INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
25–968/2026/35:350308
http://www.fspublishers.org

Review Article

Modern Approaches and Prospects of Grape (*Vitis vinifera*) Biotechnology: A Review

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Received 12 September 2025; Accepted 29 December 2025; Published online 11 February 2026

Editor: Muhammad Farooq

Abstract

Viticulture is among the significant horticultural sectors in the world, especially in Mediterranean climate zones. Grapes are mainly grown for consumption, production of raisins, technical purposes including juice and concentrate processing, organic acid and grape seed oil extraction, and winemaking. Due to their high economic value, it is important to study grape fruit characteristics such as taste, size, colour, and grapevine resistance to various diseases and stresses. By obtaining transgenic lines of grapevine, it is possible to find out and study the functions of genes associated with resistance to diseases and pests, plant development and primary and secondary metabolic products. Several studies have been conducted using various explants on grapevine transformation and regeneration processes. In particular, transformation using the biolistic method, *Agrobacterium*-mediated transformation process, and the development of somatic embryogenic tissues using anthers, unfertilised ovaries, pollen grains, and leaves as explants have been demonstrated. In these studies, grape varieties obtained by introducing new traits and characteristics into the genome of traditional varieties retain a high level of heterozygosity and are highly unlikely to undergo somaclonal mutations. In this context, the CRISPR/Cas9 genetic modification technology is an effective tool for generating pinpoint modifications of endogenous genes, conducting functional studies, and creating new varieties. Much research is being conducted in horticulture, including viticulture, on using the CRISPR/Cas9 method.

Keywords: *Vitis vinifera* L.; Gene; Abiotic stress; CRISPR/Cas9; Somatic embryogenesis; Genome editing

Introduction

Growing of the grape (*Vitis vinifera* L.) plants is expected to have been started between 8,000-11,000 BC and comprises approximately 60-80 species of *Vitis* genus (Wolkovich *et al*. 2018; Zombardo *et al*. 2022; Dong *et al*. 2023). Due to well-adaptive biological ability and essential source of raw materials for food supply, grape cultivar plantations are grown in a wide range of regions such as Mediterranean, European, Asian and American continents (Wolkovich *et al*. 2018; Savi *et al*. 2019; Gambetta *et al*. 2020). Nowadays, the total size of vineyards has increased by approximately 7.2 million ha, with annual grape production estimated at 77.8 million tons, of which 47.4% is for wine, 44.5% for consumption, and 8% for raisin production (IOVW 2025).

However, grapes industry is facing climate change issues, including increased temperature, drought, cold, and salinity, which significantly affect yield, fruit composition, and wine quality, which significantly threaten the productivity of grapevine varieties and the sustainability (Costa *et al.* 2015; Bernardo *et al*. 2018; Wolkovich *et al.* 2018; Venios *et al.* 2020; Jägermeyr *et al.* 2021; Eckardt *et al.* 2023). Developing new resistance grape cultivars is one of the effective solutions to address existing issues (Li *et al.* 2025). The efficiency of the grape breeding program depends significantly on the application used. The complex biological aspects of grapes, including the recessive nature of some genetic traits, high heterozygosity, limited hybridisation among grape species with different chromosome numbers, pollen sterility, sexual dimorphism of flowers, and time-consuming breeding cycles, may reduce the efficacy of breeding programs (Verslues *et al.* 2023; Abdullaev *et al.* 2025a). Over the last few years, several innovative techniques of grape breeding have shown high effectiveness, stimulating the integration of biotechnological, molecular, and gene-editing approaches (Ubaydullaeva *et al.* 2025).

The new biotechnological protocols have been successfully implemented into plant science, and as a result, the research has focused from anatomical and physiological studies to tissue, cellular, and molecular levels. Several

To cite this paper: Ubaydullaeva KA, HN Yusupov, AN Abdullaev, AA Bolkiev, SA Abdullaev, FI Babadjanova, JB Eshmurzaev, ZT Buriev (2026). Modern Approaches and prospects of grape (*Vitis vinifera* L.) biotechnology: A review. *Intl J Agric Biol* 35:350308. https://doi.org/10.17957/IJAB/15.2453

researchers, such as Thorpe (2007), Sussex (2008) and Vasil (2008) described developmental evaluation comprehensive reviews focusing on current applications and advances in plant biotechnology. They argued that achievements of biotechnology directly stimulated the development of gene editing tools. Moreover, grape was among one of the first perennial plants utilised in tissue culture experiments (Morel 1944), but genetic transformation of *Vitis* was reported only at the end of the 20th century by Mullins (Mullins *et al.* 1990). Nevertheless, this technique is still required for development or optimisation, mainly because of the limited regenerative attribute of grapevine tissue (Mullins *et al.* 1990).

The early reference genome of a perennial fruit crop was documented in 2007 for the homozygous grapevine genotype PN40024, which was derived from self-pollination of European species of *V. vinifera* (Jaillon *et al*. 2007; Velt *et al*. 2023). The diploid grape genome (2n = 38) is of small size, approximately 500 Mb and in addition is transformable, with the ability for micropropagation *via* somatic embryogenesis, making it a model plant among perennial fruit (Carra *et al*. 2024). Extensive investigations have been implemented to elucidate the grape genome structure, metabolic pathways, mutation regions, QTL mapping, as well as to develop molecular markers associated with agronomical traits and identify target genes based on the expression of genes for genome editing. Therefore, genes and their regions are a key factor to increase breeding program efficacy. Within review, we discuss the recent tendency of grape breeding programs, achieved results, existing issues of grape genetics and biotechnology, and new gene editing applications.

