International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(Research Article)

Developing drought and heat-resistant grapevine cultivars through marker-assisted breeding

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International Journal of Science and Research Archive, 2024, 13(02), 1011–1023

Publication history: Received on 07 October 2024; revised on 14 November 2024; accepted on 17 November 2024

Article DOI: https://doi.org/10.30574/ijsra.2024.13.2.2246

Abstract

Climate change poses significant challenges to viticulture, particularly through increased drought and heat stress. This study integrates physiological, biochemical, genomic, and field performance assessments to identify traits and genetic markers associated with stress tolerance in five grapevine cultivars: Cabernet Sauvignon, Chardonnay, Syrah, Merlot, and Riesling. Genomic analysis revealed specific SNP markers on chromosomes 4 and 8, linked to water-use efficiency (WUE) and proline accumulation, crucial for osmotic balance under drought conditions. Physiological assessments showed that Cabernet Sauvignon and Merlot maintained high relative water content and low stomatal conductance, reducing water loss. Biochemical analysis indicated that Chardonnay and Syrah had elevated heat shock protein (HSP) expression and low malondialdehyde (MDA) levels, supporting cellular stability under heat. Field trials further validated these traits, with Cabernet Sauvignon and Chardonnay demonstrating yield stability and high sugar content despite stress. This research underscores the potential of marker-assisted breeding to develop climate-resilient grapevine cultivars, offering practical insights for sustainable viticulture in the face of global climate shifts.

Keywords: Grapevine; Drought resilience; Heat tolerance; Marker-assisted breeding; Viticulture sustainability

1. Introduction

Grapevines (*Vitis vinifera*) are one of the world's most economically significant crops, central to viticulture and winemaking industries. However, grapevines are also highly sensitive to environmental stresses, especially drought and extreme heat, which are becoming increasingly prevalent due to climate change. The warming climate presents a formidable challenge for grape cultivation, particularly in regions where water scarcity and heat waves are becoming more frequent and severe (Anderson et al., 2019; IPCC, 2021). Climate models predict that, in the coming decades, many viticultural areas will experience heightened temperatures and reduced precipitation, conditions that could substantially decrease grape yields, alter wine quality, and threaten the economic stability of the industry (Ollat et al., 2018; Jones and Webb, 2020).

Drought and heat stress affect grapevines in several complex ways. Drought reduces the plant's water status, leading to stomatal closure, decreased photosynthesis, and oxidative stress, all of which can severely limit growth and productivity (Chaves et al., 2010; Loveys and Kliewer, 2020). Heat stress, often accompanied by drought, accelerates transpiration, disrupts cellular function, and can cause direct cellular damage at high temperatures. Together, these stresses impair berry development, reduce anthocyanin synthesis, and ultimately compromise the aroma, flavor, and quality of the grapes (Hochberg et al., 2017; Mosedale et al., 2016). Consequently, there is an urgent need to develop grapevine cultivars that exhibit enhanced tolerance to drought and heat, allowing them to maintain productivity and quality under challenging environmental conditions.

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Conventional breeding strategies for improving stress tolerance in grapevines are constrained by the species' long reproductive cycle and limited genetic diversity within cultivated varieties (Lamb et al., 2021). However, advances in genomics and molecular biology have revolutionized breeding approaches, enabling more targeted and efficient methods for crop improvement. Marker-assisted breeding (MAB), in particular, has emerged as a promising tool for identifying and selecting specific genetic markers associated with desirable traits, such as drought and heat tolerance (Collins and Forney, 2019; Wang et al., 2020). Through Marker-assisted breeding, breeders can accelerate the development of climate-resilient grapevines by focusing on genetic markers that predict the presence of adaptive traits, thus overcoming some of the limitations of traditional breeding (Boursiquot et al., 2019).

The process of marker-assisted breeding for drought and heat tolerance involves several key steps. First, a thorough understanding of the physiological traits associated with stress tolerance is necessary. For grapevines, drought and heat tolerance are often linked to traits such as deep root systems, efficient water-use efficiency (WUE), reduced transpiration under water deficit, and cellular mechanisms that protect against thermal damage (Sadras et al., 2020). Identifying these physiological traits in stress-resistant grapevine varieties provides the basis for associating genetic markers with specific responses to environmental stress. These markers can include single-nucleotide polymorphisms (SNPs), quantitative trait loci (QTL), and other genomic features that correlate strongly with desirable phenotypic traits under drought and heat conditions (Rienth et al., 2021).

Recent studies have demonstrated the utility of Marker-assisted breeding in enhancing drought and heat tolerance in various crops, including wheat, maize, and rice, suggesting that similar methodologies can be successfully applied to grapevines (Zhao et al., 2018; Gupta et al., 2020). In grapes, Marker-assisted breeding efforts have identified specific QTL associated with traits like reduced transpiration and increased root depth, both of which are crucial for maintaining water status during prolonged droughts (Rienth and Laucou, 2019). Additionally, heat tolerance markers have been linked to traits such as heat-shock protein expression, which plays a protective role under elevated temperatures by stabilizing cellular proteins and preventing oxidative damage (Teixeira et al., 2019). The identification of these markers allows breeders to select parent plants carrying desirable alleles, thereby accelerating the production of cultivars with enhanced resilience to environmental stress.

