Genetically Modified Organisms in the Wine Industry

Leigh Christina Berrie

A dissertation submitted in partial requirement for the Diploma of Cape Wine Master

Johannesburg 2011

Declaration

I, Leigh Christina Berrie declare that this dissertation is my own, unaided work. It is being submitted in partial fulfillment for the diploma of Cape Wine Master to the Cape Wine Academy. It has not been submitted before for any other diploma or examination to any other institute.

____

Leigh Christina Berrie

This _____________day of ________________ 2011.

ABSTRACT

Genetically modified organisms are those whose genetic material has been modified or altered using the process of "genetic engineering". The process was first performed in 1973, and has since been used in biological and medical research, production of pharmaceutical drugs, experimental medicine and agriculture. The first commercially cultivated GMO crop was available in 1996. Genetic manipulation of crops has since been used for insect resistance, herbicide resistance, tolerance to environmental conditions, viral, fungal and bacterial resistance, as well as to improve the yield, shape, colour, flavour, nutritional value and shelf-life of certain foods. In 2007, the global area of GM crops was estimated to be 114.3 million hectares with a commercial value of > US$ 44 billion.

The potential use of genetic engineering in the wine industry has been identified as a promising technology due to increasing demands from markets, consumers and environmentalists. Certain biotechnological advances which could benefit this demand include: Improved nutrient capture from soils and adaptation to adverse soil conditions; increased resistance to pathogens; enhancement of fruit quality, and germplasm development, among others. Wine biotechnology is currently practiced in all major viticultural research centres worldwide and has been used for the genetic improvement of grapevines, wine yeasts and wine bacteria. There are now two commercially available GM yeasts on the market. One such yeast has been genetically manipulated to better degrade urea during the wine making process (ECMo01). The second, has been designed to allow malolactic fermentation to proceed more efficiently (ML01).

The process of genetic engineering has however, been a very controversial topic for many years, having been named "Franken-food", and researchers being accused of "playing God" by manipulating the genetic make-up of organisms. Controversies surrounding GM crops include: potential human health impacts including allergens and transfer of antibiotic resistance markers; potential environmental impacts including unintended transfer of genes through cross-pollination, and loss of flora and fauna biodiversity; violation of natural organisms' intrinsic values and tampering with nature by mixing genes among species.

Due to the controversies and concerns surrounding GMOs, strict regulations have been implemented throughout the world with regards to GMO safety, thresholds, labeling, detection, and coexistence. A number of organizations exist who govern and monitor such regulations within each country. As well as strict regulations, there are a number of hurdles present for the commercialization of GMOs which include marketing/ public perception, traditional and cultural beliefs and patents, among others.

In order to determine the opinions of both the consumer and wine producer in the South African market on the use of genetically modified organisms in the wine industry, a survey was conducted. The majority of respondents were aware of what a genetically modified organism is, but were not aware of any GMOs being utilized in the wine industry. It was believed that GMOs could be of benefit to both the wine industry and consumer, however, the majority of wine producers felt that the use of GMOs could also be harmful to the wine industry. The majority of consumers would indeed purchase or consume a wine made using GMOs, this being in contrast to the majority of wine producers who responded that they would not. All responders were adamant that the bottle should be labeled as such, and were not prepared to pay more for a non-GMO wine. Currently in South Africa, the use of GMOs in wine production is illegal. Should the use of GMOs become legalized, only 25% of wine producers would use GMOs in wine production in the future.

ACKNOWLEDGEMENTS

The author wishes to express thanks to:

Mom, Dad, Jess and Pete, other family and friends for their support and encouragement throughout the Cape Wine Master's course. For never doubting that I would succeed.

The Wild Yeasts tasting group for years of enjoyment of many fine wines, and for their knowledge and expertise.

Various wine producers, as well as various wine consumers for their time, comments and opinions on the topic of this dissertation.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ARC	Agricultural Research Council
Bt	*Bacillus thuringiensis*
CWG	Cape Winemakers Guild
DNA	Deoxyribose nucleic acid
EFSA	European Food Safety Authority
EU	European Union
FDA	Food and Drug Administration
GEO	Genetically Engineered Organism
GFLV	Grape Fan Leaf Virus
GMO	Genetically Modified Organism
GRAS	Generally recognized as safe
HSRC	Health Science Research Council
IPGW	Integrated production of grape and wine
IPW	Integrated Production of Wine
ISAAA	International Service for the Acquisition of Agri-Biotech Applications
IWBT	Institute for Wine Biotechnology
OECD	Organization of Economic Co-operation and Development
OIV	International Organization of Wine and Vine
PEG	Polyethylene glycol
PGIP	Polygalacturonase-inhibiting protein
PUB	Public Understanding of Biotechnology
SAASTA	South African Agency for Science and Technology Advancement
SAFeAGE	South African Freeze Alliance on Genetic Engineering
SALBA	South African Liquor Brandowners Association
SET	Science, engineering and technology
TCA	Tricloracetic acid
UN	United Nations
UNECA	United Nations Economic Commission for Africa
WCSA	Wine Cellars South Africa
Winetech	Wine Industry Network of Expertise and Technology

CONTENTS

CHAPTER 1

INTRODUCTION TO GENETICALLY MODIFIED ORGANISMS (GMOs)

1. Introduction to Genetically Modified Organisms (GMOs)

1.1 Definition of a GMO

"A genetically modified organism (GMO) or genetically engineered organism (GEO) is an organism whose genetic characteristics have been altered by the insertion of a modified gene or a gene from another organism using the techniques of genetic engineering." (The American Heritage Medical Dictionary, Houghton Mifflin Company, 2007).

1.2 Background and History

The general principle of producing a GMO is to add new genetic material into an organism's own genetic material (genome). This is called Genetic Engineering and was made possible through the discovery of DNA and the creation of the first genetically manipulated organism in 1973: a bacterium, *E.coli* which was expressing a gene from another bacterium, Salmonella (Cohen *et. al*. 1973). The first company to use such technology was founded in 1978 by Herbert Boyer and was named Genentech. In the meanwhile, there were concerns from within the scientific community as to potential risks from genetic engineering, and thus it was recommended that the government oversee the technology, until it has been deemed safe (Berg *et. al*. 1975).

Genetic engineering has been used in biological and medical research, production of pharmaceutical drugs, experimental medicine and agriculture. To date, the broadest application of GMO technology is for food crops, with the first commercially cultivated GMO crop being available in 1996. Such genetic manipulation has been used in order to improve certain characteristics of the crops such as insect resistance, herbicide resistance, tolerance to environmental conditions, viral, fungal and bacterial resistance, as well as to try and improve the yield, shape, colour, flavour, nutritional value and shelf-life of certain foods. The largest share of GMO crops planted globally is owned by Monsanto Company. In 2007, Monsanto's GMO crops were planted on 246 million acres throughout the world, a growth of 13% from 2006.

Examples of GMO crops include those modified to be tolerant to the herbicides Glufosinate and Glyphosate, and GM papaya which is resistant to virus damage by Ringspot virus. GM sweet potatoes have been enhanced with protein and other nutrients, while golden rice, developed by the International Rice Research Institute, has been discussed as a possible cure for Vitamin A deficiency. In January 2008, a GM carrot was developed producing calcium, and is thought to have uses as a possible cure for Osteoporosis (Johnston *et. al*. 1994). Monsanto's GM "Triple-stack corn" is the market leader in the USA. The crop is resistant to Roundup Ready 2 herbicide, and to the insects, Corn Borer and Rootworm. US corn farmers planted more than 17 million acres of Triple-stack corn in 2007. Cotton has also been genetically modified to produce the Bt toxin, a potent insecticide.

The International Service for the Acquisition of Agri-Biotech Applications (ISAAA) estimated 13.3 million farmers to be growing GM crops in 2008. Of this, 90% were resource-poor farmers in developing countries, including 7.1 million in China (Bt cotton), 5 million in India (Bt cotton), 200,000 in the Philippines, subsistence farmers in KwaZulu Natal, South Africa, and in seven other developing countries (James, 2008). The global commercial value of biotech crops grown in 2003-2004 has been estimated to be US$44 billion (Runge, 2004). The global area (millions of hectares) of biotech crops by country (2007) is depicted in Fig 1.1.

Genetic modification of bacteria has been used for several purposes, and is particularly important in producing large amounts of pure human proteins for use in medicine (Leader *et. al.* 2008). Genetically modified bacteria are used to produce the protein insulin to treat diabetes (Walsh, 2005). Similar bacteria have been used to produce clotting factors to treat haemophilia (Pipe, 2008), and human growth hormone to treat various forms of dwarfism (Bryant *et. al.* 2007).

