

**PROGRAMME: IMPROVING GRAPEVINE, WINE YEAST AND BACTERIA FOR A QUALITY FOCUSED, MARKET DIRECTED WINE INDUSTRY (BIOTECHNOLOGY PROGRAMME)**

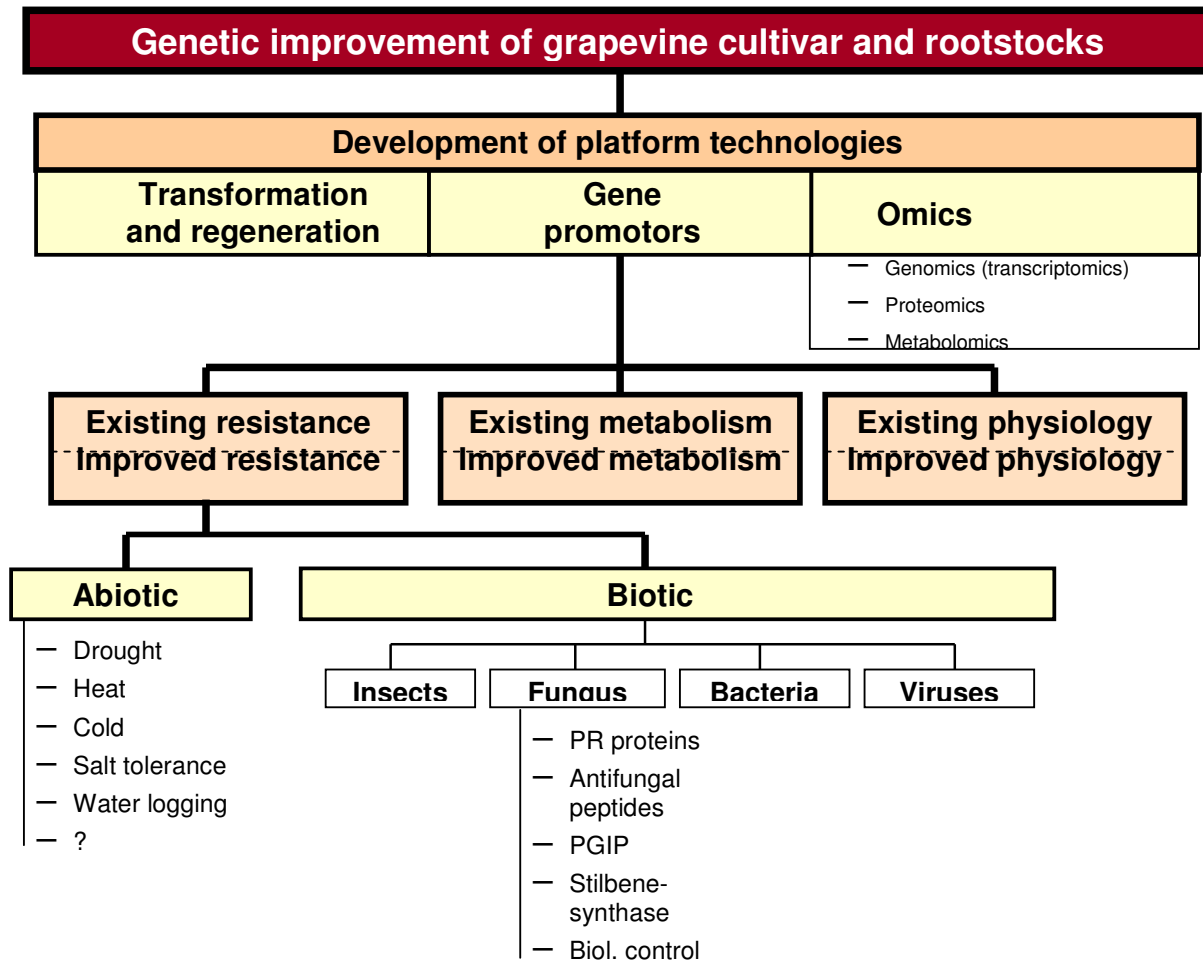
**WINETECH DEFINES A RESEARCH PROGRAMME AS:**

A number of projects with common goals that either encompass strategic benefits for the industry and/or address industry needs/problems.

**INTRODUCTION**

Biotechnology is the application of technology to living systems to make or modify products or processes for specific uses.

The components of grapevine and wine biotechnology are summarised as follows:



# Genetic improvement of wine yeast

## Development of platform technologies

### Yeast systems biology

Improved management of wine yeast cultures	Improved fermentation efficiency	Improved wine processing	Improved preservation	Improved wholesomeness	Improved sensorial quality of wine
<ul style="list-style-type: none"> <li>- Taxonomic classification</li> <li>- Strain identification</li> <li>- Culture maintenance</li> </ul>	<ul style="list-style-type: none"> <li>- Vitality</li> <li>- Viability</li> <li>- Effective sugar utilisation</li> <li>- Effective nitrogen utilisation</li> <li>- Alcohol resistance</li> <li>- Foaming</li> <li>- Resistance to antimicrobial components</li> </ul>	<ul style="list-style-type: none"> <li>- Juice yield</li> <li>- Clarification</li> <li>- Colour extraction</li> <li>- Filterability</li> <li>- Flocculation</li> <li>- Velum formation</li> <li>- Autolysis</li> </ul>	<ul style="list-style-type: none"> <li>- Bacteriocins</li> <li>- Bacteriolytic enzymes</li> <li>- Antifungal peptides</li> <li>- Antifungal enzymes</li> <li>- Zymosin</li> </ul>	<ul style="list-style-type: none"> <li>- Antioxidant</li> <li>- Ethyl carbamate</li> <li>- Low alcohol wine</li> <li>- Biogenic amines</li> <li>- Sulphur dioxide</li> </ul>	<ul style="list-style-type: none"> <li>- Esters</li> <li>- Higher alcohols</li> <li>- Terpenes</li> <li>- Phenols</li> <li>- Glycerol</li> <li>- Polysaccharides</li> <li>- Manno-proteins</li> <li>- Hydrogen sulphide</li> <li>- Bio-acidification</li> </ul>

