The tailoring of grapevine cultivars and wine yeast strains for a market-directed and quality-focussed wine industry: Novel approaches to the ancient art of winemaking

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Abstract

The widening gap between wine production and wine consumption, the shift of consumer preferences away from basic commodity wine to top quality wine, and the gruelling competition brought about by economic globalisation call for a total revolution in the "magical" world of wine. In the process of transforming the wine industry from a production-driven industry to a market-orientated enterprise, there is increasing dependence on, amongst others, biotechnological innovation to launch the wine industry with a quantum leap across the formidable market challenges of the 21st century. Market-orientated designer grape cultivars and wine yeast strains currently are being genetically programmed with surgical precision for the cost-competitive production of high quality grapes and wine with relatively minimal resource inputs and a low environmental impact. With regard to Grapevine Biotechnology, this entails 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. With regard to Wine Yeast Biotechnology, the emphasis is on the development of *Saccharomyces cerevisiae* strains with improved fermentation, processing and biopreservation abilities, and capacities for an increase in the wholesomeness and sensory quality of wine. The successful commercialisation of transgenic grape cultivars and wine yeasts depends on a number of scientific, technical, safety, ethical, legal, economic and marketing factors, and it therefore will be unwise to entertain high expectations in the short term. However, in the light of the phenomenal potential advantages of tailor-made grape varieties and yeast strains, it would be equally self-destructive in the long term if this strategically important "life insurance policy" is not taken out by the wine industry.
This overview highlights the most important examples of the way in which *Vitis vinifera* grape varieties and *Saccharomyces cerevisiae* wine yeast strains currently are being designed with surgical precision on the basis of market demand for the cost-effective, sustainable and environmentally friendly production of healthy, top quality grapes and wine.

Die immer größer werdende Kluft zwischen Weinproduktion und Weinkonsumation, die Verschiebung der Präferenz der Konsumenten vom einfachen Tischwein hin zu Topqualitäten und der mörderische Wettbewerb durch die Globalisierung der Wirtschaft rufen nach einer Neuorientierung des Mythos „Wein". Bei der Umbildung der Weinwirtschaft von einer produktionsgesteuerten Branche zu einem marktorientierten Unternehmen spielen neben anderen Aspekten biotechnische Neuerungen eine wesentliche Rolle, um die Weinwirtschaft angesichts der enormen Herausforderungen des 21. Jahrhunderts um einen Quantensprung weiterzubringen. Derzeit werden marktorientierte Designer-Reben und Weinhefestämme zur wettbewerbsfähigen Produktion hochqualitativer Trauben und Weine unter relativ geringem Aufwand und niedriger Umweltbelastung mit höchster Präzision genetisch programmiert. Was die Biotechnik der Reben anlangt, so ist die Schaffung von stressverträglichen und krankheitsresistenten *Vitis-vinifera*-Sorten mit gesteigerter Produktivität, Leistungsfähigkeit, Nachhaltigkeit und Umweltfreundlichkeit im Sinne einer verbesserten Schädlings- und Krankheitsbekämpfung, Wassernutzungseffizienz und Traubenqualität erforderlich. Hinsichtlich der Weinhefen-Biotechnik wird das Hauptaugenmerk auf die Entwicklung von *Saccharomyces-cerevisiae*-Stämmen mit verbessertem Gär-, Veredelungs- und Biopräservationsvermögen bei gleichzeitiger Erhöhung der Bekömmlichkeit und sensorischen Qualität der Weine gelegt. Die erfolgreiche Vermarktung von transgenen Traubensorten und Weinhefen hängt von einer Vielzahl von wissenschaftlichen, technischen, wirtschaftlichen, marketing- und sicherheitsspezifischen, gesetzlichen und ethischen Aspekten ab, und es ist daher unklug, sich hohen Erwartungen kurzfristig hinzugeben. Angesichts der phänomenalen Vorteile, die maßgeschneiderte Rebsorten und Hefestämme möglicherweise mit sich bringen, käme es jedoch auf lange Sicht gesehen einer Selbstzerstörung gleich, wenn diese strategisch wichtige „Lebensversicherungspolizze" von der Weinwirtschaft nicht aufgegriffen würde. Diese Übersicht zeigt die wichtigsten Beispiele auf, wie *Vitis-vinifera*-Rebsorten und *Saccharomyces-cerevisiae*-Weinhefestämme entsprechend der Nachfrage des Marktes nach einer kosteneffektiven, nachhaltigen und umwelt-freundlichen Produktion gesunder Topqualitäten von Trauben und Weinen derzeit mit höchster Präzision designt werden.

1. The metamorphosis of the wine industry in a globalised economy

It was virtually unprecedented when leading economists, sociologists and other intellectuals concurred so extensively at the start of the third millennium that the world was on the verge of an era of massive and unprecedented change – a change so dynamic and far-reaching that it will rearrange all facets of society to be virtually unrecognisable during the next 20 to 30 years. Even a traditional industry such as the wine industry will not be able to evade this unavoidable metamorphosis. In an attempt to establish a compass according to which the wine industry can prepare for the enormous challenges of the 21st century, a number of leading wine-producing regions and countries assiduously have carried out in-depth analyses of global trends in the wine business environment and have begun to plan according to the most probable scenarios. On the basis of these future scenarios, long-term strategies have been compiled and visions have been formulated to navigate the wine industries of these regions and countries through the confusing fog of shifting changes in consumer preferences and technological innovations. If the world's leading wine industries cannot remain supple and agile on their course through the potentially engulfing streams and high waves of change, the economy of those wine regions and countries could be washed unexpectedly against the sharp edges of the icebergs of the new world order. It therefore will be fatal for the welfare of numerous wine regions and countries if they do not adjust their course timeously in order to circumvent the storm clouds that are already gathering on the horizon of the new millennium.

