ISSN 1860-6768 · BJIOAM 3 (11) 1321–1456 (2008) · Vol. 3 · November 2008

Biotechnology Journal

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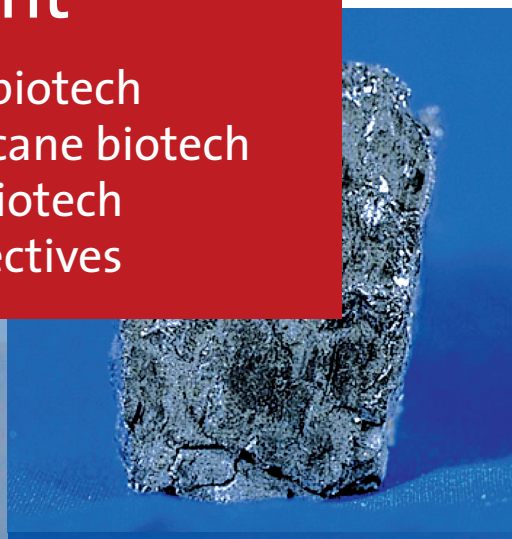

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Research Highlight

Wine biotechnology in South Africa: Towards a systems approach to wine science

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The wine industry in South Africa is over three centuries old and over the last decade has re-emerged as a significant competitor in world wine markets. The Institute for Wine Biotechnology (IWBT) was established in partnership with the Department of Viticulture and Oenology at Stellenbosch University to foster basic fundamental research in the wine sciences leading to applications in the broader wine and grapevine industries. This review focuses on the different research programmes of the Institute (grapevine, yeast and bacteria biotechnology programmes, and chemical-analytical research), commercialisation activities (SunBio) and new initiatives to integrate the various research disciplines. An important focus of future research is the Wine Science Research Niche Area programme, which connects the different research thrusts of the IWBT and of several research partners in viticulture, oenology, food science and chemistry. This 'Functional Wine-omics' programme uses a systems biology approach to wine-related organisms. The data generated within the programme will be integrated with other data sets from viticulture, oenology, analytical chemistry and the sensory sciences through chemometrics and other statistical tools. The aim of the programme is to model aspects of the wine making process, from the vineyard to the finished product.

Received 3 July 2008
Revised 21 August 2008
Accepted 29 September 2008

Keywords: Grapevine biotechnology · Systems biology · Wine biotechnology · Wine-omics · Wine science

1 Introduction

The wine industry in South Africa dates back to the arrival of Dutch settlers at the Cape peninsula in 1652 (see [1] for a useful historical introduction). Jan van Riebeeck, first Commander of the Cape, was charged by the Dutch East India Company to establish a refreshment station for Dutch ships travelling to and from their colonies in East Asia using the Cape sea route. He imported grapevine cuttings from Europe and began a small vineyard in the company gardens, now situated in the city centre of Cape Town. The first wines produced were reported to be of dubious quality, but with the arrival of French Huguenots escaping persecution in France, important viticultural and winemaking skills were brought to the Cape. Dutch Free Burghers and French Huguenots established vineyards throughout the Cape peninsula with important settlements at Constantia, Stellenbosch and Franschoek. The beginning of the Nineteenth Century saw the occupation of the Cape by the British, which proved to be beneficial to the fledgling wine industry. Wine from the Cape Colony was granted preferential access to the English import market while wine from Napoleonic France was subject to heavy import duties. This period of growth was

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Abbreviations: **DVO**, Department of Viticulture and Oenology; **endoPG**, endopolygalacturonase; **FTIR**, Fourier transform infrared; **FTMIR**, Fourier transform mid-infrared; **GC-FID**, GC-coupled to flame ionisation detection; **IWBT**, Institute for Wine Biotechnology; **KWV**, Ko-operatiewe Wijnbouwers Vereninging van Zuid-Afrika; **LAB**, lactic acid bacteria; **MLF**, malolactic fermentation; **NCED**, 9-cis epoxy di-oxygenase; **PGIP**, polygalacturonase-inhibiting protein; **ROS**, reactive oxygen species

short-lived as, towards the middle of the Nineteenth Century, the protective tariffs granted by Britain were withdrawn, which coupled with political and economic turbulence, as well as the worldwide phylloxera epidemic, resulted in collapse of the Cape wine industry. The recovery of the wine industry, however, was equally rapid, to the extent that the lack of a suitable export market, previously Britain, resulted in an overproduction that was of dire concern to farmers and consumers alike. To remedy this situation, co-operatives were formed to control price fluctuations and wine surpluses, the KWV ('Ko-operatiewe Wijnbouwers Vereninging van Zuid-Afrika' or 'Co-operative Winemakers Association of South Africa') was formed in 1918 with the task of regulating grape and wine sales through price control measures. During this period (early Twentieth Century) of market uncertainty, the first and still only university departments dedicated to viticultural and oenological research in South Africa were established at the recently formed Stellenbosch University, previously Victoria College, in the heart of the Cape winelands. It was at the university experimental farm at Welgevallen in 1925 that the viticulturist, Professor Izak Perold, crossed Pinot noir and Hermitage (Cinsault) to create South Africa's unique home-grown cultivar, Pinotage. The cultivar was painstakingly saved and propagated from seedlings and was debuted a number of years later on the South African wine market. Currently, Pinotage remains South Africa's flagship red wine cultivar with Cabernet Sauvignon and Shiraz also being widely planted. To bolster basic research in South Africa, the Oenological and Viticultural research institute was established in 1955 at Nietvoorbij outside Stellenbosch. This institute, currently part of the Agricultural Research Council of South Africa, concerns itself with basic viticultural and oenological research from a practical 'industry-based' perspective. Similarly, the Departments of Viticulture and Oenology at Stellenbosch University for much of their history have focused exclusively on improving basic viticultural and oenological practices with a view to solving industry relevant problems. With the re-entry of South Africa into the international wine market in 1994, the protective measures of the KWV were no longer desirable. The dismantling of the price and surplus control measures of the KWV, resulted in South African winemakers being subject to direct competition with other major wine producing countries for lucrative foreign markets.

