BIO Web of Conferences **235**, 01002 (2026) https://doi.org/10.1051/bioconf/202623501002
CONAVI 2024

Assessment of Intra-Varietal Diversity among Biotypes of the cv. Cannonau (*Vitis vinifera* L.)

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Abstract. Grapevine cultivars may exhibit significant intra-varietal variability resulting from somatic mutations accumulated during long-term vegetative propagation and from historical massal selection practices. Cannonau (syn. Garnacha) is one of the most important grapevine cultivars cultivated in Sardinia, where it has been grown for centuries under heterogeneous Mediterranean environments. This study aimed to assess the phenotypic variability among Cannonau biotypes under uniform environmental conditions. Eighty-three biotypes identified through a massal selection program were evaluated in a single experimental vineyard during the 2022 and 2023 growing seasons. Agronomic traits, vegetative vigor and berry composition were recorded, and multivariate statistical analyses (MFA and HCPC) were applied to explore variability patterns. The results revealed a wide range of variation in yield, vegetative vigor, berry sugar concentration and total anthocyanins. Multivariate analysis identified three groups of biotypes characterized by distinct agronomic and compositional profiles. These findings confirm the presence of significant intra-varietal diversity within Cannonau germplasm and highlight its potential for the selection of plant material suited to different viticultural and oenological objectives.

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1 Introduction

Crop domestication and subsequent improvement have historically relied on the exploitation of natural genetic diversity, which provides the basis for crop adaptation and agronomic performance under changing environmental conditions [1]. In grapevine (Vitis vinifera L.), cultivars are traditionally defined by a relatively stable set of morphological and physiological traits. Nevertheless, considerable intra-varietal variability can occur within the same cultivar. This variability mainly originates from spontaneous somatic mutations that accumulate over centuries and are preserved through vegetative propagation [2]. In addition, clonal selection and the long-term dissemination of plant material by growers have contributed to maintaining and spreading this diversity within cultivars.

In traditional viticultural regions, intra-varietal diversity represents an important component of the adaptation of grapevine cultivars to local pedoclimatic conditions and management practices. Over long periods of cultivation, the accumulation of somatic mutations together with the informal selection of superior vines has favoured the emergence of locally adapted biotypes that may differ in productivity, phenology and fruit composition.

Intra-varietal variability may have relevant agronomic and oenological implications. Previous studies have shown that clonal differences can affect ampelographic traits, phenological development, disease susceptibility [3], yield components and berry composition [4], as well as the accumulation of secondary metabolites [5]. However, the extent of intra-varietal variability is strongly cultivar-dependent. Highly mutable cultivars such as Pinot noir exhibit pronounced clonal diversification and phenotypic plasticity, whereas other cultivars, for example Cabernet Sauvignon, tend to display greater genetic and phenotypic stability [6,7]. Molecular marker studies have nevertheless demonstrated that geographically distinct accessions of the same cultivar may show measurable genetic divergence [8], suggesting that local adaptation processes can contribute to intra-varietal differentiation.

Garnacha (syn. Grenache), one of the most widely cultivated wine grape varieties worldwide and ranking among the top ten varieties in terms of global vineyard area [9], provides a particularly interesting case study of intra-varietal diversity. In Sardinia the cultivar is known as Cannonau and represents one of the most important grape varieties for regional wine production. Cannonau wines are characterized by distinctive compositional traits, including relatively slow anthocyanin accumulation in berries [10]. Considering the long history of cultivation of this cultivar across heterogeneous Mediterranean environments, a substantial level of intra-varietal variability among locally selected biotypes can be expected.

In many traditional vineyards, the historical practice of massal selection has contributed to preserving a broad spectrum of intra-varietal diversity, often wider than that maintained through modern clonal selection programs.

The present study was based on the hypothesis that Cannonau biotypes originating from different areas of Sardinia express measurable phenotypic variability in agronomic and compositional traits even when grown under uniform environmental and management conditions. To test this hypothesis, 83 Cannonau biotypes previously identified through a massal selection program were established in a single experimental vineyard and cultivated under identical agronomic practices. This common-garden experimental approach allowed the assessment of intra-varietal variability while minimizing environmental effects. Multivariate statistical analyses were applied to identify patterns of phenotypic variability among biotypes and to determine the variables contributing most strongly to their differentiation.

2 Materials and methods

2.1 Plant material and experimental setup

The trial was conducted in an experimental vineyard established in 2009 and located in the Parteolla Valley, in the municipality of Serdiana (south Sardinia, Italy). The soil, of alluvial origin and homogeneous, has an average depth ranging from 80 to 100 cm and is characterized by the following texture: 51% sand, 24.9% clay, and 24.1% silt. Soil pH was 7.44 and organic matter content was 16 g/kg.