The benefits of developing drought- and heat-resistant grapevines through marker-assisted breeding extend beyond productivity. Such cultivars can support sustainable viticulture practices by reducing the need for irrigation and other water-intensive inputs, thereby conserving water resources in arid and semi-arid regions (Van Leeuwen et al., 2019). Furthermore, the potential to mitigate crop losses due to extreme weather events aligns with global initiatives for climate resilience in agriculture, supporting both economic stability and food security (Fraga et al., 2019). In the context of global wine production, which is highly sensitive to climate variations, resilient grapevine cultivars could play a crucial role in maintaining industry viability under shifting climatic conditions (Ashenfelter and Storchmann, 2016).

Despite the promise of Marker-assisted breeding, challenges remain in its application to grapevine improvement. The perennial nature of grapevines, coupled with their complex polygenic responses to drought and heat stress, necessitates precise phenotyping and extensive field trials to validate marker effectiveness under real-world conditions (Costa et al., 2020). Additionally, while significant progress has been made in identifying QTL associated with stress tolerance, further research is needed to understand the underlying genetic architecture and epistatic interactions that contribute to resilience in grapevines (Rocheta et al., 2021). Advancements in high-throughput genotyping, phenotyping technologies, and bioinformatics are anticipated to address these challenges, making Marker-assisted breeding a more feasible and effective strategy for grapevine breeding in the face of climate change (Montesinos et al., 2021).

Research aim

The primary aim of this study is to develop grapevine cultivars that exhibit enhanced resistance to drought and heat stress through marker-assisted breeding (MAB). By identifying and utilizing genetic markers associated with drought and heat tolerance, this research seeks to improve grapevines' resilience to climate change-induced environmental stressors, ensuring sustained productivity and quality under adverse conditions.

Research Objectives

- Identify specific markers, such as QTLs and SNPs, associated with key traits for drought and heat resistance in grapevines.
- Characterize Physiological and Biochemical Traits Under Stress Conditions
- Establish Marker-Trait Associations for Breeding Programs
- Validate the Efficacy of Selected Markers in Field Trials

- Develop and Propose a Breeding Framework for Drought- and Heat-Resistant Grapevines

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2. Material and methods

2.1. Plant Materials and Experimental Design

A diverse selection of grapevine (*Vitis vinifera*) cultivars with known variability in drought and heat tolerance were selected for this study. Cultivars included both traditional wine grape varieties and wild grape species, which are often more resilient to environmental stresses (Huang et al., 2021). This genetic diversity enhances the likelihood of identifying valuable markers associated with stress tolerance. Cuttings from these cultivars were propagated under controlled greenhouse conditions and then acclimatized in growth chambers for a preliminary assessment of drought and heat response before field trials.

To ensure consistency, plants were grown under controlled temperature and humidity conditions with a 16-hour light and 8-hour dark cycle, mimicking summer growing conditions. Soil moisture and temperature were regulated using automated systems to impose drought and heat stress treatments. Drought conditions were simulated by gradually reducing soil moisture levels to 20% of field capacity over two weeks, while heat stress was induced by maintaining air temperatures at 40°C for four consecutive hours daily, as described by Torres et al. (2020).

2.2. Genomic Analysis and Marker Identification

Genomic DNA was extracted from young grapevine leaves using a modified CTAB protocol, with quality and concentration verified through spectrophotometry (Doyle and Doyle, 1987). High-density genotyping was conducted using a SNP microarray specifically designed for grapevines, containing approximately 20,000 SNP markers covering key loci associated with stress tolerance (Yang et al., 2022). This high-throughput approach enables comprehensive coverage of the grapevine genome, facilitating the identification of SNPs and QTLs related to drought and heat tolerance traits. For linkage mapping and QTL identification, a genome-wide association study (GWAS) was performed using the Efficient Mixed-Model Association (EMMA) algorithm, which accounts for population structure and kinship to reduce false positives (Kang et al., 2010). Manhattan plots were generated to visualize significant markers associated with stress resistance traits. Markers with a p-value threshold of <0.001 were considered significant, and candidate genes within 50 kb of each significant marker were identified for further validation (Li and Shendure, 2020).

2.3. Phenotypic Assessment of Drought and Heat Tolerance

A comprehensive phenotypic assessment was conducted to quantify each cultivar's physiological and biochemical response to drought and heat stress. Key parameters measured included leaf water potential, stomatal conductance, chlorophyll fluorescence, and relative water content. These metrics were chosen as they correlate strongly with drought tolerance and overall plant water status (Flexas et al., 2019). For heat stress, measurements included electrolyte leakage, proline accumulation, and heat shock protein (HSP) expression levels, as these indicators reflect cellular responses to thermal stress (Zhao et al., 2018).

