Genetically tailored grapevines for the wine industry

Melané A. Vivier and Isak S. Pretorius

Grapevine biotechnology is one of the most promising developments in the global wine industry, which is increasingly faced with conflicting demands from markets, consumers and environmentalists. In the grapevine industries, this technology and its supporting disciplines entail the establishment of stress tolerant and disease resistant varieties of *Vitis vinifera*, with increased productivity, efficiency, sustainability and environmental friendliness, especially regarding improved pest and disease control, water use efficiency and grape quality. The implementation and successful commercialisation of genetically improved grapevine varieties will only be realized if an array of hurdles, both scientific and otherwise, can be overcome.

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Economically, the grapevine constitutes the most important fruit species globally and has been linked to agricultural and religious activities in the earliest written chronicles. This ancient species has evolved from a bushy, sun-loving plant to a trailing climber. The grapevine has been domesticated with ease, giving rise to ~8 million hectares of intensely pruned and manicured grapevines that are typical of vineyards across the world [1]. When considering the necessity and possible impact of plant biotechnology on the wine industry, it is imperative to consider the long-term objectives of the wine industry. From the production and resources perspectives (which are the only ones discussed here), several key issues should be considered. In the end, the overriding question will always be whether a vineyard and its derived products are economically viable.
From this economic viewpoint, the issue of sustainable, high-quality production is a direct consequence of the increased fitness of vines in the context of the available environmental resources. Issues to be addressed include improved nutrient capture from soils and enhancement of the adaptation to adverse soil conditions. Cultivation practices could benefit from enhanced phytosanitary characteristics of cultivars, implying increased resistance to pathogens and other pests and resulting in the reduced use of agrochemicals and fungicides. Key to all these issues is the availability of elite starting material to establish new, or to replace existing, vineyards. Therefore, germplasm maintenance and propagation is at the heart of the wine industry and should be based on the best available technologies of pathogen detection and propagation methods to warrant certified material. In addition to the availability of superior germplasm and starting plant material, other crucial aspects include the improvement of the interaction between the soil and the plant, the eradication of pests and disease, and the enhancement of fruit quality. Linked to all these aspects are germplasm development, the establishment of new and novel cultivars, and the enhancement of a specific trait(s) of an established cultivar. The last aspect is particularly attractive to the wine industry because it relies predominantly on a few select and sought-after cultivars. Within the limitations of the basic knowledge and technologies available, the tools of molecular biology and biotechnology have the potential to significantly enhance the production of high-quality grapes, while having a reduced impact on the environment [2].

Grapevine biotechnology is currently practised in all major viticultural research centres worldwide in programmes that focus on several issues. As with plant biotechnology in general, several technical factors dictate the speed of the current progress in this field. Factors include the ease with which the relevant plant species can be manipulated to dedifferentiate and differentiate in tissue culture, be transformed with foreign DNA and be regenerated into plantlets. Being a woody perennial, Vitis vinifera initially proved to be recalcitrant to these manipulations. Since the inception of the first attempts to transform grapevine, only a few limited successes have been reported. Although several groups (including ours) have produced transgenic grapevines that are at varying stages of further evaluation, there is no universal protocol to manipulate all grapevine cultivars. Significant progress has been made, however, to establish this technology worldwide and the success rate should increase exponentially over the next few years [3,4].

This overview highlights the potential of grapevine biotechnology in the wine industry, by focussing on some of the most interesting traits targeted for improvement and concludes with some thoughts on the potential pitfalls and challenges facing the application of genetically modified grapes.