## Development of platform technologies

### Lactic acid bacteria

Improved management of wine yeast cultures	Improved fermentation efficiency	Improved preservation	Improved wholesomeness	Improved sensorial quality of wine
<ul style="list-style-type: none"> <li>-Taxonomic classification</li> <li>-Strain identification</li> <li>-Culture maintenance</li> </ul>	<ul style="list-style-type: none"> <li>-Viability</li> <li>-Effective malic acid utilisation</li> <li>-Effective nitrogen utilisation</li> <li>-Alcohol resistance</li> <li>-Resistance to antimicrobial components</li> </ul>	<ul style="list-style-type: none"> <li>-Bacteriocins</li> <li>-Bacteriolytic enzymes</li> </ul>	<ul style="list-style-type: none"> <li>-Ethyl carbamate</li> <li>-Biogenic amines</li> </ul>	<ul style="list-style-type: none"> <li>-Diacetyl</li> <li>-Higher alcohols</li> <li>-Volatile phenols</li> <li>-Glycerol degradation</li> <li>-Volatile sulphur</li> <li>-Bio-deacidification</li> </ul>

## OBJECTIVES OF THE BIOTECHNOLOGY PROGRAMME

This programme focuses on the improvement of grapevine, wine yeast and bacteria, and aims to promote environmentally friendly, quality driven, sustainable and cost-effective production of grapes, wine, brandy and other grape-derived products.

All projects must address one or more of the aspects listed in the overall aim of the programme and fall into any one of the following categories:

1. **The improvement of grapevine (*Vitis vinifera* and rootstock cultivars)**
  - The improvement of disease and pest resistance
  - The improvement of virus resistance and diagnosis
  - The improvement of stress tolerance, including drought resistance
  - The improvement of plant/fruit metabolism and quality characteristics
  - The improvement of nutritional value
  - The development and improvement of support technologies
  - The evaluation and risk assessment of transgenic grapevines
  
2. **The improvement of wine yeast (*Saccharomyces cerevisiae*) and bacteria (*Oenococcus oeni*)**
  - The improvement of wine yeast and bacterial culture management
  - The improvement of the fermentation performance of wine yeast and bacteria
  - The improvement of wine processing
  - The improvement of wine preservation
  - The improvement of wine wholesomeness
  - The development of yeast strains with low ethanol yields
  - The improvement of the sensorial quality of wine
  - The development and improvement of support technologies
  - The evaluation and risk assessment of transgenic wine yeast and bacteria

➤*These categories are potential targets and are not fixed. They may be adapted to include aspects that become important to the industry in future.*

## TARGETS FOR THE IMPROVEMENT PROGRAMMES

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

Desirable properties	Focus area	Examples of current and potential target genes
<b>Improved disease resistance</b>		
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 ( <i>Vv11</i> ); antifungal peptide encoding genes from plants and insects; PGIP (polygalacturonase-inhibiting protein); encoding genes from plant species, stilbene phytoalexins (stilbene synthases: <i>sts1</i> , <i>vst1</i> , <i>vst2</i> ); phenylalanine ammonia lyase: <i>pal</i> ) <i>CuZnSOD</i> (putative CuZn superoxide dismutase; detoxification enzyme-producing genes (NADPH-dependent aldehyde reductase, <i>Vigna radiata</i> -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 ( <i>virE2delB</i> ) from <i>Agrobacterium</i>
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.
<b>Improved stress tolerance</b>		
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; <i>Adh</i> (alcohol dehydrogenase) genes; SODs (cytosolic CuZnSOD, chloroplast-residing CuZnSOD, mitochondrial-residing MnSOD)
Osmotic stress and other abiotic stresses	Proline accumulation; polyamines and their role in stress	<i>Vvp5cs</i> ( $\Delta^1$ -pyrroline-5-carboxylate); <i>Vvoat</i> ( $\delta$ -ornithine aminotransferase); FeSOD, glycine betaine, antifreeze genes from Antarctic fish (freezing tolerance)
<b>Improved quality factors</b>		
Colour development	Ripening related processes and signals, anthocyanin biosynthesis and control (several genes and some promoters have been cloned); isolation of berry-specific promoters	<i>ufgt</i> (UDP-glucose:flavonoid 3-O-glucosyltransferase) and/or regulatory sequences of <i>ufgt</i> ; production of pelargonidin-based anthocyanins for novel berry colour; anthocyanin methyltransferases
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 ( <i>Vvsuc11</i> , <i>Vvsuc12</i> , <i>Vvsuc27</i> ); hexose transporters ( <i>Vvht1</i> , <i>Vvht2</i> )
Reduced browning	Oxidation reactions	Silencing of polyphenol oxidase
Seedlessness	Seed-formation; isolation of seed-specific promoters	Barnase gene

