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Composition of grape and wine from resistant vine varieties

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Abstract

Abstract: Obtaining resistant varieties to diseases without loss of organoleptic quality is a real challenge for oenology. Inter-specific hybridization of grapevines began in the 19th century and was initially aimed at introducing pest and disease resistance in offspring. Later, several breeding programmes implemented worldwide led to the development of varieties showing different characteristics such as cold-hardiness, short/long growing season, and pest resistance. *Vitis vinifera* grapes have preferred flavour characteristics for wine production, but they tend to be susceptible to pests, diseases, and extreme temperatures; species native to North America and East Asia are generally better adapted to these stressors. But these wild species tend to be low yielding and produce wines with undesirable sensory characteristics, including high acidity, low astringency, and excessive herbaceous or undesirable aromas. To be an innovative revolution during the 21st century, resistant varieties (actual and future) should be tested in different contexts for 3 major points: (i) vines can produce grapes without pesticides; (ii) grape and wine quality (chemical and safety quality) needs to be compared with parent vine sources, and a referential of chemical composition for secondary metabolite families (aromas, phenolics, potential spoilage compounds, etc.) has to be realized for the new resistant varieties after different winemaking processes; and (iii) wines need to be tested (with mapping technique) for sensory perception and classified according to their quality level after winemaking and during the ageing process.

Keywords: grapes, wine, composition, quality, resistant varieties

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Introduction

The European grapevine belongs to the botanical species *Vitis vinifera*, which is the most widely used around the world for wine production (about 7.4 million ha; FAO, 2002). Unfortunately, *V. vinifera* grapes are susceptible to various diseases, mildew (Reisch *et al.*, 2012) and insect damage, Phylloxera in particular, and despite being grafted onto resistant rootstocks, they need frequent treatments against several pathogens. Until recently, the range of pesticides available (fungicides and insecticides) ensured that winemakers enjoyed a high quality harvest while protecting yields. It is only relatively recently that the winemaking sector has become aware of the need to reduce the use of pesticides. Winemakers and their workers have often been unaware of the risks to their health. The 2001ban on the use of sodium arsenite (a carcinogenic product) in the winemaking sector to treat the main vine trunk disease (Esca) only served to confirm the general criticisms levelled at pesticides by ecologists. There were also some complaints reported by the press with regard to a slight intoxication suffered by school children when the product was sprayed near their school. The data concerning the proportion of pesticides used by the winemaking sector in Europe have also heaped further disgrace on the sector. The French Ministry of Agriculture launched the Ecophyto 2018 plan with a view to satisfying this social demand, requiring a commitment from the stakeholders to reduce the use of pesticides across the country by 50%, if possible within a time frame of 10 years. The fear of facing legal proceedings, as in the case of asbestos, for having failed to provide agricultural workers with sufficient protection against these risks further increased awareness within the sector, drawing operators' attention to all possible improvements in this sphere as well as the potential contributions of new resistant varieties. The reduced use of pesticides in the winemaking sector has therefore come to represent an essential social demand (Montaigne *et al.*, 2016). Massive use of pesticides is unsustainable from an environmental and economic point of view, and leads to pesticide resistance (Barnaba *et al.*, 2017). Inter-specific hybridization of grapevines began in the 19th century and was initially aimed at introducing pest and disease resistance in offspring (Galet, 1999). Later, several breeding programmes implemented worldwide led to the development of varieties showing different characteristics such as cold-hardiness, short/long growing season, and pest resistance (Reynolds, 2015). From a historical standpoint, innovation in wine-growing plant material has focussed more on sanitary and clonal selection since 1962 than on plant breeding. This line of research has transformed the value chain by eradicating the main viral diseases and thus supplying the entire world with plants boasting unparalleled levels of productivity and quality. The new varieties produced by the cross-breeding programmes launched in 1956, at least in France, did not have this impact. Since 1974 for the pioneering work of Alain Bouquet, and since the beginning of the new millennium for French viticulture research, the increasing social demand for sustainable development and reduced pesticide use has renewed the technological paradigms of plant breeding along with the attention paid by policy-makers and researchers to this road to progress that had been almost completely abandoned in France (Montaigne *et al.*, 2016).

