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Moving towards grapevine genotypes better adapted to abiotic constraints

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Summary

Vitis spp., both in their cultivated and wild forms, have been growing in a large diversity of environments for thousands of years. As a result, they have developed many adaptive mechanisms controlled by a range of regulatory processes. The cultivated species, *Vitis vinifera*, is quite well adapted to semi-arid conditions and its cultivation can be used to produce crops on marginal lands. However, this is threatened by climate change, which is associated with increased temperature and CO_2 atmospheric content, changes in water availability and an increased likelihood of extreme events, such as heat waves and early spring frosts. Indirect effects of climate change on solar radiation and soil minerals are also expected. Consequently, cultivated grapevines will presumably face more abiotic constraints occurring concomitantly or successively over one or more growing cycles. In addition to climate change, worldwide viticulture must reduce the use of pesticides. Adapting to climate change and reducing pesticide use are challenging, and increase the need to create new grapevine varieties that are more resistant to diseases and better adapted to abiotic constraints. For this purpose, the adaptive mechanisms of wild and cultivated *Vitis spp.* must be exploited. While major advances have already been made in exploiting wild alleles for disease resistance, the polygenic nature of adaptation to abiotic factors has slowed down research progress. To tackle this limitation, ambitious integrative strategies need to be undertaken from collection and characterization of genetic resources, investigations on genetic architecture and identification of underlying genes (including those involved in epigenetic regulation), to the implementation of new breeding technologies and the development of genomic selection. An update on the state-of-the-art regarding these aspects is presented.

Key words

phenotyping, polygenicity, climate change, *Vitis*, diversity

Introduction

The grapevine, both wild and cultivated genotypes, is present in a large range of pedo-climatic environments, from hot dry deserts to tropical climates and very cold areas (Callen *et al.,* 2016; Walker *et al.,* 2019). This implies the existence of a broad spectrum of physiological mechanisms regulating the interactions with a wide range of environments. Being a thermophilic species, *V. vinifera sylvestris*, the ancestor of cultivated *V. vinifera sativa*, was restricted to the Mediterranean regions and the refuge zones south of large mountain ranges during the quaternary ice age (Bouby *et al.,* 2013; Mariani *et al.,* 2018). For American *Vitis spp.*, water is considered as a key factor in habitat restriction. Even in desertic regions, wild grapevines will be found where water sources exist (Walker *et al.,* 2019). As a cultivated species in traditional viticulture countries, the forms of *V. vinifera sativa* most adapted to local environmental conditions have generally been selected over centuries by farmers in order to achieve specific quantitative and qualitative objectives. Growing practices have also permitted the culture of varieties outside of their region of origin, especially when they were exported to other countries in America or Asia through human migrations or commercial roads.

The ongoing climate change is threatening the suitability of actual grape growing areas. The greenhouse gas (GHG) concentrations have now reached 410 ppm of CO_2, 1866 ppb for CH_4 and 332 ppb for N_2O in 2019 (IPCC, 2021). The magnitude of recent changes is much greater than that of previous centuries or millennia. Each of the last four decades has been successively warmer than any other decade, since 1850. According to the last IPCC report (2021), the increase of global

surface temperature over land has reached, in average, 1.6 °C during the 2001-2020 period in comparison to 1850-1900. Projections for the future foresee that global surface temperature is expected to continue to rise for all GHG emission scenarii and could reach + 3.3 °C to + 5.7 °C in the most pessimistic scenario. As global warming increases, many other climate parameters will be affected by the frequency and intensity of extreme temperatures, heat waves, heavy precipitations and drought. Very wet and very dry weather events and seasons will become more frequent, but precipitation projections remain uncertain. Some mid-latitude and semi-arid regions are expected to experience high temperature increase on hot days and agricultural drought with high confidence. In addition, it is also projected that each region will face more concurrent and multiple changes in climate impact-drivers. The number of drivers affected increases with the intensity of global warming, with all regions experiencing changes in at least 5 drivers and 50 % in at least 15 drivers. This will have major consequences, among others on agriculture and food production systems (Ollat *et al.*, 2022). Indirect agronomical effects of climate change on soil properties, as well as spring frost events and biotic interactions should also be taken into account.