The tremendous growth in wine sales and production post 1994 coupled with the pressures of competing internationally has necessitated that the South African wine industry adapt to the changing international environment. This required not only updating viticultural and oenological practices and complying with international regulations, but also the development of high-level skills to further innovation in grapevine and wine science. Biotechnology, because of its cross-cutting nature as a research tool, its tremendous innovation potential and its ability to attract students from different fields to wine-related research was seen as one of the most important drivers to provide momentum to the transformation of the South African wine industry. For this reason, the wine industry, supported by funding from the national government and the University of Stellenbosch established the Institute for Wine Biotechnology (IWBT) in 1995 with Professor Isak S. Pretorius of the Department of Microbiology as founding director. The Institute is affiliated with the Department of Viticulture and Oenology (DVO) with a vision and mission to become a nationally and internationally competitive centre of excellence in wine and grapevine biotechnology that, by means of visionary training and innovative research, provides the South African grapevine and wine industry with well-trained human resources, cutting-edge technology, expert knowledge; and environmentally friendly products and practices (see Fig. 1). The IWBT is a centre of postgraduate research in biotechnology in South Africa providing training to honours, master and doctoral degree students with basic science backgrounds (such as biochemistry, microbiology, chemistry, botany, etc.), as well as those coming from the applied agricultural sciences (such as viticulture and oenology). The research portfolio covers fundamental investigations into the cellular and molecular biology of wine-related organisms (grapevine, yeast and wine bacteria) and the application of biotechnological tools to improve these organisms. These tools range from traditional methodologies such as breeding and selection to the use of genetic modification. The industry cur-

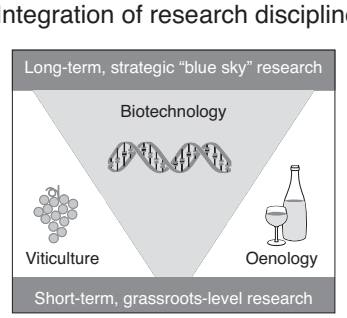

Figure 1. A schematic representing how the biotechnology programme of the IWBT integrates into the DVO to position the South African wine industry at the cutting edge of scientific innovation.

rently maintains that the IWBT and its research portfolio creates the opportunity to competitively position the South African industry and to reap the direct benefits of GM products should world market perceptions change.

This review summarises the past and current research thrusts of the IWBT, important publications, new initiatives towards integrated research, as well as an initiative to facilitate the commercialisation of IWBT research through the development of a biotech spin-off company.

2 Grapevine molecular physiology and biotechnology

The grapevine biotechnology programme of the IWBT was started 10 years ago with the aim of supplementing and strengthening the few ongoing efforts in this field in South Africa [2]. Development of suitable cultivar-specific tissue cultures, transformation and regeneration systems and the basic molecular biological tools to manipulate the grapevine became the first focus of the programme [2, 3].

Grapevine is a woody perennial and has been considered "difficult to work with" by geneticists, molecular biologists and biotechnologists. Woody perennials, such as *Vitis* spp. have successive annual cycles of vegetative and reproductive growth with intermittent dormant (winter) periods. Grapevine also exhibits extensive youth phases that significantly prolong generation times. The *Vitis* genome is of moderate size, but is considered extremely heterogeneous and quite complex. These inherent characteristics of *Vitis* spp. remain complicating factors when studying grapevine, although the recent release of the grapevine genome sequence has provided new avenues for research progress [3, 4]. The genome sequence release has provided the impetus to develop *Vitis* as the first 'model' woody perennial fruit crop [3, 4]. New scientific possibilities through the development of molecular tools (*e.g.* BAC libraries, molecular markers, genetic and physical maps) and the prospect of employing systems biology approaches have arisen [3, 4].

Targeted gene disruption to create knockout mutants and high throughput transformation procedures are both still lacking in grapevine, making systematic analyses of signal transduction pathways and epigenetic analyses difficult. Gene silencing mechanisms are also still being optimised for grapevine and virus-induced gene silencing, specifically linked to transient transformation technologies are one of the aspects targeted for development in the international grapevine research community [2, 3].

Unlike model plant transformations, grapevine transformation and regeneration is not yet routine. However, a number of cultivars and rootstocks have now been successfully transformed and the first field trials of genetically manipulated grapevine have been conducted. We have also been successful in developing transformation and regeneration platforms for *V. vinifera* and are amongst the few labs worldwide able to routinely produce transgenic grapevine tissue and plants [5].