Few examples of studies

1. French studies

In the 1970s, Alain Bouquet (INRA Montpellier) extracted genes from *Muscadinia rotundifolia* offering resistance to numerous parasites and diseases, including mildew and powdery mildew. He then applied the classic method of back-crossing with traditional high-quality *vinifera* varieties. The ResDur programme (standing for RESistance DURable, or sustainable resistance) took over the new varieties created by Alain Bouquet with the resistances derived from *Muscadinia*, which it combined with German varieties, Regent and Bronner, which drew their resistance from American or Asian *Vitis* species. The aim of this programme is to take account of the study results showing that combining several resistance genes (referred to as gene pyramiding) improves resistance and reduces the risk of it being circumvented.

Several candidate resistant varieties have been registered in the French catalogue in 2016. Other candidates should follow between 2020 and 2023. The CTPS (Standing Technical Selection Committee) responded favourably to the pressure from the professionals by offering the possibility of already planting resistant varieties. However, the philosophy of the INRA (French National Institute of Agricultural Research) is still to maintain the pyramiding system and to disseminate varieties exhibiting polygenic resistance, despite the scientific controversies surrounding this subject. On the 1st of June, 2016, 4 varieties (Muscaris B, Souvignier G, Monarch N and Prior N) got a favourable decision for a definitive inclusion in the catalogue, 7 varieties

(Bronner B, Johanniter B, Solaris B, Saphira B, Cabertin N, Pinotin N and Divico N) were temporarily included to the catalogue, and 3 varieties (Cabernet Blanc, Cabernet Cortis N and Cabernet Jura N) got a potential favourable decision for classification with the hypothesis of their denomination change. On the 29th of September, 2016, 4 varieties from the ResDur1 INRA programme - two red (IJ58 and IJ134) and two white varieties (Col-2011G and Col-2007G) - were temporarily registered. Recently (17th of January, 2017), 7 monogenic Bouquet varieties from INRA received a temporary registration in the classification within the framework of the National Observatory for the Deployment of Resistant Grape Varieties (OsCaR).

2. Italian studies

At the IGA (the Istituto di Genomica Applicata at the University of Udine), work began again at the beginning of the new millennium. Selection was based on the crossing of local or international varieties, such as Tocai, Sauvignon Blanc, Merlot and Cabernet Sauvignon, with German or Hungarian varieties already "introgressed" and known for their resistance: Bianca, Regent or 20-3. Ten resistant varieties have thus been presented for registration in the Italian catalogue: Fleurtai, Soreli, Early Sauvignon, Petit Sauvignon, Sauvignon Doré, Petit Merlot, Royal Merlot, Petit Cabernet, Royal Cabernet and Julius. In collaboration with the University of Udine, VCR (Vivai Cooperativi Rauscedo), the leading nursery cooperative in Italy, is testing the new varieties resistant to cold weather or fungal diseases (monogenic or polygenic). The new varieties are of the Chardonnay, Cabernet and Sangiovese type. Thirty-four hybrids are in the process of being tested. These varieties will compete with or complement the range of new varieties available to French winemakers. The development of contracts between the researchers and the nurseries is certainly favourable to a rapid dissemination process.