## 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 <i>GSY1</i> and <i>GSY2</i> (glycogen synthase), <i>TPS1</i> (trehalose-6-phosphate synthase), <i>TPS2</i> (trehalose-6-phosphate phosphatase)]
Improved efficiency of sugar utilisation	Hexose transporters, hexose kinases	Overexpression and modification of <i>HXT1-HXT18</i> , <i>SNF3</i> , <i>FSY1</i> and use of heterologous transporters and kinases
Improved efficiency of nitrogen assimilation	Improved utilisation of less efficient N-sources	Proline catabolism [ <i>PUT1</i> (proline oxidase) and <i>PUT2</i> (pyrroline-5-carboxylate dehydrogenase)] and use of heterologous catabolic genes
Improved ethanol tolerance	Sterol formation, membrane ATPase activity	Modification of the expression of <i>PMA1</i> and <i>PMA2</i> (ATPase), sterol anabolic genes
Increased tolerance to antimicrobial compounds	Resistance to killer toxins, sulphur dioxide, agrochemicals	Inclusion of <i>KIL2</i> (zymocin and immunity factor), overexpression of <i>CUPI</i> (copper chelatin)
Reduced foam formation	Cell surface proteins	Deletion of <i>FRO1</i> and <i>FRO2</i> (froth proteins)
Improved processing efficiency		
Improved protein clarification	Proteases	Overexpression of <i>PEP4</i> (protease A) and secretion of other proteases
Improved polysaccharide clarification	Glucanases, pectinases, xylanases, arabinofuranosidases	Overexpression of <i>END1</i> (endoglucanase), <i>EXG1</i> (exoglucanase), <i>CEL1</i> (cellodextrinase), <i>BGL1</i> ( $\beta$ -glucosidase, cellobiase), <i>PEL5</i> (pectate lyase) and <i>PEH1</i> (polygalacturonase), <i>XYN1-5</i> (xylanases), <i>ABF2</i> (arabinofuranosidase)
Controlled cell sedimentation and flocculation	Flocculins	Late expression of flocculation genes ( <i>FLO1</i> , <i>FLO5</i> , <i>MUC1/FLO11</i> ) under control of promoters ( <i>HSP30</i> ) imparting desired expression
Controlled cell flotation and flor formation	Cell wall hydrophobic proteins	Late expression of <i>MUC1/FLO11</i> under control of promoters ( <i>HSP30</i> ) imparting desired expression pattern
Improved biological control of wine spoilage microorganisms		
Wine yeasts producing antimicrobial enzymes	Lysozyme, glucanases, chitinases	Expression of <i>HELI1</i> (hen egg white lysozyme), <i>CTS1</i> (chitinase), <i>EXG1</i> (exoglucanase) and other antimicrobial enzymes
Wine yeasts producing antimicrobial peptides	Bacteriocins	Expression of <i>PEDI1</i> (pediocin), <i>LCA1</i> (leucocin) and other heterologous bacteriocin and zymocin genes
Wine yeasts producing sulphur dioxide	Sulphur metabolism and SO <sub>2</sub> formation	Overexpression of <i>MET14</i> (adenosylphosphosulphate kinase) and <i>MET16</i> (phospho adenosylphosphosulphate reductase), and deletion of <i>MET10</i> (sulphite reductase)
Improved wine wholesomeness		
Increased production of resveratrol	Stilbene synthesis	Expression of <i>4CL9/216</i> (co-enzyme A ligase), <i>VST1</i> (stilbene synthase)
Reduced formation of ethyl carbamate	Amino acid metabolism, urea formation	Deletion of <i>CAR1</i> (arginase) or expression of <i>URE1</i> (urease)
Reduced formation of biogenic amines	Bacteriolytic enzymes, bacteriocins	Expression of <i>HELI1</i> (hen egg white lysozyme), <i>PEDI1</i> (pediocin), <i>LCA1</i> (leucocin) and other bacteriocins
Decreased levels of alcohol	Carbon flux, glycerol metabolism and glucose oxidation	Overexpression of <i>GPD1</i> and <i>GPD2</i> (glycerol-3-phosphate dehydrogenase), modification of <i>FPS1</i> (glycerol transport facilitator), expression of <i>GOX1</i> (glucose oxidase)
Improved wine flavour and other sensory qualities		
Enhanced liberation of grape terpenoids	Glycosidases, glucanases, arabinofuranosidases	Overexpression of <i>END1</i> (endoglucanase), <i>EXG1</i> (exoglucanase), <i>CEL1</i> (cellodextrinase), <i>BGL1</i> ( $\beta$ -glucosidase, cellobiase), <i>PEL5</i> (pectate lyase) and <i>PEH1</i> (polygalacturonase), <i>ABF2</i> (arabinofuranosidase)
Enhanced production of desirable volatile esters	Esterases	Modified expression of <i>ATF1</i> (alcohol acetyl transferase) and other alcohol transferases, <i>IAH1</i> (esterase) and other esterases
Optimised fusel oil production	Amino acid metabolism	Deletion of the <i>ILE</i> , <i>LEU</i> and <i>VAL</i> genes
Enhanced glycerol production	Glycerol metabolism	Overexpression of <i>GPD1</i> and <i>GPD2</i> (glycerol-3-phosphate dehydrogenase), <i>FPS1</i> (glycerol transport facilitator), and deletion of <i>ALD6</i>
Bio-adjustment of wine acidity	Maloethanolic and malolactic fermentation, lactic acid production	Expression of <i>MAE1</i> (malate permease), together with <i>MAE2</i> (malic enzyme) or <i>mleS</i> (malolactic enzyme), or <i>LDH1</i> (lacticodehydrogenase)
Optimisation of phenolics	Phenolic acid metabolism	Modified expression of <i>PAD1</i> (phenyl acrylic acid decarboxylase), <i>padc</i> (p-coumaric acid decarboxylase), <i>padc</i> (phenolic acid decarboxylase)
Reduced sulphite and sulphide production	Sulphur metabolism, hydrogen sulphide formation	Deletion of <i>MET14</i> (adenosylphosphosulphate kinase) and <i>MRX1</i> (methionine sulphoxide reductase)

