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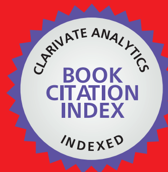

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Chapter

Somatic Variation and Cultivar Innovation in Grapevine

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Abstract

Paradoxically, continuous vegetative multiplication of traditional grapevine cultivars aimed to maintain cultivar attributes in this highly heterozygous species ends in the accumulation of considerable somatic variation. This variation has long contributed to cultivar adaptation and evolution under changing environmental and cultivation conditions and has also been a source of novel traits. Understanding how this somatic variation originates provides tools for genetics-assisted tracking of selected variants and breeding. Potentially, the identification of the mutations causing the observed phenotypic variation can now help to direct genome editing approaches to improve the genotype of elite traditional cultivars. Molecular characterization of somatic variants can also generate basic information helping to understand gene biological function. In this chapter, we review the state of the art on somatic variation in grapevine at phenotypic and genome sequence levels, present possible strategies for the study of this variation, and describe a few examples in which the genetic and molecular basis or very relevant grapevine traits were successfully identified.

Keywords: somatic variation, genome sequence variation, somatic mutations, chimerism, Meunier phenotype, fruit color variation, seedlessness, Muscat aroma, forward genomics

1. Introduction

World viticulture is based on a wide diversity of cultivars, many of them autochthonous from their cultivated areas. In fact, almost 1500 grapevine wine cultivars are listed in the statistics published by the Wine Economics Research Centre at the University of Adelaide (Australia) every 10 years [1]. However, sixteen of those cultivars already occupy more than 50% of the world vineyard surface either because they belong to the few elite cultivars that are internationally recognized and grown in multiple wine regions across the world, or because they are widely grown in their regions of origin (**Table 1**). While this pattern responds to winemaking being a classical industry in which traditional cultivars are often preferred by producers and consumers, this also leads to the use of a limited genetic diversity, which represents a risk for the adaptation of viticulture and wine making to changing environments and market demands.

Global warming is changing climatic conditions in traditional winemaking regions [2, 3]. Along with the prolonged use of grapevine in monoculture and

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Rank	Prime variety	Color	Origin	Area (hectares)	Share (%)	Cumulative share (%)
1	Cabernet Sauvignon	R	France	290,091	6.30	6.30
2	Merlot	R	France	267,169	5.81	12.11
3	Airén	W	Spain	252,364	5.48	17.60
4	Tempranillo	R	Spain	232,561	5.05	22.65
5	Chardonnay	W	France	198,793	4.32	26.97
6	Syrah	R	France	185,568	4.03	31.00
7	Garnacha Tinta	R	Spain	184,735	4.01	35.02
8	Sauvignon Blanc	W	France	110,138	2.39	37.41
9	Trebbiano Toscano	W	Italy	109,772	2.39	39,80
10	Pinot Noir	R	France	86,662	1.88	41.68
11	Mazuelo	R	Spain	80,178	1.74	43.42
12	Bobal	R	Spain	80,120	1.74	45.16
13	Sangiovese	R	Italy	77,709	1.69	46.85
14	Monastrell	R	Spain	69,850	1.52	48.37
15	Grasevina	W	Croatia	61,200	1.33	49.70
16	Rkatsiteli	W	Georgia	58,641	1.27	50.97

Selected from Wine Economics Research Centre [1].

Table 1.
Grapevine cultivars contributing to more than 50% of world vineyard surface.

globalization-related issues, global warming associates with the emergence of new pathogen and pest threats [4]. At the same time, consumers and new agriculture and food safety regulations are more and more demanding a reduction in the use of pesticides and fungicides in viticulture [5]. In this context, strategies to adapt viticulture in different regions to different models and markets are required to ensure the sustainability of the crop. Among the multiple possibilities that can be considered to this aim, strategies intending the genetic improvement and adaptation of elite and autochthonous varieties are very relevant to keep their intrinsic varietal values—these cultivars are traditionally related with wine quality and are indeed the basis of the most famous and expensive wines.

Grapevine varieties derive from the domestication of wild forms of the species *Vitis vinifera* [6]. Wild grapevines are dioecious, which obligates to outcrossing and results in highly heterozygous genotypes, a genetic feature that has been inherited by domesticated forms. This is the reason why vegetative propagation has been the preferred method to multiply selected grapevine varieties since ancient times, to keep the varietal attributes and shorten production lapses. In fact, most traditional cultivars in use nowadays derive from seeds that probably germinated several centuries ago and that have been vegetatively multiplied since that time to currently cover large vineyard surfaces as those shown in **Table 1**.

All species within the genus *Vitis* are cross-fertile and the identification of sources of genetic resistant for *Vitis vinifera* pathogens and pests mainly in other American or Asian species opened the possibility to improve grapevine varieties through classical breeding strategies. This approach has been successfully developed during the twentieth century and new resistant grapevine varieties have reached the markets with different success rates [7–10]. Furthermore, rising knowledge of the grapevine genome and the development of new genomics and molecular techniques

in the last decade have triggered a renewed interest for breeding given, the pressure to reduce the use of pesticides in viticulture [10]. Genomics-assisted breeding represents an interesting and efficient strategy that has the potential to change the role of genetic materials in viticulture and wine making [11, 12]. Still, breeding ends in new grapevine genotypes that need to be registered as new varieties with new names, what generates bureaucratic problems delaying their commercial use and hindering their acceptance by the market.