The vineyard covers an area of 2,500 m² and includes 103 biotypes of cv. Cannonau (syn. Grenache), each consisting of 11 contiguous vines. Vines were grafted onto 1103 P rootstock and spaced at 2.1 × 1.0 m.

Vines were trained using a vertical shoot positioning system and pruned according to a traditional bush vine system, leaving a maximum of six buds per vine.

For the purposes of this study, 83 biotypes were evaluated. No cluster thinning was performed in order to assess the actual yield potential of the evaluated biotypes.Weed control along the rows was carried out using mechanical cultivation. The trial was conducted over the 2022 and 2023 growing seasons; grape harvest and yield assessments were performed at commercial harvest, which took place on September 12 and September 18, respectively. For each biotype, nine out of eleven vines were harvested, and yield per vine was recorded. To determine yield components, 20 clusters were randomly selected per plot and weighed; from each cluster, 30 berries were randomly sampled to determine average berry weight. Agronomic data for each biotype were expressed as the mean of 20 biological replicates.

2.2 Fruit composition and vegetative vigour

For berry composition analysis, the nine vines were divided into three groups of three contiguous vines. From each group, 0.5 kg of berries with pedicels were randomly collected. Berries were weighed, crushed, and the resulting must was analysed for sugar content, expressed as total soluble solids (°Brix), pH, and malic and tartaric acidity according to the methods of the International Organisation of Vine and Wine[11].

Total anthocyanins were determined spectrophotometrically according to the method described by Di Stefano and Cravero[12] and expressed as mg L^{-1} of must, with absorbance measured at 700 nm and 520 nm, respectively.

During the dormant season, pruning wood weight and the number of shoots per vine were recorded in order to estimate vegetative vigour. Pruning wood weight was also used to calculate the Ravaz Index.

2.3 Statistical analysis

Descriptive statistics, including mean, standard deviation, and range, were computed for all agronomic, chemical, and morphological variables. AMulti Factorial Analysis (MFA) was performed to explore the multivariate structure of the dataset. Considering the presence of predefined groups of variables in the dataset (fruit composition and plant vigour) MFA was considered the most appropriate exploratory approach. Data were standardized prior to analysis. Then, in order to identify homogeneous groups of biotypes,a Hierarchical Clustering on Principal Components (HCPC) was applied to the individual factor scores obtained from the MFA. Clustering procedure followed the Ward's criterion. Variables significantly contributing to cluster characterization were identifiedaccording to the v-test, which measures the deviation of the cluster mean from the overall mean relative to the pooled standard deviation.The magnitude and sign of the v-test were used to determine the strength and direction of association. Statistical analyses were performed using R (version 4.5.2) in RStudio with the FactoMineR package.

3 Results

The present study characterised 83 Cannonau biotypes according to their agronomical and technological traits. The different biotypes were selected from various sites across Sardinia and then grafted and cultivated in the same vineyard in south-west Sardinia.

Table 1 reports the mean values, standard deviation, and coefficient of variation of the measured parameters during the two years of observation. Regarding yield per vine, in 2022 the lowest yield (0.32 kg vine^{-1}) was recorded in selection 2/24, whereas the highest value (3.21 kg vine^{-1}) was observed in selection 16/47 (Fig. 1). In the second year, yield ranged from 0.5 to 5.3 kg vine^{-1}, corresponding to selections 2/26 and 18/15, respectively.

Table 1. Must composition and agronomic parameters of 83 Cannonau biotypes collected in the 2022 and 2023 growing seasons. Data are expressed as mean ± standard deviation (SD); the coefficient of variation (CV, %) is also reported for each trait.

Stage	2022	2023
Total Soluble Solids (°Brix)	24,7 ±2,1 CV = 8,4	25,3 ± 3,2 CV = 12,5
pH	3,6 ± 0,1 CV = 2,9	3,8± 0,2 CV = 5,5
Malic acid (g/L)	0,6± 0,2 CV = 29,7	0.5±0,3 CV = 61,1
Tartaric acid (g/L)	5,5± 0,9 CV = 15,6	6,4± 0,7 CV = 11,2
Total anthocyanins (mg/L)	437,1± 78,4 CV = 17,9	507,9±102 CV = 20,1
Yield (kg Vine^{-1})	1,8±0,5 CV = 27,5	1,6±0,7 CV = 43,9
Cluster numbers (n°/Vine)	8,3± 1,5 CV = 17,9	7,9±2,2 CV = 27,4
Cluster weight (g)	220,9± 59,9 CV = 27,1	205,8± 50,6 CV = 24,6
Berry weight (g)	1,75 ± 0,33 CV = 18,8	1,87 ± 0,25 CV = 13,4
Pruning weight (kg)	0,51± 0,13 CV = 25,6	0,42± 0,18 CV = 36