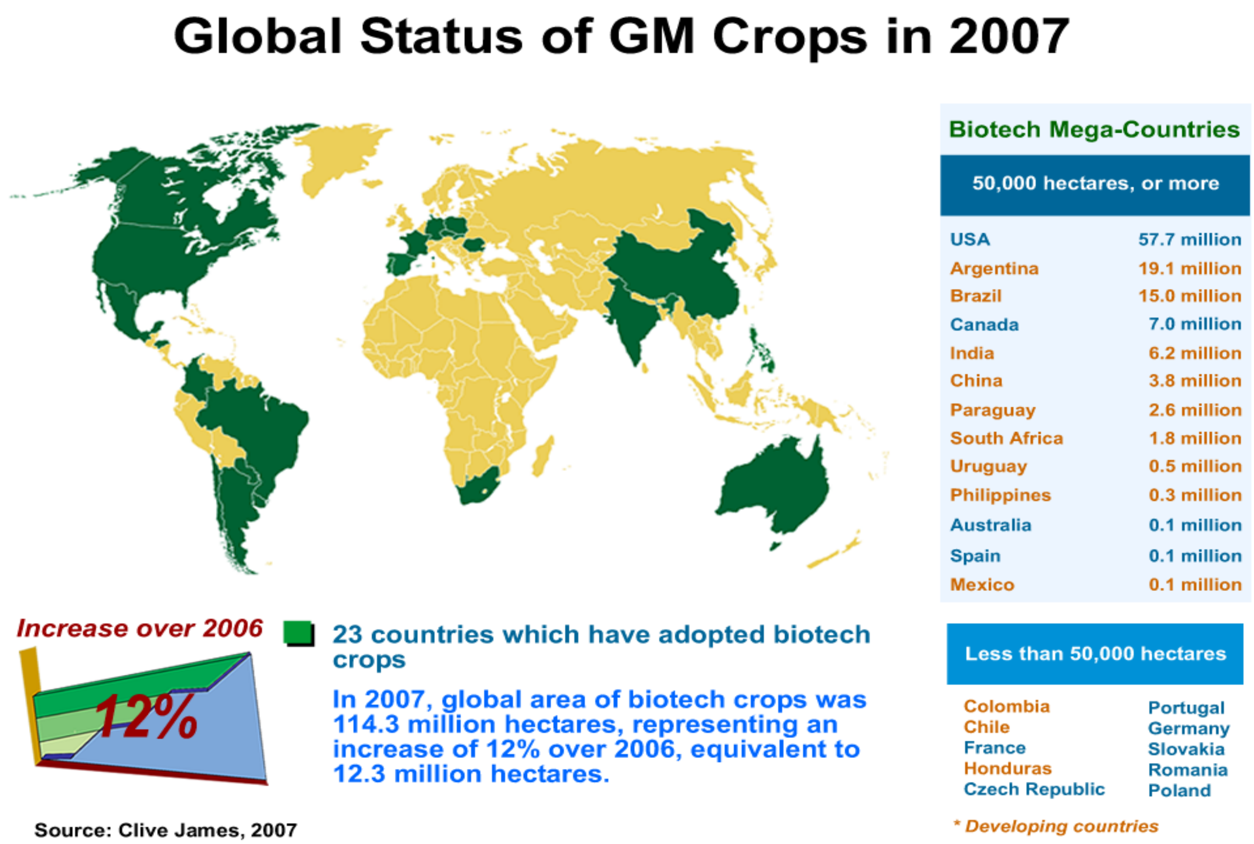

Figure 1.1. Global area (millions of hectares) of biotech crops by country, 2007.

1.3 GMOs in the wine industry

The potential use of genetic engineering in the wine industry has also been identified as being a promising technology. Historically, wine production technology has been very conservative and improvements, as in other food-production areas, have been difficult to implement. Until almost 100 years ago, viticulture has relied on plant and microbial breeding to improve the yield and quality of wine, and in the 1930's, accelerated mutagenesis was first introduced as a means of deliberately changing a plant's DNA.

The wine industry is faced with increasing demands from markets, consumers and environmentalists, and as such, long-term objectives of the wine industry need to be considered when looking at the necessity and possible impact of biotechnology on the wine industry. The most important question is whether a vineyard and its derived products are economically viable, which would rely heavily on sustainable, high-quality production. Certain biotechnological advances which could benefit this need include: Improved nutrient capture from soils and adaptation to adverse soil conditions; increased resistance to pathogens; enhancement of fruit quality, and germplasm development, amongst others.

Biotechnology has also been used to enhance certain characteristics of yeasts and bacteria used in wine production. Wine biotechnology is currently practiced in all major viticultural research centres worldwide. Today, genetically modified yeasts have been developed for the wine industry, as well as attempts to produce genetically modified (transgenic) grape vines. Concerning genetically modified yeasts, in modern wineries, reliable fermentations are essential in order to obtain consistent wine flavor and quality from the (generally used) *Saccharomyces cerevisiae*. There is a demand for wine yeast strains that are optimized for specific tasks set by winemakers, which has led to dedicated yeast breeding and genetic engineering (Pretorius and Bauer, 2002).

1.4 Objectives

The main objective of this dissertation is to discuss the potential use of genetically engineered organisms in the wine industry. Current techniques for producing GM plants will be discussed, as well as the types of GMOs available to the wine industry, both currently and in the future. Viewpoints, both locally and internationally will be reviewed from a consumer, wine producer and researcher's point of view. Various controversies surrounding the use of GMOs will be discussed, as well as determining factors influencing the possible commercialization of GMOs for future use in the wine industry.

CHAPTER 2

GENETIC ENGINEERING

2. Genetic Engineering

2.1 General Introduction

The process of producing a genetically modified organism is termed "Genetic Engineering". Genetic engineering may be applied to plants, bacteria, yeasts, animals and fungi. Whichever organisms are to be modified, the techniques are inherently the same. Genetic engineering involves three major steps: (i) the delivery of DNA into cells by a process known as "Transformation", (ii) the development of a culture system where transformed cells are efficiently regenerated into whole organisms, and (iii) transgene expression. Transformation involves the introduction of genetic material into cells, such that the material is integrated into the organism's chromosomes and will be stably inherited by subsequent generations. This stable inheritance distinguishes transformation from transient expression, where genetic material is introduced into a cell, but need not be integrated into the organism's genome. In this way, the genetic material exhibits a short-lived stability in the recipient cells. Genetic engineering has become a most promising and effective technique for the improvement of plant agronomic characteristics. Plants have been engineered for disease (Duan *et al*., 1997), pest (Kota *et al*., 1999), herbicide or pesticide resistance (Vasil *et al*., 1992), increased yield, vigour, or nutritional value (Molvig *et al*., 1997). All major crop species, including cereals and legumes, have been transformed (Christou and Ford, 1996).

2.2 Gene Transfer Systems

Over the past decade various systems have been developed for the transfer and integration of a desired gene into an organism's genome.

2.2.1 Microinjection

Microinjection involves the use of micro capillaries and microscopic devices to deliver DNA into defined cells in such a way that the injected cell can survive and proliferate (Neuhaus and Spangenburg, 1990). Only one cell receives DNA per injection and extreme skill is required. Microinjection has been successfully used to obtain stable transformation of tobacco, alfalfa, and oilseed rape protoplasts (plant cells that have had their cell walls removed) (Crossway *et al*., 1986; Reich *et al*., 1986; Neuhaus *et al*., 1987), and transformation frequencies of up to 30% have been reported.

2.2.2 Direct gene transfer

Direct gene transfer can be achieved by two methods utilizing protoplasts. One method of direct gene transfer involves the use of polyethylene glycol (PEG) which is able to permeate cell membranes, making the cells competent for DNA uptake (Potrykus *et al*., 1985). Cucumber (Wieczorek and Sanfaçon, 1995) and rice (Cornejo *et al*., 1993) have been successfully transformed using this method. A second method, known as electroporation, involves subjecting cells to the discharge of a capacitor, resulting in transient openings in the plasmalemma through which the DNA can enter. Rice was the first crop in which fertile transgenic plants resulted from protoplast electroporation (Shimamoto *et al*., 1989). Since then, a number of plant species including wheat (He *et al*., 1994) and tobacco (Spörlein and Koop, 1991) have been successfully transformed by this method.

2.2.3 Liposome fusion

DNA-containing liposomes may aid in the transportation of DNA via plasmodesmata or directly across cell membranes. The liposome encapsulation aids in protecting the DNA against intracellular nuclease activity (Caboche, 1990). The frequency of transformation mediated by liposomes is considerably lower however, than that obtained either by electroporation or by chemical agents.