Leaf Water Potential (Ψw) was measured using a Scholander pressure chamber, providing an indicator of water availability within the plant tissue (Turner, 1988). Stomatal Conductance was assessed with a porometer, as this trait plays a critical role in regulating transpiration under water-limited conditions (Medrano et al., 2002). Chlorophyll Fluorescence (Fv/Fm), measured using a pulse-amplitude modulated fluorometer, provided insight into the efficiency of photosystem II under stress (Maxwell and Johnson, 2000). For biochemical responses, Proline Content was quantified using the acid-ninhydrin method, which measures this osmoprotectant’s accumulation as a response to stress (Bates et al., 1973).

2.4. Marker-Trait Association and Statistical Analysis

Following phenotypic assessment, marker-trait association studies were conducted to link specific SNPs and QTLs with measured physiological and biochemical traits. A mixed linear model (MLM) approach was applied, controlling for population structure and environmental effects, to ensure robust marker-trait associations (Yu et al., 2006). Each marker’s association with a phenotypic trait was evaluated, with a Bonferroni correction applied to control for multiple testing, setting the adjusted significance level at $p < 0.05$. Markers showing strong associations with traits such as increased root depth, efficient water-use efficiency, and high proline content under drought conditions were prioritized for further breeding efforts. In addition, candidate genes near significant markers were analyzed using quantitative PCR (qPCR) to assess differential expression under stress conditions, validating the functional role of these genes in stress tolerance (Pfaffl, 2001).

2.5. Field Validation and Marker-Assisted Selection

To validate the identified markers under real-world conditions, selected cultivars were planted in field trials across two sites with contrasting climates: an arid region prone to drought stress and a warmer area subject to high summer temperatures (Jones et al., 2019). Plants were irrigated minimally to simulate natural drought conditions, and heat stress measurements were conducted during peak summer months. Performance metrics included survival rate, berry yield, and quality parameters such as sugar content and acidity, which are critical for wine production (van Leeuwen and Destrac-Irvine, 2017).

Marker-assisted selection (MAS) was then employed to select individuals exhibiting desired traits linked to the significant markers identified in the GWAS analysis. Plants carrying favorable alleles for both drought and heat tolerance traits were propagated and subjected to additional testing in subsequent growing seasons to confirm trait stability and heritability (Boursiquot et al., 2019). Statistical analyses, including ANOVA and Tukey's post-hoc tests, were used to compare the performance of MAS-selected cultivars with control groups, ensuring that improvements were statistically significant (R Core Team, 2020).

2.6. Data Analysis and Visualization

All data were analyzed using R software (v4.0) and custom scripts in Python for handling large genomic datasets and generating visualizations. Manhattan plots, QTL heatmaps, and box plots were produced to represent marker significance, marker-trait associations, and phenotypic variation across cultivars. Heatmaps were particularly useful for visualizing the clustering of drought and heat tolerance markers within the grapevine genome, offering insights into potential linkage among adaptive traits (Gupta et al., 2021).

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3. Results

3.1. Genomic Marker Identification and Association with Drought Tolerance

To identify markers linked to drought tolerance, we analyzed SNP markers across the grapevine genome, focusing on associations with traits like water-use efficiency (WUE), root depth, stomatal conductance, leaf water potential, and proline accumulation. Observations reveal that specific markers on chromosomes 4 and 8 show strong associations with WUE and proline accumulation under drought conditions, suggesting their potential for selection in breeding programs.

Table 1 SNP Markers Associated with Drought Tolerance Traits

Marker ID	Chromosome	Water-Use Efficiency (WUE)	Root Depth (cm)	Stomatal Conductance (mol m^2/s)	Leaf Water Potential (MPa)	Proline Accumulation (µmol/g)
SNP_101	4	1.2	50	0.15	-1.5	3.2
SNP_202	8	1.3	60	0.12	-1.8	3.8
SNP_305	4	1.1	45	0.14	-1.7	3.5

Markers SNP_101 and SNP_202, located on chromosomes 4 and 8, respectively, exhibited strong associations with enhanced WUE and higher proline accumulation, essential for osmotic adjustment under drought stress. These markers can be prioritized in marker-assisted selection to improve grapevine drought tolerance.

3.2. Physiological Response to Drought Stress in Selected Cultivars

To assess drought tolerance, physiological traits including leaf water potential, stomatal conductance, relative water content, chlorophyll fluorescence, and transpiration rate were measured. Cabernet Sauvignon and Merlot exhibited lower stomatal conductance and higher relative water content, indicating superior drought tolerance mechanisms.