**PROF FLORIAN BAUER**

**PROGRAMME COORDINATOR**

**2009**

The following projects are currently classified under this programme:

## CURRENT PROJECTS IN THIS PROGRAMME

### 1. Grapevine Biotechnology

- Improvement of disease and pest resistance

*Project status: Completed*

- ❑ The cloning and characterization of the polygalacturonase-inhibiting protein (PGIP) encoding gene in *Vitis vinifera* (IWBT 4/3)
- ❑ The cloning and molecular characterization of the coat protein gene of a South African isolate of a grapevine leafroll associated virus III (IWBT 4/5)
- ❑ The transformation of grapevine with yeast glucanase and chitinase genes (IWBT 4/6)
- ❑ The expression of antifungal peptides in grapevine cultivars (IWBT 4/7)
- ❑ A pathogen-derived resistance strategy for the broad-spectrum control of grapevine leafroll disease (IWBT 4/8)
- ❑ The isolation, cloning and characterisation of novel antifungal genes and their encoded products for use in resistance strategies against fungal pathogens of *Vitis vinifera*. (IWBT 5/08A)
- ❑ Grapevine biotechnology: The use of various antifungal genes to upregulate the plant's natural disease response (IWBT 5/08B)

*Project status: Ongoing*

- ❑ Understanding and manipulating disease resistance in grapevine (IWBT - P 08/12)

- Improvement of stress tolerance

*Project status: Completed*

- ❑ Isolation and characterisation of carotenoid pathway genes and promoters from *Vitis vinifera* as resources towards stress-tolerant grapes with superior quality (IWBT 5/09A)
- ❑ Functional analysis of central metabolic pathways with regards to roles in stress-tolerance, colour development or sugar metabolism (IWBT 5/09B)

*Project status: Ongoing*

- ❑ Metabolic engineering of grapevine towards enhanced abiotic stress resistance and improved quality parameters (IWBT - P 08/13)

- Improvement of plant/fruit metabolism

*Project status: Completed*

- ❑ Genetic manipulation of fruit metabolism in grapevine (IPB 4/3)
- ❑ The study of sugar translocation in grapevine with an over-expressed yeast invertase targeted to different cellular locations (IWBT 4/12)
- ❑ Functional analysis of central metabolic pathways with regards to roles in stress-tolerance, colour development or sugar metabolism (IWBT 5/09B)

- Development of support technologies

*Project status: Completed*

- ❑ The establishment of efficient transformation and regeneration systems for grape cultivars (IWBT 4/1)
- ❑ Somatic embryogenesis for grapevine improvement: The induction and maintenance of embryogenic cell-lines of winegrape and rootstock cultivars for use in transformation experiments (US WIN 95/1)
- ❑ The identification of grape cultivars using genetic marker technology (IWBT 4/4)
- ❑ The isolation and characterisation of viral promoters for directed expression of transgenes in grapevine (IWBT 4/9)
- ❑ The establishment of stable and synchronous embryogenic cell lines of grapevine rootstock cultivars for use in transformation systems (IWBT 4/10)
- ❑ The construction of genomic and cDNA libraries of grapevine cultivars (IWBT 4/2)

- ❑ The grapevine transformation and regeneration programme (IWBT 4/11)
- ❑ The molecular characterisation and genetic transformation of the grapevine chloroplast genome (IWBT 4/13)
- ❑ The evaluation of transgenic grapevines (IWBT5/10)
- ❑ Development of PCR-based test for identification of Black dead arm, Black foot rot and Black goo (IFT-NVB 240031)
- ❑ Molecular Diagnostic Services for identification of nematodes, insects, fungi, bacteria and virus infection – grapevines (IFT-NVB 240032)
- ❑ Analysis of grapevine leafroll-associated virus 3 (GLRaV-3) isolates by single-strand conformation polymorphism (SSCP) (WW 07/12)
- ❑ Molecular study of viruses from LN33 hybrid showing symptoms of corky bark (CB) disease (WW 07/09)
- ❑ Determination of a possible viral etiology of Shiraz disease (WW 07/07)
- ❑ PCR detection of viruses of the grapevine leafroll complex (WW 07/06)
- ❑ Evaluation of transgenes and regulatory elements from grapevine in an alternative system (IFT-NVB 240035)
- ❑ The establishment of stable and synchronous embryogenic cell lines of grapevine rootstock cultivars for use in transformation systems. (IWBT 4/10-2005)

*Project status: Ongoing*

- ❑ Isolation and characterisation of fruit-specific promoters and identification of alternative systems for transgenic plants (IPB 4/4)
- ❑ Funding support for transformation and regeneration facility for grapevine: 2008 (IWBT – P 08/14)

## 2. Yeast and Bacterial Biotechnology

- Improved wine yeast and bacterial culture management

*Project status: Completed*

- ❑ Selection, breeding, evaluation and characterisation of new wine yeasts (IWBT 1/1)
- ❑ The selection and breeding of brandy yeasts with enhanced ester formation (IWBT 2/1)
- ❑ The improvement and expansion of the microbial culture collection (IWBT 5/01)
- ❑ Evaluation of the population dynamics in wine using a non-culturing approach (IWBT 6/01)
- ❑ Establishment of the metabolic profiles of lactic acid bacteria (IWBT B – 08/10)