As in all scientific fields, the viticultural sciences need hypothesis-driven research, facilitated by experimental systems that yield repeatable results with a clear separation between cause and effect. Vineyard complexity, however, is an impediment to this very basic requirement of scientific inquiry, leading to datasets that are difficult to interpret and extract statistically significant information. Furthermore, the results obtained are usually limited to the specific vineyard and vintage under study. What is needed is a highly characterised vineyard where as many contributing factors are identified and measured. The concept of a 'model' vineyard to support hypothesis-driven research in viticultural science is one of the core drivers of a recently established integrated research programme, the Wine Science Research Niche Area (RNA) that is discussed later in the review [6].

The main thrusts of the IWBT's grapevine biotechnology programme centres around plant stress (biotic and abiotic) and the consequent effects on growth and fruit quality (see Fig. 2 for examples of genes isolated and studied in the grapevine programme). The fundamental question(s) we are trying to answer relates to the molecular regulation of stress in grapevine. Biotic stress research focuses largely on fungal diseases, such as grey rot (*Botrytis cinerea*) and the mildews (powdery and downey) [7], whereas virus research is conducted in a collaborating laboratory in the Department of Genetics at Stellenbosch University. Fungal pathogens and insect pests continue to be one of the most limiting factors in grapevine cultivation [7]. The production of fungal disease-resistant plants using transgenic technology is an attractive alternative to chemical treatments and should encourage environmentally friendly practises in the vineyard. To achieve this the IWBT has focused research efforts on chitinases [8, 9], polygalacturonase-inhibiting proteins (PGIPs) [10, 11] and antifungal peptides [12], which are known to confer disease resistance to host plants producing these proteins. PGIPs are known to confer reduced susceptibility to their respective hosts and the

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PGIP-encoding gene from grapevine (*Vvpgip1*) is no exception [10, 11]. When expressed in a model plant system such as tobacco, the protein confers a clear advantage to plants challenged with *B. cinerea*, leading to significant improvements in disease resistance [10, 11]. The mechanisms by which PGIP confers reduced disease susceptibility against *B. cinerea* and fungal pathogens are not fully understood. One mechanism thought to be involved is through the direct inhibition of cell wall maceration enzymes secreted by fungal pathogens [10, 11]. The disease protection can be clearly observed by comparing tobacco plants overexpressing a potent endopolygalacturonase (endoPG) from *Botrytis*, leading to severe tissue maceration and plant structural collapse, whereas plants co-expressing both the endoPG and the PGIP display a far less severe phenotype [10, 11]. Experimental evidence also suggests that PGIPs could have functions totally unrelated to their activity, but still important for plant defence [11]. Tobacco lines harbouring the grapevine PGIP have been shown to have an up-regulated disease resistance as well as significantly more lignin than untransformed controls (unpublished observations). This wall-associated phenotype is also reflected on a transcriptional level and genes involved in cell wall biosynthesis and structural organisation in transgenic tobacco over-expressing PGIP (unpublished observations). These results have spurred further research into identifying the nature of the direct and indirect mechanisms that may be responsible for these disease resistance phenotypes. Numerous PGIP genes have been cloned and sequenced from *V. vinifera* as well as wild *Vitis* species and are currently being investigated for improved resistance activity.

Similarly, the first antifungal peptides from grapevine (*VvAMP1*) have been cloned. Purified peptide has been tested against economically important grapevine pathogens, showing significant activity against several of them [12]. Antifungal peptides are believed to mediate their activity through targeting the membrane structures of invading fungal organisms leading to pathogen death. The exact mechanisms, in a similar vein to the PGIP mode of action, appear to be the result of direct and indirect action on cellular processes. Antifungal plant peptides and their encoding genes are abundant in most plant species and have a recognized biotechnological potential in both the medical and agricultural biotechnology sectors. These peptides typically contribute to preformed defence by developing protective barriers around germinating seeds or between different tissue layers within plant organs.

South Africa has a rich and unique floral biodiversity and some native *Brassicaceae* spp. were targeted for the potential isolation of additional novel antifungal peptides. Fourteen new plant defensin sequences from four genera of the *Brassicaceae* family present in South Africa were isolated. Members of this group are well known for their strong antifungal activity, but other activities such as met-

YEAST BIOTECHNOLOGY
Extracellular enzymes to improve processing and wine quality
STA1/DEX2/MAL5, EXG1/BGL1, BGL2, SSG1/SPR1, EXG2, PAD1/POF1, CTS1-2, PEP4, ATF1/IAH2, PGU1/PGL1, STA2/DEX1, STA3/DEX3, SGA1, LKA1, LKA2, S FA1, SFG1, RSA1, RSG1, XYS1, XYN2, *XYNC, XYN5, BGLA, XYL1.*
Modified adhesion properties (flocculation, sedimentation and turbidity in wine)
MSS10, MSS11, FLO1, FLO5, FLO8, FLO10, FLO11/MUC1, CLN1, CLN2, GPA2, ASH1, STE7, STE11, STE12, TEC1, MSN1, NRG2, ROM1, ROM2, SOK2, KSS1, PH D1, RAS2, RME1, SCH9.
Aroma compound metabolism (improved sensory qualities)
CAT2, YAT1, YAT2, CIT2, AGP2, ATF1, ATF2, BAT1, BAT2, GPD1, GPD2 , AAT10, AAT11.