The use of alternative varieties, for scion and as for rootstock, better adapted to environmental stress conditions, is a major lever to tackle the impacts of climate change on grapevine (Delrot *et al.*, 2020; Duchêne *et al.*, 2016). These could be existing varieties that are already growing under more extreme conditions. Another option would be to create new varieties that would combine traits of adaptation to abiotic stresses, appropriate berry composition, and disease resistance properties to achieve the goals of both adaptation to climate change and low environmental impact (Töpfer and Trapp, 2022).

While much information has been gathered over the past decades on the genetic control of disease resistance in grapevine (Vezzulli *et al.*, 2022) and, to a lesser extent on yield components and berry composition (Delrot *et al.*, 2020; Gomès *et al.*, 2021), knowledge regarding adaptation to abiotic stresses that could be useful for breeding purposes remains very fragmented. The complexity of the constraints and biological mechanisms of the responses, as well as the polygenic nature of their genetic architecture, largely explain the difficulties in linking phenotype to genotype for adaptive traits. However, with the increasing number of sequenced genomes, the development of new phenotyping and integrative approaches, and the prospects offered by genomic selection and new breeding technologies, we can hope to reach major progress before 2030. Recently published studies illustrate this trend and will be summarized hereafter.

A complex issue

Abiotic stresses are defined as environmental conditions that reduce growth and, for a crop, yield (Cramer *et al.*, 2011). For a cultivated species such as grapevine, adaptation should lead to the maintenance of an acceptable yield and of berry composition that allows for their transformation into a marketable product. For a perennial crop, adaptation is also related to sustainability (Ollat *et al.*, 2019). Abiotic factors include water and chemical resource (nutrient, CO_2) availability, temperature and light. The timing, intensity and duration of stress events throughout the life cycle are also parameters that strongly affect plant responses. Although elevated CO_2 is expected to enhance growth and yield in C3 species such as grapevine, some authors consider that it could also be a stressor due to its effect on the redox status of the plant (Foyer and Noctor, 2020) and its negative impacts on nutrient acquisition and assimilation (Gojon *et al.*, 2022). In addition, any stress is rarely occurring alone and climate change is characterized by multi-stress aspects (Mickelbart *et al.*, 2015), including biotic stresses. When exposed to these stresses, plants respond in a complex and dynamic way that may or may not be reversible. Responses may also be organ-specific and may not necessarily lead to better adaptation at the plant level. In addition, the response to a combination of stresses is unique and can be much more complex than the response to a single stress. Interactions can be synergetic, antagonistic, or additive, and it is usually difficult to infer the effects of multiple stresses from studies of a single stress (Suzuki *et al.*, 2014; Zandalinas *et al.*, 2021a). The most cited example is the antagonist effect of drought and heat stresses, which induce a decrease and an increase in stomatal conductance respectively, when they occur individually. The combined effect usually results in a dominant effect of drought with a more severe impact of the heat when associated to drought (Suzuki *et al.*, 2014). A compensatory effect on plant responses to heat and drought could be provided by the beneficial contribution of elevated CO_2 on net C uptake (Birami *et al.*, 2020). Recently, a large multi-stress study in *Arabidopsis thaliana* showed that a specific combination of genes results in a unique molecular signature, with the number of unique differentially expressed genes increasing with the number of environmental factors combined (Zandalinas *et al.*, 2021b). However, common pathways linked to abscisic acid (ABA) signalling, basal thermotolerance, and regulation of iron, sulfur and ROS appear to be central for multi-stress responses (Zandalinas *et al.*, 2021b). At the metabolic level, primary metabolites such as gamma-aminobutyric acid (GABA), secondary metabolites and hormones are targets for improving plant tolerance under climate change (Zandalinas and Mittler, 2022). In grapevine multi-stress studies are rare (Ollat *et al.*, 2022). Recent publications confirm the greater impact of combined stresses and the unicity of some responses (Ju *et al.*, 2021; Tan *et al.*, 2023).

