92 for total acid, 0.51–0.86 for malic acid and 0.36–0.54 for botrytis susceptibility. While re-ranking for trait-specific heritabilities occurred across populations, trends remained relatively consistent for seemingly related traits, such as tartaric acid, malic acid, and total acid content (Fig. 4).

Table 1
Multi-Year BLUP Summary Statistics for key Traits

Trait	Variety	No. Clones	Mean	CV _G %	H ²
Yield (g/m ²)					
	Auxerrois	87	1,714.82	8.13	0.60
	Müller-Thurgau	123	2,111.16	9.15	0.63
	Pinot	535	1,203.87	17.96	0.70
	Riesling	1,087	1,383.09	16.08	0.68
	Savagnin	98	975.11	12.22	0.47
Brix (°)					
	Auxerrois	87	20.16	1.03	0.50
	Müller-Thurgau	123	18.17	2.57	0.72
	Pinot	536	21.69	2.34	0.60
	Riesling	1,087	21.35	2.21	0.64
	Savagnin	98	22.53	1.29	0.52
Total acid (g/L)					
	Auxerrois	87	7.60	6.48	0.92
	Müller-Thurgau	123	7.41	4.89	0.83
	Pinot	536	7.98	8.20	0.79
	Riesling	1,087	10.19	2.60	0.40
	Savagnin	98	7.25	6.42	0.73
Tartaric acid (g/L)					
	Auxerrois	87	6.03	4.34	0.87
	Müller-Thurgau	123	5.30	3.11	0.54
	Pinot	536	5.04	7.26	0.63
	Riesling	1,087	6.41	3.88	0.45
	Savagnin	98	4.96	1.45	0.13
Malic acid (g/L)					
	Auxerrois	87	2.33	12.60	0.86
	Müller-Thurgau	122	2.81	6.88	0.76
	Pinot	536	3.84	11.29	0.71
	Riesling	1,087	4.21	4.86	0.51
	Savagnin	98	3.15	6.70	0.64
Botrytis (%)					
	Auxerrois	87	1.50	8.38	0.54
	Müller-Thurgau	123	1.11	5.65	0.36
	Pinot	532	1.07	2.32	0.37
	Riesling	1,087	2.22	8.10	0.38
	Savagnin	98	1.40	7.49	0.52

Wide Phenotypic Variance Persists Across Traits in Adjusted Clonal Estimates

The multi-year mixed model analysis revealed considerable variance in the estimated BLUPs across all traits for all clonal populations (Fig. 5). Effectively isolating the genetic signal from environmental noise and estimate uncertainty, the mixed model analysis produced BLUPs with focused variance and CV_G%. While numerically lower than raw observation values, this concentration reflects a clearer representation of the underlying genetic variation (Table 1). Trait-specific population performance exhibited clear re-ranking patterns, for example, Auxerrois and Riesling populations had the highest tartaric acid content

BLUPs (Fig. 5c), while Auxerrois and Müller-Thurgau populations showed the lowest sugar content (Fig. 5b) and highest yield (Fig. 5a). Similar to the population heritability trends, rankings remained consistent across acid content traits, whereas an inverse relationship was observed between yield and brix (Fig. 5a and 5b). Given the trait-by-trait, population-specific nature of the analysis, statistical significance tests between population BLUPs were not applicable.

Negative correlation between yield and quality traits predominates across clones

Genetic correlations were generally consistent across clonal populations (Fig. 6), where yield exhibited negative correlation with brix ranging from -0.72 to -0.94 . The relationship between acid content traits (tartaric acid, malic acid, and total acidity) and yield was generally positive, particularly for tartaric acid in Riesling and Müller-Thurgau populations, or weakly correlated, especially in Pinot. Acid content traits were predominately positively correlated, except in Riesling and Müller-Thurgau, where negative correlation (-0.66 , and -0.48 , respectively) were detected between malic and tartaric acid. A consistent negative relationship between brix and acid content traits was observed across all populations, with the exception of Pinot where a weak positive (0.13) correlation was observed between brix and malic acid. Some genetic correlations could not be reliably estimated due to dataset imbalance and sparsity or low genetic variance, particularly in populations with smaller sample sizes.

BLUPs Reveal Potential for Within-Population Clonal Selection

Visualization of within-population BLUPs for tartaric acid, brix, and yield highlights the potential for targeted clonal selection within each varietal population (Fig. 7). Using arbitrary selection thresholds (top 30% for high tartaric acid, bottom 60% for Brix, and the 30–95% range for yield) potential selections were identified across all populations. This could help to identify new clones with lower sugar, higher tartaric acid and good yield potential that could be more suitable for constantly warming climates in the future. The number of clones meeting these criteria was generally proportional to both population size and phenotypic variance for the traits. Riesling and Pinot, which exhibited the largest number of clones and the highest trait variance (Table 1; Supplementary Table 2), had 168 and 69 clones, respectively, that fit within the selection window. In contrast, Savagnin, Müller-Thurgau, and Auxerrois had fewer candidates, with 19, 16, and 8 clones, respectively. While this selection approach is a preliminary assessment, it demonstrates the potential for within-population clonal selection to enhance key agronomic and quality traits.

Discussion

This study provides a comprehensive, long-term assessment of intra-varietal phenotypic variation by examining six key traits within five commercially important clonal populations over more than a decade. By quantifying the proportion of variance attributable to the clone effect, our analysis provides unique insights into the potential for genetic improvement via clonal selection across these complex traits. Notably, the extent of phenotypic variation observed was considerable, though interestingly, it did not directly correlate with population size. For instance, Pinot exhibited the highest variance (CV% and often CV_G%; Table 1) for most traits despite being the second-largest population sampled ($n = 536$), whereas Riesling, the largest ($n = 1,087$), did not consistently show the highest variation. The high variability in Pinot, particularly for acid-related traits, may reflect historical breeding efforts aimed at modifying acidity, including the use of induced variation to reduce juice acidity. In contrast, acidity has not been a major target in traditional Riesling selection, which may partially explain the lower phenotypic variance observed. Conversely, Auxerrois, the smallest population ($n = 87$), displayed particularly high CV_G% for acid-related traits (Table 1).