The first reports related to grape biotechnology (Table 1) were carried out by Morel (1944). These early studies were related to *in vitro* tissue culture in a sanitary setting (Torregrosa *et al.* 2001; Bouquet and Torregrosa 2003). Later, artificial culture media for *in vitro* culture were developed for plant propagation, allowing tissues or organs to be developed from pre-existing meristems. During the 1970s and 1980s, these methods allowed the development of sanitary procedures against viruses and the use of grafts and varieties for vegetative and genetic improvement for *in vitro* propagation. Since the 1980s, methods for plant regeneration from shoot meristems have been developed. The initial somatic regeneration process was carried out through adventitious organogenesis, which involves triggering the formation of neo-buds (Barlass and Skene 1978).

The first work on introducing targeted genes into grapevine was successfully carried out by Hemstad and Reisch (1985) using an *Agrobacterium tumefaciens* plasmid and reported genetically modified callus and root regeneration. Cabernet-Sauvignon transgenic forms were developed from vegetative organs which are expressed ectopically, but stable plant regeneration was not possible (Baribault *et al.* 1989). Mullins *et al.* (1990) obtained the first transgenic grape, which was achieved by transforming

Table 1: Grapevine biotechnology – main achievements

Achievements	Reference
Micropropagation	Galzy (1961)
Somatic embryogenesis process	Mullins and Srinivasan (1976)
Obtaining transgenic grapevines	Mullins *et al.* (1990)
Agronomic trait manipulation	Gall *et al.* (1994)
Using CRISPR/Cas9	Ren *et al.* (2016)
DNA-free genetic editing	Malnoy *et al.* (2016)

rootstock cells with *Agrobacterium*-deactivated vectors and regenerating embryos produced by the somatic embryogenesis process. Gall *et al.* (1994) made the first attempt to insert genes of agronomic significance by introducing the Grapevine Chrome Mosaic Virus (GCMV) coat protein gene into interspecific *Vitis vinifera* x Muscadinia hybrids and rootstocks. Many grape (*V. vinifera* L.) species received advantages from the development and application of gene transformation technology over time (Lovisolo *et al.* 2010; Torregrosa *et al.* 2015; Bouquet *et al.* 2006, 2008). The *Agrobacterium*-based transformation process was also applied to microvines (Chaib *et al.* 2010), a suitable example for improving physiological as well as genetic research in the vineyard (Torregrosa *et al.* 2016). But even with all of the advancements, Vidal *et al.* (2010) report that in most grape genotypes, genetically modified plant regeneration is still an extended and difficult procedure. *V. longii* and *V. vinifera* are among the few species that have adapted to somatic embryogenesis (Gray 1992; Reisch and Pratt 1996). Different parts of the host plant were used in grape tissue culture for obtaining a new transgenic cultivar.

Unlike previous reviews, this work integrates recent advances in somatic embryogenesis with emerging CRISPR/Cas-based genome editing strategies to highlight practical bottlenecks and future directions for grapevine improvement.

Agrobacterium-based transformation

Permanent grapevine transformation efficiency, a complex and prolonged process, is influenced by the plant genotype, the type of explant used, the composition of the culture medium, and the transformation method. Grapevine transformation is most often achieved through somatic embryo co-cultivation with *A. tumefaciens*, primarily for functional gene studies (Lecourieux *et al.* 2010; Li *et al.* 2012; Nicolas *et al.* 2013, 2014; Sun *et al.* 2018; Pessina *et al.*, 2016; He *et al.* 2018). Since the genetically modified plant regeneration obtained from altered somatic embryos occurs over an extended period, certain organs can also be used to investigate gene function. This genetic transformation technique has been continuously improved to enable the description of the functions and control of multiple genes (Jelly *et al.* 2014; Chialva *et al.* 2016) (Table 2).

Agrobacterium can also transform non-embryogenic cultures in a stable way, which has been demonstrated to be especially effective in the biosynthesis of biologically active metabolites (Martínez-Márquez *et al.* 2015). Chu *et al*. (2016) employed the Sonication-Assisted

Table 2: Different methods of Agrobacterium-based transformation techniques

Technique	Explant	Varieties	Strain	Use	Authors
Syringe-based agroinfiltration	*In vitro* cultured plantlet leaves	Superior seedless	AGL1, GV3101	Repression of gene function	Urso *et al.* (2013)
Vacuum infiltration	*In vitro* cultured plantlet leaves	Thompson seedless	C58C1 (pCH32)	Enhanced expression	Visser *et al.* (2012)
		Cabernet Franc, Syrah, Zinfandel	EHA105	Engineering of virus-based vectors	Kurth *et al.* (2012)
	In vitro cultured plantlet leaves	Cabernet Franc	GV3101	Repression of gene function	Bertazzon *et al.* (2012)
		Cabernet-Sauvignon, Cinsault, Muscat, Carignan	C58C1 (pCH32)	Overexpression	Santos-Rosa *et al.* (2008)
			GV3101	Overexpression	Guan *et al.* (2011)
				Overexpression	He *et al.* (2013)
			LBA4404	Overexpression	Xu *et al.* (2014)
	Leaves	Grenache	GV2260	Viral vector engineering	Liu *et al.* (2009)
		Syrah	GV3101	Overexpression	Henanff *et al.* (2009)
Co-cultivation	Cell suspension	Gamay Red	EHA105	Promoter analysis	Gollop *et al.* (2002)
	Somatic embryos	Chardonnay	GV3101	AmiRNA activity assessment *via* cotransformation	Jelly *et al.* (2012)
		Thompson seedless	EHA105	promoters testing	Li *et al.* (2001)
		Freedom	EHA105	Enhanced expression	Tillet *et al.* (2012)
		41B	EHA105	Enhanced expression	Nicolas *et al.* (2013, 2014)
		Chardonnay, Thompson seedless	EHA101	Enhanced expression	Fan *et al.* (2008); Agüero *et al.* (2015)
		Thompson seedless	GV3101	Enhanced expression	Zhou *et al.* (2014)

