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Scientific rationale and application of clonal selection for enhancing enological properties of *Vitis vinifera L.*

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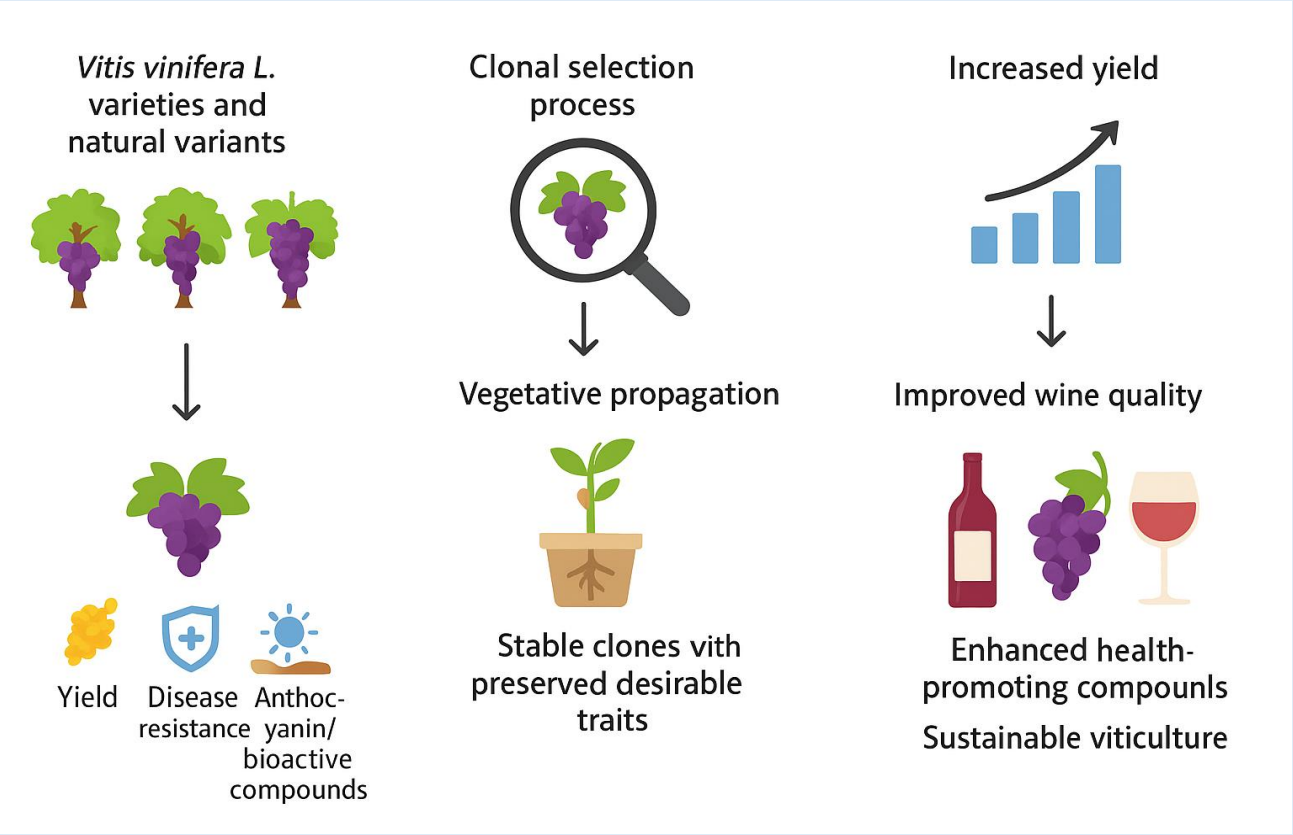
ABSTRACT

The work is devoted to a current trend – clonal selection. Clonal selection in viticulture is one of the key directions of varietal improvement, the main goal of which is to identify, evaluate and establish genetically stable subtypes within the same variety in the form of clones characterized by high productivity, qualitative improvements and adaptability. The core of this process is the identification and analysis of variants of plants of mutational origin that differ in morphological and biological economic characteristics. A clone can only be considered a subtype whose changed phenotypic characteristics are preserved at the level of vegetative generations, demonstrating hereditary stability.