The wide variance observed within Pinot, irrespective of its relative population size, likely reflects its status as one of the oldest grapevine cultivars, with an estimated vegetative propagation history spanning approximately 2,000 years (This et al., 2006). This extensive history of clonal propagation, combined with cultivation across diverse environmental conditions, has likely facilitated the accumulation of somatic mutations and epigenetic modifications. In addition, while all individuals in the population are genetically classified as clones, the Pinot group includes clones now recognized as distinct varieties, such as Pinot gris, Pinot blanc, and Pinot précoce, each with potentially different clonal selection histories and harvest timings. These differences likely contribute to the wide phenotypic variance observed, particularly for quality-related traits, where multimodal distributions were evident (e.g. Figure 1). Consequently, Pinot exhibits considerable intra-varietal phenotypic diversity compared to many other cultivars. This is evidenced not only by results of this study but also by the numerous registered clones and the well-documented emergence of distinct variants through bud sports (Vezzulli et al., 2012). In contrast, the high variance observed in acid traits within the much younger Auxerrois population is more plausibly attributed to founder effects or historical selection pressures acting on relevant pathways, rather than extensive mutation accumulation over time. Overall, the substantial phenotypic variance observed across all six traits and five populations, substantiated by the significant clonal variance components (Table 1 and Supplementary Table 3), underscores the considerable genetic potential available for targeted clonal selection within these established varieties.

Contextualising findings within existing literature

To our knowledge, six studies have examined phenotypic variation within clonal populations of grapevine, including in Cabernet Franc (Van Leeuwen et al., 2013), Grenache (Buesa et al., 2021), Malbec (van Houten et al., 2020), Tempranillo (Arrizabalaga et al., 2018; Portu et al., 2024), and White Riesling (Laidig et al., 2009). Most of these studies assessed traits such as yield, brix, and total acidity, providing a useful basis for comparison. While direct comparisons are complicated by differences in experimental design and data reporting, general trends suggest that our study exhibits comparatively high phenotypic variance for traits like yield and total acidity. This may be attributable to the larger population sizes included here, the presence of older cultivars, and the broader temporal coverage of phenotypic data (14 years), which spans a period of increasing climatic variability. Overall, these comparisons support the conclusion that the five clonal populations analysed in this study capture substantial phenotypic diversity for key breeding traits, reflecting, in part, the distinct breeding goals applied to flagship cultivars across different wine-growing regions.

A key contribution of this study is the quantification of substantial genetic variance attributable to clonal differences, with broad-sense heritability (H^2) frequently exceeding 0.5 across the five studied clonal populations. While previous grapevine clonal population studies often described phenotypic variation, few have partitioned the underlying variance components to explore the contribution of individual effects, particularly the clonal genetic component. Laidig et al. (2009), however, provided valuable estimates for Riesling, finding small but statistically significant clonal variance components (less than 1% for yield and quality traits) among 30 commercial clones, suggesting limited genetic differentiation. In contrast, our results demonstrate considerably larger clonal effects, especially within the expansive Riesling population analysed here (1,087 clones), where clonal variance accounted for 13.53% (yield) and 8.36% (brix) of phenotypic variance (Supplementary Table 2). This discrepancy likely reflects the broader genetic and phenotypic spectrum captured in our study, which included both widely cultivated and historically underutilized clones, many of which were identified during targeted surveys of heritage vineyards. Whereas Laidig et al. (2009) focused on commercial clones already subject to intensive clonal selection for target traits, our collection encompasses a more diverse representation of the clonal landscape. By providing robust heritability estimates based on 14 years of data, this study not only confirms substantial clonal genetic influence on key complex traits but also offers reliable parameters for predicting selection response in breeding.

The study also highlighted the contribution of other key variance components across the multi-year analysis, and most notably the impact of year and the year-by-field interaction for all traits. This result is not unsurprising, with several prior studies in grapevine reporting the significant impact of year-to-year variation on agronomic, phenology and quality-related traits (Costantini et al., 2008; Laidig et al., 2009; Suter et al., 2021). The high year-to-year variance observed for malic acid (ranging from 22.5–82.9% of total phenotypic variance; Fig. 3) is likely due to its metabolic instability and sensitivity to environmental conditions, with warmer seasons accelerating degradation and cooler temperatures slowing the process, resulting in substantial annual variation (Rienth et al., 2016). Gonçalves et al. (2016) demonstrated that genotype-by-environment interactions are significant in clonal populations across traits such as yield, probable alcohol, and acidity content, emphasizing its critical role in clonal selection pipelines. Notably, Gonçalves et al. (2016) also found the year effect to be substantial, with genotype performance across different years at the same site being no more correlated than across different sites, highlighting the importance of accounting for temporal variation. Consistent with this emphasis on temporal influence, our results similarly demonstrated that the combined variance components for year and year-by-location interaction substantially exceeded that of the field effect alone across the studied traits and populations.

Our analysis of genetic correlations, while acknowledging the inherent estimation challenges associated with complex, unbalanced clonal datasets (discussed below), reveals both biologically meaningful patterns and points of divergence from previous grapevine studies. Consistent negative genetic correlations were observed between yield and brix, across most populations (e.g. Riesling – 0.73; Pinot – 0.72), supporting previously reported tendencies toward a trade-off between productivity and sugar accumulation (Damiano et al., 2022). Recent polyclonal selection work by Surgy et al. (2025) similarly reported strong negative phenotypic correlations between sugar concentration and total acidity, a relationship that is also evident at the genetic level in our study, where brix was negatively correlated with both malic and tartaric acid content across multiple populations. This convergence of phenotypic and genetic signals across different populations underscores the biological significance of the sugar–acid balance in grapevine berry development and ripening.

Interestingly, we observed a strong negative genetic correlation between tartaric and malic acid content in Riesling (–0.66) and Müller-Thurgau (–0.48), which contrasts with expectations given the acid's largely independent biosynthetic pathways. As malic acid degradation is highly sensitive to temperature and other environmental factors (Sweetman et al., 2014), this unexpected relationship may reflect population-specific co-selection or environmental co-variation rather than true metabolic antagonism. Additional targeted studies under controlled conditions would be necessary to disentangle these effects. Also noteworthy is the consistently strong positive genetic correlation between tartaric acid and total acidity, suggesting tartaric acid is the dominant driver of total acidity under the sampling conditions used here. In contrast, the contribution of malic acid to total acidity appears more variable across populations. Finally, the botrytis trait showed a strong negative genetic correlation with brix in several populations (e.g., Savagnin – 0.89; Riesling – 0.95), which may reflect both physiological and sampling-based artefacts. As botrytis scoring may have varied across years and was likely confounded by harvest timing (discussed below), caution is warranted in interpreting these relationships as causal.

Multi-year BLUPs revealed that all clonal populations generally exhibited higher levels of tartaric acid relative to malic acid, except for some Pinot clones, which showed a near 1:3:1 ratio, indicating naturally higher malic acid retention in this variety. This intra-population variation suggests that certain Pinot clones possess greater malic acid stability. These findings point to opportunities for selecting clones with more favourable acid balance, a key trait for climate-adaptive breeding. Furthermore, improving our understanding of the genetic relationships between malic and tartaric acid content will enhance selection precision, enabling breeders to better predict acid profiles and optimize clonal performance across both cool and warm growing regions.