Agrobacterium-mediated Transformation (SAAT) approach, originally developed for soybean transformation by Trick and Finer (1997), to enhance the transformation efficiency of dedifferentiated *V. vinifera* cv. Monastrell cells. The transformation efficiency is increased by this technique.

The zygotic ovaries, leaves, embryos, and anther filaments are the most important plant parts in grape tissue culture for obtaining new transgenic cultivars (Scorza *et al.* 1995; Scorza *et al.* 1996; Motoike *et al.* 2001). To obtain transgenic grape cultivars *via* somatic embryos, Thomson seedless leaves were used, and a total of 700 somatic embryos yielded 0.68–2.32% transgenic plantlets depending (*Agrobacterium* alone with the pGA482GG construct resulted in the recovery of 9 transgenic plants; particle bombardment was combined with *Agrobacterium* using the pGA482GG construct, 26 transgenic plants were obtained; *Agrobacterium* and particle bombardment with the pGA482TomRSV-CP construct yielded the highest number, with 41 transgenic plants recovered) on the construct and transformation method (Scorza *et al.* 1996). Motoike *et al.* (2001) demonstrated the formation of stable embryo Niagara' and 'Fredonia' grape cultivars, which are derived from *Vitis* × *Labruscana* that were able to maintain embryonic viability for more than two years. The cultivar-specific responses to auxin (2, 4-D) and cytokinin (TDZ) highlight the complex hormonal control of cellular totipotency. The conversion rate from somatic embryos to normal plants was 15–18% despite high germination rates (56–93%), suggesting a major barrier between morphogenesis and physiological normalisation (Motoike *et al.* 2001).

Agrobacterium-based transformation is one of the main strategies used to deliver vector constructs into plant cells. This method is a technically difficult however this approach to obtain transgenic plants has not lost their appeal (Barampuram and Zhang 2011). *Agrobacterium* tumefaciens is the common strain used in grapevine transformation, but according to Hu and Du (2006) *Agrobacterium* rhizogenes strains can also be used for transformation into root hairs. The strains of *Agrobacterium tumefaciens* most often applied are C58C1 (Hamilton *et al.* 1996), GV3101 (Koncz and Schell 1986) and EHA105 (Hood *et al.* 1993). For example, genetically modified root hairs generated using A. rhizogenes are applied in functional genomic analysis (Hu and Du 2006; Gomez *et al.* 2009; Terrier *et al.* 2009; Höll *et al.* 2013), because the formation of transgenic root hairs occurs quickly (Torregrosa and Bouquet 1997).

Many temporary transformation systems have already been created to rapidly investigate the function of unknown genes. These experimental techniques are more rapid and simpler than the permanent transformation method. The technique primarily uses direct genetic transformation techniques by biolistic bombardment, electroporation, or protoplast transfection, as well as *Agrobacterium*-mediated transformation. Janssen and Gardner (1990) demonstrated that active transcription of the target gene was produced when mesophyll cells and A. tumefaciens were cocultured for a brief period of time. Various agroinfiltration approaches, including syringe and vacuum-based delivery into leaves or whole plants, have been successfully applied in grapevine, with efficiencies varying among cultivars (Table 2).

The advancement of genome editing technologies has led to a significant improvement in genetic modification techniques. These technologies, especially the CRISPR/Cas9 system, apply restriction enzymes to modify the expression and the structure of genes more precisely and specifically than previous methods. Ren *et al.* (2016) demonstrated the initial proof of the notion for grapes by altering the tartaric acid metabolism through mutations in the Chardonnay enzyme L-idonate dehydrogenase (IdnDH). From that time, a number of research teams have worked to create methods for modifying DNA without introducing genetic material from bacteria or viruses. Both functional genomics investigations and grapevine genetic enhancement could benefit from these new technologies (Costa *et al.* 2017).

Table 3: Different cloning methods

Gene cloning techniques	Limitations	Benefits
Recombinational	Higher cost compared with conventional approaches Use of supplier-defined vector sets	Facilitates large-scale and efficient vector generation Supported by extensive, publicly accessible ORF libraries
Traditional	Limitations in sequence flexibility resulting from the presence and potential translation of restriction sites	Low cost Versatile Oriented cloning
PCR	Restricted availability of vector options Relatively higher cost compared with alternative methods Lack of precise sequence control at the cloning junction Directional cloning can be technically challenging	High cloning efficiency Compatible with high-throughput applications

Cloning methodologies

One of the fundamental processes in the area of genetic engineering is the process of molecular cloning. It is the technique for separating and introducing DNA/RNA molecules into a host organism. Usually, this procedure includes the following elements: (1) a recipient vector or plasmid core that has all the elements needed for the replication process in the host cell and (2) the fragment of DNA to be inserted (Costa *et al.* 2019). Several strategies exist for inserting modules assembled individually or from multiple DNAs (Table 3).