Viticulture based on traditional varieties has relied on the phenotypic variation generated by spontaneous somatic mutations for the improvement and diversification of the crop. This variation has been traditionally selected by farmers along the history of viticulture to improve cultivars and adapt production to evolving conditions [6]. Later, along the twentieth century, this somatic variation became the basis of clonal selection. This strategy has the advantage that the derived clones keep the original cultivar name and are already mostly adapted to vineyard management practices and the wine making process as well as the market [13]. Varieties are considered to consist in groups of clones selected during vegetative propagation that share common features. When clones of the same variety have phenotypes different enough to be grown for the production of different wines, they can be considered as derived varieties [14] that could keep the name of the progenitor variety. For example, this is the case of Pinot Noir Blanc derived from Pinot or the recent Tempranillo Blanco derived from Tempranillo [15]. By the time being, the advent of new genomic and phenotypic techniques enables the identification of the origin and features of somatic mutations and the associated phenotypic variation, knowledge that can be exploited to efficiently improve the adaption of traditional cultivars to changing market and environmental demands. This strategy can be complementary to the development of new varieties by breeding and help understanding the genome diversity of traditional varieties and maintaining their production. In fact, part of the variation used in breeding programs is the result of somatic mutations selected along grapevine domestication as described in subsequent sections.

Along this chapter, we summarize what we have learned from the study of somatic variants in grapevine cultivars, their origin, their value, and the interest of their study. We also review several examples of very relevant grapevine traits that likely originated by somatic variation and that have been characterized at the molecular level and discuss how understanding the basis of this variation can now help to apply new technologies to the genetic improvement of grapevine cultivars.

2. Grapevine somatic variation

Despite vegetative propagation is used in grapevine to multiply plants that are identical to the original type, spontaneous phenotypic variation occasionally appears on some shoots (known as bud sports) as a result of somatic mutations [16]. From bud sports, the new variant phenotype can be established as a whole plant and, eventually, as a new variety, using the same propagation strategy. Bud sports can display any type of phenotypic variation at any organ, leaf, stem, bunch, berries, seeds, etc. Variation can affect reproductive traits that determine yield and quality such as fertility, cluster compactness, berry color, or flavor. Somatic variation can also affect vegetative traits including plant vigor, leaf morphology, or even disease susceptibility. There are some cultivars like Pinot Noir, Sultanina, or Italia [14, 16] for which a large number of somatic variants have been identified for multiple traits. The number of sports appearing in a given variety is expected to increase proportionally with its age and vineyard surface. In addition,

the possibility that some genotypes are more prone to generate somatic variants has not been proven but cannot be discarded.

Spontaneous somatic variation results from the combination of mutations and cellular events. Initially, mutations take place in single meristematic cells associated with the DNA replication and cell division processes. Somatic mutations accumulate at a very low frequency. However, since current plants of traditional grapevine cultivars result from millions of mitotic divisions since the germination of the original seed, they accumulate a relatively large number of mutations in their genomes (see the next paragraph). For nuclear DNA, every somatic mutation can be considered to be heterozygous as they only affect one of the two existing genome copies per cell. These somatic mutations can range from single nucleotide substitutions to nucleotide insertions or deletions or even to large DNA sequence recombinations causing chromosomal reorganizations [16]. Other infrequent alterations include the change of ploidy level of the cell, reported in different varieties [17]. Somatic epimutations altering gene expression without affecting nucleotide sequence and causing new phenotypes have so far not been described in grapevine.

Most mutations do not have any effect on gene and cellular functions since only a small part of the genome sequence is involved in coding or regulatory functions [18]. Even mutations in coding sequences do not always generate amino acid changes in the encoded protein or if they do, still in many cases they behave as silent changes. Emergent somatic mutation will only affect one of the two copies of a given gene. This makes derived phenotypic effects to be mostly expected from dominant mutations either due to gains of function or haploinsufficiency. Independent recessive mutations causing loss of functional alleles in heterozygous loci carrying a null allele could also generate phenotypic effects although at low frequency. Importantly, deleterious mutations constraining essential cell functions will not accumulate because purifying somatic selection will prevent their propagation in the plant.

Cellular events associated with the stabilization of somatic mutations are conditioned by the tissue structure of plant meristems. The grapevine shoot apical meristem is organized in at least two cell layers, the outer L1 and the inner L2, from which all the cells of the plant derive [19]. These cell layers constitute almost closed compartments with very limited cell exchange between them. Cell division and differentiation in the L1 layer gives rise to all the epidermal cells of all the plant organs, while the L2 layer generates the cells that constitute all their internal tissues. The L2 cell layer is also responsible for gamete development within reproductive flower organs. Because mutations emerge spontaneously in either L1 or L2 layers, grapevine plants are genetic chimeras that carry slightly different genetic composition in L1- and L2-derived cell lines. In addition, vegetative multiplication from cuttings along centuries contributes to select and enrich part of the variation accumulating in the plant. At the same time, because of the lack of sexual reproduction, there is no purifying selection against mutations that could have deleterious effects on gametogenesis, fertilization, zygote formation, embryo development, seed germination, or juvenile growth.

To manifest a mutant phenotype in a given plant organ, the mutation has to propagate through cell division from the original mutant meristematic cell. Initially, mutant daughter cells occupy a meristem cell layer (either the L1 or L2) or sectors of it, which subsequently gives rise to mutant organs by additional cell divisions. Once the mutant genotype is propagated in the L1, the L2, or both cell layers of a shoot apical meristem, the mutation could be transmitted by bud propagation. Periclinal chimeras with somatic mutations fixed in only one meristem cell layer are quite stable in grapevine, and indeed, some varieties like Pinot Meunier (L1 mutant) [20] or Pinot Gris (L2 mutant) [21] are chimeras that are stably maintained through

vegetative multiplication as we explain in sections below. If the mutant daughter cells colonize both meristem cell layers by migration of mutant cells to the wild type layer, bud multiplication from such mutant buds will fix the mutation in all tissues of derived plants. This is the case of white-berried variants derived from originally black-berried varieties such as Pinot Blanc [21]. Since plants do not have a separated germline, somatic mutations present in the L2 can be transmitted through sexual reproduction as far as they are not lethal in the haploid phase. Somatic mutations generating new interesting phenotypes, stabilized in grapevine plants as periclinal chimeras, or extended to all cell layers, have been selected as new clones of wine grape cultivars or as new derived cultivars [6, 14, 16].