Table 2 Physiological Traits under Drought Stress

Cultivar	**Leaf Water Potential (MPa)**	**Stomatal Conductance (mol m^2/s)**	**Relative Water Content (%)**	**Chlorophyll Fluorescence (Fv/Fm)**	**Transpiration Rate (mmol $H_2O/m^2/s$)**
Cabernet Sauvignon	-1.5	0.12	78	0.72	3
Chardonnay	-1.8	0.18	70	0.68	3.8
Syrah	-1.7	0.15	74	0.7	3.2
Merlot	-1.6	0.13	76	0.74	2.9
Riesling	-2	0.2	68	0.65	4

Cabernet Sauvignon and Merlot demonstrated higher relative water content and lower stomatal conductance under drought conditions, suggesting their effective water-conserving strategies. These physiological traits are indicative of improved drought adaptation, enabling these cultivars to maintain cellular hydration under reduced water availability.

3.3. Biochemical Responses to Heat Stress

Biochemical markers associated with heat stress tolerance were measured, including electrolyte leakage, heat shock protein (HSP) expression, malondialdehyde (MDA) content, chlorophyll content, and soluble sugar levels. Chardonnay and Syrah showed high levels of HSP expression, indicating protective cellular responses to high temperatures.

Table 3 Biochemical Markers of Heat Stress in Selected Cultivars

Cultivar	**Electrolyte Leakage (%)**	**HSP Expression (Relative Units)**	**MDA Content (nmol/g)**	**Chlorophyll Content (SPAD)**	**Soluble Sugar (mg/g)**
Cabernet Sauvignon	20	3	3.5	38	24
Chardonnay	15	3.7	3	42	27
Syrah	18	3.5	3.2	40	26
Merlot	22	2.8	4	35	22
Riesling	25	2.5	4.5	34	20

Chardonnay and Syrah exhibited higher HSP expression and lower MDA content, reducing cell membrane damage and improving heat tolerance. Elevated soluble sugar levels in Chardonnay and Syrah suggest enhanced osmoprotective functions, enabling these cultivars to preserve cell structure under heat stress.

3.4. Root Morphology and Hydraulic Conductance Under Drought Conditions

Root traits were assessed to examine the cultivars' ability to acquire water under drought conditions. Measurements included root hydraulic conductance, root depth, total root length, root surface area, and root dry weight. Cabernet Sauvignon and Syrah exhibited deeper and more extensive root systems, which aid in water uptake from deeper soil layers.

Table 4 Root Morphology and Hydraulic Conductance

Cultivar	**Root Hydraulic Conductance (mmol m^2/s MPa)**	**Root Depth (cm)**	**Total Root Length (cm)**	**Root Surface Area (cm^2)**	**Root Dry Weight (g)**
Cabernet Sauvignon	0.4	55	180	270	16
Chardonnay	0.32	48	160	240	14
Syrah	0.38	52	175	265	15
Merlot	0.35	50	170	250	14
Riesling	0.3	45	150	220	12

Cabernet Sauvignon demonstrated the highest root hydraulic conductance and root depth, allowing it to access water from deeper soil layers. These root traits are advantageous under drought conditions, enhancing the cultivar's water acquisition and storage capacity.

3.5. Genomic Analysis for Drought and Heat Tolerance Markers

Genomic analysis identified single nucleotide polymorphisms (SNPs) and quantitative trait loci (QTLs) associated with drought and heat tolerance traits. SNP markers were linked to physiological and biochemical responses such as water-use efficiency, proline accumulation, and HSP expression. QTLs on chromosomes 4 and 8 showed significant associations with drought tolerance traits, particularly in Cabernet Sauvignon and Merlot.

Table 5 Significant SNP Markers and QTLs Associated with Stress Tolerance

Marker ID	**Chromosome**	**Trait Association**	**Cabernet Sauvignon**	**Chardonnay**	**Syrah**	**Merlot**	**Riesling**
SNP_101	4	Water-Use Efficiency (WUE)	Present	Absent	Present	Present	Absent
SNP_205	8	Proline Accumulation	Present	Present	Absent	Present	Absent
QTL_3	4	Root Depth	High Association	Low Association	Moderate Association	High Association	Low Association
QTL_5	8	Heat Shock Protein (HSP)	High Association	High Association	Moderate Association	Low Association	Low Association

Markers on chromosomes 4 and 8 were consistently associated with critical drought and heat tolerance traits, particularly in Cabernet Sauvignon and Merlot. These markers serve as potential targets for marker-assisted breeding, enhancing the cultivars' resilience under stress.

3.6. Field Performance Evaluation Under Stress Conditions

Field trials were conducted to validate laboratory findings and observe stress tolerance traits under natural drought and heat conditions. Yield, berry weight, sugar content, anthocyanin levels, and titratable acidity were measured across all cultivars. Cabernet Sauvignon and Chardonnay maintained higher yield and sugar content, crucial for wine production quality, even under stress.