Concerning yield components, the number of clusters per vine showed a narrower range of variability range in 2022 compared with the following season. Cluster number varied from 5.5 to 13 clusters vine^{-1} in the first year and from 3 to 14.3 clusters vine^{-1} in 2023. Although the population mean remained similar between years (approximately 8 clusters vine^{-1}), the coefficient of variation (CV) clearly highlighted the different level of intra-varietal variability expressed by the 83 biotypes across the two seasons. Conversely, mean cluster weight showed comparable values for both the population mean and the coefficient of variation during the two-year observation period.

Although the population mean showed a higher berry sugar concentration at harvest in 2023 compared with the previous season, the variability exhibited by the biotypes under comparison was greater in the second year, with a range of variability between 14.62 and 32.78 °Brix recorded in selections 18/17 and 9/2, respectively.

Similar patterns were observed for pH, malic acidity and total anthocyanins, with coefficients of variation confirming the higher level of intravarietal variability within the Cannonau population in 2023 compared with 2022 (Table 1). With regard to total anthocyanins—which are particularly relevant for this cultivar given the genetically low color intensity of its berries—the variability range, calculated as the mean across the two seasons (Fig. 1), showed minimum and maximum population values of 322.5 and 659.5 mg L^{-1}, respectively, measured in the musts obtained from selections 11/23 and 9/2.

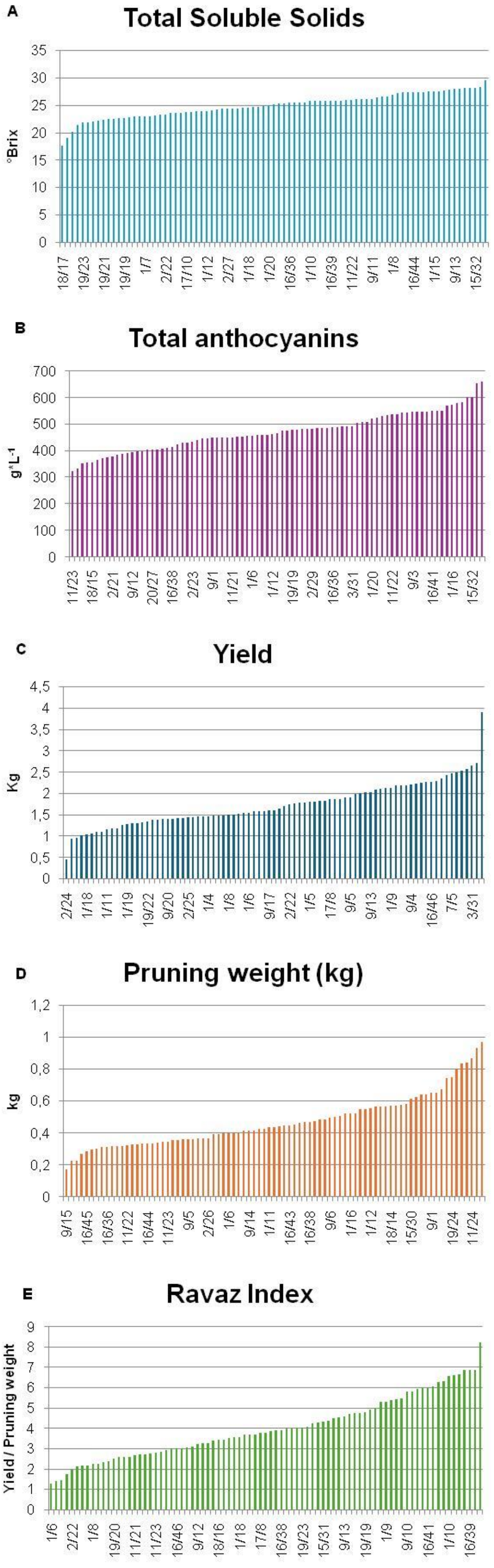

Fig. 1 Distribution of Total soluble solids (A), Total Anthocyanin (B), Yield (C), Pruning weight (D) and Ravaz index (E) in the Cannonau population. For each selection, the mean value of the trait across the 2022 and 2023 seasons is reported.

