

New Genomic Techniques in Viticulture |

Challenges, impacts and
contribution to the sector



International Organisation
of Vine and Wine
Intergovernmental Organisation



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1 | Introduction and objectives

New Genomic Techniques (NGTs) offer great opportunities for the genetic improvement of plants of agricultural interest – such as the grapevine – by increasing tolerance to parasites and diseases, and improving qualitative and nutritional aspects, thereby directly contributing to the economic and environmental sustainability of agricultural systems. NGTs are important for viticulture as they can also be used to improve traditional cultivars. While the development of new varieties is advancing in laboratories and several field trials are underway, the scientific community and the production chain must discuss international harmonised approaches to standardise cultivation, marketing and the aspects of intellectual protection related to NGTs and derived varieties.

In 2015, the International Organisation of Vine and Wine (OIV) published important guidance based on OIV activities related to biotechnology in vitiviniculture (OIV, 2015). The study provided foundational information for Member States, international standardisation bodies and other stakeholders, drawing on the application of modern biotechnology in the vitivinicultural sector, while also considering its potential impact. The main purpose of the guide, however, was to provide a factual basis for potential discussion.

In the years that followed – characterised by rapidly evolving scientific and regulatory contexts – the Experts of the OIV continued a fruitful debate, which is presented in this collective expertise document. It aims to:

- a) study the development of New Genomic Techniques (NGTs) – previously known as New Breeding Technologies – and its synergy with other current breeding and selection approaches;
- b) study the potential of developing (propagating/multiplying) plant material using NGTs;
- c) establish general principles that allow the study, evaluation and dissemination of NGTs in a sustainable vitivinicultural sector model, uniting all voluntary stakeholders of the public and private sectors (national governments, local and regional governments, companies, trade organisations, NGOs, research facilities, etc.).





2 | Evolution and current state of NGTs

2.1. Brief history of the evolution of selection and breeding techniques

In response to the challenges faced when cultivating *Vitis vinifera* L. subsp. *vinifera* of European origin in eastern America for over two centuries, grapevine breeding emerged in the late 18th century. The cultivation of this species was found to be quite difficult due to unfavourable conditions, pests and climate factors. Meanwhile, in Europe, major breeding activities emerged due to the introduction of pathogens like powdery mildew, phylloxera and downy mildew. These diseases posed a significant threat to Europe's millennia--old tradition of viticulture, forcing growers to adopt new strategies to address the problem.

To combat mildew fungi, sulphur and copper were found to be necessary, as they demonstrated useful fungicide activity in the Bordeaux mixture. Even today, an extraordinary amount of plant protection is necessary for grape production. Furthermore, breeding was also employed as a strategy to address the mildew problem. However, hybridisation led to different wine profiles, sometimes of lesser quality. Finally, at the turn of the millennium, the first cultivars showing good field resistance and high wine quality were introduced, providing a solution to the difficulties caused by mildew.

Notably, private French breeders – including Albert Seibel, Georges Couderc, Eugene Kuhlmann, Bertille Seyve, Victor Villard, among others – played a significant role in grapevine breeding. They performed thousands of crosses, resulting in tens of thousands of seedlings, from which the best grapevine genotypes were selected. Some of these genotypes showed mediocre wine quality, even though they expressed high resistance levels.

Phylloxera was identified as the reason for the destruction of vineyards in France in 1868. Within 15 years, it had spread rapidly across the country and eliminated hundreds of thousands of hectares of vineyards. The pest then spread throughout Europe, posing a heavy threat to the survival of viticulture. Unfortunately, no treatment was able to stop the pest from spreading. However, some American hybrids in the grapevine collection in Bordeaux showed resistance to phylloxera on their roots. In 1887 *V. berlandieri* was discovered in North America, which grew very well on calcareous soils. Although this species had poor rooting ability, it was crossed with other *Vitis* species in several research institutes. This led to the development of a series of rootstock cultivars with good rooting ability and adaptation to calcareous soils, marking the beginning of target-oriented rootstock breeding. Rootstocks became available at the beginning of the 20th century and provided a solution to the phylloxera disaster, thereby decreasing the

need for rootstock breeding activities. However, rootstock breeding programmes continue in various countries to this day, targeting other soil-related issues as well, e.g. salt-stress/drought resistance or root knot nematodes. Furthermore, new phylloxera strains are evolving that will require more breeding efforts for new resistance mechanisms, and research is focused on understanding the genetics of certain traits.

Recent advancements in genetic research have revolutionised the study of genomes and opened up new avenues for molecular-level analysis. The development and application of molecular markers, genetic mapping and whole genome sequencing, combined with high throughput technologies, have provided a deep understanding of the location and organisation of genetically determined traits within the genome.

In the field of grapevine genetics, molecular markers emerged as an instrumental tool for analysing genetically determined traits. The development and application of DNA microsatellite analysis using Sequence Tagged Microsatellite Sites (STMS), also known as Simple Sequence Repeats (SSRs), has been particularly successful. This type of molecular marker has proved to be reliable, comparable and robust, leading to better understanding of genetically determined traits in grapevine.

To provide a genetic framework for Quantitative Trait Loci (QTL) mapping, several genetic maps have been developed using SSR or other marker types and combinations thereof. This biostatistical analysis dissects complex traits – that are polygenic and governed by several factors – into a genetic map. It provides a rough localisation of the underlying genes and an orientation in the grapevine genome.

Single Nucleotide Polymorphism (SNP) based markers are expected to be the most accurate markers for applications in grapevine breeding. Grapevine SNPs have already been found to be abundant and useful for genetic analysis.



Alternatively, whole-genome sequencing approaches may be used, which will become standard once the bioinformatic tools for rapid and correct genome sequence assembly become generally available.

Genetic research has revealed several molecular markers associated with resistance traits, berry and wine quality, seedlessness, and other important traits. Studying the genome in more detail has led to an understanding of how traits are structured and inherited, which is essential for plant breeding.

Precision breeding of grapevine is a genetic approach that involves the selective use of only those genetic elements that encode specific desirable traits, resulting in more predictable outcomes than conventional breeding. This approach provides a targeted and efficient way to produce grapevines with specific traits that are important for the wine industry – such as resistance to disease, adaptability to limiting climate conditions, and improved fruit quality – while retaining all desirable traits associated with the production of quality wines.