3. Genome sequence variation within cultivars

Sequencing and de novo assembly and annotation of the first grapevine genomes [18, 22] provided a new body of knowledge and a new toolbox for the study of genome sequence diversity. Two different strategies were used for the first genome assemblies, a homozygous assembly based on PN40024, a partially inbred line derived from Pinot Noir, [18] or an assembly including both, consensus contigs of the two genome copies and independent contigs for each of the two haplotypes in more dissimilar genome regions of Pinot Noir (ENTAV 115) [22]. Both projects estimated a haploid genome size close to 500 Mb. More recently, long-read sequencing technologies such as PacBio are facilitating the release of haplotype-resolved assemblies, which are already available for the heterozygous grapevine cultivars Cabernet Sauvignon and Chardonnay [23, 24]. By the time being, the availability of reference genomes combined with the development of next-generation sequencing (NGS) technologies enable genome-wide analysis of the grapevine germplasm at affordable costs, which is extremely useful in genetic diversity studies as well as to search for mutations causing phenotypic variation [15, 24–26]. Although the use of these approaches to characterize somatic variation in grapevine is still scarce, an increasing number of publications are shedding light on the magnitude and type of variation that accumulates at the genome level within given cultivars.

Somatic SNV (single nucleotide variants) and small insertions/deletions (INDEL) mutations are often the result of errors in DNA replication taking place during mitotic cell division. While the frequency of INDEL may exceed that of single base substitutions due for instance to low resolution of polymerases at homopolymeric or short repeats, INDEL are more difficult to detect using high-throughput sequencing methods due to the same reason. The first attempt to detect somatic polymorphisms at a genome-wide scale in grapevine used 454 GS-FLX sequencing technology to compare three Pinot Noir clones to the sequences in the genome assemblies of the Pinot-related accessions PN40024 and ENTAV-115 [27]. In this study, mean rates of 1.6 SNV, 5.1 INDEL, and 35.2 mobile element movements per Mb were described among clones. Short-read sequencing technologies led by Illumina provide a framework to accurately detect SNV and are also useful to detect small INDEL. In this manner, genome resequencing of three clones corresponding to different morphotypes of the ancient Italian wine cultivar Nebbiolo identified between 16 and 26 clone-specific SNV per Mb of genome [28]. However, these numbers might be over-estimated considering that the validation success was 61% for a quality-trimmed sub-selection of SNV [28]. More recently, the re-sequencing of 15 clones of Chardonnay compared to a de novo genome draft assembly for this cultivar identified a much more reduced number of SNV using a stringent k-mer-based calling strategy variation [24]. The sum of SNV + INDEL ranged between 221 and 2 polymorphisms per clone (0.004–0.455 per Mb of genome), which

corresponds to at least three orders of magnitude of lower rates than in the Nebbiolo study, despite that Chardonnay accessions corresponded to diverse geographical origins and phenotypes including seedlessness and berry color variation [24]. Concerning the putative impact of these polymorphisms, a total of 21 (0.07%) and 55 (3.4%) clone-specific variants were predicted as potentially altering protein function in Nebbiolo and Chardonnay, respectively, including one nonsynonymous substitution in the *VviDXS* gene as the possible origin of the Muscat flavor of one Chardonnay clone [24, 28]. Transcriptome re-sequencing (RNA-seq) can also be useful to identify polymorphisms in coding sequences. For example, an RNA-seq study comparing the seedless somatic variant Corinto Bianco to its seeded ancestor Pedro Ximenes identified 13 polymorphisms with 100% validation rate (12 SNV and one dinucleotide), all of them being heterozygous variants [29]. This is also important to be considered since, rather than resulting from direct base substitution mutations, some of the somatic SNV detected in sequencing studies might correspond to loss of heterozygosity (LOH) in hemizygous regions generated after somatic SV.

SV involves changes in the chromosome landscape. It includes inter- and intra-chromosomal translocations, deletions, and insertions (the last two types are generally considered as SV if >1 kb) including those caused by the movement of transposable elements (TE) [30, 31]. The rapidly growing number of genomic studies in multiple species is unveiling more complex forms of SV, collectively known as chromoanagenesis, and combines several of the previous features [32]. In addition to the activity of TE, SV often relies on mistakes in replicative processes or on DNA breakage during mitosis followed by illegitimate repair mechanisms [33–35]. Although SV is generally deleterious, it can accumulate along the multiplication of grapevine cultivars behaving as recessive heterozygous due to the absence of sexual reproduction [15]. Features such as changes in copy number and breakpoint joins have been used in genomic studies to detect SV between grapevine cultivars and somatic variants [15, 25, 36–38]. By far, the most recurrently described case of somatic SV in grapevine relates to hemizygous deletions of different sizes around the grape color locus on chromosome 2 that causes loss of berry color variants (see below). Smaller SV, translocations, and inversions have also been described in somatic variants differing in ripening time [25]. Genome-wide SV studies in a higher number of clones would be required to estimate the frequency of different types of SV independently of specific phenotypes or genome regions resulting from human selection.