Change in the wine industry is not new, because, regardless of the fact that the fundamental principles of vineyard cultivation and vinification have remained largely unchanged over the past 7 000 years, the image of wine has changed completely. At the start of the 17th century, wine was regarded as the only *storable, wholesome beverage*, while today it is presented to consumers as a *universal, first-choice lifestyle beverage for moderate use*. But the core of the current bewildering rate of change lies in this modern image of wine. This has placed wine in the centre of the high-tension field between the forces of *market pull* and *technology push*, in which tradition and innovation need to find one another so that it is possible to continue to meet the demands of wine producers and the preferences of wine consumers. On the one hand, the continued existence and welfare of the producer is directly dependent on the sustainable profitability of the wine industry; on the other hand, the increasingly health- and environmentally-conscious wine consumer is looking for individualised, tailored products with a good ratio between quality and price. The balance between these two poles is increasingly moving to the side of the demanding consumer preferences, and this places the producer under increasing pressure.

One of the primary incentives that led to the traditional, *production-orientated* wine industries being forced to transform themselves into *market-driven* enterprises was the increasing gap between wine production and wine consumption in a global, "borderless" and highly competitive economic system. This universal mismatching of demand and supply is stimulated, on the one hand, by the international trend among consumers to progressively consume fewer alcoholic beverages and, on the other hand, by the rapid pace at which wine production is being expanded, particularly in the New World countries (Argentina, Australia, Chile, California, Canada, New Zealand and South Africa). The latter countries already produce about 20% of the world's wine, compared to the more than 50% of the Old World wine-producing countries (France, Italy, Spain, Portugal and Germany). The approximately 27 billion litres of wine that are produced annually from about 8 million hectares of vineyard throughout the world are more or less 5 000 million litres more than the market can absorb. This wine surplus, which fluctuates annually at between 15 and 20% of the total production, has given rise to keen competition for market share. This surplus is limited particularly to the basic bulk wine category, which indicates that, at the same time, there has been a significant shift in the preferences of the consumer from basic commodity wines to premium, ultra-premium and even icon wines. This means that the so-called wine surpluses no longer can be regarded merely as a *production problem*, but rather as a *management problem* of "market unconscious" producers who do not yet realise that it is only customer and consumer choice, and no longer the producer, that determines what are *affordable products of quality*.

The rules of the game within which the wine industry has to compete today have changed to such an extent that, in the stiff, image conscious and price sensitive wine market, *quality* is defined as *sustainable customer*

and consumer satisfaction. Successful and enterprising wine industries therefore increasingly are becoming consumer driven and customer responsive, so that wine products of outstanding quality can be offered at every price point. If a wine industry therefore strives to be globally competitive and highly profitable, it must necessarily be able to take the initiative and thereby retain the lead in a world market that demands products with strong brand names and continuous renewal with regard to wine style, quality, purity, uniqueness and diversity. In order to ensure product leadership in terms of intensity, complexity and diversity at each price point, innovation at all levels of the total value chain is no longer an option for the wine industry, but a necessity. The inculcation of a culture of innovation that permeates the entire wine industry is the only route to the consumers, who relentlessly vote with their wallets for specific ranges of wine products. This therefore demands that any profitable and socially responsible wine industry or enterprise makes a total commitment to quality, style and innovation, from the vineyard to the consumer's palate.

It therefore is clear that technological innovation is one of the cornerstones with which the successful wine industries of the 21st century can be assured of winning advances with regard to global influence and sustainable profitability. These include, amongst others, market-orientated, biotechnological innovation that is aimed at overcoming the problems in the wine industry or creating and realising new, strategically important opportunities. With the correct approach, the outcomes of biotechnological innovations can harmonise meaningfully with a complex of market, cultural, social, environmental and technical factors, without stripping the ancient art of winemaking of its charming mysticism and romanticism.

In spite of the current scepticism of some "misinformed" consumer groups about genetically modified organisms and products (the so-called GMOs and GM products), there is no doubt that the application of leading gene technology in the wine industry holds breathtaking potential. In order to be technologically equal to the tremendous consumer challenge, the world's pacesetting wine industries increasingly are focussing on the genetic programming and improvement of the two main organisms involved in the production of wine, namely the grapevine and the wine yeast.

2. The potential of genetically improved grapevine cultivars

2.1 Grapevine species and cultivars

Economically, the grapevine constitutes the most important fruit species globally and has been linked to agricultural and religious activities in the earliest writings and chronicles. This ancient species has evolved over the ages from a bushy, sun-loving plant to a trailing climber. The grapevine has been domesticated with ease, giving rise to approximately 8 million hectares of intensely pruned and manicured grapevines that are typical of vineyards across the world.

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, *Vitis vinifera*, originated in Europe, whereas more than 30 species are native to China and a further, approximately 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 approximately 5 000 true cultivars used in the wine, table and dried grape industries of the world. 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. Grapevine improvement has curiously been "untouched" by classical breeding programmes, in the sense that relatively few new cultivars became commercial successes, especially in the wine industry, where a few select and ancient cultivars are relied on for commercial production. However, breeding programmes significantly impacted on the development of rootstock varieties resistant to soil-borne pests and pathogens, as well as to negative abiotic conditions

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When cultivar improvement is considered, the table/dried grape and wine industries have different goals. The former industries market their products directly and have to provide the consumer with new, exciting and excellent quality products, 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 these industries. Some of the advantages

linked to this technology and its application in grapevine production will be discussed in the following sections.

