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RECENT ADVANCEMENTS IN GRAPEVINE (*Vitis vinifera* L.) IMPROVEMENT: INTEGRATING NOVEL GENETICS, BIOTECHNOLOGY AND BREEDING STRATEGIES

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ABSTRACT

The grapevine (*Vitis spp*.) is one of the most important fruit crops globally, driving significant economic value across the wine, fresh table fruit and raisin industries. Modern viticulture faces intensifying pressures from climate change, shifting pathogen dynamics, and strict market demands for quality and seedlessness. While traditional breeding has successfully leveraged the rich genetic diversity of the genus *Vitis*, it is severely constrained by the crops long juvenile phase, complex polygenic traits and inbreeding depression. This review synthesizes the paradigm shift from conventional breeding to advanced multi-omics biotechnological interventions. The genetic architecture of key agronomic traits, modern tissue culture methodologies like embryo rescue for seedless-by-seedless crosses and deployment of Marker-Assisted Selection (MAS) for stacking defense loci. Additionally, the development of antimicrobial peptides (AMPs) for broad-spectrum disease resistance and the optimization of abiotic stress-tolerant rootstocks. Finally, we discuss current challenges and outline future strategies for engineering resilient, high-yielding and climate-smart grapevine cultivars.
Keywords **:** Grapevine, CRISPR/Cas9, Antimicrobial peptides, Marker-Assisted Selection, Biotic and Abiotic Stress.

Introduction

Grapevine (*Vitis vinifera* L.) is a woody perennial crop of immense global economic and cultural significance, cultivated extensively across temperate, subtropical and tropical regions. According to FAOSTAT, global grapevine production exceeds 77 million tonnes annually (FAOSTAT, 2021). The utilization cascade of harvested grapes is highly specialized: approximately 71% is processed into wine, 27% is consumed as fresh table grapes, and 2% is preserved as dried raisins (Alleweldt *et al.*,1997). Due to its wider adaptability to different geographical locations *Vitis vinifera* and its hybrids are commercially cultivated *Vitis* species (Poolsawat *et al.*, 2012). During the years 2023–2024, India exported grapes (343 MT) worth 417.07 USD million to Europe, UK and Netherlands (APEDA, 2024). Maharashtra leads in terms of production and export market (accounting 67% of the nation's output). With a share of 27% in 2023–24, Karnataka accounts next largest producer (APEDA, 2024). The four different grape cultivars liked by consumers are colored seeded, coloured seedless, white seeded and white-seedless kinds and cultivars. Thompson Seedless, with its clones dominates the other cultivars, taking up 78.96% of the land. In 2024, Tas-e-Ganesh, Sharad Seedless and 2A-Clone each make up 4.5% and 3.5% of the entire area, respectively (APEDA, 2024). Beyond basic caloric and economic value, grapes are increasingly studied for their rich biochemical profiles, particularly secondary metabolites such as polyphenols, anthocyanins, and resveratrol. These compounds exhibit potent antioxidant properties, driving demand in the pharmaceutical, nutraceutical, and cosmetic industries.

To sustain this industry, grapevine genetics and breeding must evolve rapidly. Traditional objectives, maximizing yield and fruit quality (color, firmness, flavor) should be balanced against the urgent need for robust environmental resilience (This *et al.*, 2004). This review provides a comprehensive analysis of modern biotechnological tools, including CRISPR/Cas9 gene-editing platforms, Genome-Wide Association Studies (GWAS), high-throughput transcriptomics, advanced mutation breeding and multi-locus Marker-Assisted Selection (MAS) essential for introducing adaptive traits into elite grapevine germplasm.

Bottlenecks and challenges in grapevine breeding

Developing superior grapevine cultivars through conventional breeding methods is inefficient due to several biological and methodological constraints (Tello *et al*., 2019).

Extended Juvenile Phase: Grapevines require 2 to 5 years to transition from seed germination to the first reproductive phase, drastically prolonging selection cycles.

High Heterozygosity and Inbreeding Depression: One of the primary challenges in grape breeding and genetics is the genetic complexity of grapes. Grapes possess complex genome therefore, outcrossing is challenging and manipulating specific genes responsible for desirable traits also difficult (Carreño *et al*., 2015).