Over the last few decades, the development of new technologies such as whole genome sequencing using next-generation sequencing (NGS) platforms and bioinformatics has revolutionised the field of grapevine breeding. These advances have allowed for the rapid selection of plants for propagation and manipulation for various purposes. For example, by using NGS, researchers can identify specific genetic markers associated with desirable traits, enabling them to select grapevines with those markers for further breeding.

Bioinformatics tools have also been developed to analyse large datasets of genetic information generated by NGS. These tools allow researchers to identify genetic variations that are associated with specific traits and to study the genetic pathways that underlie these traits. This information can then be used to develop new grapevine varieties with improved traits that are important for the wine industry.

Overall, precision breeding of grapevine using NGS and bioinformatics is a powerful approach that has transformed grapevine breeding and has the potential to contribute significantly to the development of new grapevine varieties for the wine industry.

The development of new and superior-quality grapevine varieties with higher productivity and greater tolerance to different stress factors has been a challenging and resource-intensive process. Alternative methods for genetic transformation have been explored to overcome this challenge, as conventional breeding is unable to provide resistance to diseases or pests to known elite cultivars of *Vitis*. These cultivars were previously maintained through vegetative propagation, requiring the frequent use of pesticides to control diseases. This raised environmental and health concerns. To address these issues while improving elite cultivars, modern biotechnology proposed precision breeding. However, genetic transformation in grapevines has been challenging due to several factors.





These include genes involved in grapevine genetic transformation (i.e. selection marker genes), vectors used for gene delivery, protocols for transformation and non-chimaeric plant regeneration.

Several methods of inserting specific genes into plants with different vectors and methods have been developed in perennial crops over the past thirty years. In grapevine, physical and chemical delivery methods have been tested with transgene delivery being mediated by *Agrobacterium* and viruses. *Agrobacterium*-mediated transformation and biolistic bombardment have been used to transform several grapevine varieties. Other methods of transformation, such as electroporation or protoplast transfection, have also been attempted.

The success of a transformation is facilitated by using marker genes – including selectable marker genes and reporter genes – that ensure a more efficient selection of modified cells from non-modified cells. Antibiotic or herbicide resistance genes are often used as selectable marker genes, while reporter genes, such as green fluorescence protein (GFP), luciferase or -glucuronidase (GUS), are used to ensure the visual selection of transformed tissues and plants.

Furthermore, it is now feasible to remove the marker gene after the transgenic plant has been selected. This approach utilises transformation vectors that contain an excision system in the T-DNA. This is composed of a recombinase gene under the control of an inducible promoter and recombinase recognition sites close to the T-DNA borders (e.g. FLP-FRT, Cre-Lox). Upon induction of the recombinase enzyme, selectable marker genes can be removed from the plant genome (Dalla Costa et al. 2016; Moffa et al., 2024).

The success of genetic transformation in plants depends heavily on the efficient regeneration of transgenic plants. Several factors influence the efficiency of plant transformation and regeneration, including genotype, explant source, acceptor material, culture medium, bacterial strains, selectable markers, and selection methods. In grapevine cultivars such as Thompson Seedless, Silcora, and Chardonnay, genetic transformation was achieved through bud neoformation and shoot organogenesis. The *in vitro* organogenesis of certain grapevine cultivars and rootstocks was obtained from various types of explants – including petioles, leaf internodes, and shoot apices – while the regeneration of somatic embryos induced from a single cell could be used to avoid chimeras. Although somatic embryogenesis was used for grapevine micropropagation and genetic transformation, the induction of somatic embryogenesis is highly genotype-dependant and generally low, depending on the type of explants. Transgenesis in grapevine is therefore mostly based on the *Agrobacterium* system, and the regeneration of transformants is generally achieved with somatic embryogenesis. Improved *Agrobacterium*-mediated transformation protocols have been published to enhance fruit quality and tolerance to some abiotic and biotic factors. However, the production of regenerable grapevine material that can be used for either transgenic modifications of the grapevine genome via *Agrobacterium* or for DNA-free genome modifications via protoplast transfection remains a challenge and bottleneck.

2.2. Inventory of NGTs at the international level

2.2.1. Description and characteristics of each NGT

Genome editing enables precise genetic modifications with different purposes – such as gene inactivation – providing the opportunity to explore the function of a particular gene as well as the effects of inserting or replacing genes at specific sites on genetic improvements. Strategies involving genome editing are based on Site-Directed Nucleases (SDN), which break the DNA double strand near a specific target sequence. This break is then repaired by the cell's DNA repair mechanisms and may result in site-specific modifications such as nucleotide deletions, insertions or substitutions. Zinc Finger Nucleases (ZFNs), Meganucleases (MNs), TAL Effector Nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein (CRISPR/Cas) are examples of SDN techniques. Another strategy for genome editing is the Oligonucleotide-Directed Mutation (ODM). It is a site-specific gene modification system that uses synthetic oligonucleotides to introduce small mutations into the target genomic sequence. These oligonucleotides are introduced into cells and modify the target sequence by pairing with it and being recognised by the DNA repair mechanism.

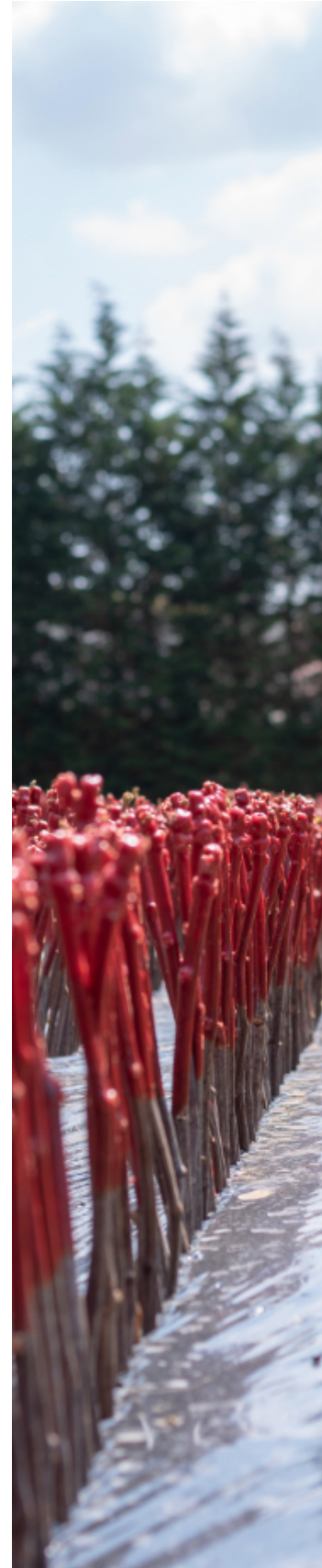
Cisgenesis and intragenesis are two more techniques used in genetic modification. Cisgenesis involves using an existing natural gene from the crop plant itself or from a sexually compatible species with its native regulatory sequences. On the other hand, intragenesis involves a gene comprising functional elements – such as a coding part, promoter and terminator – originating from different genes from the crop plant itself or from crossable species. All gene elements belong to the traditional breeders' gene pool, but intragenesis utilises a man-made genetic construct.

