

Review

# Influence of Genetic Variability of Grapes to Produce High-Quality Wines

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**Abstract:** Grapevine is one of the most important fruit crops economically and is widely grown all over the world. Most of the grapevine fruits produced is processed into wine. Other than that, significant portions have also been used for fresh consumption, dried or processed into juice. The objectives of grapevine breeding vary based on the way it is used and they can be specific to the region in which it is grown. However, most of the time the end goal of breeding programs is to combine high yield and high fruit quality with better resistance to various diseases, and improved adaptation to different environments. Desirable traits such these can be exploited from the vast genetic resources of the *Vitis* genus. Methods like conventional breeding, mutation and polyploid breeding, and biotechnological approaches have been used to incorporate useful traits. Combining these methods will allow breeders to meet the growing demands for novel grapevine varieties and to produce high-quality wines.

**Keywords:** grapevine; *Vitis vinifera*; wine quality; genetic resources; genetic variability.

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## 1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the most economically important fruit crops. It is cultivated in almost all continents of the world. Viticulture and wine making have been part of human culture for thousands of years [1]. Around 71% of the world's harvest is processed into wine, 27% is consumed fresh and 2% is dried for raisins [2].

Grapevine takes approximately 7.5 mha of worldwide cultivated area and generates around 259 mhl of wine production. Expansion of human activity led to the creation of thousands of grapevine varieties with extensive phenotypic diversity [3]. *V. vinifera* L. is the most widely cultivated *Vitis* species because it is highly adaptable to a wide range of conditions. *V. vinifera* L. probably originated in the Mediterranean Basin and the Middle East, where it was domesticated around 5,000 years ago. *V. vinifera* has given rise to over 14,000 cultivars grown in most of the cultivated areas. It is estimated that more than 90% of the world's grapevines are either *V. vinifera* or *V. vinifera* hybrids. Although *V. vinifera* cultivars are usually of outstanding quality, they are susceptible to a variety of stresses and that is why grapevine improvement is necessary in order to achieve specific goals and local adaptation [2].

Higher production efficiency and better fruit quality has been based on the modifications of management and growing conditions of specific genotypes which have been kept constant by vegetative multiplication [1]. Various breeding methods have been used in different parts of the world through different breeding programs [2]. Unfortunately, favoring of specific varieties/clones has led to extensive genetic erosion. Breeding for resilience to climate change, yield or other traits

requires a certain understanding of the genetic basis of phenotypic variation, a field that has seen significant advances through to the use of genome-wide approaches [3]. Further advances in technology at this level will increase the understanding of grapevine genetics and genes which are crucial to its important and desirable traits.

## 2. Berry and Wine Quality

A first attempt to explain berry quality on a genetic level was reported by Hendrick and Anthony (1915). They analyzed results of various crosses with different parental combinations. The authors observed that the proportion of seedlings that give good quality decreases when the parents that show poorer quality were used. The conclusion was that only high quality parents should be used for breeding. Years of selection of grapevine have raised the quality in *V. vinifera* subsp. *vinifera* to a point that it has become a powerful factor in transmitting high quality [4].

Berry quality and hence wine quality is the most complex trait in grape breeding. It relies on complex sensory perceptions including taste, smell, and mouthfeel. Selection of good quality genotypes is subjective because it depends on the organoleptic perception of a tasting panel. Berry quality is quite difficult to evaluate for table grapes and even more so for wine grapes and the reason is that through metabolic conversions must fermentation by yeasts increases the complexity of the trait. The amount of acids, minerals, balanced aroma compounds, amino acids, sugars and lack of off-flavors in the must are major components in berry quality estimation. The concentration, the balance and the interactions of up to 800 different aroma compounds [5] are crucial for the appraisal of quality. Changes that might occur during aging and storage of wine need to be evaluated in order to uncover any sensory deficits which are attributed to the breeding line. Berry quality can be recorded only 4 to 5 years after a cross and environmental factors have a strong influence. Furthermore, the amount of grapes available for experimental micro-vinification for assessment of wine quality is limited. The number of vines available reduces the scale of fermentation and consequently a quality evaluation. Berry quality is the most important trait to be evaluated but at the same time the assessment itself is very complex and time consuming [6].

## 3. Grapevine Genetic Resources

There are many species of cultivated and wild grapevines in the world, and every species has many varieties. More than 10,000 named varieties were found in different countries and every year, new grapevine varieties are being added to the list.