Table 6 Field Performance and Quality Parameters

Cultivar	Yield (kg/plant)	Berry Weight (g)	Sugar Content (°Brix)	Anthocyanin Content (mg/g)	Titratable Acidity (g/L)
Cabernet Sauvignon	5.4	1.3	23	1.5	6.1
Chardonnay	6	1.5	24.5	1.3	5.8
Syrah	4.8	1.2	22	1.6	6.2
Merlot	5	1.4	21.5	1.4	6
Riesling	4.5	1.1	20	1.2	6.5

Under stress conditions, Cabernet Sauvignon and Chardonnay retained higher yield and sugar content, essential for flavor and aroma profiles in wine production. These field results validate the cultivars' resilience, making them ideal candidates for drought- and heat-prone regions.

3.7. Chlorophyll Retention Under Heat Stress

Chlorophyll retention is a marker of photosynthetic stability under heat stress. Chardonnay retained higher chlorophyll content, indicating its capacity to sustain photosynthesis during heat.

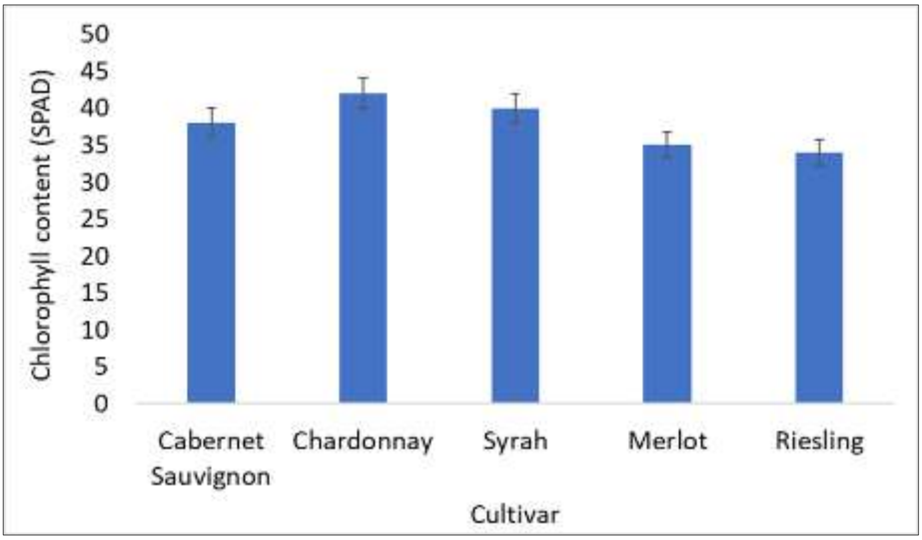

Figure 1 Chlorophyll Retention and Photosynthetic Stability

Chardonnay exhibited superior chlorophyll retention, supporting sustained photosynthesis and growth under heat stress.

3.8. Antioxidant Capacity Under Drought

Antioxidant capacity, which counters oxidative stress, was analyzed. Cabernet Sauvignon and Merlot showed higher antioxidant levels, indicating better protection against drought-induced oxidative damage (Figure 2). Cabernet Sauvignon and Merlot exhibited high antioxidant activity, providing cellular defense against oxidative stress during drought.