Breeding implications and selection strategies

The substantial phenotypic variance directly attributable to the genetic clonal effect across the five studied populations confirms the feasibility of achieving genetic gains for complex traits via clonal selection. This finding holds particular significance for the wine industry, which often exhibits conservatism towards adopting entirely new cultivars yet faces pressing demands to adapt established, high-value varieties to evolving climate challenges. Utilising the inherent diversity within existing clonal populations, as demonstrated here, offers an important pathway for enhancing adaption and performance while maintaining variety integrity. Furthermore, identifying traits with low clonal variance within these populations is equally valuable, informing targeted breeding strategies, whether exploiting natural mutations or employing advanced breeding techniques, to introduce necessary genetic diversity where it is currently lacking for future improvement goals.

Effectively harnessing the identified clonal variation for tangible genetic improvement, particularly for complex goals like climate adaptation, necessitates selecting across multiple traits simultaneously. Targeted selection for acid and sugar balance for future climate adaptation is a key example of the multi-trait selection opportunities that are apparent within the diversity present in these populations. Using a basic example of three traits with simplistic selection criteria we have demonstrated the ability to select high performing clones in all five clonal populations, while the number of selections was somewhat proportional to the population size, reinforcing the importance of large populations. While the exact quality trait configurations for adaptation of major varieties like Pinot and Riesling to future environments requires further exploration, it is apparent that it will be highly complex and multifaceted, and thus

may necessitate the use of selection indices, similar to the multi-trait genotype-ideotype distance index approached applied by Brault et al. (2024) in Rosé wine and Cognac production breeding programs.

Although the aim of selection indices is to identify the best clones based on multiple traits, a key limitation lies in the risk of improving certain traits at the expense of others. This risk is particularly pronounced, and can increase substantially, when negative genetic correlations exist among traits, a reality that is explicitly evident in the current study. As an alternative, Surgey et al. (2025) propose the use of optimization frameworks that use polyclonal selections rather than single clone selections, specifically using integer programming, to enable effective multi-trait selection in breeding scenarios. By allowing the definition of realistic minimum gains, this approach facilitates the selection of groups of clones that achieve improvements in desirable traits while avoiding losses in others. Although the authors did not explicitly address the applicability of this method to pure clonal selection, the extension of such optimization-based approaches to multi-trait clonal selection offers considerable potential for genetic improvement.

Limitations and future directions

A key limitation of this study is the lack of a replicated experimental design in the historical dataset, particularly the absence of within-field replication and the minimal within-year replication, that is further compounded by inconsistent sampling across years. While this limitation is somewhat mitigated by the extensive dataset, with most clonal populations having up to 14 years of trait data, a more structured experimental design would improve the precision of variance estimates. Implementing even a partially replicated (p-rep) design, as demonstrated by Gonçalves et al. (2022), or a multi-environment augmented p-rep design (Moehring et al., 2014), could reduce prediction error variance and enhance the efficiency of clonal selection, particularly in early-generation evaluations.

Furthermore, the sparse and spatially dispersed arrangement of plots limited our ability to effectively model local environmental heterogeneity. Consequently, uncaptured spatial effects likely inflated residual variances and uncertainty in the genetic estimates. This data structure not only increases uncertainty and shrinkage in predictions but can also compromise genetic correlation estimates derived from bivariate models. Specifically, unmodeled environmental factors co-varying between traits within plots may generate spurious covariance potentially misattributed by the model to the genetic covariance component. Such misattribution can lead to inflated estimates of genetic correlation, an effect likely exacerbated in the populations with smaller sample sizes where reliable correlation estimates were particularly challenging and not reported when reaching the boundary. Additionally, interpretation of the Botrytis bunch rot trait should be made with caution, as variation in scorer training across years and early harvest practices (especially in Auxerrois, Müller-Thurgau, and Savagnin) may have limited the expression of post-veraison infection symptoms. While this necessitates caution regarding the precise magnitude of some correlations, the general significance and directional trends observed likely remain valid indicators of underlying genetic relationships.

A further analytical limitation involves the assumption of an independent diagonal variance structure for the year-by-plot residual term, which fails to explicitly model the inherent temporal dependencies expected in perennial crops like grapevines, consequently neglecting potential auto-correlation within plots across years (Gonçalves et al., 2020). Although the simplified structure adopted in our study facilitated robust model convergence across all datasets, particularly where data sparsity precluded the stable estimation of more complex covariance structures, it likely leads to an underestimation of true year-to-year correlations within plots. Such an assumption can inflate the residual variance estimate by absorbing persistent plot-level effects into the error term, potentially obscuring systematic genotype-by-year interactions and resulting in an underestimation of the temporal stability of clonal performance. Future work incorporating appropriate temporal covariance structures, where data permit, would provide more accurate insights into clonal stability over time.

Notwithstanding the discussed limitations, this study fundamentally underscores the considerable potential for achieving genetic improvement via clonal selection within established grapevine varieties, a process potentially accelerated by integrating new breeding technologies. Beyond direct selection efforts, these clonal populations represent invaluable resources for dissecting the genetic architecture of complex traits and potentially mapping causal variants with high precision. Functionally akin to near-isogenic lines, the subtle genetic and epigenetic distinctions between clones provide a powerful framework for association analyses designed to disentangle the phenotypic contributions of specific sequence or methylation changes. Insights gained from such analyses could subsequently guide the application of highly targeted precise trait improvement using genome editing tools, such as CRISPR/Cas9 that has already successfully optimized for targeted trait modification in grapevine (Ren et al., 2016; Villette et al., 2024). Ultimately, these clonal varietal populations constitute uniquely valuable genetic material, offering pathways for advancing fundamental knowledge, however efficiently selecting improved clones remains a significant practical hurdle.

The primary constraint in traditional clonal selection is the prolonged evaluation period required to accurately calculate a clone's genetic merit for key traits, a process that typically spans 15 or more years. The application of predictive models, such as genomic selection (GS), can substantially reduce this timeframe by using genome-wide molecular markers and trained statistical models to estimate an individual's genetic merit at early selection stages, prior to physical trait measurement. Similarly, phenomic selection (PS) uses high-throughput phenotypic data, such as spectral or imaging-based traits, and trained statistical models to predict genetic merit. While PS has been studied in diverse grapevine varieties and half-diallel breeding populations (Brault et al., 2022), it has yet to be applied in the prediction of genetic merit within clonal populations of commercial cultivars. Given the high heterozygosity of grapevine, incorporating non-additive genetic effects into predictive models is likely essential for improving selection accuracy. Supporting this, Werner et al. (2023) emphasized the importance of including non-additive effects, such as dominance, in GS models for clonal breeding programs, particularly for optimizing parental selection. Their findings indicate that when dominance effects are significant, genomic predicted cross-performance (GPCP) surpasses genomic estimated breeding values (GEBVs) in maximizing genetic gain. Similar conclusions were drawn by Yadav et al. (2021) in sugarcane, and insights from both studies can be applied to clonal selection programs without sexual recombination. Since clones retain their genetic makeup, leveraging both additive and dominance effects can likely improve the accuracy of estimating clonal genetic merit, particularly at early-stages. Nevertheless, a general limitation of such population-level prediction models is their inherent difficulty in capturing the influence of extremely rare or novel mutations due to insufficient statistical power.