TE are extremely frequent in plant genomes and correspond to sequences that have the ability to replicate and insert in different locations, either indirectly through an RNA intermediate (retrotransposons or class I) or directly by cut-and-paste mechanisms (transposons or class II) [39]. The transposition of these elements generates changes in genome size and can disrupt target loci upon insertion. In addition, TE can lead to SV and genome rearrangements due to noncanonical transposition events or to homologous recombination related with their repetitive nature [39]. Altogether, TE has a high potential to impact on organismal phenotypes. While all superfamilies of TE are represented in the grapevine genome, those in class I (e.g.: Non-LTR LINEs, LTR Ty1/copia, LTR Ty3/gypsy, and other LTR) are much more numerous (>100,000 copies in total) than class II superfamilies (hAT, PIF, Mutator, and CACTA) totaling about 3000 copies in the grapevine reference genome [18, 40]. Because ca 50% of the grapevine genomes involve mobile element-like/repetitive sequences [18, 38], it is reasonable that they could be a major driver of somatic variation emerging during the extensive vegetative multiplication of grapevine cultivars. In fact, emergent phenotypes in grapevine somatic variants have frequently been associated with the movement of TE altering gene expression [41–43], although,

with the exception of color variants, their phenotypes have not been selected for production. While the use of molecular markers suggests that the TE genomic landscape can vary between grapevine clones [27, 44, 45], systematic studies are still required to determine the magnitude of somatic genome variation that accumulates associated to TE during the propagation of grapevine cultivars.

4. Nucleotide sequence variation underlying grapevine somatic variation

The availability of grapevine reference genomes and the advent of NGS technologies have paved the way for the identification of the nucleotide diversity underlying variation for relevant phenotypic traits in grapevine. Somatic variants are excellent tools for this goal, since they allow studying the mutation effect in a common genetic background when comparing somatic variants to the direct ancestor of the same cultivar. This facilitates the identification of the causal genes and gene variants. In fact, in the last years, the molecular and genetic basis of an increasing number of phenotypic traits has been elucidated using somatic variants as experimental systems.

We consider transcriptome RNA-seq comparisons as an excellent diagnosis tool for the screening of candidate genes because this technology has the potential to trace mutations that alter either gene expression or coding sequences. In our hands, the process starts with a careful phenotypic analysis comparing the progenitor normal plant and the somatic variants. Concurrently, we develop self-cross derived progenies of both genotypes for segregation analyses. The main objective of the phenotypic analysis is to understand the developmental origin of the emerged trait. In this manner, we can identify a target organ, tissue, and developmental stage in which the mutation is initially expressed and take samples of it from each variant to conduct a transcriptome comparison. The interpretation of gene biological function from the developmental and phenotypic variation can frequently be misleading since, as mentioned before, many of these mutations have dominant gain-of-function effects.

Under these premises, transcriptome comparison, both at gene expression and sequence levels, combined with the results of segregation analyses of mutant phenotype in self-cross populations of each variant can provide a preliminary identification of putative candidate genes. These candidates will have to be confirmed by directly comparing their sequences in normal and somatic variants of the same cultivar. Both in transcriptome and sequence analyses, it is important to consider the possible chimeric state of causal mutations in the somatic variants.

When the described approaches lead to the identification of sequence variation susceptible of generating the mutant phenotypic effect, it is still required to confirm that this sequence variation is the cause of the phenotype. When the responsible mutation is present in the L2 layer and can be transmitted through gametes, co-segregation of the mutant phenotype with the candidate sequence variants would support a causality relationship although it is not a definitive proof. Genetic transformation to restore normal or variant phenotypes can be a difficult and time-consuming alternative in grapevine. Other possibilities like allele-specific expression analyses or sequence characterization of a large number of variants or cultivars displaying the same phenotype have been used in different cases to proof that a candidate gene variant is in fact responsible for a relevant phenotypic effect [26, 42, 43, 46].

In the next section, we review several examples of studies taking advantage of somatic variants to understand the molecular genetics of four relevant grape traits.

4.1 Meunier phenotype

The Meunier phenotype accounts for the tomentose (hairy) phenotype of shoot tips and leaves in cultivar Pinot Meunier derived from Pinot noir. Plants derived by somatic embryogenesis from different L1 and L2 cell layers of Pinot Meunier showed different phenotypes, demonstrating that Pinot Meunier is a periclinal chimera carrying a mutant L1 line responsible for the Meunier phenotype. In addition, those plants regenerated from L1 somatic embryos displayed a new dwarf phenotype with short internodes [20]. Further characterization of those dwarf plants showed that they produced inflorescences and bunches in all nodes along the length of the shoots [47]. Grapevine nodes develop either inflorescences or tendrils that share a common ontogenetic origin from uncommitted primordia in grapevine [48]. Application of gibberellins (GAs) and GA biosynthesis inhibitors has been shown to modify tendril and inflorescence development in grapevine [49]. This phenotype suggested that the Pinot Meunier was associated with an altered response to gibberellins, what was confirmed by the high levels of active gibberellins detected in the dwarf plants paralleled by their insensitivity to the application of these hormones [47]. It is also similar to the phenotype of *gai* mutants of *Arabidopsis*, carrying mutations in GAI, a negative regulator of GA response [50]. In fact, dwarf plants derived from Pinot Meunier L1 were shown to carry a point mutation in a *GAI* homologous gene converting a leucine residue into a histidine within its conserved DELLA domain (**Figure 1**), the GA-sensitive domain unique to all members of this family of regulatory proteins [47]. The final proof confirming the role of this mutation in the origin of the dwarf phenotype came from the genetic analyses performed on self- and out-crosses of the mutant dwarf plants regenerated from the L1 of Pinot Meunier. The results showed that the mutated allele behaved in a semi-dominant manner, with homozygous mutant plants displaying a more extreme dwarf phenotype [47].

Similar hairy phenotypes have also been found in other cultivars given the names of some derived varieties like Garnacha "peluda" (hairy in Spanish), a name that refers to the tomentose phenotype. However, whether this phenotype has the same genetic and molecular basis as the Meunier phenotype has not been investigated. The Meunier phenotype constitutes a great example of how a relevant agricultural trait can be generated by a mutation in chimeric state in a somatic variant, a feature that is lost when the mutation is present in all plant cell layers. Because bibliographic references to Pinot Meunier or Schwarzriesling in Germany date back at least to the seventeenth century [51], this case itself proves the stability of periclinal chimeras in grapevine.