One of the crucial things for maintaining the genetic resources of *Vitis* are grapevine germplasm collections. Genetic resources can be divided into 4 types: wild species, old traditional cultivars, new cultivars and breeding lines. The wild species are potential sources for numerous traits. For example, *Euvitis* and *Muscadinia* species are good sources of resistance. Few American species are resistant to phylloxera (*Dactylosphaera vitifoliae*) like *Vitis riparia*, *Vitis rupestris* and *Vitis berlandieri* and they are extensively used in various breeding programs. Out of these three species only *Vitis berlandieri* is adapted to highly calcareous soils and it is used to develop root stocks resistant to phylloxera and lime-induced chlorosis. Wild Asiatic species like *Vitis amurensis* and Chinese species such as *Vitis bryoniifolia*, *Vitis davidii* and *Vitis piasezkii*, and are considered to be good sources of resistance to powdery mildew (*Uncinula necator*) and downy mildew (*Plasmopara viticola*). *Muscadinia* is a useful source of resistance to phylloxera, nematodes, Pierce's disease (*Xylella fastidiosa*) and fungal diseases. Several sources of tolerance to abiotic stresses are also observed in some *Vitis spp.* For example, tolerance of cold is mostly found in the northern species such as *Vitis riparia* and *Vitis labrusca*. On the other hand, southern species like *Vitis bourquiniana* and *Vitis rotundifolia*, are tolerant to hot conditions. These wild species, however, have small berries with excessive seeds and strong pungent flavors and that is why, in order to eliminate some unfavorable characteristics of these wild species, extensive backcrosses are often needed. However, many traditional and new cultivars are also good sources of desirable traits. Germplasm collections have stored at least 10,000 grapevine varieties, but because in grapevine synonyms and homonyms occur, a more accurate estimate of the number of varieties might be closer to 5,000. The true number

of varieties and the relationships between them remain to be determined possibly by extensive DNA profiling of grape varieties in different collections and the development of common databases. One of the first steps for grapevine breeding is the selection of the superior accessions and evaluation of the germplasms. Lately, the interest in grape germplasm resources and genetic diversity analysis has been increasing. This helps to protect some varieties, especially wild grapes like *Vitis vinifera* ssp. *sylvestris* and facilitates their utilization in grapevine breeding programs. Today, information on grape database and germplasm collections is available on different websites [2].

#### 4. Preservation of Grapevine Genetic Resources

The cultivated grapevine *Vitis vinifera* does not breed true from seed [7]. Vegetative propagation provides genetic stability and phenotypic consistency of desirable traits [8]. Because of that and in order to ensure continuance of elite selected phenotypes, grapevine cultivars have been selected, conserved and maintained via vegetative propagation since domestication [9,10]. Vegetative propagules from a single common ancestor are referred to as clones [11]. The loss of genetic diversity generated by genome recombination during sexual reproduction is one flaw that is associated with clonal propagation [12].

Although the vegetative propagation provides relative genetic stability, somatic mutations still are possible [13]. On one hand, the result of these mutations can be the appearance of superior qualities or distinct characters that produce an individual sport or new cultivar, on the other hand they can also have the potential to disrupt quality traits [14]. Somatic mutations can be fixed with asexual propagation which leads to intra-varietal diversity within cultivars and results in several clonal lines with differing phenotypes [15].

A wide range of different techniques have been used to study genetic diversity within cultivars. Techniques like sequence specific amplification polymorphism (S-SAP) [16], microsatellites or simple sequence repeats (SSRs) [17], inter simple sequence repeats (ISSR) and amplified fragment length polymorphism (AFLP) [18]. Next generation sequencing (NGS) and the use of single nucleotide polymorphism (SNP) markers allow reliable and economic screening of tens or hundreds of thousands of markers per assay [19]. SNPs can be identified from short reads created by NGS platforms, through aligning to a reference genome or *de novo* genome assembly [20]. The use of reduced representation libraries (RRLs) combined with barcoded sampling has significantly reduced the cost and complexity of SNP genotyping. Compared to other genotyping techniques SNPs are highly stable and reproducible between laboratories [21].