Abiotic stresses induce a cascade of responses and regulation pathways from the cellular to the whole plant level, which will affect the cellular homeostasis, induce metabolic reprogramming and have both developmental and functional consequences. Primary stresses can lead to secondary stresses such as osmotic and oxidative stresses, the latter being a common type of response (Carvalho *et al.*, 2015; Chaudhry and Sidhu, 2022). Depending on the genotype, these responses will be integrated at the plant level and result in a tolerant phenotype where growth, yield, harvested organ composition and survival rates are maintained. As previously mentioned (Ollat *et al.*, 2019), adaptation can result from a combination of favorable alleles that lead to a constitutive phenotype or a plastic phenotype.

Phenomics and modelling to address the complexity

Usually adaptation is not linked to a single trait whether it is integrated or not. Morphological, structural, physiological and biochemical traits characterise a genotype at a given stage in a given environment. Appropriate statistical approaches must then be implemented to analyse the relationships, covariations and trade-offs between the traits themselves, as well as with environmental variables, in order to identify the most relevant combination of traits (so-called ideotypes) associated with adaptation in a given environmental scenario (Granier and Vile, 2014). Furthermore, typical traits of interest, such as yield, result from the integration of many physiological processes that act at the interface between genotype and phenotype. Because the genetic control of such integrated traits and environmental responses is complex (as reviewed for trees by Lind *et al.*, 2018), characterizing intermediate (or endo) phenotypes, such as the metabolome, proteome, or transcriptome, can help establishing the causal link between genotype and phenotype (Großkinsky *et al.*, 2015). For grapevine, the main traits of interest involved in abiotic stress responses and adaptive processes are summarized in Table I. Most traits should be considered for their absolute values but also for their plasticity to environmental conditions (or grafting partner in the case of a grafted grapevine) which adds another level of complexity (Ollat *et al.*, 2019).

To assess this complexity, multi-scale strategies that link physiological mechanisms with plant performances are requested (Tardieu *et al.*, 2017). Phenomics is defined as the systematic study of phenotypes and refers to the characterization of phenotypes via the acquisition of high-dimensional phenotypic data (Houle *et al.*, 2010), taking into account the spatio-temporal variations from cell to canopy and short to long term scales (Tardieu *et al.*, 2017). Over the past decade, major technical improvements in sensor development, imaging and data handling have increased capacities to generate high-throughput phenotypic information. Grapevine genetic research benefits from these advances (Cadle-Davidson *et al.*, 2019). So far, they have been applied primarily to assess developmental and morphological traits such as phenology, leaf area and wood parameters (Kicherer *et al.*, 2017; Grimm *et al.*, 2019; Chedid *et al.*, 2021). Greenhouse phenotyping platforms have also been mobilized. In addition to studying the responses to water deficit (Marguerit *et al.*, 2012; Coupel-Ledru *et al.*, 2016), Dunlevy *et al.* (2022) monitored the response of rootstocks to a combination of heat and salinity treatments using a high-throughput phenotyping platform. Phenotyping based on hyperspectral reflectance has a strong potential to characterize physiological responses at high throughput (Grzybowski *et al.*, 2021). These approaches are being evaluated for grapevine (De Bei *et al.*, 2011; Rustioni *et al.*, 2016; Tosin *et al.*, 2021; Ryckewaert *et al.*, 2022), with very promising results for Near Infra-Red Spectroscopy (NIRS)-based technologies (Coupel-Ledru and Segura, personal communication). Field phenotyping, especially under various locations, is an appropriate way to assess genotype × environment interactions and multi-stress impacts. It requires high quality geo-referenced data to describe weather, soil characteristics and growing practices (Rosenqvist *et al.*, 2019). Process-based models are companion tools of phenotyping, used to dissect integrated phenotypes in a set of intermediate mechanisms with simpler genetic control and environmental effects (Vivin *et al.*, 2017). Modelling can also

Table 1: list of traits of interest to characterize the phenotype of grapevine in interaction with its abiotic environment (Gomès *et al.*, 2021; Dayer *et al.*, 2020; Carvalho *et al.*, 2019; Pettenuzzo *et al.*, 2022; Darriaut *et al.*, 2022)