Understanding the molecular basis of this phenotype opens the possibility to recreate with genome editing tools such as CRISPR/Cas9 [52] the causative single point mutation or other point mutations known to have similar effects on the DELLA domain of the GAI regulatory proteins. However, the replication of the Meunier phenotype in the same or other cultivar backgrounds will be difficult because it will require the mutation to be stable only in the L1 cell layer, something that could require more sophisticated cell culture and plant regeneration techniques. This exemplifies the specificity of phenotypes resulting from chimeric states.

To end, it is important to mention that the capacity of these dwarf plants to flower rapidly from the initiation of the first tendril makes them useful model systems for genetic studies in grapevine [53]. In this case, genome editing to recreate mutations in the DELLA gene and regenerate whole mutant plants to obtain dwarf models in cultivars other than Pinot would be more feasible.

4.2 Muscat flavor

The Muscat flavor in grapevine describes an intense floral aroma present in the berries of some specific cultivars and their derived wines. It is linked to the high accumulation of monoterpenoids such as linalool, geraniol, nerol, citronellol, and α-terpineol, all having a low olfactory perception threshold [54]. This aroma has been strongly appreciated since ancient times and a family of closely related Muscat varieties was spread from the Eastern Mediterranean area by Greeks and Romans and can still be found with different names in many locations of the world [55]. The Muscat flavor has also been found in somatic variants of cultivars like Savagnin, Chardonnay, or Chasselas [46, 56].

Genetic analyses of Muscat aroma in grapevine have been performed in biparental progenies involving Muscat varieties [57, 58] and in self-cross derived populations of Muscat Ottonel and Gewurztraminer (a Muscat flavor somatic variant of Savagnin) [59]. Muscat aroma segregated as a dominant trait and at least one common major QTL responsible for Muscat aroma was detected in all progenies, located in linkage group 5. A positional candidate gene, 1-deoxy-D-xylulose-5-phosphate synthase (*VviDXS*), was proposed to account for the terpenoid overproduction phenotype [58, 59]. This gene encodes the first enzyme of the plastidial methylerythritol phosphate (MEP) pathway, which functions upstream in monoterpene and diterpene biosynthesis. Several investigations have shown that this enzymatic reaction is a biosynthetic step of the pathway that limits terpenoid biosynthesis in plants [60].

Based on those hypotheses, Emanuelli et al. [46] re-sequenced the *VviDXS* grapevine gene in a collection of 148 grape varieties, including Muscat-aromatic as well as other aromatic and neutral accessions. Among the SNP significantly associated with the presence of Muscat aroma, they identified the putative causal SNP responsible for the Muscat phenotype. This SNP is present in all Muscat varieties and generates a predicted nonneutral substitution of a lysine by an asparagine in residue 284 of *VviDXS*. Interestingly, Muscat-like aromatic somatic variants also displayed unique nonsynonymous mutations in close positions of the same

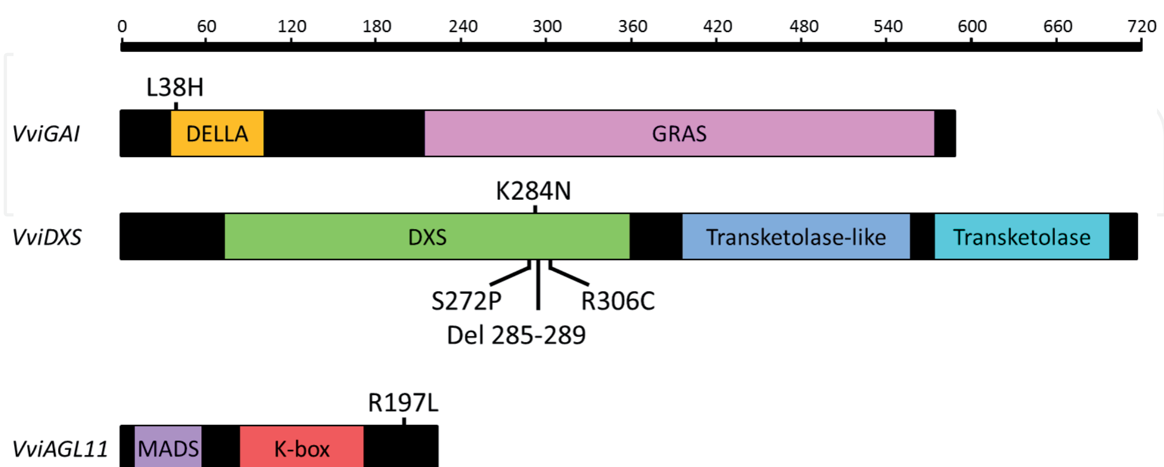

Figure 1.
VviGAI, VviDXS, and VviAGL11 proteins and mutations responsible for Meunier, Muscat, and Seedlessness traits. Protein domains are represented according to Pfam database. L38H mutation in the DELLA domain results in the lack of GA-response in pinot Meunier. K284N mutation is present in all Muscat varieties. Three additional independent mutations were identified in the same DXS domain in Muscat-like aroma somatic variants: S272P in chardonnay Musqué, deletion (Del) of five amino acids 285–289 in Chasselas Musqué, and R306C in Gewürztraminer. R197L mutation in AGL11 located in the C-terminus of the protein alters development and lignification of the seed coat.

domain of DXS protein (**Figure 1**). A serine substitution by a proline in position 272 in Chardonnay Muscat, an arginine substitution by cysteine in position 306 in Gewurztraminer, and a deletion involving five amino acids in position 285–289 on Chasselas Musqué [46]. Altogether, the correlation of independent nonlethal spontaneous Muscat mutations in this conserved DXS domain with the presence of Muscat aroma in all studied cases suggests its relevance in protein function. In fact, the Muscat amino acid substitution influences the enzyme kinetics by increasing its catalytic efficiency and it is also able to dramatically increase monoterpene levels in transgenic tobacco plants [60].