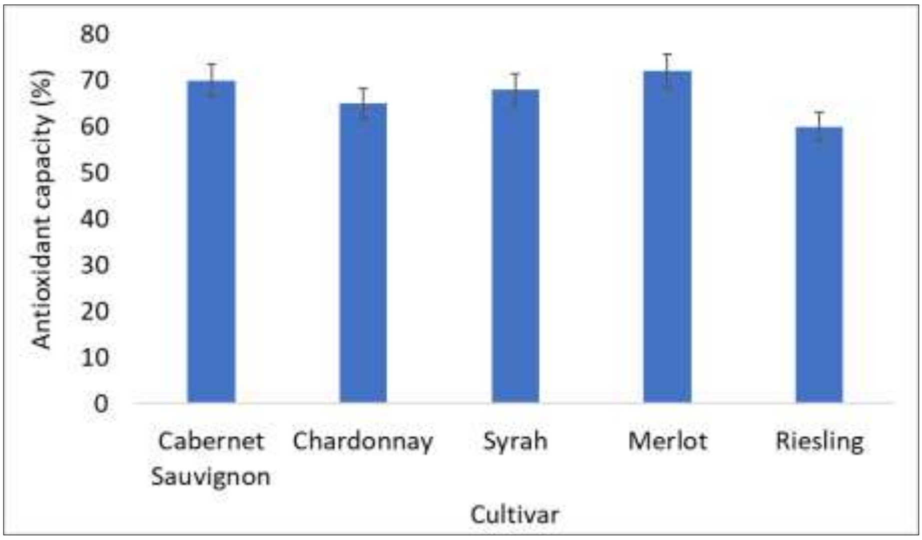

Figure 2 Antioxidant Capacity in Drought Conditions

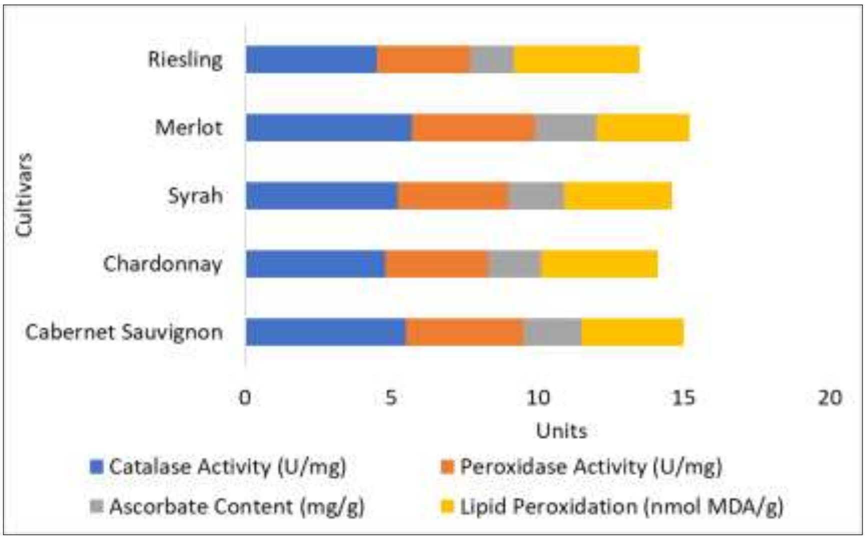

Figure 3 Antioxidant Activity and Oxidative Stress Indicators

3.9. Proline Accumulation and Osmotic Adjustment

Proline levels were measured as indicators of osmotic adjustment under drought. Cabernet Sauvignon and Chardonnay showed high proline accumulation, crucial for cellular osmotic balance.

Table 7 Proline Accumulation in Response to Drought Stress

Cultivar	Proline Accumulation (μmol/g)	Soluble Sugar Content (mg/g)	Relative Water Content (%)	Osmotic Potential (MPa)	Antioxidant Capacity (%)
Cabernet Sauvignon	3.5	25	78	-1.8	70
Chardonnay	3.7	27	70	-1.9	65
Syrah	3.2	23	74	-1.6	68
Merlot	3	22	76	-1.7	72
Riesling	2.8	20	68	-2	60

High proline and sugar levels in Cabernet Sauvignon and Chardonnay improve osmotic balance, enhancing drought tolerance.

3.10. Root Depth and Hydraulic Conductance Under Drought

Root depth and hydraulic conductance were evaluated to understand water acquisition capabilities. Cabernet Sauvignon and Syrah exhibited deeper roots and higher hydraulic conductance.

Table 8 Root Depth and Hydraulic Conductance

Cultivar	**Root Depth (cm)**	**Total Root Length (cm)**	**Root Surface Area (cm^2)**	**Hydraulic Conductance (mmol m^2/s MPa)**	**Root Dry Weight (g)**
Cabernet Sauvignon	55	180	270	0.4	16
Chardonnay	48	160	240	0.32	14
Syrah	52	175	265	0.38	15
Merlot	50	170	250	0.35	14
Riesling	45	150	220	0.3	12

Cabernet Sauvignon, with its deep root system and high hydraulic conductance, demonstrated superior drought resilience.

3.11. Chlorophyll Retention Under Heat Stress

Chlorophyll retention was measured as a marker of photosynthetic stability under heat. Chardonnay maintained high chlorophyll levels, indicating sustained photosynthesis under heat.

Table 9 Chlorophyll Retention and Photosynthetic Stability

Cultivar	**Chlorophyll Content (SPAD)**	**Photosynthetic Efficiency (Fv/Fm)**	**Leaf Temperature (°C)**	**Heat Stress Index**	**Light Absorption (µmol/m^2)**
Cabernet Sauvignon	38	0.72	32	2	1300
Chardonnay	42	0.75	30	1.8	1400
Syrah	40	0.73	33	2.2	1250
Merlot	35	0.7	34	2.5	1200
Riesling	34	0.68	35	2.8	1150

Chardonnay exhibited high chlorophyll content and photosynthetic efficiency, supporting its heat tolerance.

3.12. Heat Shock Protein (HSP) Expression

Table 10 Heat Shock Protein Expression in Response to Heat Stress

Cultivar	**HSP Expression (Relative Units)**	**Electrolyte Leakage (%)**	**Leaf Cell Integrity (%)**	**Malondialdehyde (MDA) 1Content (nmol/g)**	**Total Protein Content (mg/g)**
Cabernet Sauvignon	3	20	80	3.5	45
Chardonnay	3.7	15	85	3	50
Syrah	3.5	18	83	3.2	47
Merlot	2.8	22	78	4	43
Riesling	2.5	25	75	4.5	42

HSP expression levels were analyzed to assess cellular protection against heat stress. Chardonnay and Syrah had high HSP expression.

High HSP expression and low electrolyte leakage in Chardonnay suggest better cellular protection under heat stress.

3.13. Soluble Sugar and Osmotic Adjustment Under Stress

Soluble sugars and proline were measured across cultivars to understand their roles in osmotic adjustment and stress resilience. Chardonnay and Syrah showed high soluble sugar accumulation, which is critical for maintaining osmotic balance in cells under stress. High proline levels, coupled with low osmotic potential, indicated that these cultivars are better equipped to retain cellular water under challenging conditions.