Limited Genetic Variation: The lack of genetic variation for specific traits can hinder breeding efforts to develop resistant varieties (Fernández-Muñoz *et al*., 2022).

Polygenic Architecture of agronomic traits: Vital attributes such as quality, berry weight, and tolerance to complex stresses are governed by quantitative trait loci (QTLs) displaying minor phenotypic effects and intricate epistatic interactions (Doligez *et al.*, 2013).

Environmental Influence: Grapevine phenology and berry chemistry are highly plastic and tightly modulated by microclimate, soil typography and vineyard management, compounding the difficulty of stable phenotype prediction (Fournier-Level *et al.*, 2010).

Genotype-Dependent Recalcitrance in Tissue Culture: Somatic embryogenesis and genetic transformation efficiencies remains low (frequently dropping down to 1–10% depending on the cultivar), hindering high-throughput functional genomics (Torregrosa *et al.,* 2015).

Even though these restrictions exist, continuous research is geared towards overcoming them and enhancing the effectiveness of grapevine breeding programmes. Enhancing the speed and accuracy of trait selection in grape breeding possible with help of developments in molecular genetics, genomics, breeding techniques and CRISPR-Cas9 (Emanuelli *et al.,* 2013 and Dalla Costa *et al.,* 2019). These restrictions can be addressed, and the way opened up for the creation of superior grapevine varieties by combining these approaches with conventional breeding techniques.

Objectives of grapevine breeding and genetics

Increased Yield and Quality: Research in grapevine breeding and genetics aims to create new grapevine varieties with increased potential for yield as well as enhanced fruit quality features like flavor, aroma, color and texture (Fournier-Level *et al.,* 2010).

Disease Resistance: Using genetics and grape breeding, researchers can find disease-resistant genes and incorporate them into new grape varieties, lowering the need for chemical treatments and enhancing the sustainability of grape production (Riaz *et al.,* 2018).

Climate Change Adaptation: Grapevine breeding and genetics helps to develop new varieties that are more resistance to shifting environmental conditions, such as drought and heat stress (Doligez *et al*., 2013)

Diversity in Flavor and Aroma: Research in grapevine breeding and genetics is essential for enhancing the flavor and aroma profiles of grapes. Breeders can create new grape varieties with diverse and distinctive flavors, catering to various customer preferences and broadening the variety of wine types by studying the genetic mechanisms underlying these qualities (Duchene *et al*., 2012).

Grapevine genetic resources and Germplasm architecture

The genus *Vitis* is divided into two distinct subgenera: Euvitis (2n = 38) and Muscadinia (2n = 40). The vast pool of wild relatives and traditional landraces provides irreplaceable alleles for targeted crop improvement. The major historical targets and their source genetic resources are structured in Table below.

Table 1: Major historical targets and their source genetic resources

Traits		Specific target stress	Key Genetic Donors
Disease resistance			
Biotic Resistance	Powdery mildew (*Uncinula necator)*	*V. aestivalis, V. amurensis, V. cinerea, V. berlandieri, V. labrusca, V. riparia, V. rotundifolia, V. rupestris, V. davidii, V. piasezkii, V. quinquangularis.*	
	Phylloxera (Dactylosphaera vitifoliae)	*V. berlandieri, V. cinerea, V. × champinii, V. rotundifolia, V. riparia, V. rupestris*	
Abiotic Resistance	Low temperature	*V. adstricta, V. amurensis, V. acerifolia, V. labrusca, V. riparia, V. vinifera ssp sylvestris var tipica, var balcanica, var aberrans, V. vulpina, V. yenshanensis, „Italian Riesling,*	
	Drought	*V. candicans, V. lincecumii, V. bourquiniana, V. rotundifolia*	
	Salinity	*V. acerifolia, V. berlandieri, V. riperia, V. candicans, V. × champinii*	

V. berlandieri, is utilized to create rootstocks that are resistant to phylloxera and lime-induced chlorosis since it is acclimated to highly calcareous soils. Ex-situ conservation strategies have shifted towards multi-tiered approaches, combining standard field gene banks with *in-vitro* slow-growth tissue cultures and ultra-low temperature cryopreservation (stored at -196 °C in liquid nitrogen) to preserve genomic integrity against environmental catastrophes. *V. vinifera ssp. sylvestris*, which is an ancestor of cultivated varieties, in grapevine breeding programmes and helps protect some grape varieties from biotic and abiotic stress factors like drought, lime, pests, and diseases (Tantasawat *et al*., 2012).