The mean pruning wood weight, calculated over the two-year period, showed a particularly wide range of variability among the evaluated selections. Average values ranged from less than 20 g per vine to nearly 1 kg per vine, highlighting substantial differences in vegetative vigor among the biotypes. The Ravaz index also provided useful insights, allowing a clearer interpretation of the balance between vegetative growth and crop load across the studied selections.

The two-year averages also identified a group of clones with Ravaz index values ranging from 6 to 8, suggesting a well-balanced relationship between vegetative growth and crop load.

The results of MFA analysis highlighted thatthe 10 variables considered to characterize the 83 biotypes followed a clear pattern along the two first components, which explained the 51.1% of the dataset variability (Fig. 2). Along the first component the variables describing plant productivityclearly separated from those regarding the fruit composition, indicating that where plant productivity was higher, berries had lower values of pH, organic acids, anthocyanins, and TSS.

According to the Hierarchical Clustering on Principal Components (HCPC) analysis, performed on the basis of MFA results, the 83 Cannonau biotypes could be separated into three groups (Fig.3). Yield, Total Anthocyanins, and TSS were those variables mostly contributing to clusters determination. As shown in Fig.4, the first group (23 biotypes) was characterized by high vigour selections generally producing musts with lower pH, anthocyanin concentration and tartaric acid content. Group 2, was the largest group, comprising 39 biotypes characterized by low productivityand a reduced number of clusters and berries. Similarly to Group 1, musts had low levels of organic acids, anthocyanins and pH. On the other hand, Group 3 (21 biotypes) showed opposite must composition, yielding notably higher TSS and total anthocyanins.

4. Discussion

The experimental vineyard investigated in the present study had previously been characterized with the aim of developing innovative non-destructive approaches for monitoring berry quality [13]. In contrast to that work, which primarily focused on methodological aspects, the present study provides a quantitative assessment of the intra-varietal variability expressed by Cannonau biotypes.

The results of the present study highlight the existence of substantial phenotypic variability among Cannonau biotypes when cultivated under uniform environmental and management conditions. Because all selections were grown in the same experimental vineyard, the differences observed in agronomic performance and berry composition can be largely attributed to genotypic variation among biotypes rather than to environmental effects. These findings confirm the importance of intra-varietal diversity within traditional grapevine cultivars and underline the role that historical massal selection practices may have played in preserving this diversity.

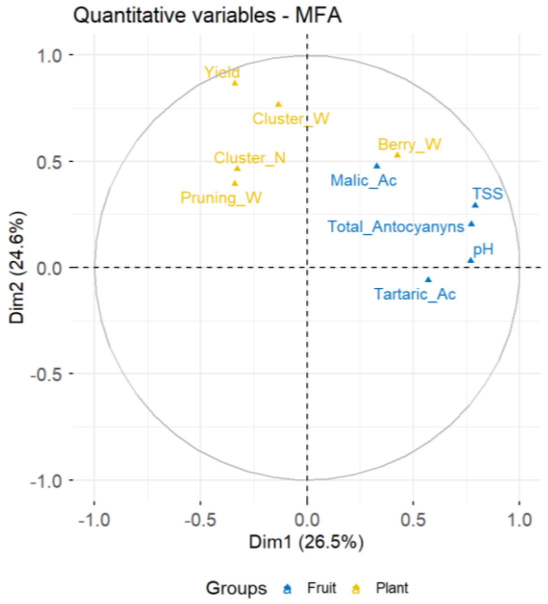

Fig. 2 Loading Plot of MFA describing the distribution along the two principal components of the two groups of Variables.

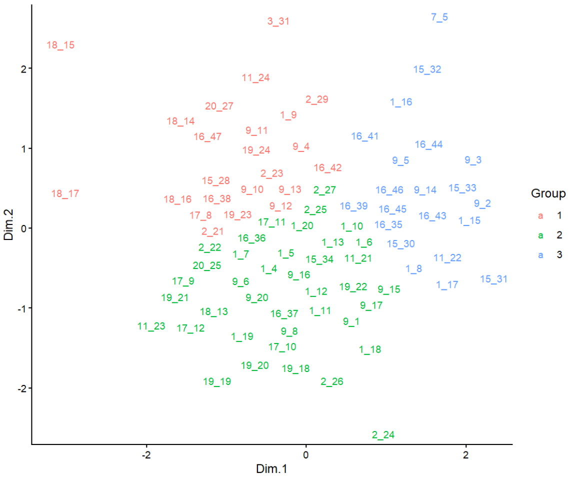

Fig.3 Scatter plot of individual samples projected onto the first two MFA dimensions. Colours highlight groups of vine selections identified through Hierarchical Clustering on Principal Components (HCPC).