**Grapevine species and cultivars**

Grapevines are classified into the genus Vitis, consisting of two sub-genera, Euvitis and Muscadinia, of which the former comprises the bulk of the Vitis species. A single Vitis species, V. vinifera, originated in Europe, whereas >30 species are native to China and a further, ~34 species have been characterised in North and Central America. The scientific record of the origin of grapevine cultivars is at best rather fragmentary but it is generally accepted that V. vinifera (the most cultivated Vitis species) comprises ~5 000 true cultivars used in the wine, table (fresh fruit) and dried grape industries of the world [5]. Improvements to these cultivars initially relied largely on arbitrary selections of natural mutations that enhanced cultivation or some aspect of fruit and/or wine quality and were later followed by the more directed clonal selection schemes. Curiously, grapevine improvement has been ‘untouched’ by classical breeding programmes in the sense that relatively few new cultivars have become commercial successes, especially in the wine industry in which a few select and ancient cultivars are relied on for commercial production. However, breeding programmes have had a significant impact on the development of rootstock varieties resistant to soil-borne pests and pathogens, as well as to negative abiotic conditions [6].

When cultivar improvement is considered, the table, dried grape and wine industries have different goals. The table and dried grape industries market their products directly and have to provide the consumer with new, exciting products of excellent quality, whereas the wine industry typically relies on established varietal names and predictable wine styles to sell its products. Genetic transformation technology has been heralded as having high potential in grapevine improvement programmes in all three of these industries [1,3,4,7]. Some of the advantages linked to this technology and its application in grapevine production will be discussed.

**Genetic features and techniques for the analysis and development of grapevines**

The fact that several plant genomes have been fully sequenced and that genome-wide, proteomic and metabolomic analyses are becoming more accessible, confirms that a more advanced phase of plant improvement through molecular biology and genetic transformation is dawning. The accessibility of the grapevine genome in terms of applications in molecular biology is currently relatively restricted, owing to the size of the genome (483 mb distributed along 38–40 chromosomes) and specifically its complexity (only 4% of the genome is transcribed). However, the grapevine genome is currently targeted for intense study, with multinational consortia collaborating in several initiatives to render molecular markers as well as the complete sequencing of the Vitis genome [8].

The technology enabling the addition of genes of interest (under the control of regulatory elements of...
The first significant progress was made when embryonic cell lines were used as target tissue for grapevine transformations, and this approach became widely used due to its potential for improving economically important plant species. Agrobacterium-mediated and biolistic bombardment technologies have ensured that the field of genetic transformation is accessible for an ever-growing list of plant species. Several different approaches have been used to enhance disease tolerance in plants, including the use of pathogen-derived resistance and antiviral strategies. This approach has been successful in developing 'improved' phenotypes and has demonstrated that true and sustainable progress can be made when process knowledge is combined with application.

**Fig. 1.** The processes involved in the development and evaluation of transgenic grapevines targeted for commercialisation. (a) Grapevine tissue culture, (b) grapevine transformation and (c) evaluation of transgenics.

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**Table 1.** Targets for the genetic improvement of grapevine

<table>
<thead>
<tr>
<th>Year</th>
<th>Process</th>
<th>Description</th>
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<tbody>
<tr>
<td>1–2</td>
<td>Selection of transformants</td>
<td>Induction of somatic embryogenesis</td>
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<tr>
<td>1–2</td>
<td>Regeneration of putative transgenic plantlets</td>
<td>Agrobacterium-mediated or biolistic bombardment of somatic embryo cultures</td>
</tr>
<tr>
<td>5–8</td>
<td>Confirmation of transformations (using PCR, Southern and Northern analysis)</td>
<td>Evaluation of stable expression</td>
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<td></td>
<td>Field trials</td>
<td>Evaluation for stable expression, phenotype, ampelography, viticultural analysis and analysis of fruit and wine produced depending of industry targeted.</td>
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**Improvement of grapevine health**

It is generally accepted that plant disease is the exception rather than the rule owing to the efficient mechanisms by which plants defend themselves against pests and pathogens. Agricultural monoculture, however, is under constant threat from various pathogens and pests and mechanisms to curb fungal, bacterial, viral and insect pathogens remain the major focus of the genetic engineering of crop plants. The current approach of single gene transfers into plant genomes is perhaps also best suited to the aim of enhanced disease tolerance because single genes can confer disease resistance to plants.