The main techniques widely recognised as **NTGs** are those listed above; however, other possibilities may be considered depending on the definition of **NTGs** adopted in different countries. Some possible techniques are mentioned below.

Epigenetic modification is a technique that involves introducing heritable changes to gene expression without altering the primary DNA sequence. RNA-Dependent DNA Methylation (RdDM) is one such technique, as is dCas9 fused with DNA methyltransferase 3A (DNMT3A) or with DNA demethylase TET.

Reverse breeding is a technique that generates genetically modified (GM) plants by introducing a transgene that suppresses meiosis transiently. The transgene is later removed by outcrossing, leaving no foreign genetic material in the plant variety.

Accelerated breeding involves introducing a transgene that shortens the juvenile phase of a plant, thereby speeding up the breeding process. The transgene is expected to be removed later by outcrossing, leaving no foreign genetic material at the end of the procedure.





2.2.2. Benefits, risks and uncertainties of these new technologies

Among the genome editing tools, CRISPR/Cas technology is considered the most efficient due to its high specificity and minimal off-target effects. Gene editing with the CRISPR/Cas9 system requires a guide RNA (gRNA) containing a protospacer sequence complementary to the target DNA sequence. The complex formed by gRNA and Cas9 nuclease scans the genome, searching for complementary double-stranded DNA. If a sequence complementary to the protospacer and a consensus protospacer-adjacent motif (PAM) is found, the nuclease generates a double strand break (DSB) in the specific gene sequence. Genome editing that uses CRISPR/Cas9 technology requires the PAM sequence downstream of the target sequence as well as proper gRNAs that are designed based on the sequences for the target genes. High-fidelity Cas9 versions further improve targeting accuracy, ensuring that only the desired genes are edited while minimising off-target edits. Enhanced Cas9 variants, such as 'nickase' Cas9 and 'dead' Cas9 (dCas9), offer greater flexibility in gene editing by making single-strand cuts instead of double strand breaks or by making no cuts at all (yet still recognising the target sequence). These modified versions are particularly useful for applications that require precise control over DNA manipulation without causing double strand breaks. dCas9 is often fused with additional protein domains to enable targeted functions such as DNA or histone methylation and demethylation, gene activation, or gene repression. This versatility makes these variants valuable tools for a wide range of gene regulation and epigenetic modifications, expanding the potential of Cas9 beyond traditional gene editing.

CRISPR/Cas12 (Cpf1) is another endonuclease that offers several advantages over other gene-editing tools like Cas9. Its precise DNA cutting ability, smaller protein size, and reduced off-target effects make it a compelling option. Cas12 creates staggered cuts, which can be more effective for certain types of edits. Additionally, its smaller size facilitates easier delivery into cells, particularly using viral vectors. Another Cas nuclease, Cas13, targets RNA instead of DNA, enabling it to cut RNA transcripts.

Despite the many advantages of CRISPR/Cas technology over ZFNs and TALENs, one downside is the occurrence of off-target mutations, influenced by parameters such as the recognition of the target, the design (sequence and secondary structure) of gRNAs, the frequency of repair events with homologous recombination, and anti-CRISPR proteins that inactivate Cas proteins.

Another limitation of using CRISPR/Cas technology relates to the delivery of system components into plant tissues. In general, gRNAs and Cas are delivered into plant cells by *Agrobacterium*, viral vectors, PEG-mediated transfection, biolistic methods, and nanoparticles. However, the structure of the plant cell wall limits the delivery of the components; thus, the system most often used for delivery into plant tissues remains *Agrobacterium*, which generally results in

the integration of foreign DNA into the grapevine genome, thereby producing GM plants. Nevertheless, the direct delivery of the purified Cas protein and gRNAs (as ribonucleoprotein complexes) into protoplasts (plant cells without cell walls), is increasingly utilised successfully for the transgene-free editing of grapevine genome. This approach has been proven to be efficient enough as to not require marker genes and yields DNA-free (i.e. non-GM) plants, but the regeneration of mature plants from protoplasts is highly genotype dependent and not trivial.

One of the key uncertainties surrounding gene-edited plants, particularly those that have been released into the environment, lies in the challenge of identifying and protecting these plants once they exhibit traits similar to naturally occurring or somatic mutations. In the case of classical indel mutations (insertions or deletions), the modifications made through gene editing are often indistinguishable from those that occur naturally, either through spontaneous mutations or through traditional breeding methods. As a result, it becomes difficult to trace and differentiate gene-edited plants from their naturally mutated counterparts, especially when they are planted and propagated. This raises concerns about regulatory oversight, traceability, and labelling requirements, as it may be challenging to enforce policies for gene-edited crops in markets where only conventional plant breeding is permitted. Research projects are currently underway within the EU's HORIZON framework to address these concerns. Additionally, this uncertainty complicates intellectual property protection and the potential enforcement of patents, as distinguishing gene-edited plants from naturally occurring variants becomes more complex. The lack of visible markers or detectable differences in these plants means that safeguards and detection methods may need to evolve to ensure transparency and accountability in the commercialisation and trade of genetically modified crops.



At present, these new technologies still depend on the limits of regeneration technologies, particularly due to genotype-specific factors. As the conditions for success depend on the genotype (varieties, clones, etc.), the successful appropriation of new elite varieties could lead to an impoverishment of the genetic diversity present in commercial vineyards.

2.2.3. Synergy between NGTs, traditional selection and breeding techniques

The successful integration of NGTs into traditional selection and breeding methods requires a comprehensive and strategic approach that takes ethical, regulatory and socioeconomic considerations into account. By combining the strengths of both approaches, researchers and breeders can enhance the development of better grape varieties that meet the changing requirements of viticulture.