The wide variability observed for yield per vine and vegetative vigor suggests that Cannonau biotypes differ markedly in their growth behaviour and crop load balance. Such differences are particularly relevant from a viticultural perspective, as the balance between vegetative growth and reproductive activity strongly influences grape composition and overall vineyard performance. In this context, the Ravaz index proved useful for identifying selections characterized by a more balanced relationship between yield and vegetative growth, which is generally considered a desirable condition for maintaining both productivity and fruit quality.

The multivariate analyses provided further insights into the structure of phenotypic variability within the studied population. The separation observed along the first MFA component between productivity-related variables and berry composition parameters suggests a trade-off between yield and fruit quality traits. Similar relationships have been reported in grapevine and are often associated with crop load effects, whereby higher yields may lead to a dilution of sugars, organic acids, and secondary metabolites in berries. This pattern may partially explain the negative association observed between productivity and compositional variables such as total soluble solids and anthocyanin concentration.

Cluster analysis allowed the identification of three distinct groups of biotypes characterized by different agronomic and compositional profiles. In particular, one group included biotypes with higher sugar concentration and anthocyanin levels, suggesting a potentially higher oenological value. Conversely, other groups were characterized by greater vegetative vigor or lower productivity. Such differentiation highlights the potential of intra-varietal selection as a strategy to identify plant material suited to specific production goals, such as maximizing yield, improving grape composition, or achieving a balanced vegetative–productive growth.

From a broader perspective, the variability observed among Cannonau biotypes may also have important implications for the adaptation of vineyards to changing climatic conditions. Mediterranean viticultural regions are increasingly exposed to higher temperatures and more frequent water deficit events, which can affect both vine physiology and berry composition. The availability of genetically diverse plant material within the same cultivar may therefore represent a valuable resource for selecting biotypes better adapted to these environmental constraints.

Overall, the results of this study confirm that traditional Cannonau germplasm preserves a considerable level of intra-varietal diversity. The characterization of this variability represents a fundamental step toward the identification of superior biotypes and the development of selection strategies aimed at improving both vineyard sustainability and wine quality.

5 Conclusion

Cannonau is one of the most important grapevine cultivars cultivated in Sardinia and represents the basis of several denomination wines. Its long history of cultivation across heterogeneous environments has likely contributed to the preservation of substantial intra-varietal diversity within traditional vineyards.

The results of this study confirmed the presence of a considerable level of variability among the evaluated biotypes for both agronomic and compositional traits. Such diversity represents a valuable genetic resource that can support the identification of plant material better suited to different viticultural and oenological objectives.

Further research will focus on the eco-physiological characterization of the most promising biotypes, particularly in relation to their responses to water deficit and high temperature conditions. This approach will contribute to identifying plant material better adapted to the challenges posed by climate change and to supporting the long-term sustainability of Cannonau viticulture in Mediterranean environments.

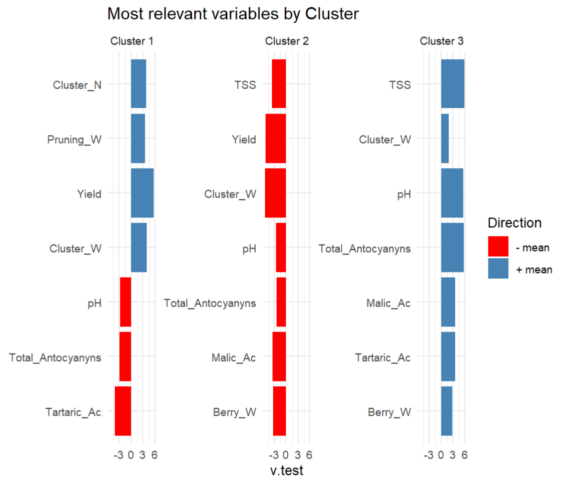

Fig. 4 Cluster-specific quantitative variables identified by HCPC. Bars represent v-test values, with sign indicating direction of deviation from the overall mean**.**

The Authors are grateful to Argiolas Farm for hosting the trial.

This study was carried out within the Agritech National Research Centre and receiVed funding from the European Union Next-Generation EU [PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022]. This manuscript reflects only the authors' Views and opinions and neither the European Union nor the European Commission can be considered responsible for them

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