2.2 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 molecular biology applications is currently much more restricted than that of wine yeast due to the size of the genome (ca. 470-483 mb divided into 38-40 chromosomes) and its complexity (only 4% of the genome is transcribed). The grapevine genome, however, 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.

The technology to add genes of interest under the control of regulatory elements of choice into plant genomes has opened up various possibilities for plant improvement and a wide range of economically important plant species have been targeted in this regard. *Agrobacterium*-mediated and biolistic bombardment technologies have ensured that an ever growing list of plant species are accessible for genetic transformation. Noteworthy, however, is the fact that the grapevine has been considered recalcitrant to genetic transformation due to various difficulties, amongst others in obtaining regenerable tissue culture systems that can withstand *Agrobacterium* or biolistic transformation and the subsequent selection regimes. The first significant progress was made when embryonic cell lines were used as target tissue for grapevine transformations, leading to several laboratories (both private and public) routinely producing transgenic grapevines. In this regard, the term “routinely” includes only a few commercially important grapevine scion and rootstock cultivars. Since 1989, when the first successful grapevine transformations were performed, the focus has gradually shifted from the development of grapevine transformation technology to the implementation of the technology in the generation of useful plant lines.

2.3 Targets for the genetic improvement of grapevine

Integrally linked to the prospect of rendering genetically improved grapevines is the use of molecular biology to study the fundamental processes in grapevine that underpin the physiological responses targeted for improvement. Initially, when limited genetic resources were available, genes with known function were introduced into plant species in the hope of developing "improved" phenotypes. This shotgun approach has taught us valuable lessons, especially about complications regarding transgene silencing, but has also proven that true and sustainable progress can be made when process knowledge is combined with application. Grapevine transformations are co-entering an exciting era with the plant sciences, as a growing list of genes and their regulatory sequences are becoming available from economically important species, including grapevine.

Improvement of grapevine health: It is generally accepted that plant disease is the exception rather than the rule, due to the very efficient mechanisms of plants defending themselves against pests and pathogens. Agricultural monoculture, however, is under constant threat by 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, since 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 due 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-pathogen 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 (PDR) and various applications thereof. In this approach, a pathogen-derived gene and its encoding product is expressed at an inappropriate time 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 PDR 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 by this approach, with varying success. Transgenic grapevines expressing heterologous antifungal and antiviral genes are currently undergoing field testing. These first "prototypes" of manipulated disease tolerance in grapevine, as in other plant species, are the beginning of a new era in plant cultivation, as 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 genes. The research has developed to the point where the disease pathways are fairly well characterised 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, since 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, 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, water transport and the role of aquaporins, as well as the regulation of these processes, are some of the areas studied actively to drive endeavours 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 for examples). The 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 of biological interaction 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/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. The hormonal, environmental and biochemical signals impacting on the key ripening processes, such as pigment production, sugar accumulation and transport, as well as aroma component formation, are studied in grapevine 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.

3. The potential of genetically customised wine yeast strains

3.1 Yeast species and strains

Yeasts are predominant during wine fermentation. In spontaneous fermentations, there is a progressive growth pattern of indigenous yeasts originating from the surfaces of grape berries and the winery equipment.

Yeasts of the genera *Kloeckera*, *Hanseniaspora* and *Candida* predominate in the early stages, followed by several species of *Metschnikowia* and *Pichia* in the middle stages, when the ethanol rises to 3-4%. The latter stages of spontaneous wine fermentations are invariably dominated by the alcohol-tolerant strains of *S. cerevisiae,* which therefore is known universally as the "wine yeast". The indigenous yeasts present in spontaneous wine fermentations are thought to produce wines with a fuller, rounder palate structure. However, spontaneous fermentations are usually protracted and the outcome is highly unpredictable. Therefore, spontaneous fermentations are only being used in some *boutique* wineries that depend more on vintage variability and that are willing to accept these risks to achieve distinct styles of wines that reflect the yeast diversity of that specific region.

In modern, large-scale wineries, where rapid and reliable fermentations are essential for consistent wine flavour and predictable quality, specially selected starter culture strains of *S. cerevisiae* with known ability are used. In addition to the primary function of these active dried wine yeast starter culture strains to catalyse the rapid, efficient and complete conversion of grape sugars (glucose and fructose) to alcohol without the development of off-flavours, today's pioneering winemakers demand starter culture strains with a whole range of specialised properties that can add value to the final product. This quest for wine yeast strains that are optimised for specific tasks set by winemakers has led to dedicated yeast breeding and genetic engineering.

3.2 The genetic features and techniques for the analysis and improvement of wine yeasts

The majority of laboratory-bred strains of *S. cerevisiae* are either haploid or diploid, whereas industrial wine yeast strains are predominantly diploid or aneuploid, and occasionally polyploid. The nucleotide sequence of the entire genome of *S. cerevisiae* is known. It has a relatively small, compact genome (ca. 13 000 kb), a large number of chromosomes (16 linear chromosomes varying in length from 200 to 2200 kb), a small number of genes (ca. 6000 protein-encoding genes), little repetitive DNA and few introns.

Powerful classical and molecular genetic methods exist with which wine yeast strains can be analysed and modified. Initially, tetrad analysis with the aid of a micromanipulator was used for the genetic identification, characterisation and mapping of yeast genes. Recently, technology has been developed to provide a direct link between the genome (full set of genes) and the transcriptome (full set of transcripts) of a wine yeast strain. The genomic sequence has been used to design and synthesise high-density oligonucleotide arrays to monitor the levels of expression of nearly all the genes of yeast cells grown under fermentation conditions. Furthermore, when the current deciphering of the function of the 6000 *S. cerevisiae* genes is completed in the near future, the entire proteome (full set of proteins) will become accessible for the unlocking of wine yeast's complex metabolome (metabolic activities and metabolites).