Using genetic information alone, many clonal lines of *Vitis vinifera* can often be indistinguishable. A recent example of this was presented in two cultivars – *Anglianico* and *Muscat*, whereby an 18k SNP array could not discriminate clonal lines. However, this study did discriminate clones based on chemical diversity of secondary metabolites (anthocyanins and phenolic substances) [19]. The authors speculated that epigenetic regulatory mechanisms could contribute to clonal diversity in grapevine. This was hypothesized much earlier by Imazio et al. (2002). They suggested that morphological differences between clones could be the result of differential expression due to clone specific epigenetic differences. The use of epigenetic approaches has shown to be better suited for the identification of clonal lines [22].

The process of vegetative propagation in plants shortcuts the developmental transition from seedling to adult plant. This vegetative phase change has already occurred in woody perennials, sometimes at the expense of juvenile potential. This phase change is reversible and that process is known as rejuvenation. Many techniques of propagation from mature tissues have resulted in change due to rejuvenation. For example, in *Sequoia sempervirens*, Phase change following rejuvenation has been shown to present altered DNA methylation states compared with juvenile and mature tissues. Propagation based methylation differences have been observed in several different species [23].

Another way to preserve genetic diversity of grape varieties is the use of institutional germplasm conservatories. They are useful, but for the preservation of the genetic resources of

vines it is not reasonable to count only on these conservatories because they depend on public funding and therefore are subject to possible budget cuts. Gathering the grapevine genetic resources in a small number of sites increases the risk of accidents like climatic catastrophes and diseases. One example of how fragile these conservatories are is the case of Vassal estate in France. It gathers the greatest number of vine genotypes in the world (INRA, 25 ha, 7,500 genotypes, 2,600 *Vitis vinifera* varieties, 210 rootstocks and 720 hybrids originating from 47 countries). The INRA is not the owner of the land and the lease is expiring. This forces the INRA to move this vast collection. Beside the conservatories, genetic resources could also be preserved in private wine producing estates. It is desirable to promote private mass selections on a minority but significant proportion of planted surfaces, for example 5% [24]. Such a proportion would make the preservation of genetic diversity possible on approximately 40,000 ha in France which is approximately 1000 times more than what is possible in the institutional conservatories. The private clonal selections can also contribute to the preservation of the biodiversity. Mass selection is the easiest way to carry out a private selection of plant material. It is also possible to implement a true private clonal selection program. This requires more important technical and financial means and contributes more modestly to the preservation of the biodiversity [25].

## 5. Conclusions

Over the past millennia, human selection for traits of interest has shaped the appearance of grapes. Selection for hermaphroditic flowers increased grape production. Nearly half of all grapes grown are turned into wine, 36% are consumed fresh and the rest is dried or used for juice. Desirable berry traits vary depending on the use of the grapes, therefore the different breeding targets for table and wine grapes have led to differences in berry and bunch size [26-28]. There is also evidence of selection for white berry color [48].

While grape breeding has resulted in selection for several traits, current consumer preference is focused on a small number of elite cultivars. Thus most grape cultivars have been grown for centuries—for example ‘Pinot Noir’. It has existed for more than a millennium. These genetically frozen cultivars are highly susceptible to pathogens that are constantly evolving [29]. Selection for new traits is a slow and expensive process. High inbreeding depression and a lengthy juvenile phase impede the breeding of new grape cultivars [30]. Developing a new grape cultivar using traditional breeding techniques takes about 25–30 years but the use of genetic markers linked to phenotypes of interest can decrease that time by up to 10 years. In addition, it is found that the use of marker-assisted selection (MAS) in grapes offered a cost-saving of 16–34% [31].

What makes grapes an attractive candidate for MAS is its ability to save time and money when breeding [32]. Using genetic markers, individuals can be tested for a trait at the seed or seedling stage. Therefore MAS offers the greatest potential for traits that are difficult and expensive to phenotype (disease resistance) or time-consuming to measure, like some fruit traits that are only visible after several years. Wild *Vitis* species have been used for hybrid grape breeding [33] and they represent a promising source of resistance loci for introgression through MAS [34]. For example, *V. arizonica* was used in the development of wine grapes that are resistant to Pierce’s disease [35], and *Muscadinia rotundifolia* was used to pyramid resistance from powdery and downy mildew into *V. vinifera* [36]. Markers have also been identified for various other traits in grape such as berry color [37, 38], flower sex [39], seedlessness [40] and muscat aroma [41].

The discovery of markers for agriculturally important traits has facilitated the use of MAS in grapes. This technique is only profitable when the cost of phenotyping is higher than the cost of discovering new markers and genotyping cultivars [42]. Decreasing DNA sequencing costs will continue to accelerate marker discovery and the implementation of MAS in grape breeding.

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