Methodological milestones in grapevine trait engineering

Developmental dynamics of seedless grape genotypes

Seedlessness is a key characteristic that improves grape marketing prospects with an objective of seedless grape with large berries suited for table consumption. It occurs *via* two mechanisms: parthenocarpy (fruit development without pollination/fertilization) (Perl *et al*., 2000) and stenospermocarpy (where fertilization occurs normally, but embryo development aborts shortly thereafter, leaving un-lignified seed remnants) (Costantini *et al.*, 2008). The inheritance of stenospermocarpy is governed by a complex genetic network. The widely accepted model posits a major dominant locus, Seed Development Inhibitor (SDI), interacting epistatically with three independent recessive genes. Molecular mapping has localized this major effect QTL to chromosome 18, identifying the MADS-box transcription factor gene VvAGL11 (Agamous-like 11) as the primary candidate driver. (Bouquet & Danglot, 1996; Lahogue *et al*., 1998; Adam-Blondon *et al.*, 2004; Doligez *et al*., 2002).

While, traditional crosses using a seedless parent as the pollen donor and a seeded parent as the pollen recipient yielded very few seedless progeny ranging from 0% to 49%, based on selected parental combination (Spiegel-Roy *et al*., 1990). Breeding for mutations: Mutant M 828 of 30 Gy, gamma radiation therapy, was noted to lack seeds in berries (Tetali *et al.*, 2020). The advent of *in-vitro* embryo rescue has revolutionized this breeding track, lifting recovery rates up to 16.7% to 92% (Ramming *et al*., 1990). Genotypic variance heavily influences embryo recovery rates; for instance, embryo rescue efficiency exceeds 68% in 'Ruby Seedless', 40% in ‘Red seedless’ whereas highly recalcitrant varieties like 'Superior Seedless' yield under 30%. (Hewstone *et al.*, 2006).

Marker-Assisted Selection (MAS) has significantly streamlined early-stage progeny screening for seedlessness in grapevine breeding programs. In F1 population derived from crosses between “Ruby Seedless” and “Sultanina”, seedless phenotypes were effectively identified using the dominant SCAR marker, SCF27. Similarly, in a "Muscat Hamburg" × "Sultani" hybrid population, Akkurt *et al*. 2019, demonstrated a robust association between a 198 bp allele from the VMC7F2 marker was strongly associated with seedless trait in early stage. The major effect Quantitative Trait Locus has been further characterized by (Cabezas *et al*., 2006) and Mejia *et al*. (2011), who mapped the VMC7F2 locus precisely 463 bp upstream of the predicted Open Reading Frame (ORF) of the VvAGL11 gene, reinforcing its functional role as the primary candidate gene regulating stenospermocarpy.

Multilevel approaches of Stress breeding under different biotic and abiotic conditions in grapevine

The grapevine (*Vitis* spp.) represents one of the most economically significant fruit crops globally, which is prized for its use in the creation of wines, juices and raisins. However, a number of environmental challenges, both biotic (black rot, downy mildew, powdery mildew, anthracnose, eutypa dieback etc.) and abiotic (drought, salt, heat, excessive moisture during ripening etc.) in nature frequently make it difficult to achieve productivity. Breeders have

concentrated on creating grapevine cultivars with improved resilience or tolerance to various stress elements in order to maintain sustainable grapes production (Jones *et al*., 2020).

To optimize grapevine performance under adverse environmental conditions, breeding programs focus on integrating genetic traits that confer pest and pathogen resistance, alongside tolerance to abiotic stresses (Guo *et al*., 2020). Substantial progress has been made in elucidating the genetic architecture underlying stress tolerance in *Vitis* species. This understanding has facilitated the development of elite cultivars through a combination of conventional breeding techniques and advanced biotechnological approaches, including marker-assisted selection (MAS) and genetic engineering (Liu *et al.*, 2012).