Larger training populations help mitigate this limitation regarding rare variants by increasing the chance they are sufficiently represented for effect estimation; simultaneously, substantial population size is a fundamental requirement for achieving high overall prediction accuracy, as GS accuracy is well-known to increase with training set size (Daetwyler et al., 2012). Aforementioned, small population sizes are a persistent challenge in grapevine, and the limitations of such are apparent in the current study. A promising solution is to connect and synchronize cross-institutional efforts by sharing phenotypic, and genotypic datasets from varietal clonal populations. By pooling data across institutes and nations, larger and more diverse training populations can be developed, improving the accuracy of predictive models for selecting superior clones, particularly for traits of moderate genetic complexity such as quality attributes and disease resistance.

Significant opportunities exist to leverage predictive breeding approaches for enhanced accuracy and efficiency of clonal selection, but their impact depends on effective integration into clonal selection programs. Yet, resource limitations make it impractical for grapevine breeders to empirically test and optimise every conceivable scenario, potentially limiting their practical benefits. Simulation-based studies provide a powerful alternative, allowing breeders to assess the effects of prediction models, like GS and PS, within selection program scenarios. Tools such as genomicSimulation (Villers et al., 2023), AlphaSim-R (Gaynor et al., 2021), and MoPS (Pook et al., 2020) enable simulations to be parameterized with real clonal selection features, using empirical estimates of variance and heritability to extend predictions beyond what is experimentally feasible.

In conclusion, this study reveals substantial intra-varietal diversity within the examined grapevine populations, with a meaningful proportion attributable to genetic differences among clones. This genetic variation represents a valuable and largely untapped resource for accelerating genetic improvement, particularly in the context of climate adaptation. Harnessing this diversity through predictive breeding and advanced multi-trait selection strategies holds considerable promise for enhancing the resilience and performance while maintaining the varietal integrity of traditional grapevine cultivars.

Declarations

Author Contribution Statement

HR conducted the primary data analysis and took the lead in drafting and editing the manuscript. The study was conceived by KPVF, who also supervised all aspects of the research. MN was involved in preliminary data exploration. HPP provided expert input on data analysis and interpretation. JS and ER coordinated the establishment and management of plant material and field trials. TS, MS, PC, JS, ER, HPP and KPVF contributed to the interpretation of results. All authors reviewed and approved the final manuscript.

Competing Interests

The authors declare no competing financial or non-financial interests.

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Data Availability

The datasets generated and/or analysed during the present study are available from the corresponding author upon reasonable request.