2.2.4 Microlaser

A microlaser beam focused into the light path of a microscope can be used to burn holes into cell walls and membranes. It was hoped that incubation of perforated cells in DNA solutions could serve as a basis for vector-independent gene transfer into walled cells (Weber *et al*., 1990).

2.2.5 *Agrobacterium tumefaciens*-mediated gene transfer

This technique for transfer of DNA into plant cells has proven to be historically the most useful, and has been widely employed for the production of transgenic crops.

During the early 1980s, several research groups engineered the Ti plasmid of the bacterium *A. tumefaciens*, in order to remove all the T-DNA oncogenic genes which lead to tumour formation. The first important discovery was that these *onc* genes are neither required for the transfer of the T-DNA to the plant cell, nor its integration into the nuclear DNA. Hence, these genes can be replaced, which not only allows the insertion of significant amounts of foreign DNA, but also removes the *onc* functions. Any foreign DNA can be transferred by this method into the genome of a dicotyledonous or even some monocotyledonous plant cells. Co-cultivation with *Agrobacterium* has been performed on various kinds of tissues such as roots, stems, leaves, meristems, cotyledons, pollen, embryos, cells, and protoplasts (McCormick *et al*., 1986; May *et al*., 1995). Stable transformation has been successfully achieved in various plant species such as *Brassica napus* L. (Pua *et al*., 1987), soybean (Hinchee *et al*., 1988) and banana (May *et al*., 1995).

2.2.6 Biolistics or particle bombardment

In 1987, John C. Sanford (Cornell University) and his co-worker Theodore M. Klein constructed a DNA particle gun that used tungsten particles to bombard cells, with a .22 calibre blank cartridge as the motive force. Researchers at Agracetus in Middleton, Wisconsin have developed a similar gun using gold particles propelled by the vaporization of a water droplet. Today, the mechanism of particle bombardment often involves the acceleration of gold or tungsten particles through a partial vacuum under the pressure of helium gas (Fig. 2.1). Particles of 1-2 microns in diameter are first coated with purified plasmid DNA and then accelerated through the walls of intact cells. The DNA is then randomly integrated into the organism's genome. Because small holes in cell walls and membranes rapidly close by themselves, the punctures are temporary and do not irreversibly compromise the integrity of the cells. Although the particles remain in the cytoplasm, they are too small to interfere with any cellular functions.

Stable transformation of various plants has been achieved using this method, such as soybean (McCabe *et al*., 1988), rice (Christou and Ford, 1996) and maize (Register *et al*., 1994). In addition, in 1995, grape (*Vitis vinifera* L.) has also been successfully transformed by this method (Scorza *et al*., 1995). Virtually every type of cell and tissue can be bombarded. Meristems, callus, pollen, leaves, seeds and suspension cells have been successfully used in the transformation of plants by particle bombardment. Transformed cells are selected by their ability to grow on medium containing an inhibitor and plants are regenerated from these clones. Advantages of this system include the fact that one shot can lead to multiple hits, the cells survive the intrusion of one particle, the genes coated on the particle have biological activity, and it is easy to use.

Figure 2.1. Particle bombardment device used in plant transformation experiments. Tissue is placed inside the chamber which is evacuated of air to form a partial vacuum. DNA-bearing particles are then accelerated into the plant tissue under the pressure of

helium gas.

2.3 Plant tissue culture

Various types of plant tissue may be used in plant genetic engineering. Ideally, single plant cells should be used in transformation experiments, such that all cells arising from the transformed cell will contain the integrated gene of interest. In this way, an entire plant with all cells containing the desired trait may be regenerated from a single cell. Leaf discs (Dodds and Roberts, 1985), callus cultures (Constabel, 1984), protoplasts (Otsuki *et al*., 1974) and cell suspension cultures (King and street, 1977) are commonly used in plant transformation experiments. Irrespective of the type of tissue, a nutrient medium containing macro- and micro-nutrients, a carbon/energy source, an iron source, and vitamins and plant hormones are used to culture the plant tissue (Aitchinson *et al*., 1977). The choice of plant tissue to be used in transformation experiments depends largely on its ability to regenerate into a whole plant, either by organogenesis or somatic embryogenesis. The ease with which a plant may be regenerated from plant tissue also varies greatly from one plant to another.

2.3.1 Leaf discs

Leaves of most plant types may be used in transformation experiments. The leaves are first surface sterilized and then cut into 0.5-1cm squares. Only the lamina of the leaf is used. Leaf discs may then be either inoculated with the appropriate *Agrobacterium* (Horsch *et al*., 1985) or bombarded with particles coated with DNA. Discs are then transferred to medium containing a selective agent (e.g. kanamycin) which will select for transformed cells. The leaf discs then undergo rapid callus formation at the cut edges of the leaf, particularly where they come into contact with the medium. If placed on appropriate regeneration medium, adventitious shoot regeneration and root development then proceed.

2.3.2 Callus culture

Callus is considered to be a mass of unorganized, loosely arranged thin-walled parenchyma cells that originate from a parent plant cell tissue that is reproducing rapidly.

Callus can be produced from cotyledons, leaves, stems and roots. In callus formation, the metabolism of cells is changed due to the stimulus of exogenous plant growth substances and active cell division commences. During this process, cell differentiation and specialization, which may have been occurring in the intact plant, are reversed, and the explant gives rise to new tissue which is composed of meristematic and unspecialized cells (Dodds and Roberts, 1985). Callus formation usually results in approximately 2-3 weeks at the cut ends of the explant and then spreads slowly across the surface of the plant tissue. Healthy callus is yellow, white or slightly green in colour. The fragile callus growth that can easily peel off is known as “friable” callus. This callus is important in the production of somatic embryos from which whole plants can be regenerated. One major problem linked with the use of callus culture is its cell variation and genetic instability resulting in histological and cytological differences which lead to the establishment of differing callus isolates, strains and lines.

2.3.3 Cell suspension culture

Cell suspension cultures consist of a rapidly dividing homogenous suspension of cells. These cultures are obtained by transferring fragments of undifferentiated friable callus into liquid medium. Single cells are then maintained by agitation. Cell suspension cultures exhibit three major patterns of growth phases after their initiation in liquid medium with the callus tissue. The first phase or lag phase is a rest period in which little cell dividing activity or minimum growth is observed. The second phase or logarithmic growth phase marks an exponential rise in cell population. In this stage, growth is linear and cell dividing is at a maximum. Immediately after this linear stage, the growth pattern enters a gradual deceleration period where a gradual decline in rate of cell division occurs. Suspension cultures have been used successfully in a number of species to generate target tissues for use in genetic transformation systems (Vasil, 1995).

2.3.4 Protoplasts

Protoplasts are plant cells that have had their cell walls removed. The first plant protoplasts to be isolated originated from the root tips of tomato seedlings, using the fungal maceration enzyme, cellulase (Cocking, 1960). Thereafter, protoplasts were successfully isolated from a variety of plant species and explants. Protoplasts can be isolated by either mechanical or enzymatic means. Mechanical isolation procedures are tedious and generally provide low yields (Bengochea and Dodds, 1986). Enzymatic procedures involve the use of polysaccharide hydrolysing enzymes such as cellulase, hemicellulase and pectinase (Stafford and Warren, 1991). Following transformation of protoplasts, regeneration is initiated by the formation of a plant cell wall. Cell wall resynthesis occurs after a lag period, during which time stress-related repair and adaptation functions take place in the protoplast. Once the cell wall has been completely regenerated, healthy protoplasts are able to undergo the first mitotic cellular divisions. Thereafter, subsequent divisions occur more rapidly, to form multicellular congregates, or microcalli. Incomplete cell wall resynthesis, or protoplast budding, is a feature observed in a number of species' protoplasts. Leaf mesophyll is frequently employed as source material for protoplast isolation, although other explants such as hypocotyl, stem, petiole, cotyledon, florets, callus, suspensions and somatic embryos may also be used (Blackhall *et al*., 1994).

Among others, protoplasts obtained from rice (Cornejo *et al*., 1993) tobacco (Spörlein and Koop, 1991) and cucumber (Wieczorek and Sanfaçon, 1995) have been successfully transformed and regenerated into fully transgenic plants.