Conventional breeding achievements against biotic and abiotic stress in grapes

Grapevines are significantly impacted by drought as an abiotic stress, especially in dry and semi-arid areas. By adopting traditional breeding methods from wild grape species like *Vitis vinifera ssp. sylvestris and Vitis berlandieri*, drought-tolerant rootstocks, have been successfully introduced. Rootstocks have increased water stress tolerance and greater water use efficiency (Coupel-Ledru *et al.*, 2021). By combining heat-tolerant grape cultivars like Regent and Solaris, researchers in Germany have employed conventional breeding to create new grape cultivars with increased heat tolerance. According to Coleman *et al*. (2009), these novel cultivars exhibit enhanced fruit set and superior photosynthetic efficiency when subjected to high-temperature environments.

Recent germplasm evaluations have identified unique disease resistance traits within native Eurasian grapevines; specifically, the *Vitis vinifera* cultivars "Kishmish Vatkana" and "Dzhandzhal Kara" from the Near East exhibit resistance to powdery mildew (*Erysiphe necator*) (Riaz *et al*., 2018), while the Georgian cultivar "Mgaloblishvili" demonstrates resistance to downy mildew (*Plasmopara viticola*) (Toffolatti *et al*., 2016).

Precision Genome Editing via CRISPR/Cas9 strategies

The development of the Type II CRISPR/Cas9 system allows for precise, site-specific targeted mutagenesis in grapevine. By delivering customized guide RNAs (gRNAs) alongside the Cas9 endonuclease, researchers can directly knock out negative regulators of immunity (susceptibility genes) or stress-sensitive motifs (Critchley *et al*., 2019).

Pathogen Susceptibility Knockouts: CRISPR/Cas-based genome editing protocols can be deployed in *Vitis vinifera* cv. 'Thompson Seedless' somatic embryos to precisely mutate host susceptibility genes. In this study, we generated extensive, targeted deletions within suspected grapevine susceptibility loci to enhance pathogen tolerance. Transgene-free editing of candidate susceptibility targets such as Auxin Induced in Root Culture 12 (VviAIR12), Sugars Will Eventually Be Exported Transporter 4 (VviSWEET4), Lesion Initiation 2 and Dimerization Partner-E2F-Like 1 (VviDEL1) *via* dual-gRNA systems has yielded exceptional disease phenotypes. Notably, VviDEL1 mutant lines display over a 90% reduction in visual symptoms following challenge with *Erysiphe necator* (Olivares *et al*., 2021).

Abiotic Stress Alteration: Advanced functional genomics utilizing CRISPR/Cas9 technology have successfully targeted several key transcription factors and transporters to enhance abiotic stress tolerance in grapevines. For instance, Wang *et al*. (2020) disrupted the VvWRKY52 transcription factor gene, which is intricately involved in drought response pathways; the resulting mutants exhibited enhanced water retention capability and superior drought resistance compared to wild-type controls. Similarly, targeting the sodium/proton antiporter gene VvSOS1, a critical determinant of salinity tolerance, yielded edited grapevines with reduced foliar sodium accumulation and significantly improved salt tolerance (Ren *et al*., 2023). Furthermore, Hussin *et al*. (2022) deployed CRISPR/Cas9 systems to manipulate C-repeat binding factor genes, which serve as master upstream regulators in cold stress signaling networks, thereby elucidating their functional role in low-temperature acclimation.

Exogenous and Endogenous Antimicrobial Peptides (AMPs)

Antimicrobial Peptides (AMPs) represent an evolutionary conserved innate defense strategy. These low-molecular-weight, cationic, amphipathic peptides typically adopt alpha-helical conformations that electrostatically disrupt the negatively charged phospholipid bilayers of invading pathogens.