The closely related genetic relationships among Muscat varieties could be interpreted as resulting from the original selection of a somatic variant in which this characteristic aroma emerged and was propagated vegetatively. Occasional hybridization of this variant with other cultivars grown in ancient times as well as more recent directed hybridizations would have generated the currently available plethora of Muscat varieties (**Figure 2**). The identification of independent non-neutral amino acid substitutions or amino acid deletions in the same protein region clearly identifies *VviDXS* as a target gene to improve Muscat aroma through breeding (**Figure 1**). Specific nonneutral amino acid substitutions are not easily obtained from mutagenesis programs. However, the current catalog of known sequence variants in *VviDXS* provides several specific amino acid changes that could be recreated through genome sequence editing to introduce the Muscat flavor trait in any desired cultivar.

4.3 Berry color

Berry color is a very relevant trait determining consumer preferences in table grapes as well as the type of wines that can be elaborated from wine grape cultivars. In this way, red and *rosé* wines are made from black-berried cultivars, while white-berried cultivars are used for making white wines. In grapevine, berry color results from the biosynthesis and vacuolar accumulation of anthocyanins in berry skin cells during the ripening process from *veraison* stage. Variation for berry color is determined by a major locus on linkage group 2 [61, 62]. This berry color locus co-localizes with a cluster of tandemly repeated *VviMybA* genes [63]. Among them, *VviMybA1* and *VviMybA2* are expressed in the berry skin of black-berried cultivars from *veraison* [64]. The function of these transcription factors is required to trigger the expression of target genes such us UDP-glucose:flavonoid 3-O-glucosyltransferase (*UFGT*), encoding the limiting enzyme activity for the anthocyanin biosynthetic pathway [64, 65]. Original wild grapevines producing black berries and berry color diversity could have emerged as a result of somatic variation and be selected as a domestication trait in cultivated out-crossed forms [66]. Black-berried cultivars carry at least one functional copy of both *VviMybA1* and *VviMybA2* linked in a functional allele of the color locus. White-berried cultivars do not synthesize anthocyanins in the berry skin and they lack functional copies of these *MYBA* genes at the color locus. Most white-berried cultivars are homozygous for the canonical null allele of the locus in which *Gret1* retrotransposon insertion in the promoter of *VviMybA1* along with a small INDEL causing a frame-shift in *VviMybA2*, respectively, causes loss-of-function in the two genes [41, 64]. Most of the diversity in berry color observed among grapevine varieties has been related to nucleotide sequence variation in this locus [67].

In addition, intracultivar variation for berry color, useful to select new derived varieties, has also been associated to variation in the berry color locus. In this way, spontaneous red-berried variants identified in white-berried table grape cultivars like Italia or in wine cultivars were shown to derive from recombination,

reverting the insertion of the *Gret1* retrotransposable element present in the promoter of *VviMYBA1* in white alleles, which at least partially recovers the expression of the gene [41, 68]. In other cases, red-berried variants emerged as new functional *MYBA* genes resulting from the recombination of nonfunctional homologous genes within the color locus [69]. On the other hand, black-berried cultivars heterozygous for the null allele occasionally display grape color variants with either red/gray or white berries depending on whether only the L2 or both L1 and L2 meristem cell layers (**Figure 3**), respectively, carry mutations at the color locus [21, 70–73]. Molecular characterization of red/gray and white berry somatic variants of Cabernet Sauvignon and Pinot Noir cultivars through Southern blots showed that the lack of berry skin anthocyanins was associated with deletion of the functional allele of the color locus [70, 74]. Later, the loss of heterozygosity along the color locus has been used to size the extent of deletions [21, 72, 73]. This heterozygosity loss has been directly related to spontaneous deletions involving the functional color locus allele and resulting in hemizygosity at the grape color locus, leaving only the null allele [15].

Altogether, these results demonstrate that intracultivar color variation appearing in either white or colored cultivars is mostly associated to structural variation at the color locus on linkage group 2, in combination with cellular events generating different chimeric situations and color patterns. Gain of color variants generally correspond to recombination events within the locus that generate gain-of-function mutations and dominant phenotypes. Loss of color variation seems to be restricted to black-berried cultivars heterozygous for a functional allele at the color locus and is associated with different deletions or complex chromosomal rearrangements eliminating this single functional copy [15]. Based on this information, bud irradiation with physical mutagens increasing the frequency of recombination and deletion could be a strategy to generate new color variants in grapevine.