Integrated traits	Developmental traits	Physiological and metabolic traits
Yield and yield components	Phenology	Photosynthesis
Vegetative biomass	Stomatal number and shape	Chlorophyll content and fluorescence
Fruit quality	Root number and diameter	Stomatal conductance
Grafting success	Root system architecture	Leaf temperature
Capacity to recruit microorganisms	Shoot and canopy architecture	Water use efficiency
…	Active leaf area	Hydraulic traits
	Callus formation	Electrolyte leakage
	….	Osmoprotectants
		ROS production
		Mineral content
		Metabolic content
		Hormone content
		Anti-oxidant production
		Anti-oxidant activities
		Heat shock proteins
		…

be used to integrate the mechanisms, make predictions and test hypotheses regarding the most relevant traits (Tardieu *et al.*, 2017). Combining modelling with phenotyping and genetic analyses in grapevine is still rare (but see Marguerit *et al.*, 2012; Coupel-Ledru *et al.*, 2014; Duchêne *et al.*, 2020). Recent studies on cold hardiness (Kovaleski *et al.*, 2018), drought responses (Dayer *et al.*, 2022) and root system architecture (Vivin, personal communication), based on modelling approaches, provide promising perspectives.

Diversity remains the key entry

Considering the large diversity within the Vitis genus, both at the intra- and inter-specific levels, occupying a wide range of habitats (Walker *et al.*, 2019), there is no doubt that adapted genotypes and favourable alleles for adaptation to adverse environmental conditions can be identified, both in *V. vinifera* and other *Vitis spp*. In addition to collecting, protecting and characterizing this diversity at the genomic level, one of the main challenges remains its phenotypic evaluation. Phenology is one of the most recorded traits across germplasms, with reports on more than 2000 *V. vinifera* varieties (Boursiquot *et al.*, 1995), or smaller panels (Parker *et al.*, 2013; Destrac-Irvine *et al.*, 2022; Koufos *et al.*, 2020). Biochemical characteristics of the grapes such as sugar, organic acid and polyphenols contents were also described on diversity panels to assess whether they could be compatible with warmer growing conditions. Bigard *et al.* (2018) reported large variations in berry size, sugar content and malic to tartaric acid ratio, even on a small panel including seedlings from an intra-vinifera progeny and traditional varieties. Suter *et al.* (2021) classified 52 *V. vinifera* varieties from a common garden experiment in Bordeaux on their sugar accumulation rate, and berry sugar content and concentration at ripeness. A large European survey for berry composition traits was also performed on about 2400 accessions growing in 20 germplasm collections over 15 countries (Rustioni *et al.*, 2019) resulting in a huge data base which could be very useful to identify cultivars of interest. However, very few investigations deal with the evaluation of genotypes in relation to abiotic stresses responses. Among the few published studies, Londo and Johnson (2014) characterized the chilling requirement and budburst rate of 27 accessions of wild *Vitis* and cultivated varieties and Xu *et al.* (2014) the heat tolerance of 47 genotypes. A recent publication focused on several leaf stomatal features, intrinsic water use efficiency (iWUE) and chlorophyll fluorescence across 49 genotypes from a *V. vinifera sativa and sylvestris* pool (Faralli *et al.*, 2022). A large variability was observed for all measured traits. No significant difference was detected between sub-species for stomatal density and chlorophyll fluorescence, whereas *V. sativa* had larger stomata and higher water use efficiency than *V. sylvestris*. No significant correlation was observed between most anatomical and functional traits, leading to the conclusion that heat tolerance is controlled independently of water use efficiency. Different strategies were identified across the panel. The combination of high iWUE and low heat stress sensitivity was associated with different stomatal responses to light and VPD. Another multi-trait study was conducted to characterize the effects of water deficit and rootstocks on grafted grapevine (Marguerit

et al., unpublished). Several traits related to aerial and root development, and plant transpiration were recorded on 55 rootstocks grafted with 'Cabernet-Sauvignon' as scion in a pot study. From the results obtained, it can be hypothesized that drought-tolerant rootstocks exhibit various strategies to respond to water deficit by combining control of development and water uptake. It appears from the published literature that the genetic resources of wild *Vitis* (except *sylvestris*) are still under exploited, despite their great potential interest for abiotic stress adaption.