CHAPTER 3

CONTROVERSIES AND BENEFITS SURROUNDING GMOs

3. Controversies and benefits surrounding GMOs

3.1 Introduction

The process of genetic engineering has been a very controversial topic for many years, having been named “Franken-food”, and researchers being accused of “playing God” by manipulating the genetic make-up of organisms. In some countries, all genetically engineered items which are available to the consumer, must be correctly labeled to indicate that genetic manipulation has taken place, and a number of press releases have caused fear about eating such foods. The safety of GMOs in the foodchain has been questioned, with concerns that new allergens could be introduced into foods, or that they could lead to the spread of antibiotic resistance (Bakshi, 2003). Scientists and governments have stated that these types of crops are safe and have no adverse health effects (Key *et. al*. 2008), however, food and environmental activist groups in many countries have discouraged their consumption, claiming that they are unnatural and therefore unsafe. Some African nations with severe food shortages have refused emergency food aid from developed countries, fearing that the food is unsafe, however, the Executive Secretary of the United Nations Economic Commission for Africa (UNECA) has encouraged nations to accept genetically modified food and expressed dissatisfaction towards the public’s negative opinion of biotechnology (Amoako, 2003).

It is due to such concerns that a number of laws and regulations have been adopted that require safety testing of any new organism produced for human consumption (König *et. al*. 2004). At the end of the 1980’s, the food safety assessment of genetically engineered foods was, for the first time, discussed at an international level. The point of the safety assessment was to determine whether the modified food was as safe as its traditional counterpart. The concept of “substantial evidence” was introduced as a means of establishing a benchmark definition of safe food. This concept was introduced by the Organization of Economic Co-operation and Development (OECD) (Plahuta and Raspor, 2007). In the case of GMO usage in winemaking, a risk assessment is compulsory (EU Regulation 1829/2003, Directive 2001/18/EC), because biotechnology products and processes should be balanced with the need to protect health, safety and the environment.

The following list mentions certain controversies and benefits surrounding the use of GMOs, some of which will be discussed in more detail below:

Controversies surrounding GM crops

- Potential human health impacts including: allergens, transfer of antibiotic resistance markers and unknown effects.
- Potential environmental impacts including: unintended transfer of transgenes through cross-pollination, unknown effects on other organisms, and loss of flora and fauna biodiversity.
- Ethical considerations such as violation of natural organisms' intrinsic values and tampering with nature by mixing genes among species.
- Objections to consuming animal genes in plants and vice versa.

Benefits of GM crops

- Enhanced taste and quality.
- Reduced maturation time.
- Increased nutrients, yields, and stress tolerance.
- Improved resistance to disease, pests, and herbicides.
- Increased food security for growing populations.

3.2 Controversies surrounding GMOs

There are a number of Anti-GM groups throughout the world who are actively discouraging the use and consumption of GM products. These groups see the development and use of GMOs as intolerable meddling with nature which has evolved naturally over long periods of time.

3.2.1 Benefits to rich companies/ countries

It has been believed that the development and acceptance of genetically modified organisms will only benefit rich research companies, and that undeveloped countries, where food aid is needed most, will not benefit from GM crops. This is because firstly, they do not have as easy access to these systems, and secondly, because they would be unable to afford the modern agricultural equipment and patent/ Intellectual property tariffs. In the past, the UN blamed food companies and accused them of violating human rights, calling on governments to regulate these “profit-driven” firms. In addition, industrialized nations have not tested GM technology on tropical plants, focusing on those which grow in temperate climates, even though the majority of undeveloped nations and people who need an extra food supply live primarily in tropical climates (Guan, 2003).

3.2.2 Cross-pollination

Another important controversy is the possibility of cross-pollination from a genetically modified crop to a conventionally bred crop, and that GM crops could proliferate. Pollen can be dispersed over large areas by wind, animals and insects. In 2007, the US Department of Agriculture fined Scotts Miracle-Gro the amount of $500,000 when modified genetic material from creeping bentgrass was found in native grasses up to 21km away from the test site (Anslow, 2008). This process, known as “outcrossing” is however, not new, and happens with any new crop variety which is naturally introduced into an environment. Due to the laws and regulations surrounding the release of GM crops, a full risk assessment is performed concerning the outcrossing of GM traits to the native plant populations before any plants would be tested or released. One issue that could result from GM-outcrossing however, is that if a patented gene is outcrossed, even accidentally, to another commercial field and the farmer deliberately selects for this GM gene, then the patent holder has the right to control the use of those crops (e.g. Monsanto Canada Inc vs. Scheiser). Due to the controversy surrounding outcrossing, certain biotechnology companies (Monsanto and AstraZeneca) have researched the production of first generation GM crops that would not generate fertile seed in the second generation. This has been termed “Terminator” technology. Similarly, the “Traitor” technology requires application of a chemical to reactivate the genetically engineered traits in the crops. Both technologies are intended to limit the spread of genetically engineered plants, however, they require a yearly fee to be paid by farmers to either reactivate the genetically engineered traits of their plants, or to purchase new seed. Both these technologies have yet to be commercialized. It should be noted that in South Africa, grapevines have no close relatives, such that there is no plant species with which they could interbreed. Grapevines are also not planed from seed, but vegetatively propagated (from cuttings and/or cuttings grafted onto rootstocks), and their flowers are functionally bisexual and largely self-pollinated, such that cross-pollination should not pose a threat in the case of GM grapevines (www.winemag.co.za; Dec 2009).

3.2.3 Increased use of herbicide

Environmentalist groups are concerned about the release of herbicide resistant GM crops, since they believe it will result in an increase in the use of the herbicide which will then be environmentally damaging. Opposing views believe that herbicide resistant crops will allow farmers to spray the crops whilst they are younger, which will thus result in the use of less herbicide. Data from the USA showed that in addition to decreased use of herbicide, there was also less soil erosion (Thompson, GM debate, UCT, 2000). However, according to the Institute of Science and Technology in London, "over 80% of the world's GM crops have been engineered to be herbicide resistant, which has led, in the US, to a 15-fold increase in herbicide use".

3.2.4 Allergens

It has been thought that genetically modified organisms would be inherently allogenic and /or harmful. There has however, been no evidence to suggest that GMOs in general are any different from "normal" foods in terms of toxicity or allogenic potential. Many of the genes used to modify plants occur naturally in plants or in the viruses and miocroorganisms that infect them, meaning that humans will have already been exposed to them.

3.2.5 Non-target beneficial insects will be killed

It is thought that beneficial insects will also be killed by feeding from plants which have been genetically modified to be resistant to certain harmful insects. In reality, the opposite appears to be occurring since the crops are not being sprayed with an insecticide, and so the beneficial insects are returning, together with bird species, some of which have not been seen in areas for many years. Such plants have been transformed with a gene encoding the Bt (*Bacillus thuringiensis*) bacterial toxin which allows the plant to have resistance to certain insects. A study was conducted in the USA to determine whether Monarch butterflies feeding from the pollen of such genetically modified plants would be adversely affected by the toxin. It was shown that inside maize fields the build-up of Bt pollen was not sufficient to pose a threat to Monarch butterflies (Thompson, GM debate, UCT, 2000).

3.2.6 Controversies surrounding GM wines

In an article entitled "GM grapevines and toxic wines" by Prof. Joe Cummins and Dr. Mae-Wan Ho, they state that "the DNA from a GM grape persists for over a year after wine fermentation, thus contradicting claims that wine fermentation eliminates DNA. GM DNA in wine carries all the risks of horizontal gene transfer and recombination, creating new viruses and bacteria that cause diseases and the triggering cancer in the case of GM DNA with strong promoters jumping into the genome of human cells. Other potential hazards from GM grapes are toxins and allergens from the transgene products, or from unexpected metabolic disturbances to the host plant".

An extensive hazards analysis was carried out by Plahuta and Raspor in 2007, comparing the hazards to human health and the environment from six wines which had been produced by different viticultural and winemaking practices, namely: organic, the integrated production of grape and wine (IPGW), biodynamic, conventional, and two modern methods using genetically modified organisms (polysaccharide degrading yeast and grapevines resistant to Grape Fan Leaf Virus (GFLV)). The polysaccharide degrading yeast strain, which is capable of degrading natural wine polymers (glucans, xylans, pectins), was produced by integrating the *Trichoderma reesei xyn2* xylanase gene and the *Butyrivibrio fibrisolvents end1* glucanase gene into the genome of the commercial wine yeast strain *Saccharomyces cerevisiae* VIN13 (Louw *et. al.* 2004). Successful field trials of GFLV-resistant grapevines have been performed (Vigne, Komar and Fuchs, 2004) where it is hoped that the elimination of GFLV will induce a strong modification of vine behaviour, an increase in cane length and leaf surface, and a dramatic increase in vigour and yield. The risk assessment was performed in 4 steps: *Hazard identification* (whether a substance is causally linked to particular health effects), *Dose response evaluation* (relationship between the magnitude of exposure and the probability of occurrence of the adverse effect), *Exposure assessment* (magnitude, frequency and duration of exposure), *Risk characterization* (quantitative assessment of the probability of an adverse affect under defined exposure conditions).