The information obtained from the analysis of the entire genomes, transcriptomes, proteomes and metabolomes of wine yeasts undoubtedly will increase the specificity of the current methods with which starter strains are genetically selected and tailored for the production of particular types and styles of wine. At the moment, the classical strain selection and modification methods, such as variant selection, mutagenesis, hybridisation (mating, spore-cell mating, rare mating, cytoduction and spheroplast fusion), are based mainly on a "shotgun" approach. With this approach, large genomic regions or entire genomes are recombined or rearranged. These methods, therefore, are not specific enough to modify wine yeasts in a well-controlled manner and they may bring an improvement in some of the yeast strain's properties, while compromising other desired traits. The only advantages of these methods are that they can be used to improve and combine traits under polygenic control and that they do not give rise to products that are included in the statutory definition of GMOs. Therefore, variants, mutants, hybrids, cytoductants and fusants are not subject to the same strict statutory regulations that pertain to GMOs and are also not treated with the same level of public suspicion as are wine yeasts that have been transformed with foreign DNA. However, genetic engineering remains the only reliable method that offers the possibility to modify an existing property, to introduce a new characteristic and to eliminate an unwanted trait without adversely affecting other desirable properties. Several effective transformation methods and plasmid vectors, as well as expression and secretion cassettes for the expression of heterologous genes and the secretion of their encoded proteins, have been developed for *S. cerevisiae*. This has offered wider applicability and a higher degree of specificity in the development of improved wine yeasts.

3.3 Targets for the genetic improvement of wine yeasts

Generally, the targets of strain development all relate to improved economics of production and wine quality. Table 2 highlights some of the improvements that can be achieved using genetically engineered wine yeasts. These targets include increasing the efficiency of the fermentation process, the processing of wine and control of microbial spoilage, as well as enhancement of the wholesomeness and sensory quality of wine.

Improvement of fermentation performance: Wine fermentations generally proceed at a rate greater than desired and are usually controlled by lowering the fermentation temperature. "Runaway" fermentations have a commercial implication, as fermentor space is reduced because of foaming and volatile aroma compounds are lost by entrainment with the evolving carbon dioxide. On the other hand, wine fermentation sometimes ceases prematurely or proceeds too slowly. The financial losses caused by "stuck", "sluggish" or "incomplete" wine fermentations are usually attributed to inefficient utilisation of fermentor space and wine spoilage as a result of the low rate of protective carbon dioxide evolution and high residual sugar content. Therefore, the predictability of fermentation and the quality of the wine are directly dependent on wine yeast attributes that assist in the rapid establishment of numerical and metabolic dominance in the early phase of wine fermentation and that determine the ability to conduct an even and efficient fermentation with a desirable residual sugar level. Many factors affect the fermentation performance of wine yeasts. Among the general targets for the improvement of fermentation performance are increased resilience and stress resistance of active dried yeast cells; improved grape sugar and nitrogen uptake and assimilation; enhanced resistance to ethanol and other microbial metabolites and toxins; resistance to sulphite, heavy metals and agrochemical residues; and reduced foam formation (Table 2).

As sterols, trehalose, glycogen and aquaporins fulfil multiple roles in increasing the survival of *S. cerevisiae* cells exposed to several physical and chemical stresses, they have important implications for the general stress tolerance, resilience, fitness and vigour of active dried wine yeast starter cultures upon reactivation. As a result, there is a strong incentive to develop wine yeast strains with a superior ability to accumulate these compounds. However, due to the complex stress response mechanisms in yeast, it is not yet clear whether the deletion of the *ATH1* trehalase gene and the modification of the expression levels of the genes involved in the metabolism of trehalose (*TPS1, TPS2, ATH1*), glycogen (*GSY1, GSY2*) and sterols (*SUT1* and/or *SUT2*), and in the synthesis of aquaporins (*AQY1, AQY2*) (Table 2) will result in an improvement in yeast viability and vitality.

An imbalance in the high levels of carbon and low levels of nitrogen in grape must is the most common cause of poor fermentative performance. Sluggish or stuck fermentations occur because nitrogen depletion irreversibly arrests hexose transport. The main focus to ensure the efficient utilisation of grape sugar (glucose and fructose) under conditions of nitrogen limitation is to increase the rate of glycolytic flux by replacing any non-functional mutant alleles of genes encoding the key glycolytic enzymes, to enhance the efficiency of hexose (especially fructose) uptake, and to alleviate the assimilation of proline and arginine (accounting for 30 to 65% of the total amino acid content of grape juice) from nitrogen catabolite repression (Table 2). In the case of incomplete fermentations, the preference of wine yeasts for glucose over fructose can lead to excessive residual fructose levels that compromise the quality of the wine. It is hypothesised that the rate of alcohol production by wine yeast is limited primarily by the rate of sugar uptake, especially the uptake of fructose in the presence of high sugar levels during the early phase of fermentation and during the final stages of nitrogen depletion coupled to nutrient limitation. Therefore, several laboratories focus on phosphorylation by the *HXK1*- and *HXK2*-encoded hexokinases and the *GLK1*-encoded glucokinase, as well as on hexose transporters encoded by *HXT1-HXT18* and *SNF3*. There is anecdotal evidence that the overexpression of the *S. pastorianus FSY1*-encoded fructose/H^+ symporter together with some of the other *HXT1-HXT18* and *SNF3*-encoded hexose transporters and the *HXK1*-encoded hexokinase (with the highest affinity for fructose, but still a significantly lower affinity than for glucose) results in improved glucose and fructose uptake during wine fermentations. Furthermore, the deletion of the *URE2*-encoded repressor of the *PUT1*-encoded proline oxidase and *PUT2*-encoded pyrroline-5-carboxylate dehydrogenase represents the first step towards the development of wine yeasts that can efficiently assimilate the abundant supply of proline and arginine in grape juice under fermentative conditions.