GRAPEVINE BIOTECHNOLOGY
Increased isoprenoid levels/general metabolism (multiple metabolic functions)
Mevalonate pathway*: VvAACT, VvHMGS, VvHMGR, VvMK,* **Deoxyxylulose 5-diposphate pathway***: VvDXS, VvDXR, VvispE, VvispF, VvispG, VvispH,* **General***: VvIPI, VvFPS, VvGPS, VvGGPS.*
Increased abiotic stress tolerance (drought, light) and/or fruit quality (flavour, aroma)
Carotenoid biosynthetic pathway*: VvPSY, VvPDS, VvZDS, VvCiso, VvLECY, VvECH, VvLBCY, VvBCH, VvZEP, VvVDE, VvNSY.* **Abscisic acid biosynthetic pathway***: VvNCED, VvCCD.*
Antifungal/disease resistance (against bacterial, fungal and insect pests)
Polygalacturonse-inhibiting proteins: *Vvpgip1, 38 pgip genes from Vitaceae and Muscadinia.* **Antifungal peptides:** *Vv-AMP1, MiAFP1-MiAFP3, HcAFP1-HcAFP6, LaAFP1-La-AFP4, LmAFP1-Lm-AFP2.* **General defense genes:** *vst1, Ntcad, CST1-2, EXG1.*

Figure 2. Examples of genes isolated in the IWBT yeast and grapevine biotechnology programmes in the last 10 years.

al tolerance and inhibition of protein translation are also known. The unique genetic resources obtained in this study have entered our biotechnology programme where improved disease resistance of grapevine is the main aim.

The utility of single genes encoding specific proteins, PGIP or antifungal peptides, with marked anti-fungal activity genetically engineered into grapevine is an extremely useful technology for the control of vineyard pathogens [2, 7]. Further research aimed at understanding the dynamics of plant-pathogen interactions and the role of proteins with antifungal activity will offer better insights into the potential of utilising this technology in plant protection strategies.

Apart from the common biotic threats, viruses, fungi and insects, grapevine plants are susceptible to abiotic stresses caused by environmental change. One common type of abiotic stress found in South Africa is drought. Southern Africa is facing progressively worsening water shortages with the result that crop productivity suffers, thus negatively influencing the regional economy. Grapevine is no exception where water stress can significantly impact factors such as canopy growth and bunch quality. Water stress also effects properties such as photosynthetic efficiency and can promote photo-oxidative stress resulting in reactive oxygen species (ROS) production. To develop grapevines with enhanced capabilities to grow under adverse conditions, in particular water stress, the IWBT grapevine programme is utilising genetic resources developed by studying the carotenoid biosynthetic pathway in grapevine. This pathway produces metabolites or compounds involved in environmental stress responses (antioxidant molecules), quality parameters of grapes (aroma molecules) and the formation of the stress hormones (such as abscisic acid). The understanding and manipulation of carotenoid metabolism in grapevine will provide insight into how grapevine deals with abiotic stresses, but can also provide alternative strategies to counteract, and/or produce novel plant material more resistant, to these stresses. Moreover, the work also aims to contribute towards a fully characterized vineyard where the cause(s) and effects of environmental stresses can be separated and studied. The metabolic pathways that will be studied are also intricately involved in quality aspects and so some of the research objectives relate to functional analysis of impact genes and their encoded products to improve flavour and aroma production in grapevine tissues and berries.

Carotenoids play a central role in plant metabolism in general, and specifically in photosynthesis where they perform a myriad of functions. In addition to being integral structural and accessory light-harvesting components of the photosynthetic apparatus, their ability to quench ROS formed under adverse conditions, thereby protecting the photosynthetic machinery against damage, is invaluable. Carotenoids also serve as important precursors for apocarotenoids. These apocarotenoids or norisoprenoids are involved in a wide range of functions in plants and can be growth regulators, pigments, flavours and aromas. Abscisic acid is an important apocaroteniod involved in growth regulation and stress responses. Norisoprenoids are frequently aromatic and volatile in nature and contribute to the varietal character of a number of important cultivars while having very low odour detection thresholds.

Work in the grapevine programme has lead to the cloning and characterisation of most of the genes encoding enzymes involved in the pathway [13–16]. The role of these enzymes have been assessed in further studies by overexpression of selected genes in tobacco and *Arabidopsis* [15]. The resultant transgenic plants have been assessed genotypically and phenotypically characterised to provide us with insights into the functional role of these genes in plants. These analyses revealed a number of parameters involved or affected during drought tolerance. Collectively, these results confirm and advance our existing knowledge of the role of carotenoid biosynthesis in drought tolerance and the specific mechanisms operating on a whole plant level.

A clear understanding of the respective genes, proteins and enzymes that contribute to the carotenoid biosynthetic pathway is needed to successfully apply genetic manipulation strategies. To support this research, a grape berry tissue culture system is being developed. The aim is to determine whether such a system would be suitable as model system to investigate berry ripening and other berry-specific processes. In summary, the current projects focus on improving our understanding of genetic networks and mechanisms responsible for the plant's response to biotic and abiotic (environmental) stresses, as well as the aroma and flavour development in grape berries and the regulation thereof. Systems biology tools are increasingly implemented into the programme as they become available.

The datasets generated will also allow careful evaluation of current manipulation strategies and support the development of improved approaches in the future.

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3 Yeast molecular biology and biotechnology

The IWBT has a long history of research projects on improving yeast strains for specific applications using molecular genetic and breeding technology [17]. Yeast projects are focused on establishing a better fundamental understanding of yeast cellular and molecular biology, and to apply this knowledge to generate new wine yeast strains. Topics of investigation include gene regulation and signal transduction networks, the molecular nature of cell wall-related phenotypes, metabolic regulation and the secretion of enzymes (see Fig. 2 for examples of genes isolated and studied in the yeast programme).