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Figures

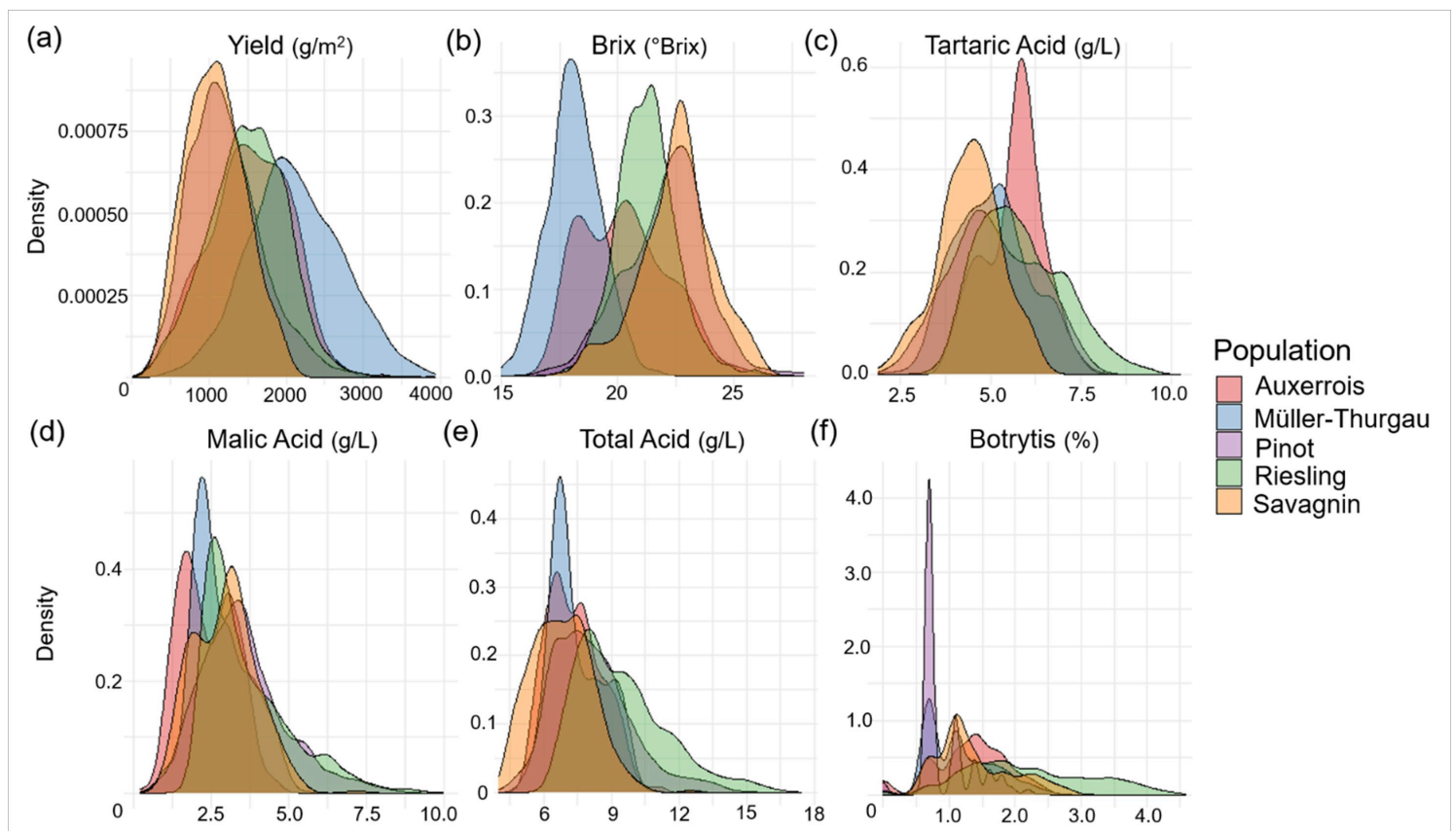


Figure 1

Frequency distribution of raw trait observations for **(a)** yield (g/m^2), **(b)** brix ($^{\circ}\text{Brix}$), **(c)** tartaric acid (g/L), **(d)** malic acid (g/L), **(e)** total acid (g/L) and **(f)** scaled botrytis infection (%) measured in the clonal populations: Auxerrois (red), Müller-Thurgau (blue), Pinot (purple), Riesling (green) and Savagnin (orange). Botrytis infection was log transformed (scaled) to analysis

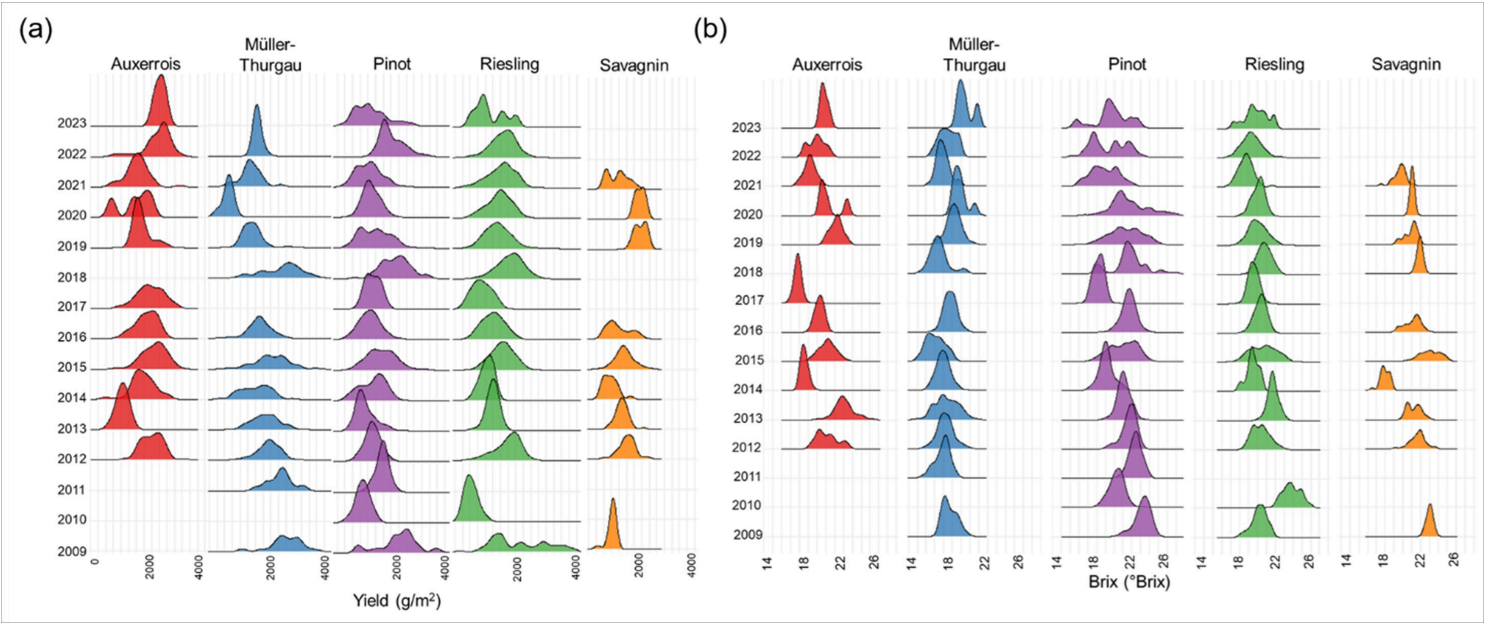


Figure 2

Frequency distribution of raw observations split by year (y-axis) for **(a)** yield (g/m^2), and **(b)** brix ($^{\circ}\text{Brix}$) across clonal populations: Auxerrois (red), Müller-Thurgau (blue), Pinot (purple), Riesling (green) and Savagnin (orange).