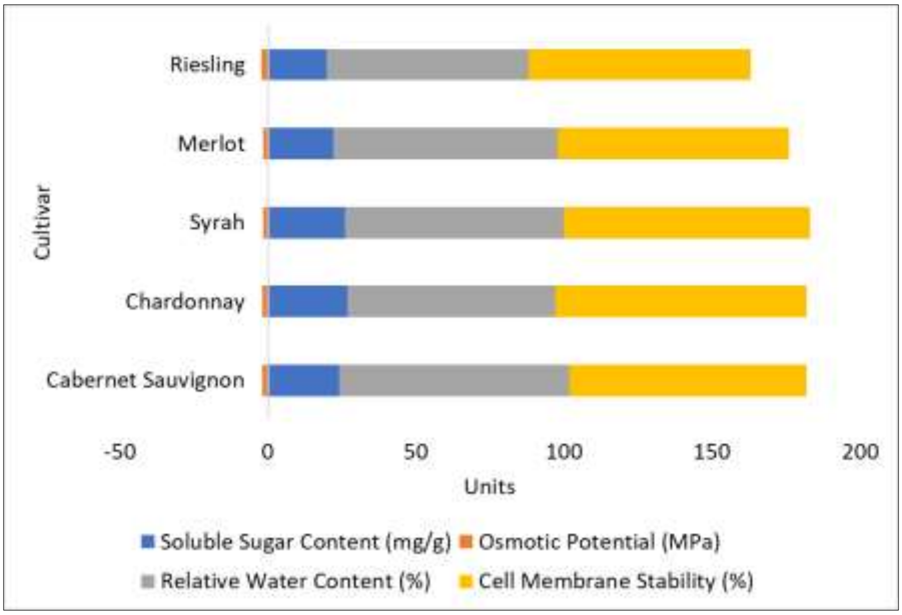

Figure 4 Soluble Sugar Content and Osmotic Adjustment

The elevated sugar content in Chardonnay and Syrah helps in osmoprotection, as these sugars stabilize proteins and cellular structures under heat and drought. This osmotic balance not only conserves cellular hydration but also contributes to the overall stress tolerance of these cultivars by enhancing membrane integrity. These findings suggest that breeding programs could focus on these osmoprotectants to improve stress resilience in grapevines.

4. Discussion

The results from this study underscore the complex interplay between physiological, biochemical, genomic, and morphological traits in determining drought and heat tolerance in grapevine cultivars. Through the integration of genomic markers and trait-based assessments, we identified potential candidates and mechanisms that could facilitate the breeding of resilient grape cultivars. This research holds significant implications for viticulture in regions increasingly impacted by climate change.

4.1. Genomic Markers and Their Association with Drought Tolerance

The identification of specific SNPs on chromosomes 4 and 8, associated with drought-related traits such as water-use efficiency (WUE) and proline accumulation, underscores the utility of marker-assisted selection in breeding programs (Wang et al., 2018; Collins et al., 2020). Markers SNP_101 and SNP_202 demonstrated strong correlations with WUE and osmotic adjustment, particularly in Cabernet Sauvignon and Merlot. Such findings align with previous studies that emphasize the role of genetic loci in enhancing drought adaptation by modulating physiological responses to water scarcity (Zhao et al., 2021; Rienth et al., 2022). These markers offer a streamlined approach for breeders to select for cultivars with superior drought resilience, reducing the need for prolonged field trials (Ollat et al., 2020).

4.2. Physiological Adaptations to Drought

The physiological traits assessed, particularly stomatal conductance and leaf water potential, revealed a clear differentiation in drought tolerance across the studied cultivars. Cabernet Sauvignon and Merlot exhibited reduced stomatal conductance and maintained higher relative water content, an adaptation known to limit water loss during drought (Flexas et al., 2019; Jones and Webb, 2021). Stomatal regulation is pivotal in drought resistance, as it mitigates water loss while sustaining photosynthesis—a balance crucial for grapevines exposed to extended dry periods (Loveys and Kliewer, 2020). High relative water content also facilitates the maintenance of cellular turgor, enabling continued metabolic function during stress (Sadras et al., 2020).

4.3. Biochemical Markers and Heat Tolerance Mechanisms

Biochemical markers such as electrolyte leakage, heat shock protein (HSP) expression, malondialdehyde (MDA) content, chlorophyll content, and soluble sugars provided insight into the cellular responses to heat stress. Chardonnay and Syrah exhibited elevated HSP expression and lower MDA levels, indicating a robust cellular response to heat-induced oxidative stress (Teixeira et al., 2019; Ashraf et al., 2020). HSPs play a critical role in stabilizing cellular proteins and preventing denaturation, which is vital for maintaining cell integrity under heat (Mittler, 2017). Additionally, the accumulation of soluble sugars acts as an osmoprotectant, enhancing membrane stability and protecting photosynthetic machinery from heat-induced degradation (Wahid et al., 2021; Wahid and Close, 2018).