- Improved fermentation performance of wine yeast

*Project status: Completed*

- ❑ Development of fructophilic wine yeast for the reduction of residual sugar in wine of wine (IWBT 1/16)
- ❑ The improvement of the fermentation performance of yeast (IWBT 5/02)

*Project status: Ongoing*

- ❑ Nitrogen metabolism, fermentation efficiency and stuck fermentation (IWBT – Y 08/03)
- ❑ Selection, breeding, evaluation and characterisation of new wine yeasts (ww10/01)
- ❑ The use of fructophilic yeasts to prevent lagging fermentations (ww10/17)
- ❑ The use of *Torulaspora delbrueckii* for wine production (ww10/21)
- ❑ Semi-industrial testing facility for newly generated yeast and bacteria (IWBT – Y 08/06)
- ❑ Bioprocess monitoring of fermentations with infrared spectroscopy (IWBT – B 08/11)

- Improved wine processing

*Project status: Completed*

- ❑ Engineering pathways for malolactic and malo-alcoholic fermentation in wine yeasts (MV01)
- ❑ The transformation of wine yeasts with tannase and laccase genes to eliminate the instability of wines caused by oxidizable polyphenols (IWBT 1/12)
- ❑ The transformation of wine yeasts with glucanase, xylanase and pectinase genes for improved clarification and filterability of wine (IWBT 1/3)
- ❑ The cloning and characterization of genes involved in flocculation and cell aggregation in sparkling wines and sherry (IWBT 1/5)

- ❑ Overexpression of the *PEP4* protease and *BGL2* glucanase genes in wine yeast for enhanced autolysis (IWBT 1/10)
- ❑ Modifying and improving yeast to increase wine-processing efficiency (IWBT 5/03)

*Project status: Ongoing*

- ❑ Enzyme secreting yeast (IWBT – Y 08/05)
- ❑ Assessing malolactic fermentation under winemaking conditions (IWBT – B 08/08)
- ❑ Effect of non-*Saccharomyces* yeast on malolactic fermentation (WW10/22)

- Improved wine preservation

*Project status: Completed*

- ❑ Control of bacterial spoilage in wine with natural (biological) antimicrobial peptides produced by lactic acid bacteria (MIC/02)
- ❑ The transformation of wine yeast with the *Pediococcus acidilactici* pediocin gene for control of spoilage bacteria (IWBT 1/4)
- ❑ Production of a fusion lysozyme enzyme by wine yeasts active against Gram-positive and Gram-negative spoilage bacteria (IWBT 1/9)
- ❑ Improving the control of wine spoilage microorganisms (IWBT 5/04)

*Project status: Ongoing*

- ❑ Developing a fast and reliable technique for detecting *Brettanomyces/Dekkera* spp. and investigating their response to the presence of sulphur dioxide (IWBT – 09/02)

- Improved wine wholesomeness

*Project status: Completed*

- ❑ Expression of a glucose oxidase gene in yeast for the production of wine with reduced levels of alcohol (IWBT 1/14)
- ❑ Enhancing the wholesomeness of wine (IWBT 5/05)

*Project status: Ongoing*

- ❑ Low ethanol yielding yeast (IWBT – Y 08/01)

- Improved sensorial quality of wine

*Project status: Completed*

- ❑ The cloning and transformation of brandy yeasts with the alcohol acetyl transferase gene for improved ester formation (IWBT 2/2)
- ❑ Engineering pathways for malolactic and malo-alcoholic fermentation in wine yeasts (MV01)
- ❑ Regulation of malate metabolism in yeast (MV03)
- ❑ Improvement of wine quality through the release of terpenes (MIC/01)
- ❑ The cloning and characterization of yeast genes involved in the synthesis of long chain fatty esters for increased flavour production in brandy (IWBT 2/4)
- ❑ The development of beta-glucosidase producing wine yeast for the release of flavour compounds (IWBT 1/6)
- ❑ Manipulation of glycerol production in wine yeast (MIC/04)
- ❑ The improvement of the sensorial quality of wine (IWBT 5/06)

*Project status: Ongoing*

- ❑ Mannoproteins: Analysis, identification and improved release (IWBT – Y 08/04)
- ❑ Metabolic engineering of yeast with grapevine genes to enhance flavour and aroma development during wine fermentation (IWBT – Y 08/07)
- ❑ Identification of important genes from lactic acid bacteria for wine production and evaluating the influence of physical and chemical wine parameters on the activity and expression of the gene (IWBT – B 08/09)
- ❑ Establishment of the metabolic profiles of lactic acid bacteria (IWBT – B 08/10)
- ❑ Understanding and controlling acid production and consumption by wine yeast strains (IWBT - Y 08/02)
- ❑ Mannoproteins: Analysis, identification and improved release (IWBT – Y 08/04)

- Metabolic engineering of yeast with grapevine genes to enhance flavour and aroma development during wine fermentation (IWBT Y – 08/07)
  - Identification of important genes from lactic acid bacteria for wine production and evaluating the influence (IWBT B – 08/09)
- Development of support technologies
    - Project status: Completed*
      - Monitoring the spreading of commercial wine yeasts in the vineyards (IWBT 1/6)
      - Development of transformation systems for wine yeasts (IWBT 1/8)
      - Spreading of wine yeast in the environment: Monitoring the occurrence of sexual and asexual DNA transfers between yeast strains (IWBT 1/15)
      - Assessing the risk associated with genetically modified wine yeast strains (IWBT 5/07)
    - Project status: Ongoing*
      - Semi-industrial testing facility for newly generated yeast and bacteria (IWBT – Y 08/06)
      - Bioprocess monitoring of fermentations with infrared spectroscopy (IWBT – B 08/11)
      - Evaluation of cell wall properties of wine-associated organisms, specifically grapevine (*Vitis vinifera*), yeast (*Saccharomyces cerevisiae*) and bacteria, in relation to berry ripening, leaf development, abiotic and biotic stresses, fermentation processes and wine properties (IWBT – P 09/01)