Genetic architecture of traits: digging more in depth

Despite the large number of genetic architecture studies performed over the past decade and reviewed by Vezzuli *et al.* (2019), only very few of the addressed traits have been associated with responses to abiotic constraints. Drought (Marguerit *et al.*, 2012; Coupel-Ledru *et al.*, 2014, 2016), iron deficiency chlorosis (Bert *et al.*, 2013), salinity (Henderson *et al.*, 2018) and cold hardiness (Su *et al.*, 2020) have been considered. However, others studies related to vegetative development (including roots, as in Tandonnet *et al.* 2018; Hugalde *et al.*, 2019), phenology (for example Delfino *et al.*, 2019) and berry composition (such as Duchêne *et al.*, 2020) are of interest in the context of climate change. Few of them only were dedicated to rootstock performance on grafted plants (Marguerit *et al.*, 2012; Bert *et al.*, 2013; Tandonnet *et al.* 2018). Among the most recent studies, innovative approaches are noteworthy.

Duchêne *et al.* (2020) combined pH modelling with an analysis of the genetic architecture of berry acidity. Using equations to describe pH from malic acid, tartaric acid and K$^+$ concentrations, this study showed that although strong QTLs were identified for malic acid, and malic to tartaric acid ratio at veraison and during ripening, they were not associated with pH variations. Reproducible QTLs were detected for pH, which co-localized with QTLs for tartaric acid, and potassium to tartaric acid ratio, but not with potassium concentration. By dissecting the pH trait with some of its causal biochemical components, this study provided important results for breeding varieties capable of maintaining high titrable acidity and low pH under warmer conditions.

For veraison date, Delfino *et al.* (2019) performed a meta-analysis combining the data from 39 genetic maps and 47 QTLs studies. Based on a consensus map (3130 SSR markers, 1922 cM), four meta-QTLs for veraison were identified on linkage groups (LG) 1 and 2 with a reduction of the confidence interval of more than 5-fold, also increasing the percentage of total variance explained. One QTL on LG2 explained up to 34 % of the total variance. Additional QTLs related to the timing of veraison were also found on LG14, 16 and 18. Combining these results with the transcriptomic data, the number of candidate genes for the control of the onset of ripening was reduced by 10 to -20 fold. Among other interesting results, links between the control of the timing of veraison and flowering were consistently detected, at both positional and transcriptional levels. This study demonstrates the power of

meta-analyses to improve our understanding of key processes and the accuracy of detecting relevant loci.

Considering that abiotic stress responses have a complex determinism and are difficult to phenotype, genome-wide approaches are considered promising tools for genetic architecture studies and genomic prediction. Increasing the density of markers and improving the computational methods to detect associations have allowed the identification of novel QTLs controlling traits related to drought responses and relevant candidate genes in a bi-parental intra-vinifera progeny (Brault *et al.*, 2021). In comparison with the work of Coupel-Ledru *et al.* (2014, 2016) using the same dataset, eight new QTLs were detected, including one for night transpiration under water deficit on chromosome 12. The percentage of explained variance per trait was also increased. Analysis of candidate genes within the confidence intervals of the newly detected QTLs resulted in the identification of relevant genes known to be involved in plant hydraulics, growth, development and photosynthesis.

So far, most genetic architecture studies in grapevine have been based on QTL mapping in biparental populations. The drawbacks of this approach are well known, including the limited allelic diversity in parents and underestimation of polygenic contribution for prediction purposes (references cited in Flutre *et al.*, 2022). Genome-wide association studies (GWAS) can overcome these limitations, but very few have been carried out in grapevine. Recently, an association study was conducted on a panel of 279 *V. vinifera* phenotyped in the vineyard for 127 traits (yield components and vegetative development, fruit composition, phenology, water use efficiency estimated by $\delta^{13}C$ on grape juice) over 5 years, including data recorded in irrigated and non-irrigated vines for each genotype (Flutre *et al.*, 2022). The detection power was greatly increased, doubling the number of QTLs detected and increasing by 70 % the number of traits for which QTLs were identified. The study yielded a mine of new information compared to investigations carried out on biparental crosses. With this approach, high broad-sense heritability (> 0.7) was obtained for most traits, except $\delta^{13}C$ for which it was only 0.37. New QTLs were detected for many traits, including malic and citric acids, and $\delta^{13}C$, encompassing relevant candidate genes. Overall, this study demonstrated how powerful association studies can be, providing appropriate methodologies for phenotyping, genotyping and data computation are implemented. Zhu *et al.* (2022) reviewed several successful GWAS studies in a range of crops, including grapevine, although results were disappointing for some traits related to abiotic stress responses. A GWAS study was conducted for cold tolerance on a panel of 118 genotypes including accessions from several wild Vitis species, *V. vinifera* and interspecific hybrids (Wang *et al.*, 2021). This work allowed to identify a phosphoglycerate kinase gene on chromosome 19 associated with cold tolerance. Running GWAS approaches on a panel including the appropriate wild *Vitis* diversity is tricky, as least in Europe, where germplasm collections do not host a large intraspecific variability. Nevertheless a GWAS study has been performed recently for traits related to root development on a diversity panel from the *V. berlandieri* species, for the purpose of rootstock breeding applications (Blois *et al.*, 2023).