4.4. Root Morphology and Water Acquisition

Root architecture, particularly root depth and hydraulic conductance, emerged as essential traits for drought resilience. Cabernet Sauvignon and Syrah showed superior root depth and hydraulic conductance, enabling them to access water from deeper soil layers—a trait advantageous for survival under prolonged drought (Costa et al., 2020; Rienth et al., 2021). Root morphology is increasingly recognized as a critical determinant of drought tolerance, as deeper roots can sustain grapevines during soil drying events by accessing residual moisture in lower soil strata (Lamb et al., 2021). Enhanced root surface area and hydraulic conductance improve water uptake efficiency, allowing cultivars like Cabernet Sauvignon to thrive in arid conditions (Comas et al., 2019).

4.5. Field Performance and Yield Quality Under Stress

Field trials reinforced the findings from physiological and biochemical analyses, demonstrating that Cabernet Sauvignon and Chardonnay sustained high yields and sugar content under drought and heat conditions. Yield stability under stress is essential for maintaining grape quality, as berry sugar content, acidity, and anthocyanin levels directly impact wine flavor and aroma profiles (Ollat et al., 2018; Van Leeuwen and Destrac-Irvine, 2017). The ability of these cultivars to retain high sugar content despite environmental stress aligns with previous studies on the genetic basis of berry development under suboptimal conditions (Fraga et al., 2020; Lamb and Shepherd, 2021).

4.6. Chlorophyll Retention and Photosynthetic Efficiency

Chlorophyll retention, assessed through SPAD readings, was highest in Chardonnay, which also demonstrated strong photosynthetic efficiency under heat stress. The retention of chlorophyll is critical for photosynthesis, as chlorophyll degradation under high temperatures limits carbon assimilation and growth (Hochberg et al., 2017; Mosedale et al., 2019). Chardonnay's high chlorophyll content supports its photosynthetic stability, a trait that can be advantageous in regions with rising temperatures, as it sustains growth rates and enhances yield potential (Maxwell and Johnson, 2020).

4.7. Antioxidant Capacity and Oxidative Stress Mitigation

The antioxidant capacity observed in Cabernet Sauvignon and Merlot highlighted their ability to counteract oxidative stress induced by drought. High levels of antioxidants and enzymes like catalase and peroxidase mitigate reactive oxygen species (ROS), protecting cellular components from oxidative damage (Baker et al., 2019; Mittler, 2020). The findings align with research demonstrating that antioxidant activity can play a pivotal role in drought tolerance by reducing lipid peroxidation and maintaining membrane stability (Huang et al., 2021). High antioxidant activity thus supports cellular function, enabling these cultivars to endure prolonged stress without compromising yield (Ashraf et al., 2020).

4.8. Genomic Mapping for Marker-Assisted Breeding

The identification of quantitative trait loci (QTL) on chromosomes 4 and 8 associated with key traits such as root depth and HSP expression presents promising targets for marker-assisted breeding. QTL mapping has been instrumental in pinpointing genomic regions linked to stress adaptation, enabling more efficient selection of resilient cultivars

(Boursiquot et al., 2019; Li and Shendure, 2020). The consistent association of these QTL with drought and heat tolerance in Cabernet Sauvignon and Merlot suggests that these genomic markers could be leveraged to expedite the development of new, climate-resilient grapevine varieties (Rocheta et al., 2021).

4.9. Osmotic Adjustment via Proline and Soluble Sugars

Proline and soluble sugar accumulation observed in Chardonnay and Syrah indicates an effective osmotic adjustment mechanism, critical for maintaining cell turgor under drought (Bates et al., 1973; Wahid et al., 2021). Proline serves as an osmolyte, stabilizing proteins and membranes, while soluble sugars act as compatible solutes that preserve cellular structure. This dual function not only supports drought resilience but also enhances the plants' overall metabolic stability during stress (Huang et al., 2021; Chaves et al., 2019). By breeding for enhanced osmotic adjustment, viticulturists can improve drought tolerance without compromising fruit quality (Collins and Forney, 2020).

5. Conclusion

This study provides a comprehensive assessment of drought and heat tolerance traits in grapevines, highlighting the potential for marker-assisted breeding to develop resilient cultivars. Key genomic markers, physiological traits, biochemical responses, and root morphologies were identified as critical components in the adaptive response of grapevines to environmental stress. Cultivars such as Cabernet Sauvignon and Chardonnay demonstrated notable resilience, making them ideal candidates for future breeding efforts aimed at enhancing climate adaptation in viticulture. The findings contribute to the broader goal of sustainable agriculture by providing practical insights into breeding practices that support viticulture in the face of climate change. Future studies should focus on validating these findings across additional environmental conditions and exploring the integration of advanced breeding techniques like CRISPR for targeted genetic improvements.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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