These fundamental projects feed into the biotechnology program, which uses traditional methodologies of breeding, selection, directed evolution and molecular modification. Applied projects in the past focused on engineering yeasts with specific enzymes useful in the degradation of diverse polysaccharides [18–21], studying the properties of yeast adhesion phenotypes [22–28] that are of interest during fermentation processes in yeast during winemaking [29], the genetics of carbohydrate source utilisation (e.g. starch, cellulose) [30–33], the metabolic engineering of aroma production pathways [34, 35], carnitine production [36, 37] and risk assessment related to the use of genetically modified wine yeast strains [38].

The IWBT has combined traditional technologies with directed evolution to generate new strains of industrial relevance. Directed evolution refers to the application of specific selection pressures over many generations of yeast growth usually in a continuous fermentation chemostat system. In such a situation strains that evolve advantageous adaptations to the selection pressure out-compete less well adapted strains. Through this approach the IWBT has been able to generate yeast strains with specific properties such as improved nitrogen efficiency or fructose utilisation. The success of the yeast breeding programme is evident in the number of valuable strains produced by the IWBT for industrial applications. An example being the successful VIN13 *Saccharomyces cerevisiae* strain marketed by Anchor Yeast and used in wine fermentations both in South Africa and abroad.

Although traditional breeding methods provide useful strains of industrial relevance, this particular approach is limited in two major ways. Firstly, such traditional methods can only improve pre-existing characteristics present in the yeast, not generate new traits that may be desirable. Secondly, experience has shown that even traits within yeast can be improved up to a specific point while further improvement is only possible through the integration of additional genetic material. A further disadvantage of these technologies is their inherent randomness, which makes outcomes unpredictable. It is also difficult to breed strains for traits that do not have an easily selectable character as is in the case of strains with specific aroma production capabilities. To overcome these limitations, techniques of molecular biology and genetic engineering are employed.

The specific aims of the current yeast strain development programme integrates systems biology-based approaches, and also continues to pursue strategies based on traditional breeding or genetic modification technology. The programme includes many of the areas that have been identified as being of commercial relevance for the yeast and wine industries. The programme can be subdivided into five major research themes, these being: (i) producing yeast with greater fermentation efficiency, (ii) improving wine processing and filtration, (iii) developing yeast that improve the aroma and flavour of wine, (iv) increasing the wholesomeness of wine, and (v) improving wine preservation. Each of these programmes support the main thrust of the yeast biotechnology programme, which is to generate and assess new yeast strains of potential value to the South African wine industry.

A focus of the current program is the generation of yeast that would yield lower levels of ethanol. South Africa is a warm climate region with the result that harvested grapes produce on average higher levels of sugar than cool climate wine regions. This results in wine with higher-than-average alcohol levels due to the greater amount of fermentable sugar in the must. Export markets are more favourable to moderate alcohol levels in wine and thus the ability to control alcohol production is necessary. To this end the IWBT developed a strategy in which the enzyme glucose oxidase is engineered into yeast to convert the excess sugar (glucose) present during fermentation into gluconolactone and so prevent excessive alcohol production [39]. In addition, during the fermentation of must, numerous partially soluble complex plant polysaccharides are extracted into the wine medium. These polysaccharides are viscous and tend to block filters as well as impede processing steps during winemaking. The general type of plant polysaccharides present can be divided into cellulose, hemicellulose and pectin polymers. A genetic engineering solution to these problems consisted of engineering polysaccharide-degrading enzymes such as polygalacturonases, xylanases and cellulases into yeast using molecular secretion systems [19–21, 40]. Thus, yeast are able to ferment the must

normally to produce wine while simultaneously degrading viscous polymers thus significantly enhancing processing steps such as filtration. Yeast engineered to degrade polysaccharides to improve filtration are also able to increase the 'fruity' bouquet of the wine. Wine yeast strains produce many of the aroma and flavour compounds that define the individual character of specific wines [41]. These compounds include in particular esters, higher alcohols and various sulphites, as well as acids, glycerol and terpenoids. Genetic and metabolic engineering strategies are able to modify the metabolic flux in a particular pathway in such a way as to favour the production of desirable compounds while concomitantly reducing undesirable ones. The power of a metabolic engineering approach is evident when considering that the overexpression of single genes are able to significantly modulate the levels of over a dozen important aroma compounds in yeast simultaneously [41–44]. Similar data have been generated for many genes, allowing a better understanding of the interactions between many metabolic pathways involved in aroma compound production [43, 44]. In addition to aroma compounds, yeast has the potential to produce compounds of human medical importance. Grape-derived compounds, such as the phenolic compound resveratrol, is linked to the health benefits of moderate wine consumption [45]. Resveratrol is positively correlated epidemiologically with reduced cardiovascular disease and so the IWBT was interested in increasing the amounts present in wine using a designer yeast strategy [45]. Two genes from the metabolic pathway needed to produce resveratrol (a coenzyme A ligase-encoding gene, *4CL216*, from hybrid poplar and the grapevine resveratrol synthase gene, *vst1*) were cloned into a laboratory strain of *S. cerevisiae* [45]. The results obtained by analytical liquid chromatography-coupled mass spectrometry demonstrated that the yeast transformants were able to produce piceid, which is the glucose-bound form of resveratrol [45]. These yeasts therefore have the ability to produce resveratrol during fermentation in both red and white wines, thereby increasing the wholesomeness of the final product. Although microbes, such as the resveratrol-producing yeast strain, impart positive attributes to wine, a common cause of wine spoilage is the growth of micro-organisms such as bacteria that have the potential to produce off-flavours [45]. A common practise to prevent such spoilage is the addition of sulphur dioxide as a preservative. While sulphur dioxide is satisfactory in this regard, and in addition also acts as an antioxidant, alternatives are desired and actively encouraged because of the potentially negative influence of the compound on aroma and health, a significant percentage of the population being hypersensitive to sulphur dioxide. The IWBT has generated yeast strains that control the growth of spoilage organisms by secreting peptides or enzymes that specifically inhibit the growth of unwanted organisms [46]. An example being the use of bacteriocin genes cloned into a laboratory strain of yeast [47]. Two bacteriocin-encoding genes, pediocin PA-1 (*pedA*) produced by *Pediococcus acidilactici* and leucocin B (*lcaB*) from *Leuconostoc carnosum*, were cloned into a multicopy episomal plasmid under the control of the alcohol dehydrogenase 1 promoter and terminator, and the yeast mating pheromone _-factor secretion signal [47]. Transformed yeast strains were shown to produce active forms of pediocin and leucocin [47]. These bactericidal yeast strains were not only able to conduct fermentations in wine, but also act as a biological control agent by inhibiting the growth of spoilage bacteria [47]. Although certain bacteria are able to cause wine spoilage, some bacteria impart beneficial properties to wine and are thus added to wine formulations to encourage growth.