Few validated genes

While the list of candidate genes potentially involved in abiotic stress responses in grapevine is long, it has mainly been established from transcriptomic studies comparing two environmental conditions, sometimes in combination with two genotypes. Most references can be found in Gomès *et al.*, (2021). As shown previously, combining genetic and transcriptomic approaches can help shorten this list (Delfino *et al.*, 2019; Wang *et al.*, 2021). Functional validation of some genes is underway, but published data are extremely scarce, especially in homologous systems. Three recent studies, published in 2021 and 2022 may be among the first to provide evidence that specific genes actually control grapevine responses to abiotic stress. Jiao *et al.* (2022) reported the functional validation in *Arabidopsis thaliana* of VaHsfC1 from *V. amurensis*. This type of transcription factor (heat stress transcription factor, *Hsf*) is known to play a central role in the process of plant resilience to high temperatures. The expression profile of VaHsfC1, one member of this family, was analysed in leaves of *V. amurensis* cuttings submitted to short-term cold and heat stresses, ABA application and salt treatment. VaHsfC1 expression was up-regulated under each stress condition, as least temporarily. Over-expression of VaHsfC1 in Arabidopsis improved heat and freezing tolerance of the transgenic lines. Genes involved in the regulatory cascade regulating plant responses to cold conditions were also up-regulated in transgenic plants submitted to low temperatures. Meanwhile, sensitivity to ABA and salt was increased, especially when germination rates were considered. While these results underline the interest of VaHsfC1 for breeding tolerant varieties to extreme temperatures, validation of this gene in grapevine is still lacking and may be a prerequisite to reach this objective.

Nerva *et al.* (2022) reported one of the first functional validation studies performed in grapevine in relation to drought responses, for *VvGST40*, a putative glutathione S-transferase (GST) gene, using a spray-induced gene silencing (SIGS) technique. GST genes have been shown to be involved in the regulation of ABA levels and antioxidant activities, leading to drought resilience in Arabidopsis. GST-treated 'Chardonnay' cuttings were submitted to a 18-day drought and recovery cycle, and compared to untreated plants. Gas exchange, gene expression and target metabolites were monitored throughout the experiment. In GST-treated plants, VvGST40 expression was constitutively down-regulated, regardless of water treatment, whereas drought induced a decrease of expression in control plants. These latter plants showed high stomatal closure under drought and rapid reopening during recovery. On the contrary, GST-treated plants displayed lower stomatal conductance before drought application, slow stomatal closure under drought and slow reopening during recovery. ABA concentration in leaves was higher in GST-treated plants before drought application, once stress was maximal, and at the end of the recovery period. The expression of genes regulating ABA synthesis and signalling was in agreement with the phenotype observed in GST-treated plants. This was also the case for resveratrol antioxidant metabolism activities and associated genes, showing strong cross-talk between ABA and antioxidant metabolism.

A gene editing approach was recently used to validate the function of *VvEPFL9-1* in grapevine (Clemens *et al.*, 2022). Epidermis Patterning Factor Like 9, also known as STOMAGEN, induces stomata formation in vascular plants. The CRISPR/Cas9 system was applied to generate mutations that resulted in a significant reduction of stomata density, confirming for the first time the role of EPLF9 in grapevine as well as in a perennial fruit plant. Compared with the wild type and under favourable water supply conditions, the modified plants showed a reduction in CO_2 assimilation rate and stomatal conductance, resulting in an improvement of intrinsic water use efficiency. Under water deficit conditions, gas exchange in the edited lines was less affected compared to the wild type, and stomatal conductance remained nearly steady for the edited plants throughout the water deficit (12 days). These results suggest that low stomata density can contribute to save water under favourable conditions, which can also be beneficial once water supply starts to be limiting.