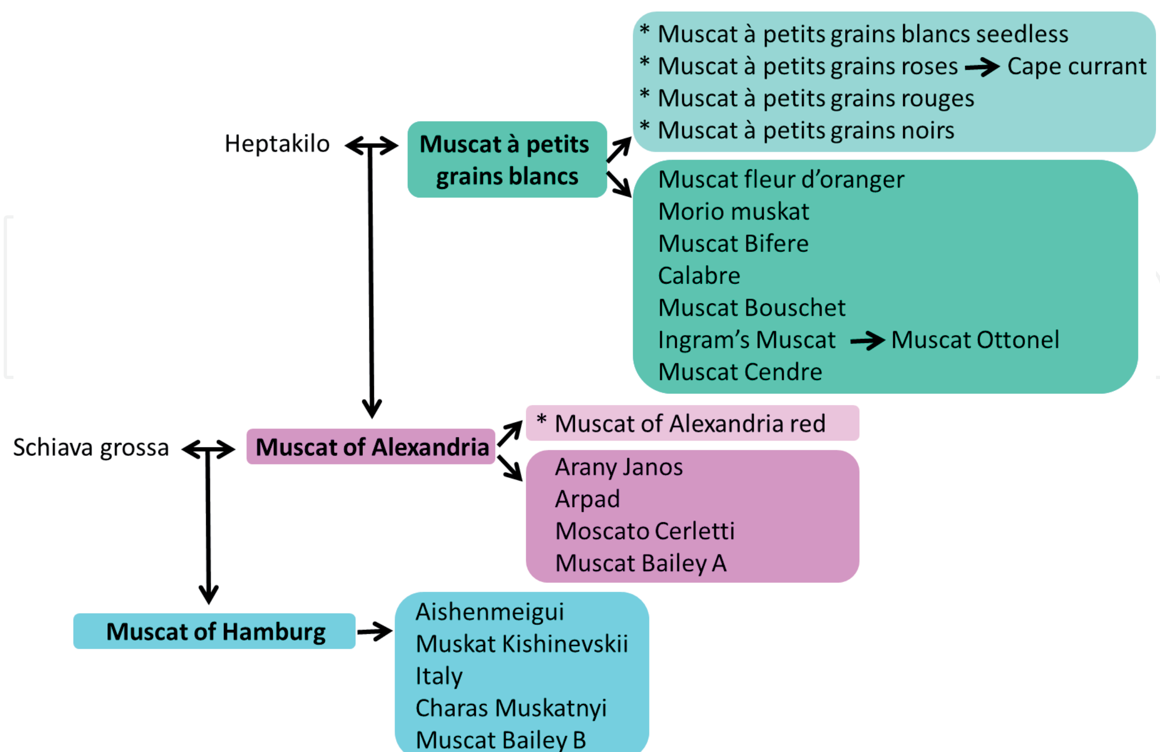

Figure 2.
Genetic relationships among Muscat varieties. Muscat à petits grains blancs is the progenitor of ancient variety Muscat of Alexandria and the putative ancestor of all the Muscat varieties. From them, additional Muscat varieties are derived by spontaneous or directed hybridizations (see [55] and Vitis *International Variety Catalog (http://www.vivc.de)) as well as through somatic variation (*).*

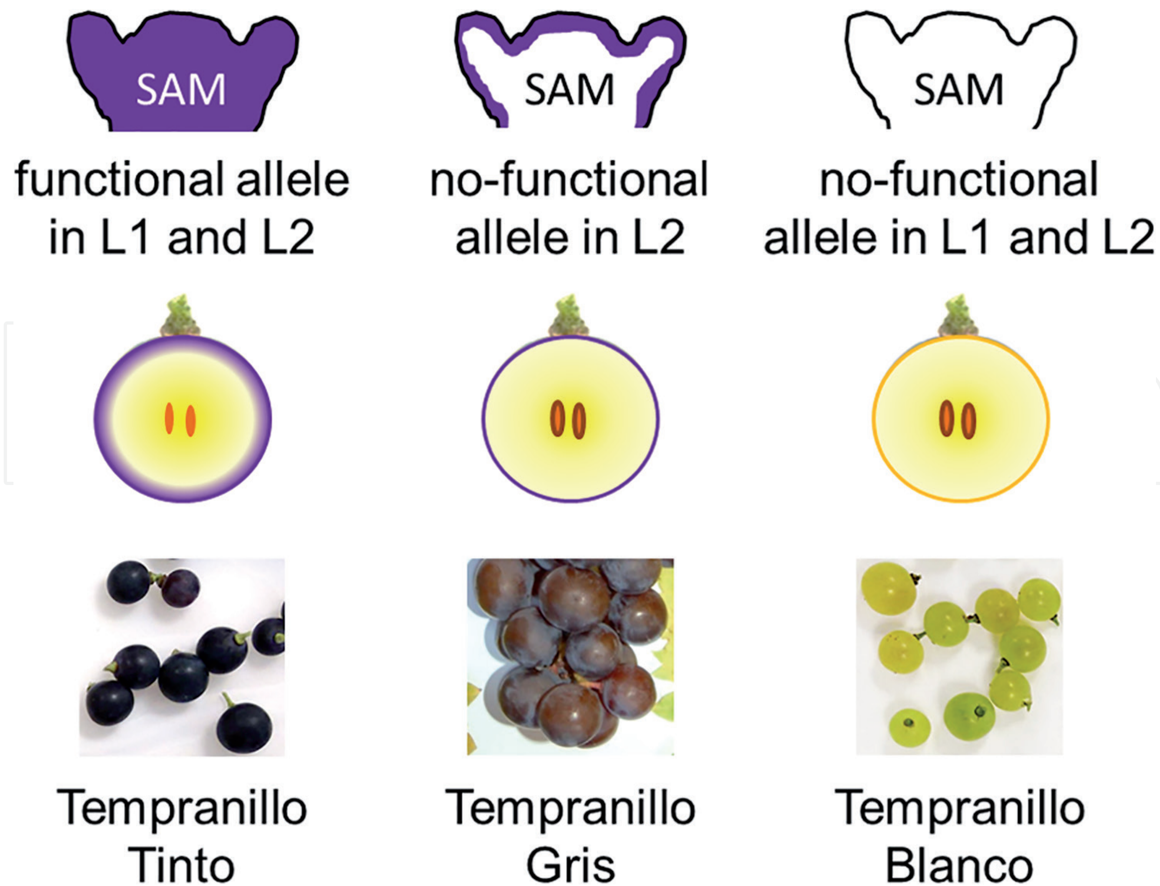

Figure 3.
Proposed genetic composition of shoot apical meristem (SAM) and berry color in Tempranillo somatic variants. L1 (outer) and L2 (inner) layers are represented in SAM, purple color indicates that cells in the layer carry a functional allele at the color locus and white color indicates the lack of functional color alleles in the cells. One functional allele in both meristematic layers is enough to develop black berries, while periclinal chimera with a mutant L2 cell layer in the SAM gives rise to gray color berries, the lack of functional alleles in both meristem layers yields white berries.