Recent investigations have highlighted the efficacy of exogenous and endogenous antimicrobial peptides (AMPs) in mitigating abiotic stress in grapevines through distinct biochemical pathways. Martínez-Esteso *et al.* (2021) demonstrated that the application of heterologous AMPs derived from diverse biological sources enhanced drought tolerance in *Vitis vinifera* by modulating the expression of

downstream stress-responsive genes and facilitating osmotic adjustment. Under salinity stress, Wang *et al.* (2022) characterized the protective mechanisms of a specific grapevine AMP, VvAMP1, which enhanced cellular resilience to ionic and osmotic imbalances. Furthermore, Kapoor *et al.* (2023) elucidated the functional role of VvAMP2 in heat stress mitigation, establishing that this peptide safeguards cellular integrity during thermal stress by upregulating the synthesis of protective heat shock proteins (HSPs).

Magainin and their synthetic analogues comprise a class of short (typically 21–26 amino acids), cationic, amphipathic alpha-helical peptides that exhibit broad-spectrum antimicrobial activity. These biopolymers have demonstrated robust inhibitory efficacy against major fungal pathogens in grapevine, notably suppressing the causative agents of botrytis bunch rot (*Botrytis cinerea*) and powdery mildew (*Erysiphe necator*) (Kristyanne *et al.,* 1997; Smith *et al*., 2001; Alan *et al.*, 2002 and Li *et al*., 2006).

Pierce's disease, a devastating vascular pathology in grapevines caused by the xylem-limited bacterium *Xylella fastidiosa*, is vector-transmitted by xylem-feeding leafhoppers (Cicadellidae), commonly referred to as sharpshooters. To mitigate this threat, transgenic resistance strategies have been developed utilizing antimicrobial peptides (AMPs) and polygalacturonase-inhibiting proteins (PGIPs). PGIPs are cell-wall-associated glycoproteins that selectively inhibit microbial endo-polygalacturonases (PGs), which are enzymatic virulence factors responsible for the degradation of host structural tissues (Vasanthaiah *et al.*, 2010). Additionally, transgenic expression of the lytic peptides "Thompson Seedless" and "Shiva-1" in grapevine host tissues has been shown to significantly enhance defense responses and restrict *X. fastidiosa* proliferation.

Mutation Breeding and Somaclonal Induced Variation

Mutation breeding is established as a highly effective method for altering specific target traits while preserving the elite genetic background of a cultivar, though a primary challenge involves minimizing collateral chromosomal abnormalities. Optimizing the mutagenic dose is critical to maximize the mutation frequency of the desired trait while preventing extensive genomic disruption and cell death from high-dose nuclear ionization (Coban, 1998). As documented by Mahure *et al*. (2010), elevated radiation doses induce mitotic inhibition, disrupting cell division and elongation through the inactivation or quantitative reduction of endogenous auxin levels, which subsequently impairs plant establishment and survival rates. Consequently, lower optimal dosages ranging from 10 to 20 Gy are preferred to mitigate these adverse effects; based on post-irradiation survival and growth kinetics, the median lethal dose (LD 50) for gamma irradiation has been established at 15–20 Gy for "Red Globe" and 15–25 Gy for "Muscat" grapevines.

Both physical and chemical mutagenesis protocols have successfully generated climate-resilient grapevine germplasm by inducing target genetic variations. Gamma irradiation has been deployed to generate novel genetic variability in grapevines, leading to the selection of stable mutants exhibiting enhanced water-use efficiency (WUE), superior drought tolerance, and stable crop productivity under water-deficit conditions (Ahmad *et al.,* 2018). Similarly, chemical mutagenesis utilizing ethyl methanesulfonate (EMS) has been applied to grapevine populations to disrupt thermal stress susceptibility pathways (Bota *et al.,* 2022). Screenings of these EMS-mutated cohorts have successfully identified distinct heat-tolerant mutants that display significantly higher photosynthetic efficiency, robust antioxidant enzyme activity, and minimized cellular damage under thermal stress compared to non-mutated wild-type controls (Li *et al.,* 2021).

Marker-Assisted selection and Multi-Locus Stacking

Rather than relying on time-consuming phenotypic evaluations under field conditions, modern breeding programs rely on high-density linkage maps constructed via Genotyping-by-Sequencing (GBS). This strategy allows breeders to track and combine multiple distinct resistance loci into single elite lines.