- NGTs can be used to introduce specific genetic changes quicker, complementing the longer and more unpredictable traditional breeding cycles.
- Traditional breeding methods often involve complex genetic backgrounds. NGTs can be used to fine-tune specific traits without disrupting the overall genetic makeup achieved through traditional breeding.
- Traditional breeding may have already developed varieties with good general traits. NGTs can then be applied to enhance specific characteristics in these varieties.
- Traditional breeding often relies on the diversity present in wild or landrace varieties. NGTs could help preserve and enhance specific desirable traits without compromising overall genetic diversity once the technologies are implemented in a larger range of genotypes.
- Traditional breeding may have identified varieties adapted to specific regions. NGTs can then be applied to further enhance these varieties for specific environmental challenges.
- Traditional breeding may inadvertently introduce undesirable traits. NGTs can be used to eliminate or reduce these traits, ensuring the development of more desirable varieties.
- Traditional breeding may have identified traits of interest without a clear understanding of the underlying genetics. NGTs can help validate these associations and provide insights for more targeted breeding strategies.
- Traditional breeding is often limited to crossing within the same species. NGTs can overcome these limitations by allowing the introduction of genes from related, sexually compatible species, thereby expanding the genetic resources available for breeding. Depending on the definition of NGTs used, in some countries genes could be introduced even from incompatible related species without being considered traditional GM.



2.3. Key technical and scientific terms and definitions related to NGTs

NGTs (New Genomic Techniques): Advanced biotechnological methods that enable more precise and targeted modifications to the genetic material of organisms than traditional/classical breeding techniques.

CRISPR/Cas: A powerful and widely used genome editing tool in which a nuclease (Cas9) and a guide RNA are employed to introduce precise changes in the DNA sequence of an organism.

Gene Editing: The targeted modification of an organism's DNA using various molecular biology techniques, including CRISPR/Cas, to achieve specific changes in its traits. When multiple loci are modified at the same time, the terms Genome Editing or Multiplex Gene Editing are used.

Cisgenesis: The transfer of genes between organisms that could potentially occur naturally through traditional breeding methods.

Intragenesis: The process where a target gene is attached to a regulatory element of a host species or a close relative. This regulatory element may not have been derived from the same locus as the gene sequence itself, unlike in the case of a cisgene.

Genome Modification: A broad term referring to the alteration of an organism's genetic makeup, which may involve techniques such as gene editing, gene insertion or gene deletion in order to achieve desired traits.

Gene Insertion: The introduction of a foreign gene into an organism's genome, often to confer specific traits such as resistance to pests or diseases.

Gene Deletion: The removal of specific genes from an organism's genome, typically to eliminate undesirable traits or characteristics.

Genetic Engineering: The manipulation of an organism's genetic material using biotechnological methods to achieve specific traits or characteristics.

Off-Target Effects: Unintended changes in the genome that potentially occur during the application of gene-editing techniques, affecting regions other than the intended target but with a high sequence similarity.

Transgenic Organism: An organism that has foreign genes inserted into its genome through genetic engineering, which are not removed through traditional breeding methods, such as crossing, or other techniques like recombinase-mediated excision.

Targeted Mutagenesis: The intentional induction of mutations in specific genes to achieve desired traits without introducing foreign genetic material.

Phenotype: The observable characteristics or traits of an organism resulting from its genetic makeup and environmental influences.

Genotype: The genetic makeup of an organism, including the specific alleles present in its genome.

Molecular Marker: A specific DNA sequence used to identify the presence of a particular gene or trait, often employed in marker-assisted breeding and selection.

Marker-Assisted Breeding (MAB): A breeding approach that utilizes molecular markers to assist in the selection of individuals with desired traits, helping to speed up the traditional breeding process.

Ethical Considerations: Reflection on the moral implications and societal impacts of using NGTs, including considerations of biosafety, justice, equity and potential unintended consequences.

Biosafety: Measures and practices implemented to prevent unintended harm to the environment or human health resulting from the use of genetically modified organisms.

GMO (Genetically Modified Organism): A broad term referring to an organism whose genetic material has been altered using genetic engineering techniques. GMOs are distinguished from NGT-derived organisms where the latter are produced without introducing and retaining foreign DNA in the organism.



3 | Inventory of legislation on NGTs at the international level

3.1. Inventory by continent (focus on vitivinicultural countries)

NGTs not regulated as GMOs or case by case decision	Argentina, Brazil, Canada, Chile, China, Colombia, Ecuador, Ethiopia, India, Japan, Kenya, Nigeria, Paraguay, Russia, Switzerland, United Kingdom, United States
Only NGT1 type not regulated as GMOs, all other NGTs still considered GMOs	Australia
Under evaluation	Bangladesh, Indonesia, Norway, Uruguay
NGTs regulated as GMOs	Austria, Belgium, Bulgaria, Croatia, Czechia, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Slovenia, Lithuania, Latvia, Estonia, Finland, Sweden, South Africa, New Zealand

3.1.1. European Union

The European Commission has proposed a new regulation for plants developed using specific NGTs methods. This proposal, which was introduced on 5 July 2023, forms a part of a broader legislative package aimed at bolstering the EU's Farm to Fork and Biodiversity strategies.

The proposal's key goals include:

- ensuring a robust level of human and animal health and environmental safety,
- directing advancements to aid sustainability objectives across various plant types, notably within the agricultural-food sector,
- fostering a conducive atmosphere for research and innovation, particularly for small and medium-sized enterprises (SMEs).

The scope of the proposal encompasses plants with genetic alterations within their species (targeted mutagenesis) or from compatible plant species (cisgenesis, including intragenesis). However, transgenic plants (those with genetic material from incompatible species) will continue to be regulated under existing GMO laws.

Significant aspects of the proposal are:

The proposal outlines two separate processes for market entry of NGT plants.

For NGT plants that could naturally occur or be produced through traditional breeding ('category 1 NGT plants'), there would be a verification system, based on specified criteria in the proposal. NGT plants satisfying these standards would be regarded as conventional plants and thus exempt from GMO laws. Details about category 1 NGT plants would be accessible through seed labelling, a public database and relevant plant variety catalogues.

Conversely, 'category 2 NGT plants', which fall outside the first category, would remain under existing GMO regulations. These would undergo risk evaluation and authorisation before market release, including tracking and GMO labelling. There's an option for a voluntary label indicating the purpose of the genetic alteration. The risk assessment, detection and monitoring procedures would be tailored to their specific risk profiles, and regulatory incentives would be provided for NGT plants that contribute to sustainability.