These approaches, which have become a precedent, have led to the fact that in the future, the need for the use of clonal selection has been persistently emphasized in the field of viticulture. The main goals of clonal selection are: the separation of high-yielding clones, improving fruit quality, identifying early ripening clones, and other targeted improvements. The results obtained in various wine-growing countries of the world confirm the effectiveness of clonal selection in terms of realizing the advantages of vegetative variability of grape varieties. In many cases, clones of regionalized grape varieties exceed the productivity of their original versions by 1.5–2 times. This is due to both the

quantitative increase in the harvest and the improvement of qualitative indicators. Thus, clonal selection is one of the effective methods of increasing grape productivity.

Keywords: grapevine varieties, grapevine clones, clonal selection



Graphical Abstract: Scientific rationale and application of clonal selection for enhancing enological properties of *Vitis vinifera L*.

INTRODUCTION

Clonal selection in viticulture is a pivotal strategy for enhancing grapevine performance, focusing on the propagation of genetically and phenotypically stable clones that exhibit superior agronomic, phenotypic, and biochemical traits. This method not only aims to improve grape yield and wine quality but also enriches the functional properties of grape-derived products, aligning with the increasing consumer demand for health-promoting foods [1,2]. Bioactive compounds such as polyphenols, anthocyanins, and resveratrol contribute to antioxidant, anti-inflammatory, and cardioprotective effects, making clonal selection relevant for the development of functional grape products [1,2]. Integration of molecular-genetic tools enhances the precision of clone characterization, facilitates detection of genetic and epigenetic variability, and accelerates varietal improvement while promoting environmentally sustainable viticulture [1–2].

In viticulture, a “clone” refers to the vegetative progeny of a single plant, selected for verified varietal identity and desirable traits [2]. Clonal differences may

arise from genetic mutations, epigenetic modifications, somatic variations, or viral infections. Deakin et al. define a clone as vegetative progeny resulting from a germinal mutation that differs from the original plant in at least one characteristic and is maintained throughout vegetative propagation [1]. The term “clone” derives from the Greek klon, meaning “branch,” reflecting asexual reproduction from a single parent plant.

Clonal selection began in Germany in the 19th century and spread to France, Italy, and Spain during the 20th century. Early programs focused on healthy plant propagation and yield improvement. Modern programs increasingly emphasize grape and wine quality, sometimes at the expense of productivity, and select spontaneous phenotypic variants affecting quality, such as Tempranillo Blanco, recently registered in DOCa Rioja [3,4].

Old grape varieties are not genetically uniform; all plants originating from the same parent are considered clones [4]. Vitis vinifera encompasses a large population of varieties and clones, with more than 3,000 registered worldwide [5]. Countries with developed viticulture conduct clonal selection alongside studies of genetic diversity and variability among clones. Popular varieties such as Cabernet Sauvignon, Chardonnay, Riesling, Pinot Noir, and Tempranillo have multiple registered clones evaluated for yield per developed shoot, phenological traits, berry composition, sugar and acid content, and polyphenol levels. Figures 1–5 summarize the most widely used clones of these varieties, highlighting their agronomic, phenotypic, and biochemical traits [6,7].

This review presents a novel perspective by integrating genetic, epigenetic, and biochemical analyses to identify superior clones with enhanced functional properties, rather than focusing solely on yield or traditional phenotypic traits. The application of modern molecular-genetic tools, combined with high-throughput phenotyping and evaluation of somatic and epigenetic variations, represents a scientific innovation that allows more precise and efficient clone selection [1–2,6]. These approaches facilitate adaptation to changing ecological and climatic conditions while accelerating the development of improved varieties.

From a practical standpoint, effective clonal selection can improve wine consistency and quality, increase the content of health-promoting bioactive compounds, and support the development of functional grape products and nutraceuticals. Selecting clones with enhanced resistance to pests and diseases reduces reliance on chemical fertilizers and pesticides, promoting environmentally sustainable viticulture. These benefits strengthen the competitiveness of local wine markets and align with contemporary consumer trends favoring healthy and sustainable products [1–2,5,7].