It was actually shown that the highest risk level was in the case of biodynamic wines, however the differences were not significant. The authors stated that DNA from GMOs is equivalent to DNA from existing food organisms which form part of human diet and that the human body processes all DNA in the same way. DNA also gets broken down during digestion which reduces the likelihood of intact genes being transferred to gut micro flora. DNA and proteins are mostly removed from wine through the processes of fining and filtration and self-clarification. The authors also stated that there is no evidence that recombinant proteins in newly developed foods are more allergenic than traditional proteins (Lehrer, Horner and Reese, 1996).

3.2.7 Groups against GMO usage in South Africa

In South Africa, a group known as SAFeAGE (South African Freeze Alliance on Genetic Engineering) has been formed who has strong views against the use of genetically modified organisms. They claim 10 reasons why South Africa does not need GM foods, which include the following (www.safeage.org):

- GM foods won't solve the food crisis – GM companies benefit from increased profits, but the poor remain hungry.
- GM crops do not increase yield potential – GM has not increased the yield potential of any commercialized crops.
- GM crops increase pesticide use – Official data from the US shows this.
- There are better ways to feed the world – GM crops have little to offer global agriculture, because better alternatives are available.
- Other farm technologies are more successful – e.g. Integrated Pest Management, organic methods for controlling pests, and Marker Assisted Selection for plant breeding.
- GM foods have not been shown safe to eat – GM foods have undergone little rigorous and no long-term safety testing.

- GMOs are in animal feed without consumers' consent – Meat, eggs and dairy products from animals raised on GM feed have been imported into Europe and do not have to be labeled.
- No one is monitoring the impact of GM foods on health – Americans have eaten GM foods for years with no ill effects, but they are unlabelled and no one has monitored the consequences.
- GM and non-GM cannot co-exist – GM contamination of conventional and organic food is increasing.
- We can't trust GM companies – Big biotech firms have a terrible history of toxic contamination and public deception.

3.3 Benefits surrounding GMOs

Genetically engineered plants have been engineered to possess several desirable traits, including resistance to pests, herbicides or harsh environmental conditions, improved product shelf life, and increased nutritional value. The first commercial cultivation of genetically modified plants was in 1996, since then plants have been modified to be tolerant to herbicides, to be resistant to virus damage as in papaya resistant to Ringspot virus, and to produce the *Bt* toxin, a potent insecticide.

3.3.1 Benefits to the environment

Although GMOs are thought by some to be harmful in that they will lead to an increased use of herbicides or insecticides, it is also believed by many that the inverse will indeed be true, and that a reduction in the use of these chemicals will actually be the case. At a media Round Table discussion held by the Department of Science and Technology, South Africa, in June 2008, the South African Agency for Science and Technology Advancement (SAASTA) claimed that there will be a reduction in the exposure to pesticides and herbicides with the use of GM crops, and that the environment would be healthier overall (www.pub.ac.za). SAASTA's mandate is to "Promote public awareness, appreciation and engagement of Science, engineering and technology (SET)" and they are the official vehicle for facilitating the promotion of SET in the SA society. SAASTA also state that costs will be reduced in the form of water, labour and fuel; CO_2 emissions will be reduced; there will be a reduction in yield losses from pest damage and a reduction in post harvest losses; there will also be a reduction in the loss of top soil due to a reduction in tillage.

3.3.2 Increase in food security/ quality

At the same round table discussion mentioned above, SAASTA stated that the development of GMO crops could lead to increased yield and thus also an increase in food security. They believe that more food could be generated from less land and healthier livestock. There could also be an increase in the usage of marginal lands such as dry areas, which were previously unusable. SAASTA believe that the quality and safety of seeds and crops will be improved, in that identification and reduction of allergens will be possible by gene "knockout" technology. Certain crops can also be enriched for micronutrients, thus improving nutritional quality of food products.

3.3.3 Benefits surrounding GM wines

3.3.3.1 Grapevines resistant to pests and diseases

Grapevine health is threatened by several fungal, viral and bacterial diseases. Substantial advances in the development of resistant, genetically modified grapevine lines have been made in Chile, France, Germany, South Africa and the U.S.A. (Vigne, *et.al.* 2004). Increased resistance to pathogens could result in the reduced use of agrochemicals and fungicides and will have a positive impact on conservation of the environment and production costs.

3.3.3.2 Benefits of yeast genetic engineering

In modern wineries, reliable fermentations are essential in order to obtain consistent wine flavour and predictable quality. There is thus a demand for wine yeast strains which are optimized for specific tasks set out by winemakers. In general, five major targets for the genetic improvement of wine yeast strains have been identified which could add benefit to the wine industry: 1). Efficiency of the fermentation process; 2). Processing of wine; 3). Wholesomeness; 4). Sensorial quality; and 5). Control of microbial spoilage (Pretorius and Bauer, 2002). In a study where genetically modified yeast strains were compared to non-GM yeast strains, it was found that the differences between them were no more than the normal variation usually observed between yeast strains bred from different parents (Bauer *et. al*., 2004). Benefits of the genetically modified yeast, ML01, which is commercially available in some countries include: reduced wine processing time, reduced risk of spoilage, and reduced health risks (Bauer, Media Round Table 2008). It is believed that genetic modification of yeasts could also lead to the following benefits:

- Increased levels of healthy substances
    - Phenolic compounds
    - Resveratrol (stilbenes)
- Reduced levels of unwanted compounds
    - Carcinogens e.g. Ethyl carbamate
    - Neurotoxins e.g. Biogenic amines
    - Asthmatic chemical preservatives e.g. Sulfites

3.3.3.3 Improvements in Lactic Acid Bacteria

Lactic acid bacteria, especially *Oenococcus oeni*, are used for malolactic fermentation of wine. In addition, they also play a very important role in wine making due to the characteristic aroma profile they create in the wine. Lactic acid bacteria have been found to produce antimicrobial agents that can inhibit the growth of spoilage lactic acid bacteria, and thus might decrease the levels of sulphur dioxide used in wine. The complete genome sequence of *Oenococcus oeni* was published in 2005 (Mills *et.al*, 2005), and direct genetic improvement protocols for this bacteria are being developed. Countries involved in the improvement of lactic acid bacteria strains for wine making includes Australia, France, Germany, Italy, South Africa and the USA.

CHAPTER 4

GMOs IN THE WINE INDUSTRY

4. GMOs in the Wine Industry

4.1 General Introduction

Vitis vinifera is thought to have originated in Europe and consists of approximately 5000 cultivars used in wine, table and dried grape industries around the world (Jackson, 1994). Initial improvement of grapevines was reliant on random selection of natural mutations which led to an improvement in cultivation and/or some aspect of the fruit quality. Grapevine improvement has remained relatively untouched however by classical breeding programs, and few new cultivars e.g. Pinotage, have become commercial successes. In comparison, classical breeding has played a major role in the development of rootstock varieties which are resistant to soil-borne pests and pathogens (Mullins, 1992). Plant genetic engineering has been proposed to have potential for grapevine/wine improvement in the wine industry (Vivier and Pretorius, 2000).

4.2 Genetically modified grapevines

Vitis vinifera consists of 38-40 chromosomes, the entire DNA sequence of which has recently been determined (Jaillon *et. al*. 2007). Worldwide, all major viticultural research centres are carrying out some form of grapevine biotechnology, including Australia, Chile, France, Germany, Italy, South Africa, Spain and the USA. Initial studies of plant genetic engineering proved difficult since *Vitis vinifera* is a woody perennial, and hence difficult to genetically manipulate (recalcitrant). Only a few limited successes have been reported and there is still no universal protocol for genetically modifying all grapevine cultivars (Vivier and Pretorius, 2002). The first significant progress was made in 1989 using embryonic cell suspensions derived from the plant's anthers as the target tissue for grapevine transformations. Both the techniques of *Agrobacterium*-mediated transformation and particle bombardment have been used for such experiments (Perl and Eshdat, 1998).

Research has been conducted into the genetic modification of grapevines for fungal, viral and bacterial resistance. Field trials have been underway from 1994-2004 in the EU (France 4; Italy 1; Germany 1), and from 1995-2008 in the USA (53 trials). Trials are also being carried out in other countries such as Canada, South Africa and Australia.

There were 25 field test releases in USA between 1999 and 2005, and a small number of commercial releases are expected any time now. The bulk of the test releases were of GM grapes resistant to diseases including powdery mildew, *Botrytis*, *Agrobacterium*, *Clostridium*, *Xylella*, nepovirus and closterovirus. The majority of these applications were from Cornell, California and New York State Universities, the rest were from vintners or wine research companies.