4.4 Seedlessness

Grapevine seedlessness is one of the best examples of cultivar innovation resulting from original somatic mutation in table grapes. Somatic variants defective in seed development appeared spontaneously along the history of grapevine cultivation and they have been propagated vegetatively [6]. Seedless variants in grapevine are classified into two major classes: (i) parthenocarpy, when fruits are set and develop without fertilization resulting in small berries free of seeds [75] and (ii) stenospermocarpy, when fertilization and embryo formation is not altered but later seed development is aborted [76]. Parthenocarpic varieties have been widely used for the production of Corinto seedless raisins, but, as their sterility makes sexual transmission of the causal mutation impossible, the use of this trait remains limited to those genotypes in which parthenocarpy appeared spontaneously. Recent work in Corinto Bianco, a parthenocarpic variant derived from Pedro Ximenes cultivar [75], has pointed out to meiotic alterations precluding the development of viable gametes as the origin of the mutant phenotype [29]. On the other hand, an ancient somatic mutation producing a stable stenospermocarpy phenotype likely emerged in a white-berried oriental cultivar known as 'Kishmish,' also known as 'Sultanina' or 'Thompson Seedless' [77]. Since the mutation responsible for stenospermocarpy has a lower impact than parthenocarpy in berry size and does not lead to sterility (pollen is fertile and embryos can also be rescued

from seed traces), it has become the major source of seedlessness in table grape breeding [13, 78, 79] (**Figure 4**).

The stenospermocarpy phenotype has been associated with abnormal development of the inner ovule integument [80], which ends in impaired development and lack of lignification of maternal seed coat tissues [81]. Genetic analyses of seedlessness trait in several F1 progenies derived from at least one stenospermocarpic progenitor identified segregations that could be explained by the presence of a dominant locus named Seed Development Inhibitor (*SDI*) interacting with several recessive loci [82, 83]. Later, quantitative genetic analyses identified the *SDI* locus as a major QTL on linkage group 18, explaining up to 70% of the phenotypic variance for different seed variables [84–87]. Based on co-localization of this QTL with a grapevine homolog of the Arabidopsis MADS-box transcription factor gene *AGAMOUS-LIKE11* (*AGL11*), responsible for ovule morphogenesis and seed coat differentiation [88], *VviAGL11* was considered the best candidate gene for the *SDI* locus [86, 87]. More recently, using an independent positional study combined with targeted sequencing in a large collection of seeded and stenospermocarpic grapevine cultivars, a single nucleotide missense mutation in *VviAGL11* was identified as the causal origin of the dominant seedless phenotype [26]. This mutation causes the substitution of a conserved arginine 197 into leucine (**Figure 1**), which could disrupt the function of multimeric complexes containing *VviAGL11* proteins in a dominant manner. Interestingly, amino acid sequence variants of oil palm *AGL11* homologs have also been selected in this crop to reduce the level of seed coat lignification [89]. Apart from the relevant application of the identification of the causal point mutation in *VviAGL11* to develop efficient marker-assisted selection strategies for seedless grape breeding, this information paves the way to the development of targeted genome editing for the genetic improvement of seedless table grapes. Stenospermocarpic seedlessness could also be useful in black-berried wine grapes as a way to avoid the negative effects of unripe seeds in the sensory quality of red wines [90]. Ripening imbalance between pulp and seeds can become a problem under climate change conditions [3], what could be addressed with the use of

Figure 4.
Red globe and crimson seedless fruits.

seedless wine varieties. Finally, editing of *AGL11* homologs could also be useful to generate seedlessness in other fruit crops.

5. Final considerations on the use of somatic variation

The application of NGS to the study of somatic variation in grapevine is increasing our knowledge on the nucleotide sequence variation underlying phenotype variation. By direct comparison of somatic variants, this technology has the potential to identify causal candidates at the gene and gene variant levels. Regardless, genetic and molecular approaches are still required to confirm the role of those candidates. So far, NGS approaches have been used to unravel widely used classical phenotypes as those described along the chapter. When combined with genome edition technologies, they constitute new tools for the genetic improvement and adaptation of traditional elite grapevine wine cultivars.

The first conclusion that comes out from the review of currently available information in grapevine is that due to the essential heterozygous condition of emergent somatic mutations, only dominant mutations can generate somatic variant phenotypes. More frequently, these dominant mutations involve gains of function resulting from either SNV that generate nonneutral amino acid substitutions [26, 46, 47] or gene overexpression and misexpression caused by transposon insertions [42, 43] or recombinations [41, 69]. Loss of function mutations has also been described but so far only in the case of SV that unmasks the effect of recessive null alleles present at the color locus in cultivars that are heterozygous for functional and null alleles of the responsible *MYBA* genes [15]. Another interesting conclusion relates to the particular relevance that chimeric expression of the mutations can have in the generation of specific cultivars such Meunier or the gray-berried variants. These examples show once more how the same mutation can lead to different phenotypes depending on the meristem cell layers affected.

Dominant gain-of-function mutations identified in grapevine somatic variants exemplify how new gene functions can be created by mutations changing expression to different cell types, developmental stage, or transcription levels, o by the alteration of a key amino acid in functional protein domains. While the effects of loss of function mutations are generally easy to predict when the function of the affected genes is known, gain of function is much more unpredictable and represents a source of innovation that can create new possibilities for genetic improvement. Their dominant nature makes them especially useful not only for the improvement of traditional cultivars but also to breed new cultivars. Systematic screening of the large clonal germplasm hosted in old vineyards and collections of ancient accessions of traditional cultivars can unveil very relevant information and variant traits to be exploited in conventional, genomics-assisted, or genetic engineering-mediated breeding.

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Conflict of interest

The authors declare no conflict of interest.

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