Despite extensive research, several limitations exist. Heterogeneity in study designs, clone evaluation methods, and analytical approaches complicates comparisons across studies. Many reports focus primarily on yield or wine quality rather than biochemical or functional traits. Standardized protocols for molecular and biochemical evaluation of clones are limited, and the use of clonal selection for functional food development remains an emerging area requiring further investigation [1–14].

Italy, as a leading wine producer in the European Union, produces approximately 4.45 billion liters annually, owing to its cultural traditions, favorable pedo-climatic conditions, and extensive germplasm. As of December 1, 2022, 2,072 grapevine cultivars are registered in the Italian Catalogue of Grapevine Varieties (http://catalogoviti.politicheagricole.it) [8–14]. These examples demonstrate the importance of clonal selection in optimizing grape productivity, wine quality, and functional properties.

In summary, clonal selection represents a multifaceted approach that strengthens the scientific and technological foundation of modern viticulture. By enabling the selection of high-performing, locally

adapted clones, it improves grape and wine quality, supports functional grape product development, promotes sustainable viticulture, and aligns with contemporary consumer preferences for healthy and environmentally sustainable products [1–14]. Figures 1–5 summarize the most important clones and illustrate their practical relevance in modern viticulture.

CABERNET SAUVIGNON ENTAV-INRA 169 ENTAV-INRA 170 UCD 7 UCD 8

DSIR MASS SELECTION KWV 15 LC 10

Figure 1. The most popular clones of Cabernet Sauvignon grape variety [7]

CHARDONNAY ENTAV-INRA 121 ENTAV-INRA 548 ENTAV-INRA 809 ENTAV-INRA 1066

B 95 2/23 UCD 6 UCD 15 MENDOZA

Figure 2. The most popular clones of Chardonnay grape variety [7]

RIESLING ENTAV-INRA 49 GM 110 GM 198-19 GM 239-10 GM 94

Figure 3. The most popular clones of Riesling grape variety [7]

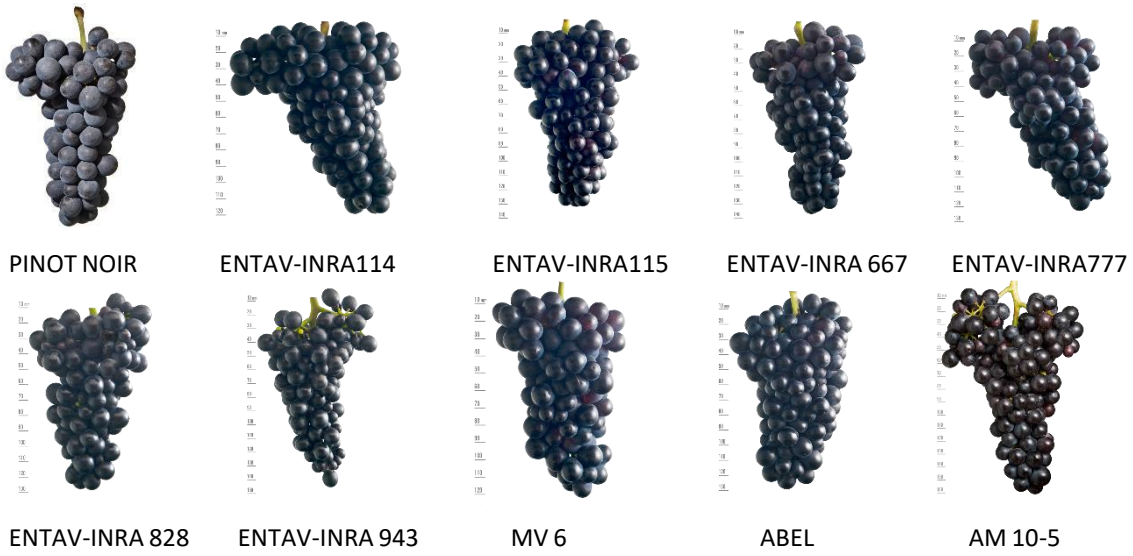


Figure 4. The most popular clones of Riesling grape variety [7]