Traditional cloning typically uses a cutting endonuclease to create DNA fragments with complementary sequences that a ligase enzyme can join. The insert gene and the vector are prepared by cutting with two unique restriction enzymes. The polymerase chain reaction (PCR) discovery PCR fragments designed with restriction sites at their ends for cloning purposes required ligation with the vector, thus facilitating the application of restriction cloning for PCR cloning (Costa *et al.* 2019).

According to the research of Gibson *et al.* (2009), using a DNA ligase to seal nicks, a DNA polymerase to extend annealed single-stranded gaps, and a 5′–3′ exonuclease to generate overhangs, this technique is a single-step isothermal reaction of the *in vitro* recombination process.

Another method of the continuous cloning process is Golden Gate assembly, which breaks down DNA outside the recognition site by applying a type II-S restriction enzyme (Engler *et al.* 2008, 2009). There are various benefits to this technique. For example, no extra DNA is added, and restriction enzymes avoid marking the newly formed overhang sequence. Additionally, it is possible to put together several fragments in a particular order due to the overhangs' fragment-specific sequencing.

Applying integrases or recombinases, recombinant cloning has established a lot of demand as part of plant genetic engineering studies in the last few decades. Without the application of restriction enzymes or ligases, these enzymes enable the transfer of the DNA fragment between vectors. It has been found that multi-round gateway technologies (recombinases) can deliver multiple transgenes in a single reaction (Vemanna *et al.* 2013).

Physical and chemical transfer techniques

The biolistic approach, sometimes referred to as particle bombardment, is a physical technique established in the 1980s to introduce gold or tungsten microparticles coated with a genetic delivery that contains specific genetic information into plant cells (Klein *et al.* 1987). Biolistic methods can introduce genetic modifications into the nuclear, plastid, or mitochondrial genomes, in contrast to transformation methods *via Agrobacterium*. Despite that, the effect of this method is limited, and the success rate varies depending on the plant species or cultivars.

The first biolistic transformation processes in the grapevine started in the 1990s-2000s. The first line of genetically modified grapevines, *Vitis* Hybrid Chancellor, was reported by Kikkert *et al.* in 1996. Then, applying the microprojectile bombardment technique provided a consistent and effective platform for stable transformation and plant regeneration (Vidal *et al.* 2003). The biolistic approach proved valuable in short-term screening of a variety of plant components in grape, including suspension cultures, leaf fragments and embryogenic tissues (Cunningham *et al.* 2018) (Table 4).

Electroporation – this technology was initially created for protoplast transformation and subsequently used on whole cells of wheat and rice (Shimamoto *et al.* 1989; He *et al.* 1994). In this method, exposing cells to a strong electric field enables the introduction of genetic material into the cell by causing a transient hole in the cell membrane. Over the past decade, electroporation protocols for plant transformation have been optimised and standardised for various species (Barampuram and Zhang 2011).

The polymer form of ethylene oxide is used in the PEG (polyethylene glycol)-mediated transformation technique to help transmit DNA inside the protoplast. Using this technique, transformation is initiated by incubating protoplasts with DNA in the presence of divalent cations. PEG treatment of protoplasts decreases membrane stability, thereby allowing DNA molecules to penetrate into the plant cell. Suspension cultures generated from PEG-treated protoplasts of *Cabernet-Sauvignon* grapevine have been widely applied for analyses of subcellular distribution of proteins (Hichri *et al.* 2010), control of promoter activity (Saumonneau *et al.* 2012), protein–protein complex

Table 4: An overview of the chemical and physical techniques for delivering DNA

Delivery technique	Negative effects	Objectives	Delivery contents	Challenges
		Physically mediated techniques		
Particle gun–based delivery	Injury to recipient tissues and the delivered material Low penetration depth Random integration	Calli, embryos, leaves	DNA, siRNA, miRNA, ribonucleoproteins, large size	Transformation success is strongly dependent on plant species and cultivar Transformation success is strongly dependent on plant species and cultivar The use of embryos as targets is limited by the demanding regeneration process required
Electroporation	Potential injury to the recipient tissue Non-selective passage of molecules through induced pores Non-selective passage of molecules through induced pores	Protoplasts Meristems Pollen grains	Nucleic acids (DNA, siRNA, miRNA)	Restricted capacity for transporting genetic material
		Chemically mediated techniques		
PEG-mediated delivery	High density can induce cytotoxicity	Species amenable to protoplast regeneration Protoplasts	Nucleic acids (DNA, siRNA, miRNA)	The regeneration process is inefficient in the majority of plant species when using transient transformation approaches