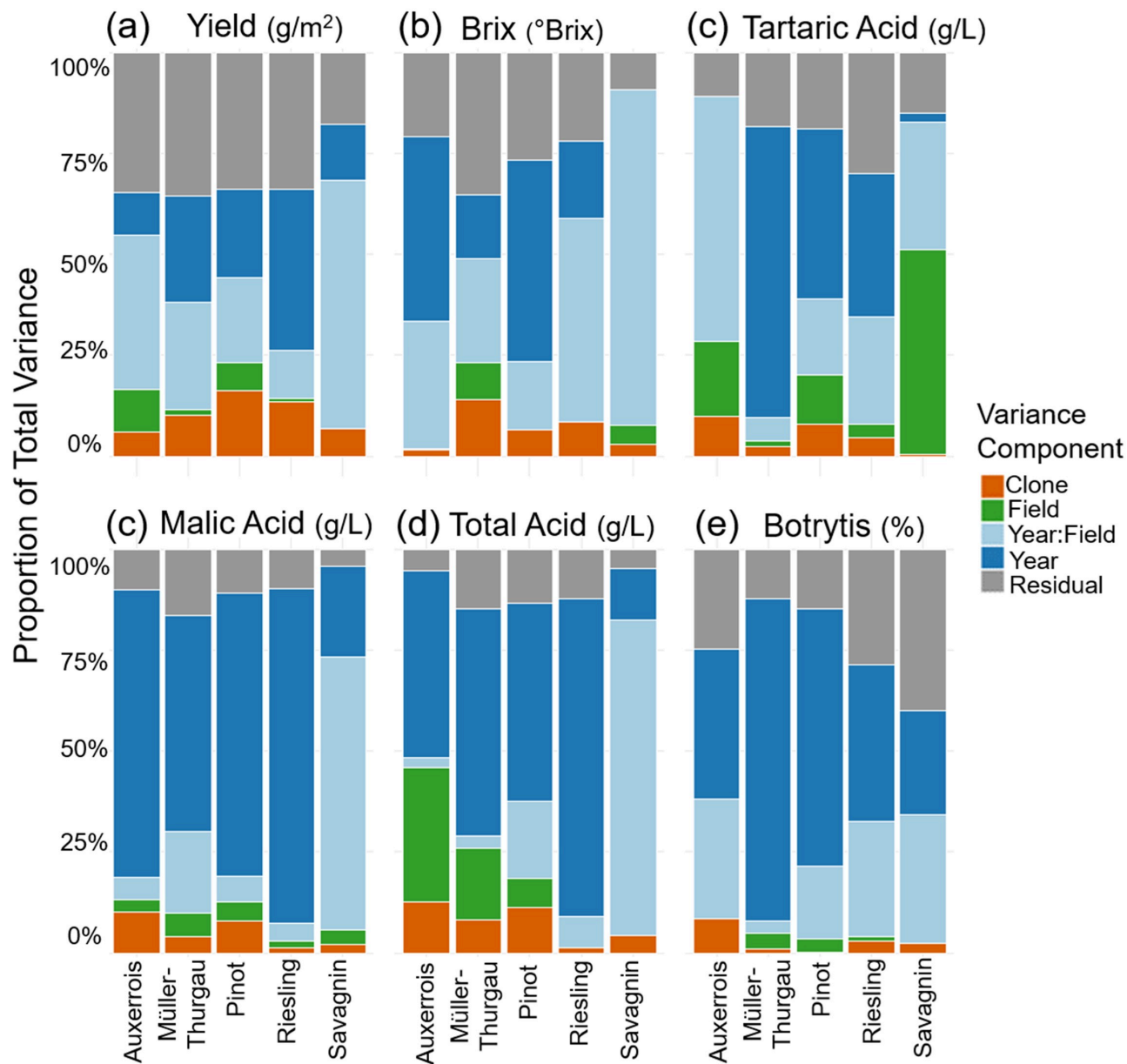


Figure 3

Proportional contribution of variance components to total phenotypic variance for six traits across all clonal populations. Each bar represents a varietal clonal population, Auxerrois, Müller-Thurgau, Pinot, Riesling, and Savagnin, and variance components are shown as percentage contributions to total variance (full bar) and include clonal (orange), field (green), year-by-field interaction (light blue), year (dark blue) and residual (grey) effects. Each full bar represents 100% of the total variance for the random model effects for (a) yield, (b) brix, (c) tartaric acid, (d) malic acid, (e) total acid and (f) botrytis bunch rot.

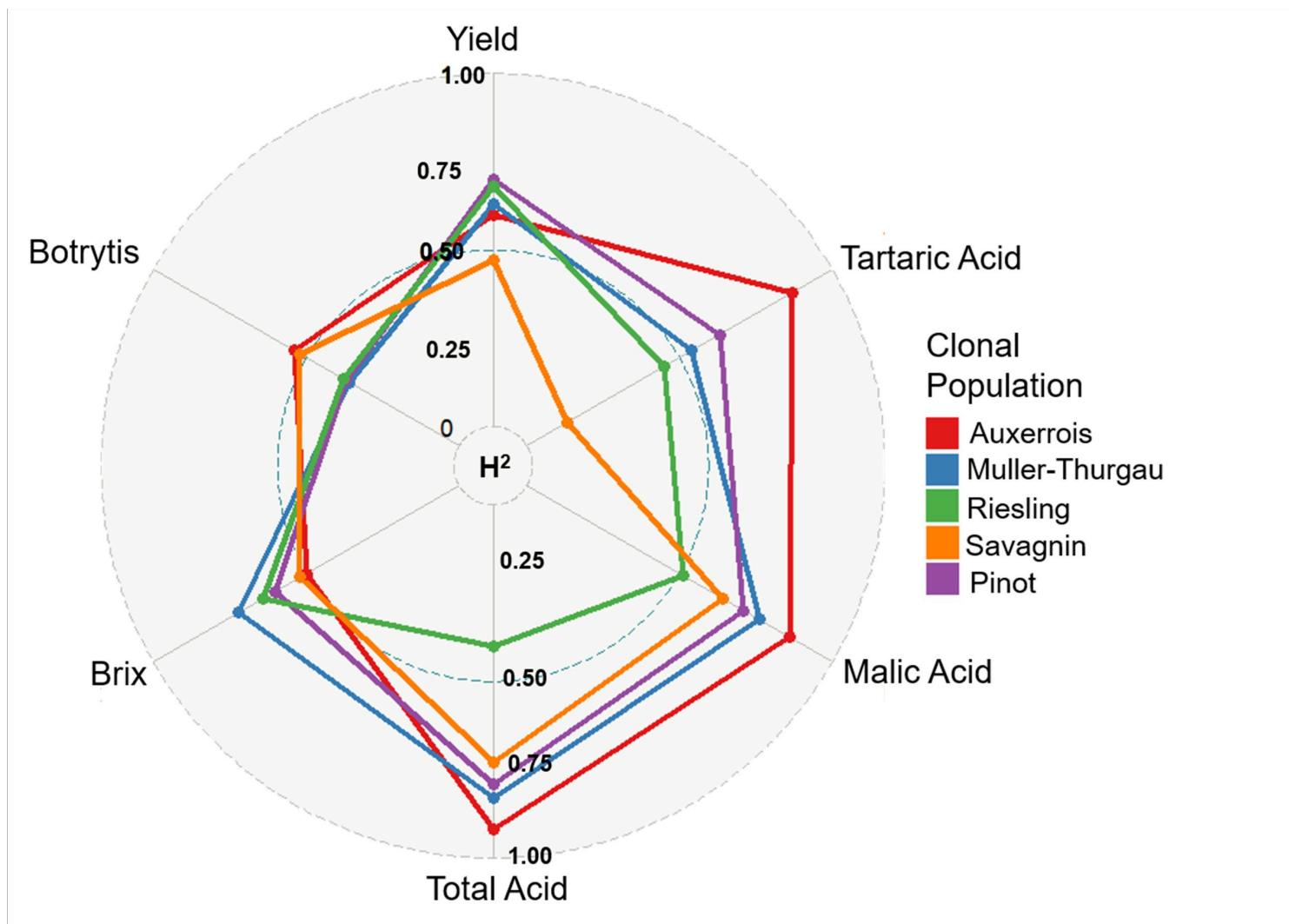


Figure 4

Radar graph of broad-sense heritability (H^2) for six traits across all clonal populations, where each trait H^2 of a population is connected with a coloured line: red, blue, green, orange and purple for the Auxerrois, Müller-Thurgau, Riesling, Savagnin and Pinot populations, respectively. The heritability value increase from the inner to outer circle, from 0.00 to 1.00, with 0.50 represented by a thin blue dotted line.