These results open up prospects for improving heat and drought tolerance in grapevines and confirm the potential of biotechnologies for functional genomics approaches, as well as for plant breeding, although there is a long way before any of these genes are included in a breeding program.

What's next

However, given the complexity of the task, there is still a long way to go before the release of better adapted grapevine genotypes. Real efforts have to be undertaken and four important priorities can be considered: 1-Epigenetics as a source of diversity and regulatory processes for plasticity and acclimation; 2-Genomic selection; 3-Bioengineering and 4-Extended phenotype and the role of microbiome. Genetic engineering approaches will not be discussed here, but the reports of functional validation studies presented in the previous section show the feasibility and the potential value of genome editing and double-stranded RNA induced-silencing.

Global epigenomic reprogramming of plants under abiotic stress is now clearly demonstrated (Gallusci *et al.*, 2022; Fortes and Gallusci, 2017). Epigenetic regulations and memory are now considered of major importance for crop adaptation to climate change (Guarino *et al.*, 2022). Furthermore natural epigenetic variation is likely involved in phenotypic diversity and plasticity of plants, both of which are important traits for adaptation. The main characteristics of cultivated grapevine, being propagated vegetatively, with a high intra-varietal diversity and significant environmental phenotypic plasticity, make epigenetics most likely very promising for grapevine improvement (Fortes and Gallusci, 2017). A few recent published studies in grapevine provide experimental evidence that such mechanisms are involved in the regulation of many processes such as rootstock-scion interactions (Rubio *et al.*, 2022), phenotypic plasticity and clonal diversity (Varela *et al.*, 2021). In addition, abiotic stresses such as UV-B and drought applied one year have been shown to affect the epigenetic landscape of grapevines, over at least two seasons (Marfil *et al.*, 2019). Genes associated with histone modifications were identified and belonged to the main category of differential-ly expressed genes found under combined heat and drought stress (Tan *et al.*, 2023). It is now important to analyse how epigenetic regulations and memory can be mobilized to drive acclimation processes and clonal diversity within ancient and newly bred varieties.

Genomic selection which relies on high-density genotyping is presented as a promising tool for breeding complex traits that are under the control of many genes. It is of particular interest in perennial plants to speed up breeding cycles and consequently increase the genetic gain, which is highly requested to address current challenges in agriculture (Voss-Fels *et al.*, 2019). Potential interests in grapevine are currently under investigation and appear promising (Brault *et al.*, 2022). Brault *et al.* (2021) evaluated genomic prediction methods for drought responses traits. A predictive ability of 0.68 on average was reported for traits with high heritability such as night transpiration under water deficit.

In the context of environmental interactions, an additional player increases the level of complexity, but also the range of adaptive processes. The plant-associated microbiome is known to enhance tolerance to abiotic stress and may contribute to adaptation to adverse conditions (Darriaut *et al.*, 2022). Grapevine genotypes, both scion and rootstock, have been shown to affect the rhizosphere bacterial community (Vink *et al.*, 2021; Berlanas *et al.*, 2019). In addition to identifying the bacteria and fungi that contribute to abiotic stress tolerance, the mechanisms underlying the microbiome-host relationship and the genetic determinism of the ability of grapevine genotypes to recruit beneficial microorganisms should be addressed. For example, the ability to produce root exudates and the molecular components of nutrient uptake could be keys factors in these interactions (Rodriguez *et al.*, 2019).

Conclusions

As the impact of climate change on vineyards become more and more visible, improving our knowledge about the mechanisms underlying responses to abiotic stresses in grapevine must be defined as a priority, despite the complexity of the issue. Modern approaches such as multi-omics and systems biology, high throughput phenotyping and genotyping, genome wide association studies, modelling are available to address this complexity and should be mobilized in a more systematic way to address the relationships between genotype and phenotypes. The availability of an increasing number of genomic sequences of *Vitis spp.* is another very important resource to address environmental challenges. New breeding strategies such as genomic selection or plant engineering should also be source of optimism. We hope it will stimulate scientific efforts on these issues in the grapevine community for the next decades.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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