According to Riaz *et al*. (2008), the Pierce's disease resistance loci identified in the breeding lines F8909-08 and F8909-17 were designated PdR1h and PdR1a, respectively. By constructing genetic linkage maps for these two populations, we successfully identified simple sequence repeat (SSR) marker alleles tightly linked to *Xylella fastidiosa* resistance, providing robust molecular tools for marker-assisted selection (MAS) and targeted introgressive breeding.

The marker-assisted breeding could help to overcome the phylloxera resistance. A single allele known as Resistance to Daktulophagia Vitifoliae 2 (RDV2), which provides resistance to grape phylloxera, was found in the *V. cinerea* accession, according to a heritability study (Smith *et al*., 2018).

To identify and characterize heat-tolerant genotypes within grapevine breeding programs, Marker-Assisted Breeding (MAB) is routinely

integrated with functional physiological evaluations (Zinelabidine *et al.,* 2021). Beyond thermotolerance, MAB frameworks are heavily utilized to discover and validate candidate genes associated with low-temperature stress responses, providing critical genomic insights for the development of cold-hardy cultivars (Liu *et al.,* 2012). Furthermore, this molecular approach has facilitated the mapping of major Quantitative Trait Loci (QTLs) linked to salinity tolerance mechanisms, establishing a robust foundation for breeding salt-resilient *Vitis* germplasm (Wang *et al*., 2022).

Multi-Omic Integrations: Transcriptomics and Metabolomics

Recent advancements in omics technologies within the field of plant science have enabled researchers to gain a comprehensive, high-resolution understanding of the molecular pathways governing crop resilience to abiotic stress. It is possible to modify metabolic pathways to increase the stress tolerance of grapevines by studying the metabolic changes that occur under stress (Negri *et al*., 2017). Stress-responsive genes, regulatory networks, and molecular mechanisms involved in stress tolerance have been discovered using transcriptomic analysis (Rocheta *et al.,* 2016).

Ectopic expression of heterologous and homologous genes has proven highly effective in mitigating both abiotic and biotic stresses in grapevines. For instance, the co-expression of the *Medicago sativa* ferritin gene and the *Arabidopsis* C-repeat binding factor 1 (CBF1) transcription factor significantly enhanced abiotic stress tolerance in transgenic grapevines (Jin *et al.,* 2009). Similarly, constitutive expression of the endogenous *Vitis vinifera* CBF4 transcription factor in the rootstock hybrid 'Freedom' conferred a high level of freezing tolerance (Tillett *et al*., 2012). In terms of biotic defense, screening pathogenesis-related (PR) proteins revealed that the transgenic expression of a rice chitinase gene in the cultivars 'Neo Muscat' and 'Pusa Seedless' successfully boosted localized resistance against powdery mildew and anthracnose (Yamamoto *et al*., 2000).

Complementing these functional genomic strategies, high-throughput transcriptomics has revolutionized our understanding of grapevine physiology. This approach has successfully elucidated the global gene expression networks regulating both vegetative and reproductive ontogeny in *Vitis* species, while simultaneously mapping the precise transcriptomic signatures activated in response to complex biotic and abiotic stress matrices (Venturini *et al*., 2013).

Advanced Rootstock Breeding Architecture

Rootstock breeding has made substantial advancements in bolstering grapevine resilience to adverse environmental conditions through the selection of climate-resilient root system architectures. In viticultural regions prone to water scarcity, specific rootstock cultivars such as 'Richter 110', 'Ramsey', and '101-14 Mgt' are widely deployed due to their superior drought-adaptive mechanisms and water-use efficiency (Fort *et al.,* 2017). For regions characterized by high soil salinity, the 'Salt Creek' rootstock serves as a benchmark for salt exclusion and ionic tolerance, maintaining vine productivity under osmotic stress. Furthermore, rootstocks like 'Freedom' have demonstrated enhanced buffer capacity against extreme temperature fluctuations, making them particularly effective in preserving scion physiological performance across variable macroclimates (Ollat *et al.,* 2018).