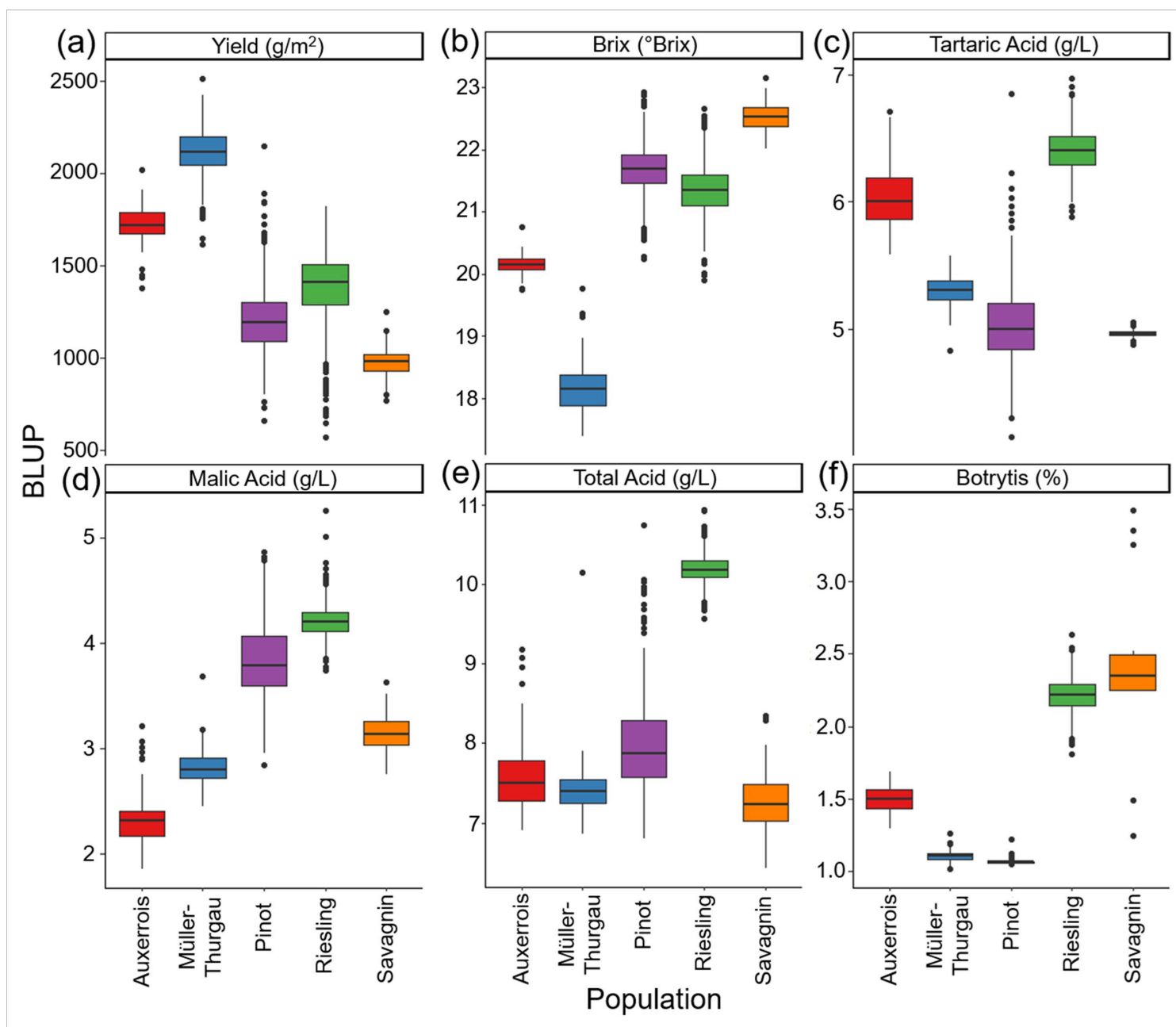


Figure 5

Boxplots of best linear unbiased predictors (BLUPs), estimated from the multi-year linear mixed model within each clonal population for six traits: **(a)** yield (g/m²), **(b)** brix (°Brix), **(c)** tartaric acid (g/L), **(d)** malic acid (g/L), **(e)** total acid (g/L) and **(f)** scaled botrytis bunch rot (%). As BLUPs were predicted separately per population, formal statistical comparisons between populations are not appropriate. Populations are colour-coded as follows: Auxerrois (red), Müller-Thurgau (blue), Pinot (purple), Riesling (green), and Savagnin (orange).