3. Brazilian studies

V. vinifera is the grape most used for winemaking throughout the world; in Brazil, wines elaborated from American grapes (mostly *V. labrusca*) and/or hybrid grapes obtained from crosses between *V. vinifera* and American/hybrid species have mostly surpassed those made from *V. vinifera* grapes. These wines, known as "table wines", represent more than 80% of all the wines produced in Brazil, the production being over 210 million liters in 2012 (IBGE, Banco de dados agregados, 2013). This is probably due to the fact that in several Brazilian regions, the climatic conditions are unfavourable for the growth of *V. vinifera*, since the ripening and harvest of the grapes occurs during the rainy season (Hamada *et al.*, 2008). Some red wines have been produced from *V. labrusca* and/or hybrid grapes: Ives (*V. labrusca*), Isabella (*V. labrusca*), Máximo (hybrid grape from Syrah and Seibel 113432), Sanches (hybrid grape from Máximo and IAC 577-8), Seibel 2 (hybrid grape from Alicante Bouschet and *V. lincecumii*); and one wine sample produced from a *V. vinifera* grape known as Barbera (Camarão Telles Biasoto *et al.*, 2014). The acceptability and preference drivers of red wines produced from *V. labrusca* and hybrid grapes were explored. In their conclusions the authors found that in general wines containing the *V. labrusca* grape Ives showed more intense aroma/flavour notes described as sweet, grape, grape juice, blackberry and roses. In turn, wines produced with the hybrid grape Máximo were characterized by a greater intensity of earthy/mushroom, vegetative/green beans, woody and yeast sensory notes. The aroma and flavour notes described as grape, grape juice, blackberry and sweet, and the aroma note described as alcohol can be considered as preference drivers for red wines amongst the consumers who participated in the test. On the other hand, the majority of the consumers did not like the sensory notes described as earthy/mushroom, yeast and vegetative/green beans found in some wines, notably those elaborated with the *V. vinifera* Barbera and Máximo grapes. Overall, consumers also disliked the aroma/flavour notes described as dried fruit, such as raisin and fig. Together with the wine produced with Seibel 2 grapes, all the wines containing the hybrid grape Máximo showed the highest total phenolic contents as compared with the wines that did not contain these grapes, in spite of the fact the beverages were produced in different wineries. Significant positive correlations were found between total phenolic compound content and "seed" flavour, astringency, bitterness and body. The wines elaborated exclusively with the hybrid grape Seibel 2 or containing the *V. labrusca* varieties Ives and Isabella were preferred by the majority of the consumers. Thus, these represent promising grapes for the production of quality wines in Brazilian regions where the environmental conditions are not good for the growing of *V. vinifera* grapes. The wines elaborated solely with the hybrid grape Máximo were appreciated by a minor segment of consumers. Therefore, the use of this grape in Brazilian wines deserves additional studies.

3. Other studies

The distribution of free and glycosidically bound phenols in the pulp, skin and seed of 4 hybrid (red, Cabernet Cantor and Prior; white, Muscaris and Solaris) and 2 European grape varieties (red, Merlot; white, Chardonnay) and the phenolic profiles of wines produced from hybrid varieties was investigated (Barnaba *et al.*, 2017). Authors mentioned that due to lack of information about glycosidically bound simple phenols in grapes, it was only possible to confirm the data reported for European grapes in hybrids (Perestrelo *et al.*, 2012; Di Lecce *et al.*, 2014) for gallic acid-hexose, gentisic/protocatechuic acid-hex, p-coumaric acid hexose and p-carboxyphenol-hexose. The glycosylated phenolic profiles of wines from hybrids corresponded with the 70% of those reported by Barnaba *et al.* (2016) for European wines, although in the former the number of detected glycosylated phenolic compounds was more than twice as high, possibly due to hydrolysis phenomena occurring during the ageing of the latter (Primitivo and Negroamaro wines).

Variation of grape and wine composition in the frame of disease-resistant varieties

To model grape and wine quality from resistant vine varieties, it is necessary to understand the genetic, chemical and biochemical basis of wine quality attributes related to consumer preferences for colour, aroma, taste and texture. For this purpose, it is also necessary to measure grape and wine quality, including attributes that are desirable and undesirable to consumers. A last aspect concerns the identification of viticultural and winemaking practices that produce grape and wine with desirable characteristics. *V. vinifera* grapes have preferred flavour characteristics for wine production, but they tend to be susceptible to pests, diseases, and extreme temperatures; species native to North America and East Asia are generally better adapted to these stressors. For example, *V. riparia* can tolerate winter temperatures down to -40 °C and *V. muscadinia* is resistant to several diseases capable of devastating *vinifera* (e.g., Pierce's disease caused by an insect-transmitted bacteria) (Reisch *et al.*, 2012). However, these wild species tend to be low yielding and produce wines with undesirable sensory characteristics, including high acidity, low astringency, and excessive herbaceous aromas. Native American grape (*Vitis*) species have many desirable properties for winegrape breeding, but hybrids of these non-*vinifera* wild grapes with *V. vinifera* often have undesirable aromas. Other than the foxy-smelling compounds in *V. labrusca* and *V. rotundifolia*, the aromas inherent to American *Vitis* species are not so well characterized. Some key odorants in wine produced from the American grape species *V. riparia* and *V. cinerea* were characterized in comparison to wine produced from European wine grapes (*V. vinifera*). Grape-derived volatile compounds with fruity and floral aromas were at similar potency, but non-*vinifera* wines had higher concentrations of odorants with vegetative and earthy aromas: eugenol, cis-3-hexenol, 1,8-cineole, 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP). Concentrations of IBMP and IPMP were well above sensory threshold in both non-*vinifera* wines. IBMP and IPMP were surveyed in 31 accessions of *V. riparia*, *V. rupestris* and *V. cinerea*. Some accessions had concentrations of >350 pg/g IBMP or >30 pg/g IPMP, well above concentrations reported in previous studies of harvest-ripe *vinifera* grapes. Methyl anthranilate and 2-aminoacetophenone, key odorants responsible for the foxiness of *V. labrusca* grapes, were undetectable in both the *V. riparia* and *V. cinerea* wines (<10 µg/L; Sun *et al.*, 2011).