On 24 January 2024, the Committee on Environment, Public Health and Food Safety established its stance on the Commission's proposal regarding NGTs, with a vote of 47 in favour, 31 against and 4 abstentions. The Members of the European Parliament (MEPs) concurred with the proposal's structure of two categories and two regulatory frameworks for NGT plants. They agreed that NGT 1 plants, considered analogous to conventional ones, should be exempt from GMO legislation, while NGT 2 plants would be incorporated into the GMO framework. Furthermore, MEPs reached a consensus that all NGT plants should continue to be banned in organic production, pending further analysis of their compatibility.

On 7 February 2024, The European Parliament adopted amendments to the proposal, with 307 votes in favour, 263 against and 41 abstentions. In the next phase, inter-institutional negotiations should take place.





3.1.2. North, Central and South America

The regulatory approaches to NGTs in various American countries, including the United States and Canada, highlight diverse strategies tailored to the unique agricultural and legal contexts of each nation.

Argentina: As a leader in the regulation of NGT crops, Argentina's approach involves assessing whether biotechnological products involve a new combination of genetic material. Products not resulting in new genetic combinations are deemed conventional. Argentina's Resolution No. 21/2021 and its annexes provide specific guidelines for this evaluation.

Brazil: In 2018, Brazil adopted Resolution No. 16/2018, marking a significant step in the regulation of NGTs. This resolution likely employs a case-by-case evaluation, although the specific criteria for assessment are not detailed in the provided documents.

Chile: Adopted a consultation procedure in 2017, suggesting a more interactive approach to regulating NGTs, where stakeholders may have opportunities to provide input on each case.

Colombia: Followed with its Resolution No. 00022991 in 2018, indicating a move towards a more structured regulatory framework for NGTs, though the specifics of their criteria are not elaborated upon in the documents.

Paraguay: Established a process in 2019 to determine whether NGT-derived crops fall under GMO regulations. This process involves a case-by-case analysis focusing on the use of genetic engineering techniques and the creation of new genetic combinations. The approach emphasizes thorough evaluation without relying on predetermined lists of techniques.

United States: The United States Department of Agriculture (USDA) announced in 2018 that genome-edited plants would not be regulated differently if they could also be developed through traditional breeding methods. This indicates a product-based approach, focusing on the characteristics of the end product rather than the process used to develop it.

Canada: Employs a unique approach with its Plants with Novel Traits regulations. This framework assesses plants based on the novelty of the traits they exhibit rather than the method used to produce these traits, reflecting a product-based perspective like that of the United States.

These countries exhibit a range of regulatory approaches to NGTs, from Argentina's pioneering guidelines to the product-based frameworks in the United States and Canada. The emphasis varies from process-based criteria in countries like Paraguay to a focus on the end product's traits in the United States and Canada.



3.1.3. Asia

In Asia, there is a mix of approaches, with some countries having clear regulations for NGTs, including gene-editing (GE) products, others in the process of developing policies, and a few still without specific frameworks. The status in specific Asian countries is outlined below:

China: Currently developing regulations for GE products. China's government organisations are responsible for GE monitoring, and there is progress towards the deregulation of GE products.

India: Regulatory landscape evolving with a focus on modernising legislation to include NGTs. India has revised genetic application rules in agriculture, excluding certain gene editing classifications from GMO regulations.

Japan: Implemented a regulatory framework for GE products, focusing on product-based regulation. Regulates GE products based on the presence of foreign DNA and specific rules for assessing risks.

Pakistan, Thailand, the Philippines, Malaysia, Indonesia, Taiwan, South Korea: These countries are at various stages of developing regulations for GE produce. Some have yet to develop comprehensive policies, while others are in the process of updating their frameworks to include GE technologies.

3.1.4. Africa

The regulatory framework for NGTs in Africa varies among countries, with specific regulations and agencies overseeing the development and commercialisation of NGT crops. Here is a detailed summary of the regulatory framework for NGTs in select African countries:

Kenya: Has the Biosafety Act of 2009, which comprehensively covers the regulation of NGT products. This act includes provisions for the contained use, environmental release, import, export and transit of NGT crops. Additionally, the Seed and Plant Varieties Act and the Seeds and Plant Varieties Regulations provide further oversight for NGT crops. The National Biosafety Authority serves as the Competent Authority for biosafety regulations in Kenya. The Kenya Plant Health Inspectorate Service (KEPHIS) and the Ministry of Agriculture are also involved in regulating NGT products. Kenya has established various committees such as the Scientific Advisory Committee, National Performance Trial Committee and National Variety Release Committee to ensure the safe development and release of NGT crops.

Nigeria: Operates under the National Biosafety Management Agency Act of 2015, which was revised in 2019 to strengthen regulations for NGT products. The country also enforces the National Agricultural Seeds Act and the National Seed Act to govern NGT crops. The National Biosafety Management Agency (NBMA) serves as the National Biosafety Authority in Nigeria. The National Agricultural

Seeds Council (NASC) under the Federal Ministry of Agriculture and Rural Development also plays a crucial role in overseeing NGT products. Nigeria collaborates with various entities, including the Nigeria Agricultural Seed Council, National Agricultural Quarantine Service, Nigeria Customs Service and other regulatory bodies to ensure compliance with biosafety regulations.

Eswatini: Regulatory framework includes the Biosafety Act of 2012 (currently under review), the Plant Control Act of 1981 (also under review), and the Seeds and Plant Varieties Act of 2000. These regulations collectively govern the development and commercialisation of NGT products. The Eswatini Environmental Authority and the Seed Quality Control Services under the Ministry of Agriculture are responsible for overseeing biosafety regulations related to NGT crops. Eswatini has established the National Biosafety Advisory Committee and the National Variety Release Committee to ensure the safe deployment of NGT crops within the country.

Ethiopia: Operates under the Biosafety Proclamations to regulate NGT products within the country. These proclamations provide a legal framework for the development and commercialisation of NGT crops. The regulatory oversight for NGT products in Ethiopia is managed by the relevant government authorities responsible for biosafety regulations.