Another thrust to improve the fermentation performance of wine yeasts is to increase their resistance to toxic microbial metabolites (e.g. ethanol, acetic acid, medium chain fatty acids, etc.), zymocins (yeast-derived

killer toxins), chemical preservatives (e.g. sulphite) and agrochemicals containing heavy metals (e.g. copper) (Table 2). For example, modification of the expression of the *SUT1*, *SUT2*, *PMA1* and *PMA2* genes results in increased sterol accumulation and cell membrane ATPase activity, thereby increasing the resistance to ethanol. Also, the genetic, mycoviral determinants and other genes encoding killer toxins (zymocidal peptides) and immunity factors can be incorporated into wine yeasts to make them insensitive to the zymocins of contaminating wild yeasts. With respect to resistance to agrochemicals, an increase in the copy number of the *CUP1* copper chelatin gene enables wine yeasts to tolerate higher levels of copper residues in the grape must.

Improvement of processing efficiency: The main objectives of fining (addition of adsorptive compounds followed by settling or precipitation) and clarification (e.g. sedimentation, racking, centrifugation, filtration, etc.) during wine processing include the removal of excess amounts of certain components and microbial cells to achieve clarity and to ensure the physicochemical stability of the end product. The fining and clarification of wine often include expensive and laborious practices that generate large volumes of lees for disposal, thereby causing a loss of wine and removing important aroma and flavour compounds from the remaining wine. In order to minimise the disadvantages of these harsh fining and clarification practices, an increasing spectrum of relatively expensive commercial enzyme preparations (e.g. proteases, pectinases, glucanases, xylanases, arabinofuranosidases, etc.) are frequently added to the grape must and wine. As an alternative strategy to the addition of costly enzyme preparations that often contain unwanted contaminating or side activities, wine yeasts are being developed to secrete proteolytic and polysaccharolytic enzymes that would remove haze-forming proteins and filter-clogging polysaccharides respectively. To this end, the overexpression of several bacterial, fungal and yeast genes resulted in the development of proteolytic, pectinolytic, glucanolytic and xylanolytic wine yeast strains (Table 2).

A second target for the improvement of clarification and filtration aims at efficiently removing all yeast cells from the liquid phase of the tank or barrel. Regulated expression of the flocculation genes is important to guarantee a high suspended yeast count for a rapid fermentation rate during the fermentation process, while efficient settling is needed to minimise problems with wine clarification at the end of sugar conversion. Yeast flocculation is especially important for the production of bottle-fermented sparkling wine, and the controlled onset of yeast flocculation at the appropriate time during sparkling wine production can simplify this costly process. The expression of the *FLO1* flocculin gene, linked to the late-fermentation *HSP30* promoter, can be induced by a heat-shock treatment, confirming that controlled flocculation is indeed possible during fermentation. Cell aggregation also plays a key role in the production of flor sherry, during which a related cellular process results in the flotation of the yeast cells, thereby forming a velum (biofilm) on the surface of the wine. By placing the *MUC1* (also known as *FLO11*) mucin gene under the control of the *HSP30* promoter, the formation of the biofilm can be promoted at the end of fermentation, thereby simplifying the development of the flor.

Improving biological control of wine spoilage microorganisms: Uncontrolled microbial growth before, during or after wine fermentation can alter the chemical composition of the end product, thereby detracting from its sensory properties of appearance, aroma and flavour. Healthy grapes, cellar hygiene and sound oenological practices are the cornerstones of the winemaker's strategy against the uncontrolled proliferation of spoilage microbes. Added safety is provided by the addition of chemical preservatives, such as sulphur dioxide, dimethyl dicarbonate, benzoic acid, fumaric acid and sorbic acid, which control the growth of unwanted microbial contaminants. However, excessive use of these chemical preservatives is harmful to the quality of the wine and is confronted by mounting consumer resistance. Consumer preferences have shifted to products that are less heavily preserved with chemicals, less processed, of higher quality, more natural and healthier. Therefore, biopreservation with yeast-derived metabolites (e.g. formation of SO_2 or hydrogen peroxide during wine fermentations), antimicrobial enzymes (e.g. lysozyme, chitinases, endoglucanases, etc.) and peptides (zymocins and bacteriocins) is currently being considered as an alternative strategy to chemical preservation. However, the use of purified antimicrobial enzymes and bacteriocins is expensive, resulting in an increase in retail costs. This problem might be circumvented by expressing effective antimicrobial enzymes and peptides in wine yeast starter culture strains, thereby addressing the wine industry's call for wines of higher quality and purity. To this end, the hen egg white lysozyme gene (*HEL1*), the *Pediococcus acidilactici* pediocin gene (*PED1*) and the Leuconostoc carnosum leucocin gene (*LCA1*) have been used to engineer bactericidal yeasts. The antifungal yeast *CTS1*-encoded chitinase and *EXG1*-encoded exoglucanase have also been expressed in *S. cerevisiae*. The main approach in the construction of zymocidal strains entails the inclusion of a combination of mycoviral killer toxin determinants of *S. cerevisiae* (e.g. a K_1/K_2 double

killer) and zymocin-encoding genes from other yeasts (e.g. *Hanseniaspora*, *Kluyveromyces*, *Pichia*, etc.) into wine yeasts. The ideal would be to incorporate all of these antimicrobial activities into a single wine yeast, thereby counteracting all contaminating spoilage bacteria (e.g. *Acetobacter*, *Gluconobacter*, *Lactobacillus*, *Pediococcus*, etc.), yeasts (e.g. *Bretannomyces*, *Pichia*, *Zygosaccharomyces*, etc.) and moulds (*Aspergillus*, *Botrytis*, *Penicillium*, *Trichoderma*, etc.) in winemaking.