For example, table 1 gives representative reported concentrations of some key wine components that differentiate typical red *vinifera* varieties from *V. riparia* and *V. labrusca* (Concord) (Waterhouse *et al.*, 2016).

Anthocyanidins are widely present in the plant kingdom, mainly in the form of glycosides, especially in black/red grape skins, where they are responsible for the colours red, blue, and purple depending on the

Table 1 - Representative concentrations of key wine components in different *Vitis* varieties

Wine component	*Vitis vinifera*	*Vitis riparia*	*Vitis labrusca*	Correlation/Threshold
Titratable acidity (g/L as tartaric)	5-6,5	35	9,5	sourness
Condensed tannin (mg/L catechin equivalents)	500-700	<50	<50	astringency / colour stabilization
3-Isobutyl-2-methoxypyrazine (ng/L)	mars-17	56	ND	“Green pepper ” detection threshold = 2 ng/L
Methyl anthranilate (µg/L)	0,06- 0,6	ND	600- 3000	“Grape Kool-A id ” detection threshold = 300 µg/L

pH of the cell compartment (Burns *et al.*, 2002). The most common anthocyanidins are pelargonidin, cyanidin, delphinidin, peonidin, and malvidin, but these compounds are only present under their glycosylated forms, called anthocyanins. Anthocyanidins are also capable of conjugation with hydroxycinnamic acids (e.g., p-coumaric acid, caffeic acid) and other organic acids (e.g., malic and acetic acids). Unlike other species, North American *Vitis* species have significant levels of diglycosylated anthocyanins in positions C3 and C5; *V. vinifera* contains only traces of these and is characterized by the presence of a majority of anthocyanin monoglucosides, particularly malvidin-3-O-glucoside and its acylated derivatives. Anthocyanins are present in red grapes at about 500-3000 mg/kg, but can reach higher values in "dyers" cultivars such as Alicante Bouschet (5000 mg/kg) in which the anthocyanin concentration in pulp is also high (Teissedre *et al.*, 2013).

We previously compared grape seed proanthocyanidin composition of Croatian *V. vinifera* spp. and different American *Vitis* spp. (Ćurko *et al.*, 2012). Important significant differences were observed not just in concentration of monomeric and oligomeric flavan-3-ols, but also in structural characteristics such as mDP (mean Degree of Polymerization) and %G (% of Galloylation). Significantly higher concentrations of monomeric and oligomeric flavan-3-ols were observed in both cultivars of *V. vinifera* spp. *V. doaniana* and *V. champinii* showed significantly high %G, which was not previously reported in the literature. Since there has been a lack of studies comparing differences in proanthocyanidin composition between species of the genus *Vitis*, this research provides basic data for future studies.

For total anthocyanins and anthocyanin 3,5-diglucosides, differences amongst red, white and pink grapes can largely be explained by allelic variation in a transcription factor (VvmybA1) responsible for the expression of a 3-O-glycosyltransferase (UDGT) involved in the final step of anthocyanin biosynthesis (This *et al.*, 2007). Similarly, anthocyanin 3,5-diglucoside production is not observed in *vinifera* due to a mutation in a 5-O-glycosyltransferase gene (Janvary *et al.*, 2009). Differences in methoxy-pyrazine (MP) formation between Cabernet Sauvignon (high MP) and Pinot Noir (low MP) could be explained by allelic variation in VvOMT3, a methyltransferase involved in the final step of MP biosynthesis (Dunlevy *et al.*, 2013). For monoterpenes, the higher monoterpene content of Muscat varieties could be explained by variations in the 1-deoxy-d-xylulose 5-phosphate synthase gene (*VvDXS*). Analogous genes are known to be responsible for the first steps of monoterpene biosynthesis in other plants (Emanuelli *et al.*, 2010).