In South Africa, genetically modified grapevines have recently been planted at Welgevallen, an experimental farm at the University of Stellenbosch. It was at this farm in 1925 that viticulturist, Professor Izak Perold, crossed Hermitage and Pinot noir to create South Africa's unique home-grown cultivar, Pinotage. In addition to Welgevallen, the Oenological and Viticultural Research Institute was established in 1955 at Nietvoorbij, outside Stellenbosch. This institute forms part of the Agricultural Research Council (ARC) of South Africa and is involved with basic viticultural and oenological research from a practical "industry-based" perspective. In 1995, the Institute for Wine Biotechnology (IWBT) was also established, supported by funding from the national government and the University of Stellenbosch, with Professor Isak Pretorius of the Department of Microbiology, as the founding director. This institute's vision is to become a nationally and internationally competitive centre of excellence in wine and grapevine biotechnology, and offers postgraduate research for honours, masters and doctoral degrees (Moore *et. al.* 2008). In South Africa, Winetech (Wine Industry Network of Expertise and Technology) has also been established, which is an organization performing research into various aspects of genetically improving organisms for the wine industry. Their program involves "Improving grapevine, wine yeast and bacteria for a quality focused, market directed wine industry". The mission of Winetech is "To provide the South African wine industry with a sustainable basis of forefront technology and human resources in order to strengthen both local and international competitiveness and profitability". The members of Winetech are SALBA, VinPro and WCSA. Winetech claims that all research projects in their program that will involve the use or production of genetically modified organisms will be conducted in facilities that have been registered in terms of Regulation 4 of the Genetically Modified Organisms Act, 1997 (Act No.15 of 1997) with the National Department of Agriculture, Directorate: Genetic Resources.
The components of their program are shown below (Fig 4.1.):

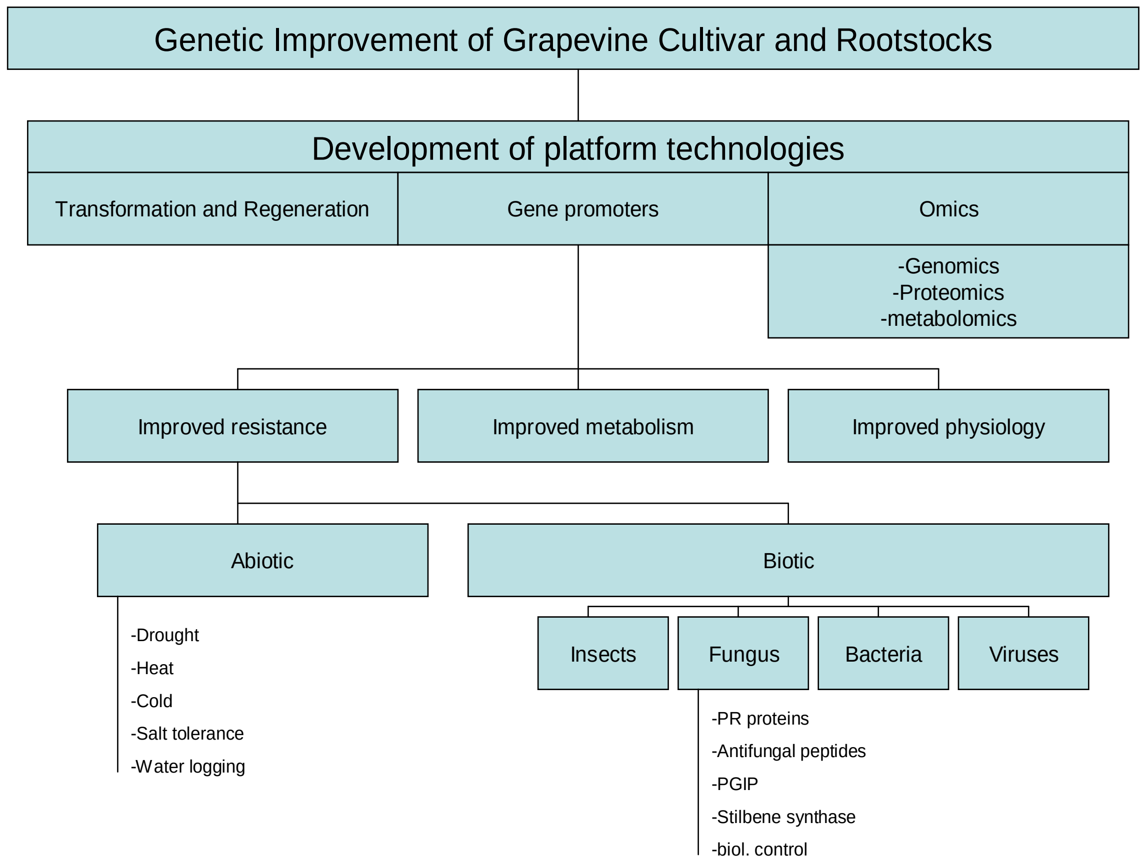

Figure 4.1. Winetech's program for genetic improvement of grapevine cultivars and rootstocks

4.2.1 Fungal resistance

Since 1999 there have been field trials with gene-modified fungal-resistant grape vines in two areas in Germany, the Pfalz and Franken. The trials were planned to last for at least 10 years, and examined mainly the varieties Riesling and Chardonnay, for which it had not been possible previously to breed fungus-resistant vines (e.g. against grey mould, powdery and downy mildew). The trials however, were suspended at the beginning of 2005. Recently however, new trials have been initiated in Germany where the vines have been modified with genes from barley, to protect the grapes from fungal infections. The Institute of Wine Cultivations said there would be no noticeable difference in the wine's taste. The first wine made from these altered grapes will most likely only be available in several decades (Food and Drink weekly, Aug 1999). In the

USA, the following universities have research projects underway for fungal resistance in grapevines: University of California Davis, University of Florida, Cornell University, and the University of Missouri.

In South Africa, a field trial for fungal resistance is being carried out on the Welgevallen Experimental Farm in Stellenbosch. One hundred non-transgenic and virus-free 238 US Vit8-7 rootstocks have been planted and onto these, transgenic Chardonnay or Sultana has been grafted. The grape vines have been genetically modified with a grape gene (Vvpgip) which protects against fungal pathogens, and a grape gene (VvNCED) which protects against water stress. The vines will be monitored for at least 5 seasons as to their performance (www.winemag.co.za; December 2009). The IWBT is largely focusing on fungal resistance to grey rot (*Botrytis cinerea*), as well as powdery and downy mildew. They believe the production of fungal disease resistant plants using transgenic technology is an attractive alternative to chemical treatments and should encourage environmentally friendly practices in the vineyard (Moore *et. al.* 2008). The IWBT is focusing on the use of chitinases (Carstens *et. al*. 2003), polygalacturonase-inhibiting proteins (PGIPs, as mentioned above) (Joubert *et. al.* 2006) and antifungal peptides (DeBeer *et. al.* 2008) to establish fungal resistance in transgenic grapevines. The following projects in the Winetech program, together with the IWBT, have been completed:

- The cloning and characterization of the polygalacturonase-inhibiting protein (PGIP) encoding gene in *Vitis vinifera.* (IWBT 4/3)
- The transformation of grapevine with yeast glucanase and chitinase genes. (IWBT 4/6)
- The expression of antifungal peptides in grapevine cultivars. (IWBT 4/7)
- The isolation, cloning and characterization 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)

The following projects in the Winetech program, together with the IWBT are still ongoing:

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

4.2.2 Virus resistance

Grapevines with genetically engineered resistance to Fanleaf degeneration caused by a virus are being tested in field trials in Colmar (Alsace, France). The virus is transmitted by nematodes and is the most widespread nepovirus involved in grapevine degeneration.

In South Africa, Winetech established a “Grapevine Virus research program” in 2000 which “aims to alleviate the serious virus disease problems in the South African wine industry by thorough characterization of grapevine viruses, to develop strategies to manage the diseases they cause, to prevent further spread of these diseases, and to use the latest technologies for the establishment of genetic virus resistance in wine grape cultivars in the longer term” (www.winetech.co.za). The following projects are included in this program and have been completed:

- The establishment of efficient transformation and regeneration systems for grapevines. (IWBT 4/1)
- The grapevine transformation and regeneration program. (IWBT 4/11)
- The molecular characterization and transformation of the grapevine chloroplast genome. (GENUS 2/2)
- The establishment of stable and synchronous embryogenic cell lines of grapevine rootstock cultivars for use in transformation systems. (IWBT 4/10-2005)
- The evaluation of transgenic grapevines. (IWBT 5/10)
- 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)
- A pathogen-derived resistance strategy for the broad-spectrum control of grapevine leafroll disease. (IWBT 4/8)

4.2.3 Resistance/ tolerance to abiotic stress

Apart from biotic threats such as viruses, fungi, bacteria and insects, grapevines are also threatened by abiotic stresses such as drought, heat, cold, water logging and salt. Grapevines can be affected significantly by water loss in the form of factors such as canopy growth, bunch quality and photosynthetic efficiency. The IWBT is researching into the development of grapevines with enhanced capabilities to grow under adverse conditions including water stress, and are studying the carotenoid biosynthetic pathway of grapevines in this regard. This pathway has been found to produce compounds involved in environmental stress responses (Moore *et. al.* 2008). Examples of genes which have been isolated in the IWBT biotechnology program include:

- Carotenoid biosynthetic pathway: *VvPSY, VvPDS, VvZDS, VvCiso, VvLECY , VvECH, VvLBCY, VvBCH, VvZEP , VvVDE , VvNSY* .
- Abscisic acid biosynthetic pathway*: VvNCED, VvCCD.*

These genes have been used in the following completed projects:

- Isolation and characterization 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)
- Metabolic engineering of grapevine towards enhanced abiotic stress resistance and improved quality parameters. (IWBT - P 08/13) – project ongoing.