Table 5: Common successful somatic embryogenesis protocols in *V. vinifera*

Application	Growth regulators	Explant types	Reference
Methodological framework development	2,4-D 6.5 μM + BA 7 μM	Immature anthers	Vasanth and Vivier (2017)
Methodological framework development	NOA 5 μM + BA 0.9–4.5 μM	Leaves, anthers	Stamp and Meredith (1988)
Methodological framework development	2,4-D 4.5 μM + BA 9 μM	Whole flowers, anthers, ovary	Martinelli *et al.* (2003)
Methodological framework development	2,4-D 4.5 μM + BA 9 μM	Whole flowers, anthers, ovary	Gambino *et al.* (2007)
Influence of parameters	2,4-D 9.0 μM + BA 4.4 μM	Unopened and fully opened leaves, petioles	Dhekney *et al.* (2011)
Genetic modification for improved disease tolerance	NOA 5 μM + BA 4.44 μM + phenylalanine 5.0 m*M*	*In vitro* leaves	Nookaraju and Agrawal (2012)
Gene transfer approaches	2,4-D 4.52 μM + BA 4.4 μM: 2,4-D 4.52 μM + NOA 2.5 μM + 4-CPPU 5 μM	Floral explants	Saporta *et al.* (2014)
Optimisation of transformation procedures for difficult-to-transform genotypes	2,4-D 4.5 μM + BA 8.9 μM	Immature inflorescences	Forleo *et al.* (2021)
Preservation of embryogenic potential during long-term culture	2,4-D 9 μM + IAA 6 + BA 4.4 μM + GA3 1.8 μM	*In vitro* leaves	Tsvetkov *et al.* (2014)
Mutagenesis	2,4-D 9 μM + BA 4.4 μM then NAA 5.4 μM + BA 4.4 μM +	Leaves	Kuksova *et al.* (1997)
Optimisation of experimental approaches	NOA 5 μM + BA 4.5 μM + several amino acids	*In vitro* leaves	Nookaraju and Nookaraju (2013)
Optimisation of experimental approaches	2,4-D 9 μM + BA 9 μM	Leaves	Tsolova and Atanassov (1996)
Optimisation of experimental approaches	2,4-D 0.45 μM + BA 4.5 μM	Leaves	Das *et al.* (2002)
Optimisation of experimental approaches	IAA 5.7 μM or IBA 0.5 μM	Leaves, petioles	Martinelli *et al.* (1993)
Optimisation of experimental approaches	2,4-D 9 μM + BA 4.4 μM, then NAA 10.7 μM + BA 0.9 μM	Leaves, petioles	Robacker (1993)

2,4-D: 2,4-dichlorophenoxyacetic acid; BA: 6-benzyladenine; 4 CPPU: N-(2-chloro-4-pyridyl)-N′- phenyl urea; IAA: indole-3-acetic acid; IBA: indole-3 butyric acid; NAA: 2-naphtaleneacetic acid; NOA: 2 naphthoxyacetic acid; GA3: gibberellic acid

(Saumonneau *et al*. 2008) and DNA–protein complex (Marchive *et al*. 2013). The predominant method for introducing CRISPR/Cas9 ribonucleoproteins into Chardonnay protoplasts is PEG-mediated transformation (Malnoy *et al.* 2016).

Somatic embryogenesis

In somatic embryogenesis (SE), bipolar plant forms arise from individual somatic cells without undergoing meiosis or fertilisation, producing plantlets that are clonal copies of the parental plants. This complex process can proceed *via* either direct or indirect somatic embryogenesis. Nevertheless, it is sometimes hard to differentiate between both ways, because each of these ways can happen at the same time from the same type of explant. Indirect somatic embryogenesis is the most commonly used method, starting with the development of a normal callus, involving a mass of cells that proliferate at various levels, and there are many examples of this process in grapevine (Krul and Worley 1977; Martinelli *et al.* 1993; Zhu *et al.* 1997; Salunkhe *et al.* 1999; Nakajima *et al.* 2000; Perrin *et al.* 2004; Prado *et al.* 2014; Bertini *et al.* 2019). The specimen reacts to both intrinsic and external stimuli that change its internal hormone levels while plant cells are dedifferentiating and differentiating. The above information provides validity to the ideas that auxins are crucial for cell polarisation and for the initiation and subsequent development of embryos (Souter and Lindsey 2000; Wabnik *et al.* 2010; Rodríguez-Sanz *et al.* 2015; Nic-Can and Loyola-Vargas 2016; Pérez-Pérez *et al.* 2019).

The SE process application has enabled the introduction of many practical and scientific developments. For example, this method allows for the genetic and hygienic enhancement of commercially important varieties and provides insight towards the fundamental mechanisms of biological processes (Goussard and Wiid 1992; Gambino *et al.* 2009; Acanda *et al.* 2015; Bouamama-Gzara *et al.* 2017; Catalano *et al.* 2021; Forleo *et al.* 2021). Although CRISPR technology has revolutionised genetic engineering, its effective application remains limited in many plants due to the lack of efficient *in vitro* regeneration systems (Zhang *et al*. 2021). In grapevine, however, this limitation has been overcome through the use of somatic embryogenesis (SE) (Li *et al*. 2020; Wan *et al*. 2020; Paul *et al*. 2021; Ren *et al.* 2021; Tu *et al.* 2022; Fizikova *et al.* 2024).

The restricted embryogenic potential of certain varieties represents the main factor restricting the effective use of SE. If *in vitro* somatic embryo regeneration is not possible using commonly used protocols, it is called recalcitrant (Horstman *et al.* 2017). This recalcitrant affects both embryo differentiation and the following steps of the regeneration process, from embryo germination to plant acclimation. Although the SE process can be performed in several plant organs or tissues (Carra *et al.* 2024) (Table 5), selecting the appropriate form of explant is essential (Fiore *et al.* 2002). Successful application requires identifying the best period for explant collection, the developmental phase, and the plant organs most responsive to embryogenesis. In numerous plant species, the developmental stage of the cells is critical, with younger cells typically regarded as the most favourable for embryogenic culture initiation (Carimi and Pasquale 2003; Carimi *et al*. 2005; Bonga *et al.* 2009).