## COMPLETED PROJECTS

COMMITTEE	PROJECT / PROJECT LEADER	PROJECT TITLE	BUDGET	BEGIN DATE	END DATE
Plantbiotechnology	IWBT 4/2 - M Vivier	The construction of genomic and cDNA libraries of grapevine cultivars	Completed	1999	2002
Plantbiotechnology	IWBT 4/3 - M Vivier	The cloning and characterization of the polygalacturonase-inhibiting protein (PGIP) encoding gene in <i>Vitis vinifera</i>	Completed	1999	2002
Plantbiotechnology	IWBT 4/4 - M Vivier	The identification of grape cultivars using genetic marker technology	Completed	1999	2002
Plantbiotechnology	IWBT 4/5 - M Vivier	The cloning and molecular characterization of the coat protein gene of a South African isolate of a grapevine leafroll associated virus III	Completed	1999	2002
Plantbiotechnology	IWBT 4/6 – M Vivier	The transformation of grapevine with yeast glucanase and chitinase genes	Completed	1999	2004
Plantbiotechnology	IWBT 4/7 – M Vivier	The expression of antifungal peptides in grapevine cultivars	Completed	1999	2004
Plantbiotechnology	GENUS 1 / 2 - J Burger	A pathogen-derived resistance strategy for the broad-spectrum control of grapevine leafroll disease	Completed	1999	2004
Plantbiotechnology	IWBT 4/11 – M Vivier	The grapevine transformation and regeneration programme	Completed	2001	2003
Plantbiotechnology	IWBT 4/12 – FC Botha & M Vivier	The study of sugar translocation in grapevine with an over-expressed yeast invertase targeted to different cellular locations	Completed	2002	2005
Plantbiotechnology	IWBT 4/14 – M Vivier	The evaluation of transgenic grapevines	Completed	2002	2007
Microbiology	IWBT 1/1 – P van Rensburg	Selection, breeding, evaluation and characterisation of new wine yeasts	Completed	1998	2002

Microbiology	IWBT 1/3 – P van Rensburg	The transformation of wine yeasts with glucanase, xylanase and pectinase genes for improved clarification and filterability of wine	Completed	1997	2001
Microbiology	IWBT 1/4 – M du Toit	The transformation of wine yeast with the <i>Pediococcus acidilactici</i> pediocin gene for control of spoilage bacteria	Completed	1997	2002
Microbiology	IWBT 1/5 – F Bauer	The cloning and characterization of genes involved in flocculation and cell aggregation in sparkling wines and sherry	Completed	1998	2002
Microbiology	IWBT 1/6 – P van Rensburg	The development of beta-glucosidase producing wine yeast for the release of flavour compounds	Completed	1998	2002
Microbiology	IWBT 1/8 – F Bauer	Development of transformation systems for wine yeasts	Completed	1998	2002
Microbiology	IWBT 1/9 – M du Toit	Production of a fusion lysozyme enzyme by wine yeasts active against Gram-positive and Gram-negative spoilage bacteria	Completed	1999	2002
Microbiology	IWBT 1/10 – P van Rensburg	Overexpression of the <i>PEP4</i> protease and <i>BGL2</i> glucanase genes in wine yeast for enhanced autolysis	Completed	2000	2002
Microbiology	IWBT 1/14 – P van Rensburg	Expression of a glucose oxidase gene in yeast for the production of wine with reduced levels of alcohol	Completed	2002	2007
Microbiology	IWBT 1/15 – F Bauer	Spreading of wine yeast in the environment: Monitoring the occurrence of sexual and asexual DNA transfers between yeast strains	Completed	2002	
Microbiology	IWBT 1/16 – F Bauer	Development of fructophilic wine yeast for the reduction of residual sugar in wine of wine	Completed	2002	2004
Brandy & Distillation	IWBT 2/1 – M du Toit	The selection and breeding of brandy yeasts with enhanced ester formation	Completed	1997	2002
Brandy & Distillation	IWBT 2/4 – F Bauer	The cloning and characterization of yeast genes involved in the synthesis of long chain fatty esters for increased flavour production in brandy	Completed	1998	2002
Plantbiotechnology	IPB 4/3 – FC Botha	Genetic manipulation of fruit metabolism in grapevine	Completed	1999	2002
Plantbiotechnology	GENUS 2/2 – J Burger	The molecular characterisation and genetic transformation of the grapevine chloroplast genome	Completed	2002	2005
Plantbiotechnology	240031 – S Safodien	Development of PCR-based test for identification of Black dead arm, Black foot rot and Black goo	Completed	2001	2004
Plantbiotechnology	240032 – M Arendse	Molecular Diagnostic Services for identification of nematodes, insects, fungi, bacteria and virus infection – grapevines	Completed	2001	2004
Plantbiotechnology	240035/2005 – A Burger	Evaluation of transgenes and regulatory elements from grapevine in an alternative system	Completed	2005	2006