Knowledge of the genes controlling fruit quality and other traits should assist grape breeders in their efforts to produce new varieties (Waterhouse *et al.*, 2016). However, how can the gene expression of phenolics and aroma precursors be affected by new disease-resistance genes introduced in *Vitis* species?

Assays carried out to improve the quality of wine from Fungus Resistant Grapes varieties (FRG) were primarily meant to reduce the occurrence of foxy flavours with the use of different winemaking processes (Pedneault and Provost, 2016). In 1974, carbonic maceration (CM) was found to efficiently reduce foxy flavours in red Concord (*V. labrusca*) wines (Fuleki, 1974). CM was initially developed to reduce oxidation reactions occurring spontaneously in grapes in order to preserve fruit flavours (Paul, 1996). In organic wine production, restricting contact between berries and air using CM may be of particular interest because organic grapes have been shown to carry twice as much polyphenol oxidase activity compared to conventional grapes (Núnez-Delicado *et al.*, 2005). White FRG wines such as Chardonel, Solaris and La Crescent generally present desirable floral notes that may be related to compounds such as C13-norisoprenoids (e.g., ß-damascenone) and monoterpenes (e.g., linalool) located in the berry skin (Cadwallader *et al.*, 2009; Savits, 2014; Liu *et al.*, 2015). In fact, extended skin maceration (24 h cold-soak and 30 h on-skin fermentation) significantly improved the intensity of floral notes in Solaris wines, but also increased green vegetable notes (Zhang *et al.*, 2015). Conversely, short cold-soaks (3-8 h) did not improve the aroma intensity of Traminette wine (Skinkis *et al.*, 2010). A recent study reported for the first time the presence of 3-mercaptohexanol in the FRG variety Cayuga, at a concentration of 195 ng/L (Musumeci *et al.*, 2015). This compound is a highly odour potent thiol (odour perception threshold: 60 ng/L) that produces a grapefruit aroma in white wine (Musumeci *et al.*, 2015).

Future research and alternatives

The question for winemakers in a near future is not which variety of resistant vine should they plant, but rather what choice of resistant variety for what types of wines: Generic, Protected Geographical Indication, Protected Origin Appellation?

1. A first important issue is the quality of the wine from the considered resistant variety

Several parameters can be involved in quality composition. Quality is the result of complex interactions between non-volatile (e.g., phenolics, polysaccharides, etc.) and volatile compounds (e.g., aromas, alcohols, etc.) as perceived in the nose and in the mouth. pH is also an important point for quality as some of these new resistant varieties can bring low acidity that can be a problem for wine perception, preservation and stability. The quality of a wine is in part a reflexion of what is perceived whether by a winemaker, an expert, or a consumer. Wine quality can, therefore, be defined as that wine style that is "liked/preferred" by a defined population of consumers.

2. A second important issue is the question of new names for the new resistant varieties

An approach is that of the new Italian varieties that are qualified by a double name, one of which is none other than the name of an outstanding and renowned variety, for example Merlot Kanthus, Cabernet Volos, Sauvignon Kretos, Cabernet Jura and Cabernet Cortis. On the one hand, the authorities want to protect consumers and not mislead them. On the other hand, the experience of the new cross-bred varieties of the 1980s, rejected by the promoters of varietal wines, makes professional leaders aware of the interest of playing on this renowned reputation. The idea they would like to promote is that it is more or less the same variety or an equivalent variety to which it has been added a fungal-disease resistance. What are other approaches on this point?

To be a future, innovative revolution during the 21st century (as the solutions provided to the phylloxera crisis in the 19th century), resistant varieties (actual and future) should be tested in different contexts for 3 major points:

(i) Vines can produce grapes without pesticides;

(ii) Grape and wine quality (chemical and safety quality) needs to be compared with parent vine sources, and a referential of chemical composition for primary and secondary metabolite families (aromas, phenolics, potential spoilage compounds, etc.) needs to be realized for the new resistant varieties after different winemaking processes; and

(iii) Wines need to be tested (with mapping technique) for sensory perception and classified according to their quality level after winemaking and during the ageing process.

In the context of climate change, we should also consider drought incidence for future resistance vine variety selection, as it should affect grape composition and wine quality.

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