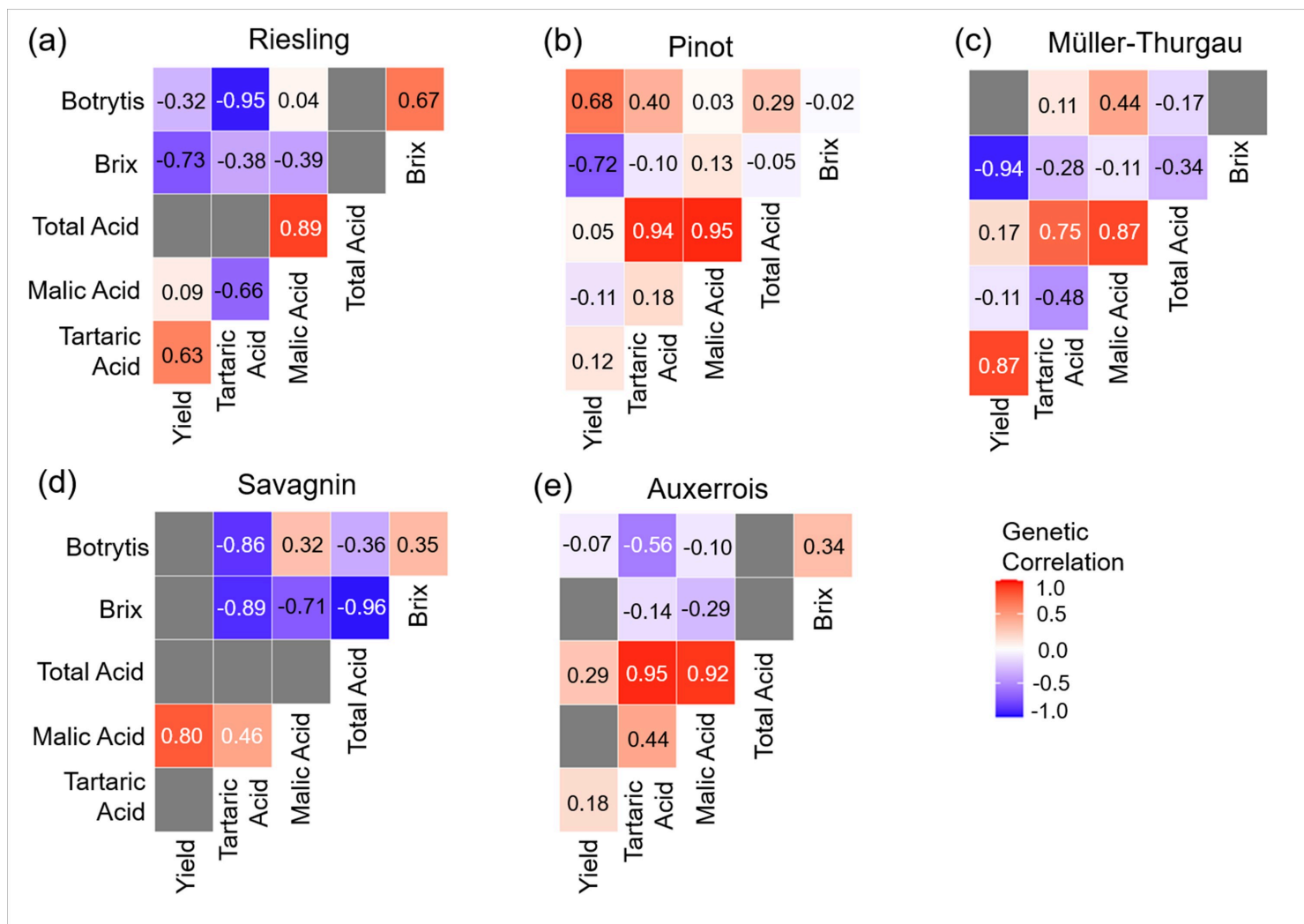


Figure 6

Heatmaps of pairwise genetic correlations between traits within each varietal clonal population: **(a)** Riesling, **(b)** Pinot, **(c)** Müller-Thurgau, **(d)** Savagnin, **(e)** Auxerrois. Correlation strength and direction are colour-scaled from strong negative (−1; blue) to strong positive (1; red), with zero correlation represented in white. Grey tiles indicate trait combinations where genetic correlations could not be reliably estimated.

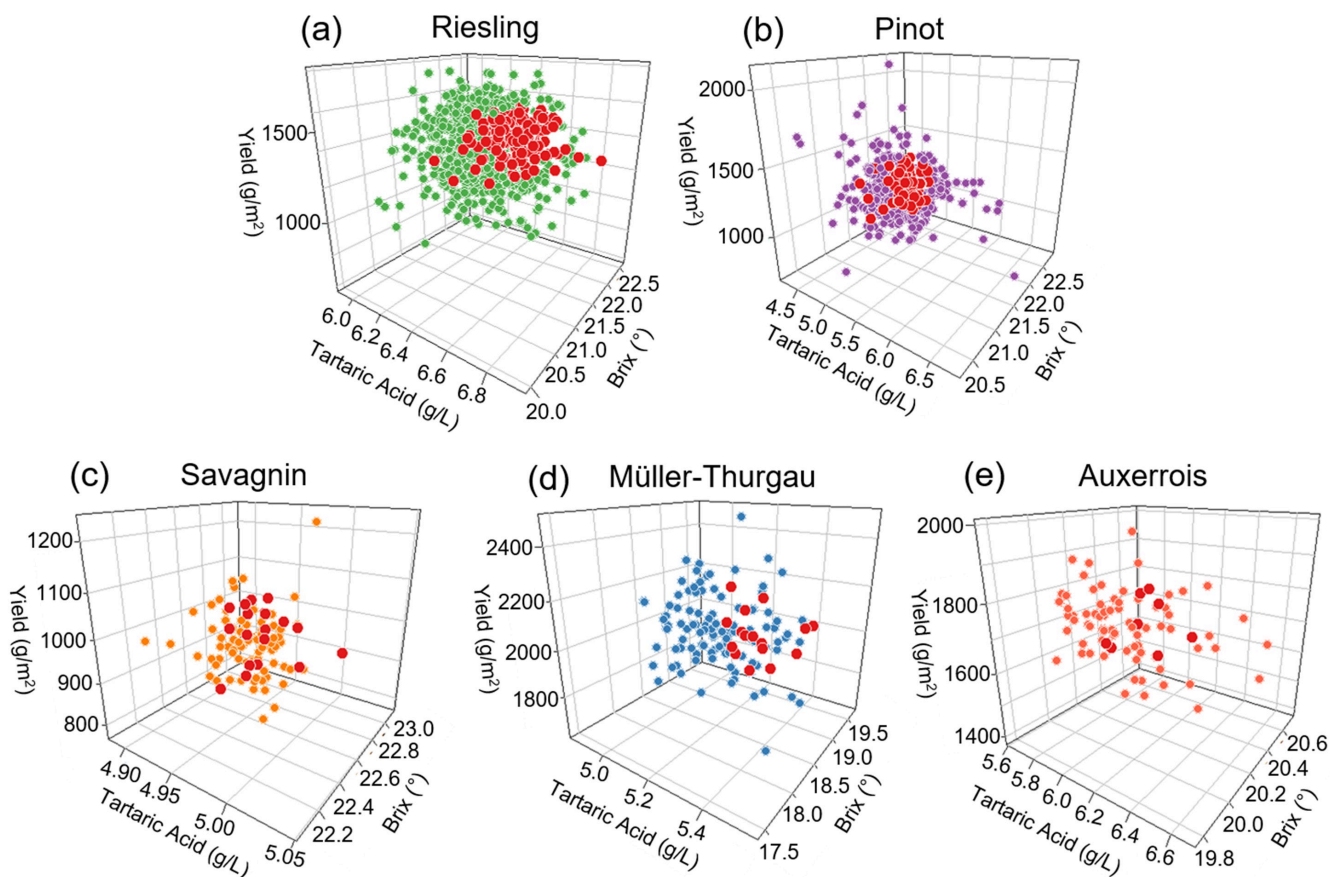


Figure 7

Three-dimensional bi-plots displaying multi-year BLUPs for yield (z-axis), tartaric acid (x-axis), and brix (y-axis) across five varietal clonal populations: Riesling (a; green), Pinot (b; purple), Savagnin (c; orange), Mümuller-Thurgau (d; blue), and Auxerrois (e; coral red). Clones highlighted in red meet the theoretical selection criteria: top 30th percentile for tartaric acid, bottom 60th percentile for brix, and within the 30th to 95th percentile for yield. A total of 168 Riesling, 69 Pinot, 19 Savagnin, 16 Müller-Thurgau, and 8 Auxerrois clones met these selection thresholds.

Supplementary Files

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- [RobinsonIntravarietalvariationSuppMaterials.docx](#)
- [RobinsonIntravarietalvariationSuppTables.xlsx](#)