4 Lactic acid bacteria biotechnology

Lactic acid bacteria (LAB) have historically been associated with food and beverage fermentations as they occur naturally in the starting materials used [46]. Lactic acid bacteria also occur in must and wine and perform the secondary fermentation, known as malolactic fermentation (MLF). The process of MLF includes a reduction in acidity, resulting from the degradation of L-malic acid to L-lactic acid with the concomitant release of carbon dioxide. LAB isolated from grapes and wines are from the genera *Lactobacillus, Leuconostoc, Oenococcus* and *Pediococcus*. *Oenococcus oeni* is used commercially in malolactic starter cultures, as they are the LAB best adapted to wine conditions. Although MLF is primarily performed to reduce wine acidity, especially in cooler climate regions, it is also considered beneficial to the wine's sensory quality due to flavour modification. Additionally MLF provides microbial stability, since malic acid, which can serve as a carbon source to support the growth of potential spoilage LAB, is degraded [48]. In addition, LAB produce antimicrobial agents that protects the finished product by inhibiting spoilage bacterial growth [49]. These bacteria are able to compete for nutrients during fermentation and can produce antimicrobial compounds such as organic acids, ethanol, hydrogen peroxide and bacteriocins [46, 50]. A wide range of LAB have the ability to

produce bacteriocins and the fundamental knowledge gained on the biochemical and genetic characteristics of these molecules in the last decade has increased their potential application as biopreservatives in the food and beverage industries [46, 50].

Internationally, rapid progress has been made in the last 10 years in the development of tools for the genetic modification of LAB. The major target of the LAB strain development programme of the IWBT is to select for strains that are better adapted as starter cultures for MLF and to better understand the physiology, diversity and performance of strains under winemaking conditions. Advances have been made in the development of transformation systems involving the development of integration and amplification vectors, selection criteria and food-grade heterologous expression systems. The genome sequences of several LAB have become available contributing to the study of genes and operons in these important bacteria. Research efforts at the IWBT are focused on (i) specific enzymes that are involved in the production of wine aroma compounds, (ii) bacteriocins that can be used as alternative to chemical preservatives, (iii) investigating the role of LAB in bitterness, (iv) production of biogenic amines, and (v) compounds causing off-flavours in wine. The research findings will be useful in the identification of target genes for the future selection of improved starter cultures or the direct genetic improvement of LAB strains used in MLF.

The first aim involves the screening of natural wine LAB strains, as well as commercial starter cultures for enzymes important in the production of wine aroma compounds. The enzymes focused on include _-glucosidases, proteases, esterases, glucanases, citrate lyases and enzymes involved in the production of off-flavours such as volatile sulphur and phenols [51, 52]. Thus it is important to know if this aroma enhancing potential is realised under MLF conditions and to what extent MLF influences wine flavour composition [51]. Genetic screening was employed to assess the presence of bacteriocin-encoding genes. PCR revealed the presence of the plantaricin encoding genes *plnA*, *plnEF*, *plnJ*, *plnK* in *Lb. plantarum* strains. Four putative bacteriocin-encoding genes in the genome of *O. oeni* were identified and sequenced [49]. It is also known that certain LAB have the potential to affect the wholesomeness of wine by producing biogenic amines [53, 54]. Biogenic amine production is strain related due to the possession of the specific biogenic amine decarboxylase gene, which is influenced by winemaking parameters [55]. Therefore, a major thrust was to investigate to assess gene distribution and homology amongst strains.

Another contribution made by rare strains of LAB is the ability to cause bitterness in wine. Certain LAB possess the ability to degrade glycerol, leading to the formation of acrolein, an intermediate that is able to react with phenolic groups of anthocyanins, forming a bitter complex [56]. The key enzyme involved is glycerol dehydratase (*gdh*) that fortunately is not widespread in nature. From 240 natural LAB isolates only 26 contained the gene and they belonged to *Lb. plantarum*, *Lb. pentosus*, *Lb. hilgardii*, *Lb. paracasei*, *Lb. brevis* and a *Pediococcus* spp. To our knowledge, this is the first report of the presence of the GD gene in *Lb. plantarum*, *Lb. pentosus* and *Lb. paracasei* [56]. The main aims of the LAB research programme is thus oriented towards developing tools to investigate suitable LAB strains for commercial use in the South African wine industry.