Figure 5. The most popular clones of Tempranillo grape variety [7]

Clon Selection: The German scientist H. Fröhlich was one of the pioneers of clonal selection. In his fieldwork, he singled out bushes of the Sylvaner variety that demonstrated high yields, vigorous growth, high-quality bunches, and healthy dark green leaves. One of these bushes, selected in 1876 and propagated in 1900, later became known as the Sylvaner Fröhlich clone. L. Mittmann, after seven years of study in 1927, reported that the yield of this clone exceeded that of the unimproved variety by an average of 25%, with significantly higher sugar content in the juice. Although limited in scale, Fröhlich's work became widely studied in Germany and beyond [15].

Clonal selection in viticulture began in the late 1950s, initially aimed at establishing virus-free vine populations from healthy mother plants (Lacombe et al., 2004). Over time, selection criteria expanded to include agronomic and enological traits, such as yield, grape sugar content, polyphenolic composition, and wine sensory characteristics [16, 17].

Clonal selection, based on mutational variability, is now widely used worldwide to increase yield, enhance resistance to environmental stressors, and improve grape quality. The best adaptive traits and production potential of clones are usually expressed under the local soil and climatic conditions in which they were isolated. The cultivation of clones of classic wine varieties, adapted to local conditions, enables high-quality harvests with reduced production costs [18]. Numerous studies have highlighted the potential of clonal selection in exploiting vegetative variability in grape varieties [19–24].

Clonotype selection, distinct from mass or polyclonal selection, and clonal selection—typically conducted in three or four stages—are among the most widely applied intra-varietal selection methods in viticulture. Clonotype selection begins with a preselected

base population screened for phytosanitary quality and groups individuals based on intra-varietal variability expressed through key traits, such as flower morphology. This method, shown to be more effective than mass selection for specific varieties, was first introduced by Kozma in 1948 for 'Furmint' and 'Kadarka' cultivars. The clonal selection approach, adapted initially from German practices, was further developed in Hungary, where Márton Németh established a four-step procedure later simplified to three steps by Ottokár Luntz [25–27].

Each method has advantages and limitations. Clonotype selection retains more genetic diversity, supporting long-term adaptability, but the improvement process is slower. For genetically eroded varieties in small populations, clonotype selection can provide sufficient propagation material to restore the variety relatively quickly. Clonal selection, in contrast, delivers faster results and earlier clone release but reduces somatic genetic diversity, potentially limiting environmental adaptability. Regarding virus elimination, clonal selection is preferable, as virus removal is required from only a single genotype, whereas eliminating viruses from a clonotype group is impractical or costly [26].

In practical viticulture, clonal selection often relies on correlations between morphological and agronomic traits. For instance, although 'Vignoles' wine is in high demand, its production is limited by susceptibility to grey rot, linked to compact bunches. Selection has prioritized looser-clustered clones. Similar approaches apply to 'Juhfark', while in 'Kéknyelű', slightly denser clusters are selected to improve fertility [28].

Molecular tools increasingly support clonal identification and selection, though their use is more limited than in crossbreeding programs. ATR-MIR spectroscopy combined with partial least squares discriminant analysis (PLS-DA) distinguishes 'Tempranillo' clones by origin and vintage, while PLS regression predicts soluble solids, pH, and titratable acidity. SSR and inter-SSR markers have identified 'White Riesling' clones, and AFLP and S-SAP markers assess intra-varietal diversity in Croatian grape varieties. However, correlating genetic markers with functional agronomic and enological traits remains a challenge [29, 30].

The grapevine genome is dynamic, with many mutations being clone-specific. In a study of 86 'Riesling' clones using ten AFLP primer combinations, 38.5% of polymorphic markers were single-mutation events, and 17% were locus-specific, suggesting site-dependent mutation patterns. Somatic variation is valuable for identifying useful traits but poses risks, including field instability of selected clones [26].

Productivity: Grape productivity indicators are fundamental for characterizing any variety [31]. Increasing vineyard yield requires high-quality planting material derived from selected clones. Clonal selection identifies economically valuable variations arising from mutational variability, certifies them, and propagates them vegetatively. Improving a competitive assortment relies on individuals expressing optimal economic and biological traits in their selection environment. Selection aims to eliminate deficiencies while increasing yield and improving the quality of "basic varieties." Clones may exhibit enhanced resistance, yield, and quality or, conversely, weaker traits [32].