South Africa: Was the first country on the African continent to regulate commercial GM crops under the GMO Act (Act 15 of 1997), which is administrated in the Department of Agriculture, Land Reform and Rural Development (DALRRD); it has continued to do so successfully for more than a quarter of a century. At the end of 2021, DALRRD declared NBT crops to be regulated as GMOs. An immediate appeal by organised commercial Agriculture was lodged, but more than a year later the appeal was rejected by the responsible minister in spite of the Appeals Board recommendation that NBTs should be regulated differently. South Africa therefore remains the only country on the continent that regulates NBTs as GMOs.





3.1.5. Oceania

The regulatory landscape for NGTs in Oceania, which primarily includes Australia and New Zealand, reflects a varied approach to the regulation and acceptance of these technologies. Australia and New Zealand are actively updating their regulatory frameworks to adapt to advancements in New Genomic Techniques (NGTs), such as gene editing.

Australia's Gene Technology Regulator oversees the regulation of gene technologies, including NGTs. The country is considering amendments to the Australia New Zealand Food Standards Code to clarify definitions related to gene technology and new breeding techniques. The goal is to ensure that foods produced using these technologies are regulated based on the risks they pose, accommodating new advancements while maintaining safety standards.

New Zealand is also in the process of updating its gene technology regulations to support scientific advancements and promote economic growth. The government has introduced the Gene Technology Bill 2024, which aims to establish a modern regulatory framework for gene technology and genetically modified organisms (GMOs). This legislation seeks to replace the existing regime with a more flexible system that emphasizes risk-proportionate regulation and efficient decision-making processes, aligning New Zealand with that of Australia, where SDN-1 organisms are exempt.

3.2. Key legal and regulatory terms and definitions related to NGTs

The terms used in this document are employed solely for the purposes of this document and shall not be construed as having any legal or normative effect beyond its specific scope of application.



Genome Editing: A type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism using engineered nucleases – also known as “molecular scissors”.

Site-Directed Nucleases (SDNs): Enzymes that create breaks at specific locations in the DNA, enabling targeted genetic modifications. They include ZFNs, TALENs and CRISPR/Cas systems.

Zinc Finger Nucleases (ZFNs): A type of SDN that uses engineered proteins to target specific DNA sequences for editing.

Transcription Activator-Like Effector Nucleases (TALENs): Similar to ZFNs, these are engineered proteins that bind to specific DNA sequences to induce targeted genetic modifications.



Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9: A versatile and widely used genome editing tool derived from a naturally occurring bacterial defence system.

Oligonucleotide-Directed Mutagenesis (ODM): A technique that involves introducing synthetic DNA or RNA to guide specific changes in the organism's genome.

RNA Interference (RNAi): A biological process where RNA molecules inhibit gene expression, typically by causing the destruction of specific mRNA molecules.

Base Editing: A form of gene editing where single nucleotides in the DNA sequence are chemically altered without making double-stranded DNA breaks.

Prime Editing: A newer form of genome editing that combines a CRISPR/Cas9 system with a reverse transcriptase enzyme to directly write new genetic information into a targeted DNA site.

Gene Silencing: The regulation of gene expression in a cell to prevent or lower the expression of a certain gene.

Gene Knockout: A genetic technique in which one of an organism's genes is made inoperative ('knocked out' of the organism). This is often done to study gene function.

Gene Knock-In: Introducing a gene into a particular locus within the genome. This can be used to study gene function.

Genetically Modified Organism (GMO): Depending on the country and the used techniques, a broad definition for an organism whose genetic material has been altered using genetic engineering techniques. NGT-derived organisms are often considered distinguished because they can be obtained without the use of foreign DNA.

Bioinformatics: The science of collecting and analysing complex biological data such as genetic codes.

Synthetic Biology: An interdisciplinary branch of biology and engineering that involves designing and constructing new biological entities, such as enzymes, genetic circuits and cells, or redesigning existing biological systems.

Regulatory Framework: The combination of laws, regulations and guidelines governing the research, development, production and marketing of products derived from NGTs.

Biosafety: Refers to the prevention of large-scale loss of biological integrity, focusing both on ecology and human health.



Bioethics: The study of the ethical issues emerging from advances in biology and medicine. It is also moral discernment as it relates to medical policy, practice and research.

Risk Assessment: The process of evaluating the potential risks that may be involved in a projected activity or undertaking, such as the release of a genetically modified crop into the environment.

Intellectual Property Rights (IPR): Legal rights granted to inventors and creators to protect their inventions, designs and artistic works from unauthorised use by others for a certain period.

Patent: A form of IPR that gives the patent holder the exclusive right to exclude others from making, using, offering for sale, or selling the invention within a jurisdiction for a limited period in exchange for public disclosure of the invention.

Biotechnology Regulation: The body of laws and guidelines that govern the use and deployment of biotechnology, including NGTs.

Environmental Release: The intentional or accidental dispersal of genetically modified organisms into the environment outside of controlled environments, like laboratories or contained facilities.

Food and Drug Administration (FDA): A US government agency responsible for regulating food and pharmaceutical products, including those developed through NGTs.

European Food Safety Authority (EFSA): The agency of the European Union that provides independent scientific advice on food-related risks.

Cartagena Protocol on Biosafety: An international agreement aimed at ensuring the safe handling, transport and use of living modified organisms resulting from modern biotechnology that may have adverse effects on biological diversity, taking into account risks to human health.

Genetic Diversity: The total number of genetic characteristics in the genetic makeup of a species. It serves an important role in evolution and adaptation.

Agrobacterium-mediated Transformation: A method of plant transformation using the naturally occurring bacterium *Agrobacterium tumefaciens* to transfer genetic material into plant cells.

Bioreactor: A manufactured or engineered device or system that supports a biologically active environment, often used in systems for growing cells or tissues in the context of cellular agriculture or regenerative medicine.



Biopharming: The production of therapeutic proteins and other drugs through genetically engineered plants or animals.

Off-target Effects: Unintended alterations to the genome caused by genome editing tools, such as changes in DNA sequences at locations other than the intended target site.

Public Consultation: A process where feedback is sought from the public or interested stakeholders on regulatory, ethical or policy issues related to NGTs.

Molecular Breeding: The use of molecular biology tools in plant and animal breeding to select and manipulate traits.

Genomic Selection: A form of marker-assisted selection in plant and animal breeding where genetic markers covering the entire genome are used to predict the performance of an organism.

Allele: One of two or more versions of a gene. An individual inherits two alleles for each gene, one from each parent.

Epigenetics: The study of heritable phenotype changes that do not involve alterations in the DNA sequence.

Phenotype: The set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.