5 Chemical-analytical research

Since 2000 the IWBT has systematically acquired analytical instrumentation and developed resources to put the institute in the position to maintain the forefront cutting-edge analytical capacity needed in the biotechnological environment. Today, the IWBT has an analytical facility that provides full support for metabolomic and wine analytical approaches, based on HPLC, capillary electrophoresis (CE), GC coupled to flame ionisation detection (GC-FID), GC-MS and a range of spectroscopic instruments. The laboratory is also developing several high-throughput analytical tools.

The volatile composition of South African wines was determined with GC-FID and a combination of GC-FID and Fourier transform infrared (FTIR) spectra have been modelled by chemometric techniques to discriminate between the major wine cultivars. HPLC is used for quantification of sugars, ethanol, organic acids as well as for phenolic compounds in wine.

Fourier transform infrared (FTIR) spectrometers designed specifically for applications in the grape and wine industries are recent additions to the arsenal required to handle the demands on modern wine chemistry. The IWBT was the first research institution to venture into this technology by acquiring a GrapeScan FTIR spectrometer in 2002. Since then, the IWBT has played an important role in introducing the technology and its potential benefits to the South African wine industry, in assisting with the implementation thereof and in facilitating training of users by both local and international experts. This methodology is now used with great success for routine wine and grape analysis, and it also

plays a strong supporting role for research at the IWBT and has established itself as a research focus in its own right. The first non-routine application of FTIR at the IWBT was to establish a calibration for the analysis of glycerol in wine [57, 58]. This analytical tool was used to conduct an industry-wide survey of the glycerol levels in premium South African wines and to test the hypothesis that the glycerol concentration in this category wine is positively linked to quality [57, 58]. FTIR spectroscopy is also a valuable tool in the IWBT yeast biotechnology programme by speeding up the screening of fermentation profiles of desirable variants within a population of wine yeast strains and to evaluate wine yeasts that have been genetically modified. FTIR spectroscopy established for quantitative analysis in wine is also now optimised for monitoring grape quality during ripening, yeast and must during alcoholic fermentation and bacteria causing MLF. Rapid detection of spoilage and the identification of wine-associated bacteria using FTIR-coupled attenuated total reflectance (ATR) is conducted with the long-term aim of establishing spectral libraries for future research projects. Fourier transform mid-infrared (FTMIR) spectroscopy has also been used to establish fermentation profiles of yeast strains developed in a classical breeding programme for the enhanced production of glycerol [59]. FTMIR spectroscopy is firmly established in the IWBT as an analytical tool for evaluation of the volatile fermentation profiles of yeasts during breeding and fermentation. FTMIR spectroscopy has also been optimised for monitoring quality control parameters (sugar content, pH and titratable acidity) during grape ripening [60] and the current focus is on bioprocess monitoring including both alcoholic fermentation and MLF. The IWBT has recently acquired an near infrared (NIR) spectrometer, which has expanded the matrix that can be analysed to include not only liquids, but also semi-solids and solids. These latter applications currently include the development of quantitative calibrations on anthocyanin content in grape-skin homogenates and quality parameters of whole grape berries (such as browning potential). Future research applications include cell wall biospectroscopy focused on grape berry ripening processes, plant-pathogen interactions and yeast mannoprotein production.

To investigate and correlate the large amount of data generated using these spectroscopic approaches with established chemical reference methods requires advanced statistical support. The extraction and interpretation of the relevant 'underlying' information in analytical datasets requires extensive application of chemometric techniques. Modern instrumentation are frequently established with customized software packages that includes multivariate quantification, classification and cluster analysis tools [61, 62]. The modern trend to apply chemometric techniques to analytical and instrumental signals has opened up the possibility in biotechnology to establish algorithms that can be used for investigating trends, monitoring bioprocesses and for the prediction of future outcomes related to biological processes as well as chemical and sensory profiles. The IWBT is at the forefront of these initiatives with active international collaborators including industrial and academic research partners to support the development of spectroscopic and chemometric tools in the South African wine environment. The IWBT has also been instrumental in establishing the South African Chemometrics Society (SACS) with the assistance of local academics and international chemometric experts. Thus the IWBT plans to continue to be at the forefront of initiatives to integrate wine science research in South Africa into a coherent framework through the sustained development of the necessary instrumentation, statistical software tools and expert supported training of industry and academic stakeholders.

6 SunBio

SunBio is an exciting new venture that presents an opportunity to realise the commercial potential of the IWBTs research activities by establishing a spin-off company from Stellenbosch University. SunBio aims to establish a sustainable product development process that will combine the research output and intellectual property generated by the IWBT with sound commercialisation practices. The company will focus on the areas of genetic enhancement technologies, conventional development of unique yeast and bacterial strains, and development of niche service offerings (chemical and microbiological support) to the wine industry. SunBio was initiated by funding obtained from Cape Biotech Trust, a Biotechnology Innovation Centre (BIC) established by the South African Department of Science and Technology.