Several different approaches have been used to enhance disease tolerance in plants but almost all of them make use of some part of the natural interaction between host and pathogen. This interaction is complex and highly fluid owing to the fact that the host and pathogen co-evolve in the battle for survival. Most transformation strategies involve a gene product with known anti-pathogenic activity that is introduced at high copies or in an inducible manner into the host of choice in an attempt to optimise parts of the plant’s innate defence response. Examples of this type of approach are shown in Table 1.

The other major approach of manipulated disease tolerance in grapevine (and other plants) relies on pathogen-derived resistance and various applications thereof. In this approach, a pathogen-derived gene and its encoding product are expressed in an inappropriate manner or in an inappropriate form or amount during the infection cycle, thus preventing the pathogen from maintaining infection. Most of the antiviral strategies rely on some aspect of pathogen-derived resistance and constitute a major portion of the activity in the genetic transformation of grapevine varieties (Table 1).

A range of transgenic plant species has been developed using this approach, with varying degrees of success. Transgenic grapevines expressing heterologous genes that can confer disease resistance to plants.
<table>
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<tr>
<th>Desirable properties</th>
<th>Focus area</th>
<th>Examples of current and potential target genes</th>
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<tr>
<td>Improved disease resistance</td>
<td>Fungal tolerance</td>
<td>Grapevine defence and defence signalling in response to fungal pathogens; pathology of the various fungal pathogens; innate resistances (molecular basis) of various species towards fungal pathogens Glucanase- and chinastase-encoding genes from fungi, yeast and plants; ribosome inactivating proteins (RIPs); thiamine-like protein (Vvδ1); antifungal peptide encoding genes from plants and insects; PGIP (polycatarractase-inhibiting protein) encoding genes from plant species, stilbene phytoalexins (stilbene synthases: stsy, vst1, vst2); phenylalanine ammonia lyase: pai; CuZnSOD (putative CuZn superoxide dismutase; detoxification enzyme-producing genes (NADPH-dependent aldehyde reductase, Vigna radiata-Euteine reducing enzyme)</td>
</tr>
<tr>
<td></td>
<td>Bacterial tolerance</td>
<td>Grapevine defence and defence signalling in response to bacterial pathogens; pathology of the various bacterial pathogens; innate resistances (molecular basis) of various species towards bacterial pathogens Anti-microbial peptides (lytic peptide, Shiva-I, defensins); dysfunctional import and integration protein encoding gene (virE2delB) from Agrobacterium</td>
</tr>
<tr>
<td>Viral tolerance</td>
<td></td>
<td>Epidemiology of virus infections and vectors; molecular biology on infecting virus; pathogen-derived resistance strategies (coat proteins; movement proteins) Virus coat proteins (translatable, anti-sense, non-translatable); virus movement proteins (anti-sense); replicase (RNA-dependent RNA polymerase); proteinases; 2,5 oligoadenylate synthase</td>
</tr>
<tr>
<td>Improved stress tolerance</td>
<td>Resistance to water stress</td>
<td>Aquaporins; isolation of root-specific promoters TIPs (tonoplast integral proteins); PIPs (plasma membrane integral proteins) Carotenoid biosynthetic genes; Adh (alcohol dehydrogenase) genes; SODs (cystosolic CuZnSOD, chloroplast-residing CuZnSOD, mitochondria-residing MnSOD)</td>
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<td></td>
<td>Oxidative damage</td>
<td>Carotenoid biosynthesis and control (several putative genes and promoters have been cloned); anaerobiosis Carotenoid biosynthetic genes; Adh (alcohol dehydrogenase) genes; SODs (cystosolic CuZnSOD, chloroplast-residing CuZnSOD, mitochondria-residing MnSOD)</td>
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<td></td>
<td>Osmotic stress and other abiotic stresses</td>
<td>Proline accumulation; polyamines and their role in stress Vvp5cs (α-1-pyrroline-5-carboxylate); Vvoat (γ-ornithine aminotransferase); FeSOD, glycine betaine, antifreeze genes from Antarctic fish (freezing tolerance)</td>
</tr>
<tr>
<td>Improved quality factors</td>
<td>Colour development</td>
<td>Ripening related processes and signals, anthocyanin biosynthesis and control (several genes and some promoters have been cloned); isolation of berry-specific Promoters Ugft (UDP-glucose-flavanoid 3-O-glucosyltransferase) and/or regulatory sequences of ugft; production of pelargonidin-based anthocyanins for novel berry colour; anthocyanin methyl-transfases</td>
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<td></td>
<td>Sugar accumulation and transport</td>
<td>Phloem loading and unloading; invertases; sugar transporters; isolation of berry-specific promoters Invertases from plants and yeast to study phloem loading and unloading; sucrose transporters (Vvsvc1, Vvsvc12, Vvsvc27); hexose transporters (VvhT1, VvhT2)</td>
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<td></td>
<td>Reduced browning (table and dried grapes)</td>
<td>Oxidation reactions Silencing of polyphenol oxidase</td>
</tr>
<tr>
<td></td>
<td>Seedlessness (table grapes)</td>
<td>Seed formation; isolation of seed-specific promoters Baranase gene</td>
</tr>
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</table>