4.2.4 Quality traits and plant development

Australia currently has 4 GM field trials underway. They have field-tested grapes which have had their composition modified of components such as sugar content, colour and grape size. It is believed that modifying flower and fruit development may allow an increase in the harvest. These trials were applied for by the CSIRO Plant Industry in South Australia.

In South Africa, IWBT are using their research into the carotenoid biosynthetic pathway to also investigate quality parameters of grapes. These metabolic pathways have been found to also be involved in quality aspects and are thus being investigated as to their ability to improve flavour and aroma compounds in grapes. Carotenoids serve as important precursors for apocarotenoids, which are involved in a wide range of functions in plants including pigments, flavours and aromas. The following projects have been completed through Winetech and IWBT involving the improvement of plant/ fruit metabolism:

- 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)

4.3 Genetically modified Yeasts

Yeasts are single-celled organisms that are classified as fungi. According to American Tartaric Products, the first genetically modified wine yeast, ML01, was released in 2005 by Springer Oenologie (a division of Lesaffre Yeast Corporation) (Napa Valley Register, Erica Martenson, December 2006). This yeast was first available only in North America where GMOs were unregulated, but has since spread to other countries. The yeast was developed by Dr. HJJ van Vuuren from the Wine Research Centre, University of British Columbia, Vancouver, Canada, and was modified by inserting two foreign genes, one from the pombe yeast, a yeast found in Africa and used to make beer, and one from the bacteria *O. oeni*, so that the alcoholic and malolactic fermentations, could occur at the same time. This may thus be a convenience to winemakers, especially those producing large quantities of wine. In 2003, the FDA designated the yeast as GRAS (generally recognized as safe), however, Professor Joseph Cummins, genetics professor at the University of Western Ontario, has stated that wine yeasts are unstable, and that by genetically altering them, it could lead to unexpected toxicity in the final product. There has also been concern that should certain wineries choose to use ML01, the GM wine yeast could contaminate native and traditional wine yeasts through the air, surface waste and water runoff. Many wineries in the Napa Valley are very particular about their choice of wine yeast, and contamination of these other yeast strains would be undesirable to them.

There are now two commercially available GM yeasts on the market. One such yeast has been genetically manipulated to better degrade urea during the wine making process (*Saccharomyces cerevisiae* strain ECMo01). The benefit of such a characteristic is that the wine contains less ethyl carbamate, a chemical considered by some regulatory bodies to be a human health risk. In January 2006, this yeast strain was declared GRAS by the FDA. The second GM yeast (ML01, mentioned above), has been designed to allow malolactic fermentation to proceed more efficiently, thereby producing fewer biogenic amines, such as histamines, which cause headaches and asthmatic-type reactions in some people. Other experiments to genetically modify yeasts include improvement in culture maintenance and the viability of cells, improved yield of fermentable sugars and an improved assimilation of nitrogen. They have also involved an improved tolerance to anti-microbial substances, a reduction in the formation of foam, improved flocculation ability, and an improvement in wine processing with the use of polysaccharide degrading yeast strains which are capable of degrading natural wine polymers (glucans, xylans, pectins) (Barre *et. al*. 1992; Pretorius, 2000; Vivier and Pretorius, 2000). In addition, some yeasts have been genetically modified in order to correct wine acidity (Dequin *et. al.* 1999).

In South Africa, Winetech is also performing research into various aspects of genetically improving yeasts for the wine industry. The components of their program are shown below:

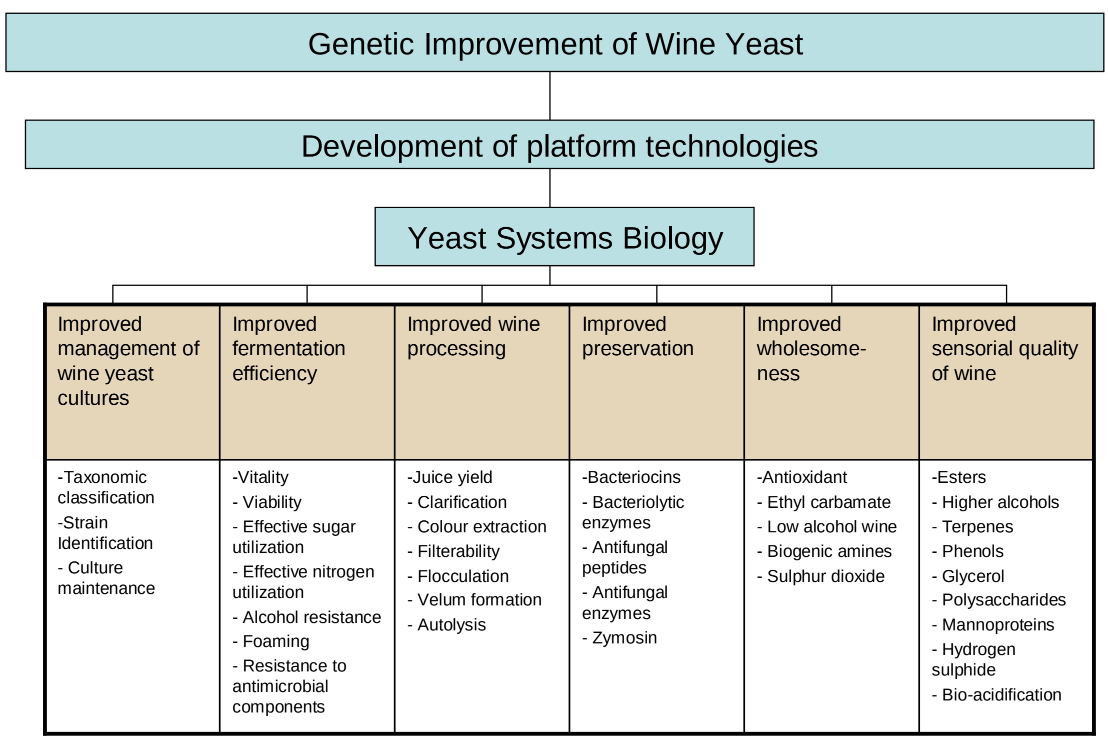

Figure 4.2. Winetech’s program for genetic improvement of wine yeast

A main focus of the current program is the generation of yeast that would yield lower levels of ethanol. With South Africa being a warm climate region, harvested grapes produce, on average, higher alcohol levels than a cool climate region would. The IWBT has thus been researching into the development of genetically modified yeast containing the enzyme glucose oxidase which converts excess sugar during fermentation into gluconolactone, and in thus doing, prevents excessive alcohol production (Malherbe *et. al*. 2003). A second project has involved the introduction of polysaccharide degrading enzymes such as polygalacturonases, xylanases and cellulases into yeast cells. During fermentation, grape polysaccharides are extracted into the wine medium and tend to block filters and can impede certain processing steps during winemaking. Thus, the generation of a yeast which would be able to both ferment the must as well as degrade polysaccharides would be beneficial to the wine industry.

Yeasts which are capable of degrading polysaccharides may also be able to increase the "fruity" bouquet of a wine. Wine yeast strains produce many of the aroma and flavour compounds which lead to the characteristics of specific wines, such as esters, higher alcohols, acids, glycerol and terpenoids (Lambrechts and Pretorius, 2000). Genetic modification of certain metabolic pathways may thus be able to favour production of certain desirable compounds. In addition to aroma compounds, yeast has the potential to produce compounds of human medical importance such as resveratrol, which has been positively correlated with reduced cardiovascular disease (Becker *et. al*. 2003). The IWBT has incorporated a co-enzyme A ligase encoding gene, 4CL216, and the grapevine resveratrol synthase gene, *vst1*, into *Saccharomyces cerevisiae* in order to increase the amounts of resveratrol produced in wine (Becker *et. al.* 2003). Lastly, the IWBT has also genetically engineered yeast cells which are capable of controlling the growth of spoilage bacteria by secreting enzymes which specifically inhibit the growth of unwanted organisms (Du Toit *et. al.* 2000).