Simultaneously, targeted breeding and clonal selection have successfully delivered rootstocks capable of combating destructive biotic pressures, most notably phylloxera (*Daktulosphaira vitifoliae*) (Migliaro *et al*., 2018). These phylloxera-resistant rootstocks employ multi-layered defense strategies, including a thickened root epidermis to deter penetration, the synthesis of endogenous allelochemicals or toxins, and the induction of a localized hypersensitive response (HR) at the insect feeding site to arrest pest development. To accelerate the integration of these complex biotic and abiotic defense traits, Marker-Assisted Selection (MAS) has been heavily adopted within modern rootstock breeding frameworks (Pap *et al.,* 2021). By facilitating the high-throughput, early-stage identification of favorable alleles, MAS circumvents the logistical bottlenecks of prolonged phenotypic evaluations, significantly shortening the breeding cycle for multi-stress resilient viticultural germplasm.

Post-Harvest Physiology and Shelf-Life Extension

A primary bottleneck in viticultural supply chains involves preserving fruit quality metrics during post-harvest storage and transit. Functional genomics has successfully identified key gene networks regulating post-harvest senescent processes, specifically those governing cell wall degradation, carbohydrate metabolism, and localized stress responses; prioritizing these targets enables breeders to select for extended shelf life and minimized decay susceptibility (Prasad *et al*., 2014). For instance, exposing grape berries to a

thermal pretreatment of 38 °C for 10 hours upregulated heat shock protein 70 (Hsp70) expression, which directly correlated with decreased solute and electrolyte leakage during subsequent chilling stress (Zhang *et al.,* 2005). Application of transcriptional and translational inhibitors namely actidione D (cycloheximide) and CHX demonstrates that this acquired cold tolerance is dependent on the de novo transcription and synthesis of Hsp70, though the precise downstream biochemical mechanics of Hsp70 mediated cryoprotection require further elucidation.

Beyond abiotic stress mitigation, specific small heat shock proteins function as critical modulators of plant innate immunity and biotic defense pathways. Heterologous overexpression of the grapevine chaperone gene VvHSP24 in *Arabidopsis thaliana* significantly increased the transcript abundance of *Nonexpressor of Pathogenesis-Related Genes 1* (NPR1) and salicylic acid (SA)-responsive marker genes (*PR1*, *PR2*, and *PR5*), thereby enhancing host resistance against *Botrytis cinerea* (Wang *et al.,* 2021). These data indicate that VvHSP24 operates via an NPR1-dependent signaling cascade to orchestrate defense against necrotrophic fungal pathogens. Furthermore, evidence suggests that during beta-aminobutyric acid (BABA)-induced priming, the VvHSP24 chaperone may undergo specific post-translational modifications necessary to activate the VvNPR1 transcriptional complex, subsequently triggering downstream pathogenesis-related (PR) gene expression and establishing the observed resistance phenotypes (Li *et al*., 2021).

Future perspective and challenges:

Pangenomics-Assisted Breeding: Moving beyond a single reference genome toward a species-wide Vitis pangenome will unlock structural variants, copy number variations (CNVs), and hidden alleles associated with complex quality and defense traits.

Cisgenic and Transgene-Free Systems: To help clear strict regulatory hurdles surrounding Genetically Modified Organisms (GMOs), future efforts should focus on cisgenics (transferring genes exclusively between cross-compatible Vitis species) or using pre-assembled Cas9 protein-gRNA complexes (Ribonucleoproteins, RNPs) to generate transgene-free edited vines.

Machine Learning and High-Throughput Phenotyping: Integrating automated field phenotyping (using drones equipped with hyperspectral cameras) with genomic selection models will allow breeders to accurately predict field performance early in the selection cycle, bypassing traditional field evaluation bottlenecks.

Conclusion

The sustainable future of global viticulture depends on our ability to quickly breed next-generation grape varieties. While traditional crossbreeding and rootstock selection provided the initial foundation for global production, they are simply too slow to keep pace with accelerating climate change and evolving pathogens. Successfully navigating these challenges requires a unified approach: combining the deep diversity of wild germplasm collections with the speed and precision of Marker-Assisted Selection, multi-omic discovery, and CRISPR/Cas9 gene editing. By unlocking these genetic tools, we can secure the long-term sustainability, yield, and exceptional quality of this globally vital crop.

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Chithra M.: Writing original draft; Sharmela P.: Reviewing and editing.

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