SunBio research projects are designed to generate a large number of hybrid and/or recombinant wine yeast strains. For some projects this will involve the development of gene expression cassettes and yeast strains that are able to enrich wines with antioxidants, and nutritional supplements geared toward the health and wellness market segment. SunBio, also aims to make the fermentation process more efficient through the pro-

duction of yeast strains with enhanced levels of key fermentation enzymes, and reducing the reliance on sulphur dioxide during the fermentation process through the use of bacteriocins.

These research and technology developments will be an important strategic advantage for the South African wine industry in the global market. SunBio seeks to actively commercialise the novel technologies at the IWBT, thus contributing towards the global competitiveness of the South African wine industry.

7 Functional wine-omics and future perspectives

Biotechnological innovation has defined the activities and goals of the IWBT since its inception and will continue to be our core focus. The competitiveness of the global wine industry, the increasing focus on and adoption of new technologies in viticulture and oenology, the ability to virtually interact and share knowledge in the research community, as well as strategic opportunities and technological advancements in general necessitate an integrated approach to wine and grapevine research. Biotechnology will only be useful when integrated with the disciplines of viticulture and oenology, and when supported by the fundamental biological sciences, including biochemistry, chemistry, genetics, microbiology and botany. Furthermore, technological advances create exciting opportunities to incorporate new methods and approaches in the wine sciences. Very basic research questions remain unanswered and hypothesis-driven research remains difficult in various fields of wine sciences due to the inherently high heterogeneity in the test systems.

How do we then move forward towards a scientific qualification of vine and wine production while optimally employing new technologies, creating exciting and novel opportunities for the modern Wine Sciences? The newly approved Wine Science Research Niche Area (RNA) aims to make progress in this regard and proposes to integrate emerging technologies into the study of wine and wine organisms. As a methodological core the RNA has been built around "Metabolomics and Metrics of Wine and Wine Organisms" (see Fig. 3 for a diagrammatic summary of the Wine Science Research Niche Area).

This theme represents a multidisciplinary, integrated and, in its scope, internationally unique effort that combines the power of new cutting-edge scientific tools of global molecular analysis (genomics, transcriptomics, proteomics and metabolomics) with the existing physiological, biochemical, genetic and molecular research at the IWBT. In the biological sciences, many approaches today are based on global analysis tools, also referred to as Omics (genomics, proteomics, metabolomics), and on the holistic integration of data from different disciplines to further the understanding of the whole (systems biology). The techniques, tools and thought patterns employed by those approaches are clearly of major relevance to wine science, and need to be implemented on a large scale.

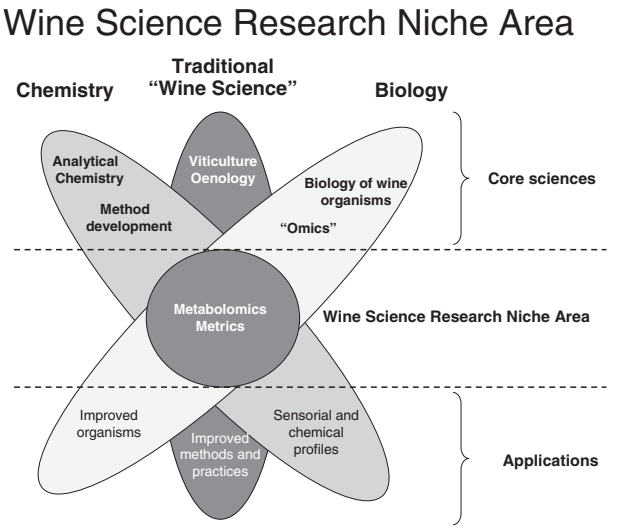

Figure 3. A diagram representing the 'Wine Science Research Niche Area' programme coordinated between the IWBT, DVO, Food Science and Chemistry at Stellenbosch University. The model reveals how core disciplines of biology, chemistry, viticulture and oenology are connected *via* (chemo)metric and metabolomic techniques to applied products and measurable outcomes (*e.g.* improved sensory profiles and fermentation processes).

The specific research problems to be addressed are all directly related to issues that have been identified by the South African wine industry as the most relevant problems threatening the future viability of the industry. More particularly, the proposal covers those aspects where biotechnological approaches can provide a significant competitive edge to the South African wine industry. These aspects are (i) environmentally friendly production practices (disease-resistant grapevines), (ii) reduced production costs (yeast with improved fermentation efficiency and improved resistance to nutrient stress), (iii) the production of wine of consistently high quality (better colour, flavour and aroma), and (iv) providing increased health benefits through metabolic engineering of yeast and other wine-associated microorganisms. Furthermore, to contribute to the long-term economic viability of these approaches, potential risks that may be associated with the release of GM organisms have to also be assessed.

A holistic approach towards wine science should attempt to integrate all scientific disciplines that are relevant to the understanding of wine-associated organisms. The two traditional wine sciences, viticulture and oenology, need to be combined with new approaches developed in the chemical, biological and other sciences, in particular, analytical chemistry and biotechnology. On a scientific level, combined approaches promise to deliver a significantly improved understanding of all the biological processes that determine industrially and commercially relevant parameters, cost efficiency and product quality. Besides the scientific and commercial benefits, South African science and the wine industry stand to gain in image and international appreciation as being innovative and forward looking. The IWBT with its integrated research programme entitled "Functional Wine-omics" will continue to develop the tools and expertise required to be at the frontier of research into the systems biology of wine.

The authors have declared no conflict of interest.

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