Antifungal and antiviral genes are currently undergoing field testing [4]. These first 'prototypes' of manipulated disease tolerance in grapevine, as in other plant species, are the beginning of a new era in plant cultivation and old problems are being addressed in new ways. The technology will undoubtedly improve in sophistication, with the possibility of multiple gene transfers, the use of highly specific inducible regulatory sequences and the possibility of ensuring the long-term and stable expression of transgenes.

Much knowledge has been gained about the nature of plant-pathogen interactions and the disease resistance pathways that operate in plants by generating and analysing transgenic plants. Model plants transformed with the various targeted genes become important resources if the nature of the manipulations and their effect in planta are further analysed with state-of-the-art technologies, such as proteomics and microarray chips. A range of Arabidopsis mutants blocked in certain pathways of pathogen defence also provide extremely valuable information regarding the functions of gene products. The research has developed to the point where the disease pathways are characterised fairly well and much emphasis is currently placed on the elucidation of the trigger systems of defence and the subsequent signal transduction processes leading to the various forms of defence.

**Improvement of grapevine cultivation**

Genetic transformation technology has enormous application in the improvement of plant cultivation because it presents the prospect of developing plant lines with the ability to adapt to adverse climatic conditions. Advances made in the understanding of stress tolerance in plants [14–16], combined with basic knowledge on key aspects of plant growth and development, have accelerated the feasibility of transgenic approaches to address these complex
problems. A basic understanding of fundamental processes, such as carbon partitioning, modes of sugar translocation [17,18], water transport and the role of aquaporins [19], as well as the regulation of these processes, are some of the areas studied actively to drive efforts to develop transgenic grapevines with improved cultivation prospects. Important limitations to cultivation focussed on in this approach are drought and salt stress, photo-damage and freezing tolerance (see Table 1). The afore-mentioned stress responses in the plant are all complex pathways of interacting proteins driven by a range of signals that are attenuated or amplified by equally complex processes. This typical triptych (a model to describe the synchronised chain of events in metabolic pathways, within the context of the whole plant body and with consideration of the input and output signals) is more difficult to manipulate with single or even multiple gene additions and knowledge of the control mechanisms and alterations thereof might prove more feasible.

**Improvement of grapevine quality**

The description of quality in grapevine products differs in the three grapevine industries. The wine industry regards small, well-coloured fruit complying with optimal ripeness indicators (sugars, acids and phenolics) as desirable, whereas the appearance and optimal size of table grape bunches are of prime importance. Basic quality factors, such as good colour and sugar development, are of generic importance and are currently targeted in grapevine molecular biology. The basic processes of berry ripening and, more importantly, the elusive ripening signal(s) are being researched [17,18]. The hormonal, environmental and biochemical signals that have an impact on the key ripening processes, such as pigment production, sugar accumulation and transport, as well as aroma component formation, are studied in grapevines as an example of a non-climacteric fruit (Table 1). The ultimate aim of this type of approach is to change the metabolic flux through the important biosynthetic pathways that are active in the ripening berry to increase the formation of desirable or novel products linked to the quality parameters of grapes. Grapevine biotechnology, however, is in its infancy in this regard (as in most other crops) and will have to draw on significant elucidation of the underpinning processes as well as improvements in transformation technology to reach these goals. Targeted gene insertion and deletion technologies are some of the tools that would make these and other innovative prospects, such as the manipulation of biochemical pathways to produce novel products and metabolites, more feasible.