Improvement of the wholesomeness of wine: It is generally accepted that moderate wine drinking can be socially beneficial and can be effective in the management of stress and the reduction of coronary heart disease. The principal protective compounds found in wine include the phenolic compounds, resveratrol, salicylic acid and alcohol. However, prudent wine drinkers are increasingly fastidious about the presence of undesirable compounds in wine. These unwanted compounds include suspected carcinogens, such as ethyl carbamate, neurotoxins, such as biogenic amines, and asthmatic chemical preservatives, such as sulphites. The most finicky among these fussy wine drinkers are even concerned about high levels of alcohol in wine. When wine yeast strains are developed, it therefore is of the utmost importance to focus on these health aspects and to develop yeasts that may enhance the benefits (e.g. production of resveratrol, carnitine, etc.) and reduce the risks (e.g. eliminating ethyl carbamate and biogenic amines, and reducing the levels of alcohol) associated with moderate wine consumption.

With regard to the production of resveratrol during fermentation, progress has already been made by constructing a wine yeast that expresses the *4CL9/216* co-enzyme A ligase and *VST1* stilbene synthase genes. The development of a bactericidal yeast, which is deleted for the *CAR1* arginase gene (blocking the secretion of urea, the precursor for the formation of ethyl carbamate) or which is transformed with heterologous urease genes (enabling the degradation of urease) would reduce the levels of added sulphite, yeast-derived ethyl carbamate and bioamines formed by bacterial contaminants.

Improvement of the sensory quality of wine: The single most important factor in winemaking is the organoleptic quality (appearance, aroma and flavour) of the final product. The endless variety of flavours stem from a complex, completely non-linear system of interactions among many hundreds of compounds. The bouquet of a wine is determined by the presence of a well-balanced ratio of desirable flavour compounds and metabolites and the absence of undesirable ones. With the exception of terpenes in the aromatic grape varieties and alkoxypyrazines in the herbaceous cultivars, perceived flavour is the result of absolute amounts and specific ratios of many of these interactive compounds, rather than being attributable to a single "impact" compound. Subtle combinations of trace components (accumulated secondary metabolites) derived from the grapes usually elicit the characteristic flavour and aroma notes of wine, whereas the products of yeast fermentation (e.g. esters, alcohols, etc.) contribute to the generic background flavour and aroma, as well as to the complexity and intensity of the aroma and taste of the final product. Yeast can also be responsible for the production of unwanted byproducts, such as hydrogen sulphide.

There is an obvious need for the development of wine yeasts that could impart specific desirable characteristics to a wine (Table 2). To this end, significant progress has been made in the construction of yeasts producing colour- and aroma-liberating enzymes (e.g. pectinases, glycosidases, glucanases, arabinofuranosidases, etc.) and ester-modifying enzymes (e.g. alcohol acetyl transferases, esterases, isoamyl acetate hydrolysing enzyme, etc.). Furthermore, yeasts producing optimal levels of glycerol (the overexpression of *GPD1*, *GPD2* and *FPS1*, together with the deletion of the *ALD6* acetaldehyde dehydrogenase gene), fusel oils (e.g. isobutyl alcohol, isoamyl alcohol, etc.), and phenolic acids (modified expression of the yeast *PAD1* phenyl acrylic acid decarboxylase gene, as well as the expression of bacterial *pdc* ρ-coumaric acid decarboxylase and *padc* phenolic acid decarboxylase genes) have been developed. In addition, wine yeasts carrying disrupted alleles of the *MET14* adenosylphosphosulphate kinase or *MRX1* methionine sulphoxide reductase have been constructed.

The bioadjustment of acidity in wine can be achieved by recombinant wine yeasts containing combinations of genes cloned from *Schizosaccharomyces pombe* and lactic acid bacteria. A wine yeast that contains the *S. pombe mae1* malate permease gene and the *mae2* malic enzyme gene converts malic acid to ethanol (maloethanolic fermentation), whereas a transformant carrying the *mae1* gene together with the *Oenococcus oeni* (*mleA*), *Lactococcus lactis* (*mleS*) or *Lactobacillus delbrueckii* (*mleS*) malolactic enzyme gene converts malic acid into lactic acid (malolactic fermentation). The maloethanolic wine yeast would be preferred for low pH wines from the cooler wine-producing regions, while the malolactic wine yeast would provide the

best solution for high pH wines from the warmer regions. In the case of high pH wines, the production of additional lactic acid during fermentation can be achieved by incorporating the *Lactobacillus casei LDH1* lacticodehydrogenase gene into the malolactic wine yeast strain. These yeasts also preclude the requirement for the use of bioamine-forming malolactic bacteria in red wine and certain styles of white wine that are required to undergo malolactic fermentation.