Most registered clones are about 1.5 times more productive than the original plantings. A challenging stage in clonal selection is distinguishing mutation-based from modification-based changes. Evidence suggests modification variability can persist for many years and propagate vegetatively, representing long-term modifications. Therefore, yields, crop quality, and phytopathological characteristics must be regularly monitored, even in early vegetative generations [33].

Environmental Influence on Viticulture: Viticulture is highly sensitive to environmental conditions. Grapevine growth and development depend on a complex system known as terroir, which includes climate, soil, geography, grape variety, and cultural practices.

Terroir has a significant impact on wine quality, particularly in traditional European wine regions. While Vitis vinifera adapts to diverse environments, local conditions profoundly influence physiology and wine quality. Research increasingly focuses on climatic components of terroir and their impact under climate change [34].

Key environmental factors include light, temperature, water and air regimes, and soil properties. Interactions among these factors are context-dependent, with terrain and supply levels affecting outcomes [35, 36]. Phenological monitoring of budburst, flowering, and ripening helps evaluate varietal suitability and informs selection and breeding decisions [37–40].

As a climate-sensitive crop, Vitis vinifera is particularly vulnerable to the effects of climate change, including global warming and extreme weather events. White grape varieties are particularly affected, with elevated temperatures accelerating ripening and reducing acidity. Climate change also increases yield and quality fluctuations in autochthonous varieties, complicating clonal selection programs [26].

Quantitative Composition of Anthocyanins: Anthocyanins are the main pigments in red and purple grape berries, accumulating primarily in skins and influencing varietal identity and wine quality. Beyond coloration, anthocyanins form polymeric pigments during aging, enhancing color intensity and stability. Structural modifications and interactions with tannins are critical for long-term color stabilization [41-44].

During ripening, anthocyanin biosynthesis is influenced by grape variety, climate, soil, yield, irrigation, and canopy management, resulting in variability in pigment composition and wine color [45-47]. Absolute anthocyanin content can vary widely within a variety across seasons [48–50], but relative proportions of individual anthocyanins remain stable, reflecting genetic determination. Consequently, anthocyanins are reliable markers for varietal identification, with multivariate analyses successfully differentiating cultivars [51–55].

Anthocyanin Fingerprint and Clonal Variation: The anthocyanin fingerprint of young red wines, typically determined by HPLC, is a valuable tool for verifying varietal authenticity [56–58]. Genetically distinct clones with unique enological traits exist within many varieties. While some clones of Cabernet Sauvignon and Pinot Noir show similar anthocyanin content [59], others exhibit significant differences under identical environmental conditions [60]. Two-dimensional NMR of polyphenolic extracts has also been proposed for distinguishing clones [61-65].

Data on differences in anthocyanin profiles among clones remain limited. Studies on six Tempranillo clones over three vintages, grown in the same vineyard, showed slight clonal differences, but year-to-year variation was more pronounced. Both clone and vintage influenced composition, with climate playing the largest role. Discriminant analysis grouped samples by harvest year, highlighting the dominance of environmental variability over clonal variation [44].

CONCLUSIONS

Clonal selection is an effective strategy for improving grapevine productivity, wine quality, and environmental adaptability. Stable clones enhance yield, disease resistance, and anthocyanin composition. While reducing genetic diversity, clonal selection ensures uniformity, virus-free material, and consistent enological outcomes. Modern molecular tools improve clone identification precision, though environmental conditions remain decisive. Clonal selection is thus crucial for sustainable improvement of Vitis vinifera L. under changing climatic and market conditions.

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reported in this manuscript.

Authors' contributions: Conceptualization, AS., ZH., and KK.; GD, AA, NS, EG, AN, BVK,JB, HM conducted the majority of the literature search and wrote the initial draft; MD, EK, EG,ZH, KK, MB contributed to data organization, figure creation, and manuscript editing; All authors contributed to the revision process and approved the final manuscript.

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