The best results on the SE process in grapevine are usually obtained using floral explants (Fig. 1A-E; Table 5). Due to the involvement of several auxin biosynthetic routes, the inflorescence represents an ideal system for somatic embryogenesis studies (Aloni *et al.* 2006; Cucinotta *et al.* 2021).

The stage of explant development significantly influences the effectiveness of somatic embryogenesis. Moreover, protocol efficiency is highly dependent on both the appropriate level of exogenous auxin and the incubation period (Vidal *et al*. 2009; Cardoso *et al*. 2010). As reported by Vidal *et al*. (2006), anther-derived embryogenic cultures are best initiated at the early flowering phase, while ovary-derived cultures respond more efficiently at subsequent developmental phases. Prado *et al.* (2010) classified three stages of flower development. Gribaudo *et al.* (2004) reported that R1 and R2 stages correspond to stages V and VI, while R3 represents the late binucleate microspore stage (Fig. 2).

Somatic embryos undergo a series of well-defined developmental stages. In dicotyledons, including grapevine, somatic embryogenesis proceeds through the globular, heart, torpedo, and cotyledonary or plantlet stages (Zimmerman 1993; Carimi *et al.* 2005; Martinelli and Gribaudo 2001; Abdullaev *et al.* 2025a), as summarized in Fig. 1E. These stages reflect progressive tissue differentiation and reserve

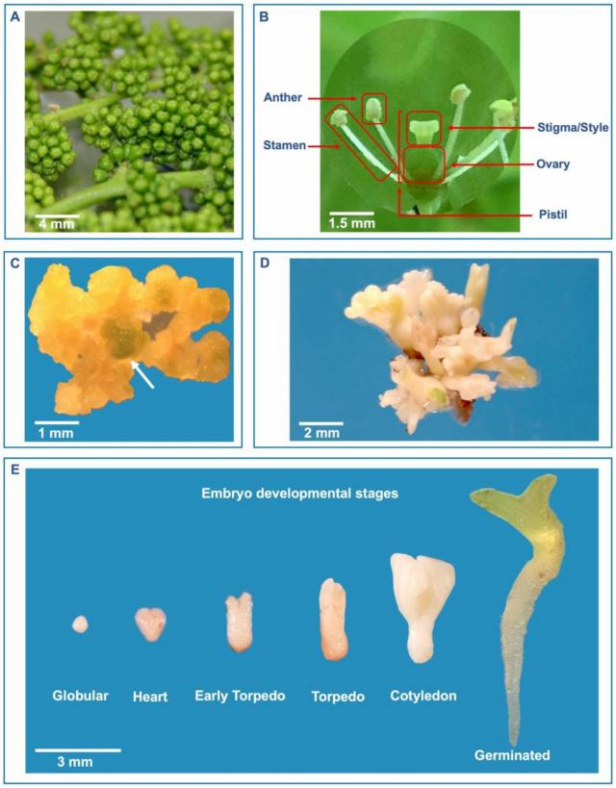

Fig. 1: Somatic embryogenesis and floral tissue–mediated plant regeneration in *Vitis vinifera* (**A**) Inflorescence, (**B**) different floral explants, (**C**) callus induction from floral tissue, (**D**) regenerated somatic embryos and (**E**) developmental stages of somatic embryos

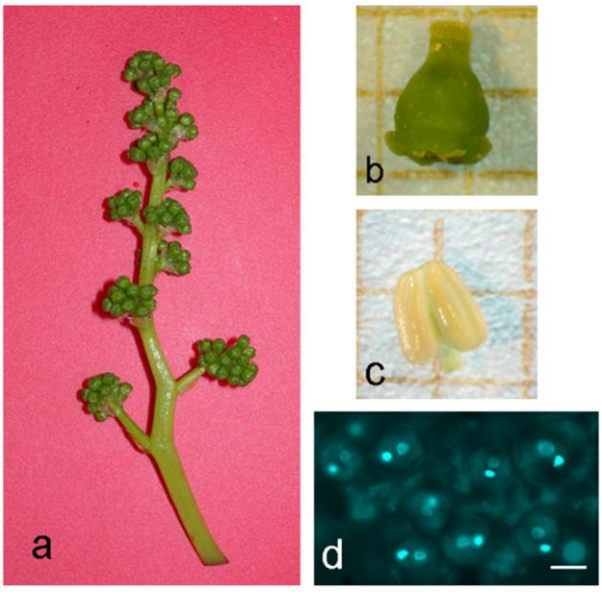

Fig. 2: Developmental stages of grapevine floral explants used for somatic embryogenesis: (**a**) inflorescence, (**b**) ovary, (**c**) anther explants and (**d**) DAPI-stained binucleate microspores at stage R3 in cv. Mencía

accumulation, ultimately leading to embryo germination and plantlet formation (Joshi and Kumar 2013).