4. The commercialisation of genetically improved grapevine cultivars and wine yeast strains

4.1 Challenges facing the commercialisation of designer grapevines and wine yeasts

Scientific and technical hurdles: Despite the strong and persuasive scientific case for the use of gene technology in the improvement of grapes and wine, the wine industry has entered the 21st century without a transgenic grapevine variety or a recombinant wine yeast being used on a commercial scale. However, considerable progress has been made over the last few years to overcome the technical hurdles in defining the wine industry's requirements genetically and improving grapevine varieties and wine yeast strains accordingly. The development of genetic transformation methods for *S. cerevisiae* (in 1978) and *V. vinifera* (in 1989), and the advent of technology with which entire genomes, transcriptomes, proteomes and metabolomes can be analysed, have undoubtedly opened up new horizons for the wine industry. However, it is important to note that the information and technology that currently exist for model plant and yeast systems have yet to be expanded to the much more complex genomes of grapevine and industrial wine yeasts before all of the requirements and concerns of the producers, consumers and regulatory authorities can be addressed satisfactorily. The recent promising "prototypes" of genetically engineered grapevine cultivars and wine yeasts have brought these objectives within the realms of possibility.

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*. The concept of "substantial equivalence" is widely used in the determination of safety by comparison with analogous conventional food and beverage products. When substantial equivalence can be demonstrated, no further safety considerations usually are necessary. When substantial equivalence is not convincingly shown, 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 GM products and the release of GMOs usually require a number of 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.

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

Political and economic hurdles: It is well known that economies are driven by different forces and therefore go through life cycles. For example, in terms of resources, the *Industrial Economy* was 'the economics of scarcity', because everything that fuelled the economy was in short supply and available to only a few nations. 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 the 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. Therefore, it can be expected that the commercialisation of genetically improved grapevines and wine yeasts 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 a great deal on label integrity and product identity. Therefore, it is of the utmost importance that genetically improved grapevines and yeasts 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. 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.

This debate on the naming issue entails a number of factors, such as the source of gene(s) introduced into a particular grapevine, the "true-to-typeness" of the transgenic vine when compared to the original cultivar/clone and the organoleptic and sensory qualities of the resulting wine. Given the immense marketing value contained in varietal names, there is an urgent need for consensus that genetically modified 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 may 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. 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 and recombinant wine yeasts 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: The future application of gene technology in the wine industry will have to overcome some more specific hurdles. Foremost, national and, even more relevant, 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 may accelerate the tendency to standardise wines in order 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 commercial users of transgenic grapevines and recombinant wine yeasts that existing, desirable characteristics have not been damaged, that the requirements of beverage legislation are met and that the engineered cultivar and strain 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 or recombinant wine yeast, they would be in a strong position to implement such a vine or yeast, because most of the wine enterprises are fully integrated agro-industries that could exert direct control over the development of new, specialised 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 GMOs have spread more readily than good sense or wise science, and far enough to masquerade in the cultural folklore as truth. 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 GMOs 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. They also doubt that there is sufficient legal and practical protection against accidents involving GMOs.
It is clear that consumer education is essential to remove this fear of the unknown. Scientists must consistently inform the public and remain open about experiments, research and products. The consumer should be reassured of first-class, transparent regulatory systems and the meticulous implementation of biosafety legislation with clear technical standards and definitions with respect to GM products. The consumer should be persuaded by proper risk assessment and clear demonstration of safety, and thus be empowered to make informed decisions. Assurance must be given that GM wine and other grape-derived products will not be "force-fed" down consumers' throats for profit when there is no clear advantage for the consumer.

4.2 Future prospects

The tailoring of grapevine cultivars and wine yeast strains will undoubtedly help the wine industry meet the technical and consumer challenges of the 21st century. Over the last few years, considerable progress has been made in improving grapevines and wine yeasts. However, due to a multitude of complex scientific, technical, economic, marketing, safety, legal and ethical issues, no transgenic grapevine variety or wine yeast has been used on a commercial scale to date. Given the current deeply rooted concerns of consumers and traditionalists, it might border on "commercial suicide" if any winery were to prematurely pioneer the first wine made from transgenic grapes or fermented with recombinant yeast in the market. However, it is also clear that it will be equally self-crippling if the phenomenal potential of gene technology, which could propel the wine industry with quantum leaps into the inevitable era of "*designer"* products, was to be ignored. There is vast potential benefit to the wine consumer and producer alike in the application of gene technology. That benefit will be realised, however, only if the application is judicious, systematic, and done with high regard for the unique nature of the product. The first GM wine products should unequivocally demonstrate organoleptic, hygienic and economic advantages for the wine producer and the consumer. Furthermore, wine's most enthralling and fascinating aspect, its diversity of style, should never be threatened by the use of tailored grapevines and wine yeasts. In fact, gene technology should rather be harnessed to expand the diversity of high quality wines.

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This paper represents a combined version of two recent reviews [References by Pretorius & Bauer (2002) and Vivier & Pretorius (2002)]. As a result of the limited space allocated to this overview article, all the literature references that were consulted are not listed here. To consult the finer details of the information contained in this article, the reader is referred to the publications listed below, as well as the references contained in them.