Quantitative Trait Loci (QTL) Mapping: A technique that uses statistical methods to link complex phenotype traits to specific regions of the genome.

Somatic Cell Nuclear Transfer (SCNT): A laboratory strategy for creating a viable embryo from a body cell and an egg cell. It is used in cloning and in the production of embryonic stem cells for research.

DNA Sequencing: The process of determining the precise order of nucleotides within a DNA molecule.

Proteomics: The large-scale study of proteins, particularly their structures and functions.

Metabolomics: The scientific study of chemical processes involving metabolites – the small molecule substrates, intermediates, and products of metabolism.

Gene Therapy: A technique that uses genes to treat or prevent disease by inserting a gene into a patient's cells instead of using drugs or surgery.



Biomarkers: Biological measures of a biological state. They are often used to measure the progress of disease or the effects of treatment.

Functional Genomics: The study of gene and protein functions and interactions.

Pharmacogenomics: The study of how genes affect a person's response to drugs. This relatively new field combines pharmacology and genomics to develop effective, safe medications and doses tailored to a person's genetic makeup.

Transcriptomics: The study of the transcriptome – the complete set of RNA transcripts produced by the genome at any one time.

Gene Drive: A genetic engineering technology that can propagate a particular suite of genes throughout a population in a non-Mendelian way. It can be used to prevent the spread of diseases or pests but has significant ecological implications.

Biocontainment: Methods or procedures used in laboratories and facilities to prevent unintended release of potentially dangerous biological agents or organisms.

Precision Agriculture: A farm management approach that uses information technology – such as GPS guidance, control systems, sensors, robotics, drones, autonomous vehicles, variable rate technology and GPS-based soil sampling – to optimise field-level management with regard to crop farming.

4 | Comparative perspective on social, economic and environmental aspects

4.1. Social aspects

Acceptance and public perception: Social acceptance of NGTs varies globally, influenced heavily by public perception of genetic modification technologies. Unlike traditional GMOs, NGTs often do not involve the insertion or retention of foreign DNA into the plant, which may positively influence public acceptance. However, concerns about 'playing God' and tampering with natural processes persist. Education and transparent communication about the safety and benefits of NGTs are crucial in shaping public opinion.



Ethical considerations: NGTs raise ethical questions, including concerns about biodiversity, the naturalness of food products, and animal welfare. Legal frameworks often incorporate ethical considerations into their guidelines, balancing technological advancement with ethical concerns.

Health and safety: NGTs have the potential to address health issues, such as allergen-free foods or crops with enhanced nutritional profiles. However, public concerns about potential health risks play a significant role in the regulatory landscape. Consequently, some countries still maintain laws requiring rigorous safety testing of NGT products similar to those for OGMs.

Cultural and societal impact: The integration of NGTs in agriculture can affect cultural practices, especially in regions where traditional farming is a cornerstone of society. Regulations may need to address these cultural impacts, ensuring that technological advancements do not erode cultural heritage or social structures.

Legal and regulatory concerns: The regulatory landscape for NGTs is complex and varies by region. Laws and regulations impact the development and deployment of these technologies and influence public perception and acceptance. For instance, strict regulations in the European Union contrast with more tolerant policies in the Americas and parts of Asia.

4.2. Economic aspects

Agricultural productivity: NGTs offer significant potential for increasing agricultural productivity, which can have far-reaching economic impacts. Enhanced crop yields, reduced losses due to pests and diseases, and improved nutritional profiles can contribute to food security and economic growth, especially in developing countries.

Market dynamics: The development of crops with novel traits through NGTs can lead to new markets and economic opportunities. However, the global trade of such products is influenced by international agreements and national regulations, affecting market access and competitiveness.

Investment and research: The economic landscape of NGTs is shaped by investments in research and development. The legal framework plays a pivotal role in attracting or deterring investments. Intellectual property rights, patents and the legal protection of biotechnological inventions are essential for encouraging innovation and economic growth.

Cost implications: While NGTs can reduce costs related to pest control and crop losses, the initial research and development, along with the regulatory approval process, can be expensive. This cost aspect influences the affordability and accessibility of NGT-derived products.

Trade regulations: International trade laws and agreements significantly impact the economic aspects of NGTs. Discrepancies in regulations between countries can lead to trade barriers, affecting global market dynamics.



4.3. Environmental aspects

Biodiversity: NGTs have the potential to both positively and negatively affect biodiversity. While they can be used to develop crops that are more resilient to changing environmental conditions, there is concern about potential unintended consequences on ecosystems and natural genetic diversity. Regulatory frameworks often include environmental risk assessments to mitigate these risks.

Sustainability: NGTs can contribute to sustainable agriculture by developing crops that require fewer inputs like water, fertilisers and pesticides. However, regulations must ensure that such developments do not lead to monocultures or other practices detrimental to long-term sustainability.

Climate change resilience: NGTs hold promise in developing crop varieties that are more resistant to extreme weather conditions associated with climate change. Legal frameworks are evolving to support research in this area while ensuring environmental safety.

Gene flow and containment: The potential for gene flow from NGT-modified organisms to wild relatives or non-modified crops is a significant environmental concern. Regulatory measures often include containment strategies and monitoring to prevent unintended gene flow.

Soil health and ecosystem services: NGTs can be used to develop plants that interact beneficially with soil microorganisms, enhancing soil health and ecosystem services. For example, these technologies can modify genes that regulate the production of specific root exudates, such as sugars, amino acids, and secondary metabolites, to selectively stimulate beneficial microbial communities. Additionally, genes that control signaling pathways with arbuscular mycorrhizal fungi can also be modified. Regulatory frameworks need to account for long-term ecological impacts to ensure that these technologies contribute to environmental health.

In conclusion, the development and application of NGTs have far-reaching social, economic and environmental implications. The legal and regulatory frameworks governing these technologies play a crucial role in shaping their impacts and ensuring that their benefits are realised while mitigating potential risks.



5 | Examples of current and potential uses of NGTs in the vitivinicultural sector

NGTs, especially genome editing technologies, have shown promising applications in the vitivinicultural sector, particularly in grapevine improvement. Here are some examples of current and potential uses of NGTs in the vitivinicultural sector:

Enhancing disease resistance: Genome editing can be used to enhance disease resistance in grapevines. For instance, the knockout of specific genes like WRKY52 has been shown to improve resistance to pathogens such as *Botrytis cinerea* (Wang et al., 2018).