Several factors influence the somatic embryos' formation and maturation, and genetic or epigenetic modifications in DNA could be the cause of anomalies. Extremes in temperatures, drought, salt, heavy metals, mutagenic chemicals and plant hormones can cause DNA alterations (Garcia *et al.* 2019). ABA metabolic pathway, among other factors, is essential for grapevine somatic embryo maturation (Acanda *et al.* 2020). Long-term preservation of embryogenic cultures while retaining their potential is a significant benefit, despite inherent variations (Matsuta and Hirabayashi 1989; Perl *et al.* 1995; Motoike *et al.* 2001; Croce *et al.* 2005; Martinelli and Gribaudo 2009; Dhekney *et al.* 2011). The SE process is influenced by several intrinsic and extrinsic factors, which make it technically challenging to perform. The following factors significantly affect the embryogenesis process: the proper explant, culture media, plant hormones, cultivar type, sugar source, and gelling agent, in addition to abiotic factors (Prado *et al.* 2010; Carra *et al.* 2016; Bidabadi and Jain 2020).

Although all varieties of many species have different capacities for embryogenesis, SE competence in grapevine is strongly genotype-dependent. For instance, when comparing the embryogenic potential of eight grapevine cultivars, an approximately 50-fold difference was observed (Carra *et al.* 2016). Although many methods have been published, the methods for successfully implementing this process for some varieties still need further improvement (Gambino *et al.* 2021; Pathirana and Carimi 2023). Several scientists have carried out various studies on the genotype dependence of the SE process in grapevine (Gribaudo *et al.* 2004; Martinelli and Gribaudo 2009; Oláh *et al.* 2009; Carimi *et al.* 2013; Carra *et al.* 2016; Pathirana and Carimi 2023).

Explant type and nutrient medium composition are key determinants of somatic embryogenesis efficiency in grapevine. Also, the effects of basal nutrients on grape root length and shooting were studied (Ubaydullaeva *et al.* 2023a, b; Abdullaev *et al.* 2025b). Although a range of basal media and supplements has been reported across laboratories, variations in salt formulation, carbon source, and selected additives primarily reflect protocol optimization rather than fundamental differences in the SE process; therefore, representative media compositions and explant–response relationships are summarized in (Table 5).

Induction and regeneration rate of somatic embryos can also be strongly impacted by cultural conditions. A certain researcher suggests that a two-week dark culture phase increases the regeneration rate (Zhang *et al.* 2011). The application of activated carbon (López-Pérez *et al.* 2005), chilling as a pretreatment (Li *et al.* 2008), cotyledon removal (Li *et al.* 2008; Dhekney *et al.* 2011) and pH adjustment (Park *et al.* 2001) are additional methods used to increase regeneration effectiveness. The result may also be impacted by the form of culture medium used, whether it is liquid or solid. Liquid cultures are preferred during the initial induction phase; however, on solid media, structured embryogenic calli grow more rapidly (Jayasankar *et al.* 2005).

CRISPR/Cas technology for precise genome modification

As a highly specific technique, genome editing enables targeted genetic modifications. In plant genetic engineering, one of the most notable advancements has been the application of programmable nucleases to achieve precise genome editing (Kim and Kim 2014). Enzymes such as these, known as nucleases, cleave DNA at particular genomic regions. Resulting DSBs are resolved by cellular repair mechanisms, such as NHEJ, which may generate genetic alterations, or HDR, which employs donor DNA with homologous sequences.

Accurate genome modifications in the past 15 years have been largely achieved through three classes of programmable nucleases: ZFNs, TALENs, and CRISPR/Cas systems. The CRISPR/Cas9 system is recognised as an effective, precise genome engineering technology because of its simplicity and efficacy (Puchta and Fauser 2014). Initially identified as the adaptive immune system of *Streptococcus pyogenes*, it has been extensively used in studies to generate targeted gene alterations. Guide RNAs, consisting of a DNA-matching spacer and a Cas9-binding scaffold, mediate the formation of the functional complex in this system (Oost *et al.* 2014) (Fig. 3).

These novel approaches could be especially helpful in the grape industry, as they develop accurate and small modifications in specific genotypes, including elite varieties that are desirable for the wine market, as is the case with conventional breeding. Modified grapevines have been successfully produced using the CIRSPR/Cas9 technique (Osakabe *et al.* 2018) (Fig. 4).

Targeted editing of the phytoene desaturase gene *via* CRISPR/Cas9 in "Neo Muscat" somatic embryos produced plants exhibiting albino leaves (Nakajima *et al.* 2017). Recently, mono- and bi-allelic forms of the genetically modified Thompson seedless cultivar with an alteration in the WRKY52 transcription factor were also obtained (Wang *et al.* 2018). Malnoy and colleagues (2016) attempted to obtain edited non-transgenic grapes by directly transforming gRNAs and purified Cas9 into Chardonnay protoplasts; however, fully developed plants were not developed.

Technical challenges – The commonly employed *S. pyogenes* Cas9 is constrained by three major issues: off-target activity, dependence on an NGG PAM next to the target site, limiting targeting options, and its large molecular weight (~160 kDa). Currently, multiple Cas variants derived from SpCas9 amino acid modifications demonstrate improved specificity, accuracy, and expanded PAM recognition. In addition, several SpCas9 orthologs from different bacteria exhibit distinct PAM sequence requirements and reduced structural complexity (Cebrian-Serrano and Davies 2017).