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Table 1. Targets for the genetic improvement of grapevine cultivars and rootstocks

Desirable properties	Focus area	Examples of current and potential target genes
Improved disease resistance		
Fungal tolerance	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 chitinase-encoding genes from fungi, yeast and plants; ribosome inactivating proteins (RIPs); thaumatin-like protein (*Vvtl1*); antifungal peptide encoding genes from plants and insects; PGIP (polygalacturonase-inhibiting protein); encoding genes from plant species, stilbene phytoalexins (stilbene synthases*: stsy*, *vst1, vst2*); phenylalanine ammonia lyase: *pal) CuZnSOD* (putative CuZn superoxide dismutase; detoxification enzyme-producing genes (NADPH-dependent aldehyde reductase, *Vigna radiata*-Eutypine reducing enzyme)
Bacterial tolerance	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*
Viral tolerance	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.
Improved stress tolerance		
Resistance to water stress	Aquaporins; isolation of root-specific promoters	TIPs (tonoplast integral proteins); PIPs (plasma membrane integral proteins)
Oxidative damage	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, mitochondrial-residing MnSOD)
Osmotic stress and other abiotic stresses	Proline accumulation; polyamines and their role in stress	*Vvp5cs* (Δ^1-pyrroline-5-carboxylate); *Vvoat* (δ-ornithine aminotransferase); FeSOD, glycine betaine, antifreeze genes from Antarctic fish (freezing tolerance)
Improved quality factors		
Colour development	Ripening related processes and signals, anthocyanin biosynthesis and control (several genes and some promoters have been cloned); isolation of berry-specific promoters	*ufgt* (UDP-glucose:flavanoid 3-*O*-glucosyltransferase) and/or regulatory sequences of *ufgt*; production of pelargonidin-based anthocyanins for novel berry colour; anthocyanin methyl-transferases
Sugar accumulation and transport	Phloem loading/unloading; invertases; sugar transporters; isolation of berry-specific promoters	Invertases from plants and yeast to study phloem loading/unloading; sucrose transporters (*Vvsuc11, Vvsuc12*, *Vvsuc27)**; h***exose transporters (*Vvht1*, *Vvht2*)
Reduced browning (table and dried grapes)	Oxidation reactions	Silencing of polyphenol oxidase
Seedlessness (table grapes)	Seed-formation; isolation of seed-specific promoters	Baranase gene

Table 2. Targets for the genetic improvement of wine yeast strains

Desirable properties – Focus areas – Examples of potential target genes

Improved fermentation performance

Improved general resilience and stress tolerance

Stress response, sterol, glycogen and trehalose accumulation

Modification of glycogen or trehalose metabolism [for example acting on *GSY1* and *GSY2* (glycogen synthase), *TPS1* (trehalose-6-phosphate synthase), *TPS2* (trehalose-6-phosphate phosphatase)]

Improved efficiency of sugar utilisation

Hexose transporters, hexose kinases

Overexpression and modification of *HXT1-HXT18*, *SNF3*, *FSY1* and use of heterologous transporters and kinases

Improved efficiency of nitrogen assimilation

Improved utilisation of less efficient N-sources

Proline catabolism [*PUT1* (proline oxidase) and *PUT2* (pyrroline-5-carboxylate dehydrogenase)] and use of heterologous catabolic genes

Improved ethanol tolerance

Sterol formation, membrane ATPase activity

Modification of the expression of *PMA1* and *PMA2* (ATPase), sterol anabolic genes

Increased tolerance to antimicrobial compounds

Resistance to killer toxins, sulphur dioxide, agrochemicals

Inclusion of *KIL2* (zymocin and immunity factor), overexpression of *CUP1* (copper chelatin)

Reduced foam formation

Cell surface proteins

Deletion of *FRO1* and *FRO2* (froth proteins)

Improved processing efficiency

Improved protein clarification

Proteases

Overexpression of *PEP4* (protease A) and secretion of other proteases

Improved polysaccharide clarification

Glucanases, pectinases, xylanases, arabinfuranosidases

Overexpression of *END1* (endoglucanase), *EXG1* (exoglucanase), *CEL1* (cellodextrinase), *BGL1* (β-glucosidase, cellobiase), *PEL5* (pectate lyase) and *PEH1* (polygalacturonase), *XYN1-5* (xylanases), *ABF2* (arabinofuranosidase)

Controlled cell sedimentation and flocculation

Flocculins

Late expression of flocculation genes (*FLO1, FLO5, MUC1*/*FLO11)* under control of promoters (*HSP30*) imparting desired expression

Controlled cell flotation and flor formation

Cell wall hydrophobic proteins

Late expression of *MUC1*/*FLO11* under control of promoters (*HSP30*) imparting desired expression pattern

Improved biological control of wine spoilage microorganisms

Wine yeasts producing antimicrobial enzymes

Lysozyme, glucanases, chitinases

Expression of *HEL1* (hen egg white lysozyme), *CTS1* (chitinase), *EXG1* (exoglucanase) and other antimicrobial enzymes

Wine yeasts producing antimicrobial peptide

Bacteriocins

Expression of *PED1* (pediocin), *LCA1* (leucocin) and other heterologous bacteriocin and zymocin genes

Wine yeasts producing sulphur dioxide

Sulphur metabolism and SO_2 formation

Overexpression of *MET14* (adenosylphosphosulphate kinase) and *MET16* (phospho adenosylphosphosulphate reductase), and deletion of *MET10* (sulphite reductase)

Improved wine wholesomeness

Increased production of resveratrol

Stilbene synthesis

Expression of *4CL9/216* (co-enzyme A ligase), *VST1* (stilbene synthase)

Reduced formation of ethyl carbamate

Amino acid metabolism, urea formation

Deletion of *CAR1* (arginase) or expression of *URE1* (urease)

Reduced formation of biogenic amines

Bacteriolytic enzymes, bacteriocins

Expression of *HEL1* (hen egg white lysozyme), *PED1* (pediocin), *LCA1* (leucocin) and other bacteriocins

Decreased levels of alcohol

Carbon flux, glycerol metabolism and glucose oxidation

Overexpression of *GPD1* and *GPD2* (glycerol-3-phosphate dehydrogenase), modification of *FPS1* (glycerol transport facilitator), expression of *GOX1* (glucose oxidase)

Improved wine flavour and other sensory qualities

Enhanced liberation of grape terpenoids