Plantbiotechnology	GENUS 1/3 – J Burger	The isolation and characterisation of viral promotors for directed expression of transgenes in grapevine	Completed	2001	2005
Plantbiotechnology	IWBT 5/08 A – M Vivier	The isolation, cloning and characterization of novel antifungal genes and their encoded products for use in resistance strategies against fungal pathogens of <i>Vitis vinifera</i>	Completed	2003	2007
Plantbiotechnology	IWBT 5/08 B – M Vivier	Grapevine biotechnology: The use of various antifungal genes to upregulate the plant's natural disease response	Completed	2003	2007
Plantbiotechnology	IWBT 5/09 A – M Vivier	Isolation and characterisation of carotenoid pathway genes and promoters from <i>Vitis vinifera</i> as resources towards stress-tolerant grapes with superior quality	Completed	2003	2007
Plantbiotechnology	IWBT 5/09 B – M Vivier	Functional analysis of central metabolic pathways with regards to roles in stress-tolerance, colour development or sugar metabolism	Completed	2003	2007
Plantbiotechnology	IWBT 5/10 – M Vivier	The evaluation of transgenic grapevines	Completed	2003	2007
Plantbiotechnology	240048 – S Safodien	Development of a technique for the detection of <i>Eutypa lata</i> from grapevines by PCR-RFLP assay	Completed	2006	2007
Microbiology	IWBT 5/01 – P van Rensburg	The improvement and expansion of the microbial culture collection	Completed	2003	2007
Microbiology	IWBT 5/02 – F Bauer	The improvement of the fermentation performance of yeast	Completed	2003	2007
Microbiology	IWBT 5/03 – P van Rensburg & F Bauer	Modifying and improving yeast to increase wine-processing efficiency	Completed	2003	2006
Microbiology	IWBT 5/04M du Toit & P van Rensburg	Improving the control of wine spoilage micro-organisms	Completed	2003	2007
Microbiology	IWBT 5/05 – M du Toit & P van Rensburg	Enhancing the wholesomeness of wine	Completed	2003	2007
Microbiology	IWBT 5/06 – P van Rensburg & F Bauer	The improvement of the sensorial quality of wine	Completed	2003	2007
Microbiology	IWBT 5/07 – F Bauer	Assessing the risk associated with genetically modified wine yeast strains	Completed	2003	2006
Microbiology	WW10/20 – H du Plessis	Characterisation and optimisation of malolactic bacteria for commercialization	Completed	2005	2007
Plantbiotechnology	IWBT 4/10-2005 – P Goussard	The establishment of stable and synchronous embryogenic cell lines of grapevine rootstock cultivars for use in transformation systems	Completed	2005	2009
Microbiology	IWBT 6/01- C Witthuhn, H Nieuwoudt & P van Rensburg	Evaluation of the population dynamics in wine using a non-culturing approach	Completed	2006	2008

## RUNNING PROJECTS 2010

COMMITTEE	PROJECT/ PROJECT LEADER	PROJECT TITLE	BEGIN DATE	END DATE
Plantbiotechnology	IPB 4/4 – J Kossmann	Isolation and characterisation of fruit-specific promotors and identification of alternative systems for transgenic plants	2002	2010
Microbiology	WW10/01 – R Hart	Selection, breeding, evaluation and characterisation of new wine yeasts	Continuous	
Microbiology	WW10/17 – N Jolly	The use of fructophilic yeasts to prevent lagging fermentations	2004	2007
Microbiology	WW 10/21 – N Jolly	The use of <i>Torulaspora delbrueckii</i> for wine production	2008	2010
Microbiology	WW 10/22 – N Jolly	Effect of non- <i>Saccharomyces</i> yeast on malolactic fermentation	2009	2012
Microbiology	IWBT – Y 08/01 – F Bauer	Low ethanol yielding yeast	2008	2010
Microbiology	IWBT – Y 08/02 – F Bauer	Understanding and controlling acid production and consumption by wine yeast strains	2008	2010
Microbiology	IWBT – Y 08/03 – F Bauer	Nitrogen metabolism, fermentation efficiency and stuck fermentation	2008	2010
Microbiology	IWBT – Y 08/04 – F Bauer	Mannoproteins: Analysis, identification and improved release	2008	2010
Microbiology	IWBT – Y 08/05 – F Bauer	Enzyme secreting yeast	2008	2010
Microbiology	IWBT – Y 08/06 – F Bauer	Semi-industrial testing facility for newly generated yeast and bacteria	2008	2010
Microbiology	IWBT – Y 08/07 – F Bauer	Metabolic engineering of yeast with grapevine genes to enhance flavour and aroma development during wine fermentation	2008	2010
Microbiology	IWBT – B 08/08 – M du Toit	Assessing malolactic fermentation under winemaking conditions	2008	2010
Microbiology	IWBT – B 08/09 – M du Toit	Identification of important genes from lactic acid bacteria for wine production and evaluating the influence	2008	2010

Microbiology	IWBT – B 08/10 – M du Toit	Establishment of the metabolic profiles of lactic acid bacteria	2008	2010
Microbiology	IWBT – B 08/11 – M du Toit	Bioprocess monitoring of fermentations with infrared spectroscopy	2008	2010
Plantbiotechnology	IWBT – P 08/12 – M Vivier	Understanding and manipulating disease resistance in grapevine	2008	2011
Plantbiotechnology	IWBT – P 08/13 – M Vivier	Metabolic engineering of grapevine towards enhanced abiotic stress resistance and improved quality parameters	2008	2011
Plantbiotechnology	IWBT – P 08/14 – M Vivier	Funding support for transformation and regeneration facility for grapevine	2008	2011
Microbiology	IWBT – P 09/01	Evaluation of cell wall properties of wine-associated organisms, specifically grapevine ( <i>Vitis vinifera</i> ), yeast ( <i>Saccharomyces cerevisiae</i> ) and bacteria, in relation to berry ripening, leaf development, abiotic and biotic stresses, fermentation processes and wine properties	2009	2011
Vinification	IWBT – 09/02	Developing a fast and reliable technique for detecting <i>Brettanomyces/Dekkera</i> spp. and investigating their response to the presence of sulphur dioxide	2010	2012