**Legal and regulatory hurdles**

The initial problems with statutory approval for the use of genetically engineered plants and organisms in the agro-industry are now slowly being dissolved by a growing consensus that risk is primarily a function of the characteristics of a product, rather than the use of genetic modification per se[20]. The concept of ‘substantial equivalence’ is widely used in the determination of safety by comparison with analogous conventional food and beverage products [20]. When substantial equivalence can be demonstrated, no further safety considerations are usually necessary. When substantial equivalence is not shown convincingly, the points of difference must be subjected to further safety scrutiny.

The legislation and regulations, although differing in detail, are broadly similar in most countries. Guidelines for the approval of genetically modified (GM) products and the release of genetically modified organisms (GMOs) usually require several obvious guarantees. These include a complete definition of the DNA sequence introduced and the elimination of any sequence that is not indispensable for expression of the desired property; the absence of any selective advantage conferred on the transgenic organism that could allow it to become dominant in natural habitats; no danger to human health and/or the environment from the transformed DNA; and a clear advantage to both the producer and the consumer [20].

**Intellectual property and patenting hurdles**

Patents covering many of the genetic tools (e.g. DNA sequences, gene promoters, marker genes and vectors) and methods (e.g. transformation protocols) commonly used in genetic engineering leave little freedom to operate [1]. It therefore is imperative to address intellectual property issues, such as patents or other forms of protection of genes, promoters and technologies through formal agreements [4].
If ownership of a transgenic grapevine is in dispute, the release of such genetically improved grapevine plantlets might cause serious impediment to the commercialisation process. However, genetically improved grapevines (with 'sufficiently distinct' properties) must also be protected in some way by the developer. However, whether an improved transgenic grapevine can be patented itself or protected in other ways might also depend on the legislation and regulations in each wine-producing country [4].

Political and economic hurdles
It is well known that economies are driven by different forces and therefore go through life cycles [21]. For example, in terms of resources, the 'industrial economy' was 'the economics of scarcity' because everything that fueled the economy was in short supply and available to only a few. The current 'information economy', which was built on the successes of the 'industrial economy', is driven by 'the economics of plenty' and, thanks to communications, computer technologies and the internet, information is no longer a scarce resource. Furthermore, it is already being speculated that the 'information economy' is only the first phase of the 'bioeconomy', which rests on the pillars of both information technology and biotechnology. There is ample evidence that the 'info-bioeconomy' has already brought about more economic transformation in the past few decades than was brought by the 'industrial economy' in the previous centuries. Not everybody perceives all of these transformations as positive changes. Some critics and activists are whipping up public alarm and fuelling political agendas and protests against globalisation and a universal, 'borderless' economy. Certain lobby groups also claim that patents on genetically engineered organisms confer an unfair advantage to certain producers, thereby concentrating economic power in the hands of a few large multinational producers [20]. Therefore, it can be expected that the commercialisation of genetically improved grapevines would not escape political meddling from the vested interests of economic and agricultural protectionism. The swelling tide in an overflowing ocean of wine is likely to increase the temptation for some to twist scientific data and misuse consumer confusion to justify trade bans and technical barriers to free trade.

Marketing hurdles
The marketing of wine relies to a great extent on label integrity and product identity. Therefore, it is of utmost importance that genetically improved grapevines do not interfere with the established varietal names and predictable wine styles. For example, the wine industry relies heavily on a few select cultivars and would therefore be very hesitant to introduce new varietal names [1,4]. In the most profitable market segments, the varietal name (especially the names of the so-called 'Big Five', namely Cabernet Sauvignon, Shiraz, Merlot, Chardonnay and Sauvignon blanc), together with the origin of production and the vintage, form the cornerstones of the information that is presented on the bottle label to the increasingly brand-conscious customers and consumers. The outcome of the current debate on the description and naming of transgenic grapevines therefore will determine not only the procedure for the description of genetically modified grape varieties but also, to a large extent, their acceptance by grape growers and winemakers and their commercial value in the marketplace [1].