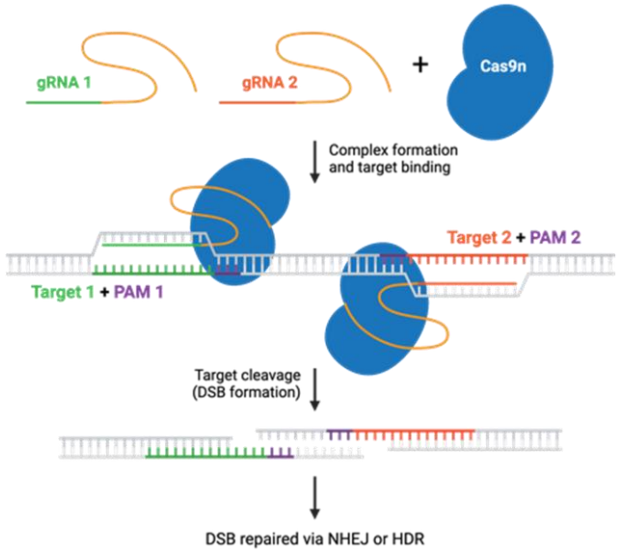

Fig. 3: Schematic overview of CRISPR/Cas9-mediated genome editing showing target recognition, double-strand break formation, and DNA repair *via* non-homologous end joining (NHEJ) or homology-directed repair (HDR). PAM: protospacer adjacent motif

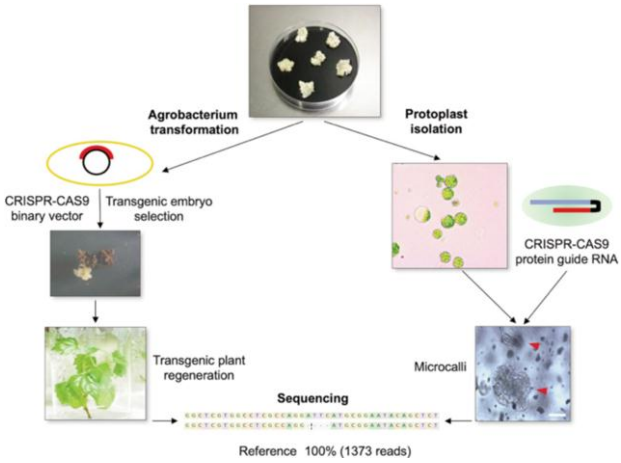

Fig. 4: Overview of current CRISPR/Cas9 genome editing strategies applied in grapevine, including Agrobacterium-mediated transformation and DNA-free ribonucleoprotein delivery approaches

Metje-Sprink *et al.* (2019) summarised new methods as well as advancements in DNA-free genetic modification, which mainly involve the transformation of protein-RNA complexes (RNPs) into protoplasts *via* electroporation, PEG-mediated transfer, and particle bombardment. The limitations of these strategies, when compared with *Agrobacterium*-mediated systems, are their reduced efficiency, the lack of enrichment mechanisms for modified plants, and the necessity of sequencing for detection. However, recent studies have investigated strategies to exclude foreign DNA when using traditional *Agrobacterium*-based transformation (Costa *et al.* 2019a). Because crossbreeding and progeny testing compromise varietal genome preservation, grapevine research should instead focus on developing and assessing DNA cassette elimination methods (Costa *et al.* 2019b).

The potential towards the genetic enhancement of grapevine with CRISPR/Cas may be associated with refining homology-directed knock-in approaches to achieve accurate donor DNA insertion. Currently, in contrast to gene knockout (mutations at the splice site), the knock-in method is still very challenging. However, it might be beneficial to introduce a gene of interest or replace alleles. The donor sequence can include either a resistance gene from wild relatives or an allelic variant with enhanced or reduced activity compared to the endogenous allele.

Finally, the CRISPR/Cas system can be applied to study plant biology and generate genetic variation to obtain new allelic variations with agronomic and qualitative benefits.

Conclusion

In conclusion, grapevine is a globally significant crop that faces growing exposure to abiotic stresses, including drought, salinity, and elevated temperatures, as a consequence of climate change. Conventional breeding approaches for producing climate-tolerant grapevine varieties are limited in efficiency due to genetic complexity and the lengthy timelines involved. The application of CRISPR/Cas9 is highly significant for improving tolerance to abiotic stresses in grapevine. This method is implemented systematically by integrating the somatic embryogenesis process and *Agrobacterium*-mediated transformation protocols. However, problems, such as off-target effects and low regeneration efficiency in elite varieties, still exist. Future researches are focused on developing optimised protoplast-based editing methods, field trials, and strategies for obtaining non-transgenic plants. Such innovative approaches will help ensure the sustainability of the viticulture sector in the face of climate change.

Acknowledgements

We gratefully acknowledge the financial support of the Academy of Sciences of the Republic of Uzbekistan and the Center for Genomics and Bioinformatics. The outcomes of this research will aid in expanding fundamental understanding and capacity in this area. Special thanks are extended to the Academy of Sciences for their continued support.

Author Contributions

HNY, ANA, AAB, SAA, FIB, JBE and KhAU: Formal analysis, data curation, investigation, methodology, visualization, resources, validation, writing original draft. KhAU, HNY and ANA: Writing, data curation and experiment design. KhAU: Conceptualization, investigation,

methodology. HNY and ANA: Writing , review, editing and project administration.

Conflict of Interest

The authors report no potential conflicts of interest.

Data Availability

The data generated in this study can be obtained from the corresponding author upon reasonable request.

Ethics Approval

Not relevant to the present study.

Funding Source

Funding for this study was provided by the Academy of Sciences of the Republic of Uzbekistan and the Center for Genomics and Bioinformatics.

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