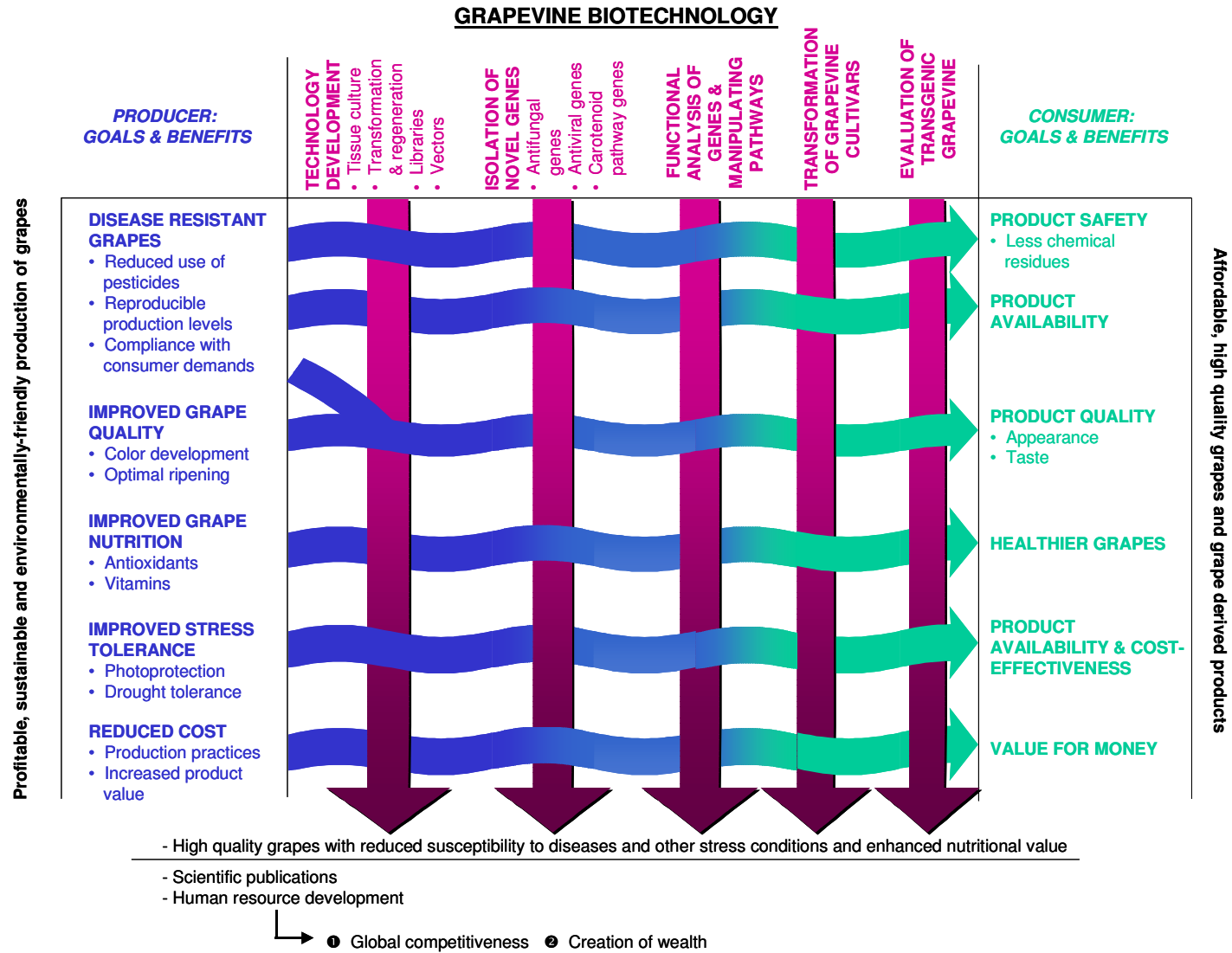
## **PROGRAMME COMMITTEE**

FF Bauer (Chairman)  
JH Booyesen  
S Safodien  
H Nel  
H du Plessis  
N Spreeth  
M du Toit  
M Vivier  
T Oosthuizen  
PG Goussard  
J Kossmann  
J Rees  
E Rybicki  
D Shepherd  
M Louw  
E le Roux (Chairman Viticulture committee)  
I Waller (Chairperson Oenology committee)  
M Lambrechts (Chairman Microbiology committee)  
E Wolf (Chairman Plant Biotechnology committee)  
H v Rensburg (Chairman Plant Protection committee)  
P van Rensburg (Chairman Vinification committee)  
J Burger (Chairman Vine Virus programme)  
E Archer (Chairman Optimal Ripeness Programme)  
V Carey (Chairperson Terroir Programme)

## **GENERAL**

- **The program members will meet at least once a year to discuss research progress and possible problems that have been experienced since the previous reporting occasion.**
- All the research projects in this programme that will involve the production of genetically modified organisms (GMOs) will be conducted in compliance to the following:
  - (i) Participation in, and adherence to the decisions of the Executive Council for Genetically Modified Organisms established by section 3 of the Genetically Modified Organisms Act (Act 15 of 1997).
  - (ii) The stipulations of the local GMO legislation, Act No 15 of 1997.

# BENEFITS OF THE PROPOSED OUTCOMES



## WINE YEAST BIOTECHNOLOGY