Improving fruit quality: NGTs can be employed to modify traits related to fruit quality – such as sugar content, acidity levels, and aroma compounds. This can lead to the development of grapevines with superior taste profiles and enhanced sensory characteristics.

Increasing abiotic stress tolerance: Genome editing techniques can help in developing grapevines that are more resilient to environmental stresses like drought, heat and salinity. This can contribute to sustainable viticulture practices in the face of climate change. An example of the application of CRISPR/Cas9 in grapevines to mitigate water stress impact can be found in Clemens et al. (2022).

Modifying flowering and ripening time: By targeting genes involved in flowering and ripening processes, NBTs can be used to manipulate the timing of these developmental stages in grapevines. This can facilitate better management of harvest schedules and optimise fruit maturation.

Creating novel varieties or clones: Genome editing offers the possibility of creating novel grapevine varieties or clones – depending on how the regulatory frame evolves – with unique traits that are not naturally occurring. This could include varieties/clones with improved nutritional profiles, novel flavours, or resistance to specific pests and diseases.

Reducing environmental impact: By developing new grape varieties – or new clones of existing varieties – that require fewer chemical inputs for pest and disease control, NGTs can contribute to sustainable viticultural practices and reduce the environmental footprint of grape production. An example of this application can be found in Giacomelli et al. (2023).

Accelerating breeding programmes: Genome editing technologies can expedite the traditional breeding process by enabling precise and targeted modifications in the grapevine genome. This can significantly shorten the time required to develop new grape clones/varieties with desired traits.

These examples highlight the diverse applications of NGTs in the vitivinicultural sector, offering exciting opportunities for innovation and advancements in grapevine cultivation and wine production.

More specifically, NGTs are being applied and explored to improve grapevine varieties, helping them cope with climate change and other challenges. The current and potential uses of NGTs in grapevines include enhancing grape quality, disease resistance, and metabolic pathways through biotechnological advancements. Butiuc-Keul and Coste (2023) highlight the potential of various biotechnologies – including genome editing and molecular biology – for grapevine genetic improvement, emphasizing the importance of integrating these new technologies with classical breeding techniques. Moreover, NGTs serve as powerful tools for functional genomics studies, enabling knock-out or targeted mutagenesis to precisely modulate gene expression, which is invaluable for this field of scientific research.

Specific applications of CRISPR/Cas9 technology have been demonstrated in various studies. For example, Ren et al. (2016) successfully used CRISPR/Cas9 to achieve targeted mutagenesis in the 'Chardonnay' wine grape variety, focusing on the *IdnDH* gene, which is crucial for wine production. This study represents a significant advancement in understanding functional genomics in grapevine and in developing new varieties through precise genetic modifications. Malnoy et al. (2016) demonstrated a method for editing grapevine protoplasts and potentially creating transgene-free edited grapevine plants in the 'Thompson Seedless' variety using CRISPR/Cas9 ribonucleoprotein complexes, an important technique for generating non-GMO grapevine lines. This was achieved later by Scintilla et al. (2022) in 'Crimson Seedless' and 'Sugraone', and Najafi et al. (2022) in 'Thompson Seedless'. Additionally, Ren et al. (2023)





effectively used the CRISPR/LbCas12a system for targeted gene editing – such as TMT1 and DFR1 in 41B grape cells – showcasing CRISPR technology's potential to alter specific metabolic pathways like flavonoid biosynthesis in grapevine. CRISPR-based genome editing has since been expanded to the use of nucleases with different characteristics, e.g. the Cas13 variants that target RNA instead of DNA. An example of such an application was the use of CasRx to introduce resistance to the ubiquitous grapevine virus A, though this was just a pilot study in *Nicotiana benthamiana* plants (Spencer et al. 2023).

In Europe, NGTs, especially genome editing, are used to create grapevine clones resistant to fungal diseases like powdery and downy mildew. Approaches include knocking out susceptibility genes in commercial cultivars, such as those in the MLO and DMR6 gene families. These methods maintain the variety's integrity and accelerate the breeding process, although challenges remain in identifying suitable target genes and developing efficient delivery of the CRISPR/Cas machinery and plant regeneration protocols. Despite these hurdles, promising results have been observed, including the development of DNA-free methods for obtaining edited grapevine plants, marking significant strides towards sustainable viticulture (Scintilla et al., 2022; Najafi et al., 2023; Moffa et al., 2024). The assessment of the first resulting DNA-free edited grapevines has commenced. However, soon after it started, it suffered deliberate sabotage from unknown vandals that destroyed all the plants – similar to what had occurred a few months prior in an NGT rice trial.

The development of gene-edited winegrape cultivars/clones with beneficial alterations of high-value traits has begun in Australia.

6 | Discussion and Conclusion

The evolution of NGTs in viticulture reflects a response to pressing challenges such as disease resistance, climate change and the need for sustainable agricultural practices. The advent of genome editing tools like CRISPR/Cas9 has opened new possibilities for precise and efficient breeding, allowing for the enhancement of desired traits such as disease resistance, fruit quality, and abiotic stress tolerance.

The synergy between NGTs and traditional breeding methods is another focal point. NGTs offer precision, complementing the more gradual process of conventional breeding. This combination can potentially lead to the creation of grapevine varieties that are not only high-yielding and disease-resistant but also adapted to various climatic conditions and consumer preferences.

The regulatory landscape of NGTs across different continents highlights the varying approaches and challenges of integrating

these technologies into existing legal frameworks. The legislation surrounding NGTs is critical in shaping their development and application, influencing everything from research and innovation to market access and public perception.

The social, economic and environmental aspects discussed above underscore the multifaceted impact of NGTs. While these technologies promise significant advancements in productivity and sustainability, they also raise concerns about biodiversity, ethical considerations, and public acceptance. Balancing these factors is crucial for the responsible development and use of NGTs in viticulture.

NGTs represent a significant leap forward in the field of viticulture, offering novel solutions to longstanding challenges. However, their successful implementation requires a careful and balanced approach. This involves integrating NGTs with traditional breeding methods, navigating complex regulatory landscapes, addressing ethical and societal concerns, and considering the environmental impact. As the sector continues to evolve, ongoing research, collaboration among stakeholders, and informed public dialogue will be key to harnessing the full potential of NGTs in viticulture. The future of viticulture – influenced by these technologies – seems promising, but it requires careful stewardship to ensure that benefits are maximised while risks and concerns are adequately addressed.





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