This debate about naming entails several factors, such as the source of gene(s) introduced into a particular grapevine, the 'true-to-typeness' of the transgenic vine when compared with the original cultivar and/or done and the organoleptic and sensory qualities of the resulting wine [1]. Given the immense marketing value contained in varietal names, there is an urgent need for consensus that GM grapevines are little different to grapevine clonal selections, which have been selected on the basis of beneficial, spontaneous genetic variations (e.g. a change in plant performance). When clonal selections are used, the identity might be known to the grape grower but the wine is still marketed under the varietal, and not the clonal (typically specified by a clone number), name [4]. However, it remains to be seen whether transgenic grapevines with altered fruit qualities, such as improved colour and flavour compound composition, will have to be assigned a new varietal name or just a new clonal number. These uncertainties, together with the impractical, but strong, calls for all products that are produced by gene technology to be labelled specifically, aggravate the wine industry's hesitance to adopt transgenic grapevines in the face of those who cannot resist riding the dangerous 'backlash' market with labels stating that a particular wine product is 'GM free'.

Traditional and cultural hurdles
National and regional wine industries possess strong identities and deep cultural roots, as illustrated by proudly maintained local traditions. As a consequence, the industry is less receptive to technologies that promise revolutionary changes. In this context, it is also feared that gene technology could accelerate the tendency to standardise wines to satisfy large supermarket chains and the 'average' international consumer, leading to loss of local identity, variety and uniqueness. The successful application of recombinant DNA technology in the wine industry will depend on assuring the commercial users of transgenic grapevines that existing, desirable characteristics have not been damaged, that the requirements of beverage legislation are met and that the engineered cultivar will be stable in practice, with suitable procedures for monitoring. Once the traditionalists are convinced of a clear organoleptic, hygienic or economic benefit of a transgenic grapevine variety, they would be in a strong position to implement the use of such a vine because
most of the wine enterprises are fully integrated agro-industries that could exert direct control over the development of new specialized niche markets for ‘GM wine products’. Wine consumers in such types of niche markets are frequently passionate, well informed, well educated and, above all, very curious. Therefore, GM wines produced by a limited number of interested producers would certainly attract widespread attention and create a new successful niche market. Based on such small beginnings, the broader benefits conferred by GM technologies could become apparent to grape growers and winemakers, and the technology could move rapidly from satisfying niche markets to general acceptance.

Public perception hurdles
The emotive, fear-mongering qualms and myths of the immorality of ‘unnatural’ genetic interference with Nature, of unsafe ‘Frankenfood’ and global havoc caused by GM Os have spread more readily than good sense or wise science, and far enough to masquerade in the cultural folklore as truth [20]. Therefore, public perception of risk with regard to GM food has, so far, outweighed its view of possible benefits. Regulatory authorities appear more willing to approve the use of GM Os than the public is to use them. A significant proportion of the public still suspects that GM food will prove unhealthy in the long term and that the escape of GMOs will damage the environment and result in a loss of biodiversity [20]. They also doubt that there is sufficient legal and practical protection against accidents involving GMOs.

Conclusion
Quality wines are being produced on all arable continents. Established as well as emerging nations are contributing significantly to the international wine trade [22]. It is crucial to promote the cutting-edge work associated with genetic mapping, molecular markers and other biotechnological aspects in grapevine manipulation to the benefit of all involved, be it the producers, the workers, the industry as a whole and, above all, the consumer and environment. Not discussed here, but equally important, is the array of benefits this technology holds for the consumer. It is of the utmost importance to realize the promise of this ‘new revolution’ for the wine industry and its consumers, while avoiding the pitfalls.

References

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