The following projects concerning **improved fermentation** performance of wine yeast have been completed through Winetech:

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

The following projects are ongoing:

- Nitrogen metabolism, fermentation efficiency and stuck fermentation. (IWBT – Y 08/03)
- Selection, breeding, evaluation and characterization of new wine yeasts. (ww10/01)
- The use of fructophyllic yeasts to prevent lagging fermentations. (ww10/17)
- The use of *Torulaspora delbrueckii* for wine production. (ww10/21)

For **improved wine processing**, the following projects have been 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)

The following projects involving improved wine processing are 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)

For **improved wine preservation**, the following projects have been completed:

- 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)

For **improved wine wholesomeness**, the following projects have been 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)
- Low ethanol yielding yeast. (IWBT – Y 08/01) – project ongoing.

For **improved sensorial quality** of wine, the following projects have been completed:

- The cloning and transformation of brandy yeasts with the alcohol acetyl transferase gene for improved ester formation. (IWBT 2/2)
- 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)

The following projects are 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)
- Understanding and controlling acid production and consumption by wine yeast strains. (IWBT - Y 08/02)

4.4 Genetically modified wine bacteria

Lactic acid bacteria have historically been associated with food and beverage fermentations as they occur naturally in the starting materials used. Lactic acid bacteria occur in must and wine and perform the secondary fermentation known as malolactic fermentation. They are also considered beneficial to the wine's sensory qualities due to flavor modification, and have been found to control spoilage bacteria. *Oenococcus oeni* is a lactic acid bacteria used commercially in the wine industry. Internationally, rapid progress has been made in the last 10 years in the development of tools for the genetic modification of lactic acid bacteria. Countries involved in the improvement of lactic acid bacteria strains for wine making include Australia, France, Germany, Italy, South Africa and the USA. Major targets of the IWBT are to select for strains that are better adapted as starter cultures for malolactic fermentation. They have also been investigating specific enzymes that are involved in the production of wine aroma compounds, and bacteriocins that can be used as an alternative to chemical preservatives. The genome sequences of several lactic acid bacteria have recently become available contributing to the study of genes of these bacteria. Winetech's program for genetic improvement of wine bacteria can be found in figure 4.3.

Genetic Improvement of Wine Bacteria

Development of platform technologies

Lactic Acid Bacteria

Improved management of cultures	Improved fermentation efficiency	Improved preservation	Improved wholesome-ness	Improved sensorial quality of wine
-Taxonomic classification -Strain Identification - Culture maintenance	- Viability - Effective malic acid utilization - Effective nitrogen utilization - Alcohol resistance - Resistance to antimicrobial components	-Bacteriocins - Bacteriolytic enzymes	- Ethyl carbamate - Biogenic amines	-Diacetyl - Higher alcohols - Volatile Phenols - Glycerol degradation - Volatile sulphur - Bio-acidification

Figure 4.3. Winetech's program for genetic improvement of wine bacteria.

The following projects concerning genetic improvement of wine bacteria have been completed through Winetech:

- Establishment of the metabolic profiles of lactic acid bacteria. (IWBT B – 08/10)
- Control of bacterial spoilage in wine with natural (biological) antimicrobial peptides produced by lactic acid bacteria. (MIC/02)

- Improving the control of wine spoilage microorganisms. (IWBT 5/04) – project ongoing.
- 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) – project ongoing.

CHAPTER 5

LEGISLATION AND REGULATIONS SURROUNDING GMOs

5. Legislation and regulations surrounding GMOs

5.1 South Africa

South African parliament passed the GMO Act (No. 15) in May 1997, and the Regulations in November 1999. Contraventions of the Act can result in a fine or imprisonment of up to four years. Implementation of this Act is measured through import, export, production, release and distribution of GMOs. The Act also lays down criteria for risk assessment and details the measures to evaluate and reduce potential risks. Monitoring and regulation of GMOs must be enforced due to the government signing the Cartagena Protocol on Biosafety (2003).

With regards to labeling of foodstuffs in SA, a food product obtained through certain techniques of genetic modification shall not be sold unless the foodstuff is labeled to inform the consumer of the genetic modification (Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972)). This regulation holds true if the following criteria apply:

- the composition differs significantly from the corresponding existing foodstuff;
- the nutritional value of such a foodstuff differs significantly from the characteristic nutritional value of the corresponding existing foodstuff;
- the mode of storage, preparation or cooking of such a foodstuff differs significantly from that of the corresponding existing foodstuff;
- the foodstuff contains an allergen;
- a foodstuff is derived from:
    - plant material containing animal nucleic acid(s) or protein(s) derived from a human or from an animal;
    - animal material containing animal nucleic acid(s) or protein(s) derived from a human, or from a different taxonomic animal family.

In South Africa, should an application be submitted for a GM crop field trial, the following guidelines are applicable (GMO Amendment Act 23 of 2007):

- A Public Notification must be placed in 3 local newspapers in the area where the permit release is contemplated.
- Details of the name of the applicant, background, objective, location and general information (predominantly scientific) must be present on the application.
- A call for comments from interested parties must be allowed, with a minimum of 30 days within which to provide comments to the Registrar.

Section 33 of the Constitution entitles South Africans to procedurally fair administrative action. This means that people affected by administrative decisions must be given advanced notice of these decisions, and must be given the opportunity to make representations to the decision-maker. In addition, article 23 of the Cartagena Protocol obliges South Africa to ensure that there is public participation and awareness concerning the use of GMOs, and to consult the public in decision-making processes.

In South Africa, GM cotton and yellow maize which are resistant to insects and herbicides have been commercially available since 1997, the latter amounting for about 2% of the total crop. A number of other GM crops are currently undergoing field trials (Maize – drought tolerance, sugarcane – antimicrobials, potato – insect resistance, cassava – improved starch content). In 1988, trials of insect resistant cotton were conducted in the Makatini flats of KwaZulu Natal, and showed a significant decrease in the use of insecticides and an increase in yield of between 18-23% (Thompson, 2000, University of Cape Town GMO debate). The total SA area planted with GM crops is as follows (Clive James, 2007; www.pub.ac.za):

- White maize: 1, 040 million hectares, 62% crop
- Yellow maize: 567 000 hectares, 52% crop
- Soybean: 144 000 hectares, 80% crop
- Cotton: 10 000 hectares, 90% crop *(90% small scale farmers)*
- <u>Total Area : 1,8 million hectares</u>

In the SA wine industry, it is illegal to use GM products. The Wine and Spirit Board has recently announced that it will not allow the use of GM grapes or GM organisms in the production of certified wines, until it is internationally acceptable. The genetically modified yeast, ML01, has been imported into South Africa by Warren Chem Specialities, however in 2007, an application to allow usage of the yeast was denied. Experiments which are being conducted by the Institute for Wine Biotechnology (IWB) at

Stellenbosch University have been appealed against by 3 environmental lobby groups (SAFeAGE, Earthlife Africa, and the Africa Centre for Biosafety), primarily because they believe that adjacent vines will become contaminated.

5.2 New World countries

5.2.1 USA and Canada

The USA, Canada, Argentina and Brazil are the major cultivators of GM-crops worldwide, and export them as food and feed. In the USA, the concept of "substantial equivalence" has facilitated the science-based safety testing of GM-crops and, once declared substantially equivalent to its conventional counterpart, a GM crop may be grown and marketed under normal food-safety regulations, in the same manner as the parent plants. As a consequence, and in contrast to the EU, the USA has no GMO thresholds or obligatory GMO labelling. Attempts to introduce state legislation on GMO labeling, e.g. in Oregon, have been unsuccessful.

In the USA and Canada, the GM yeast (ML01) has been approved by the FDA and is commercially distributed on the market. It is said that wine made with this kind of yeast distinguishes itself not only through its better taste and colour stability, but also through a lower concentration of histamines. The extent of actual use of ML01-yeast by US-American wine producers is unknown. Many of them, especially from California, have joined a declaration saying that they are not using GM organisms in their wine. For example, Mendocino County became the first state to ban the production of GMOs in 2004, with a 57% majority vote. Other counties in California such as Trinity and Marin have also imposed bans on GM crops, however for Butte, Lake, San Luis Obispo, Humblodt and Sonoma counties, this was denied. The following wine producers in the U.S have been identified as not using GM yeasts: Atlas Peak Vineyards; Beaulieu Vineyards, Beuna Vista Carneronos, Chappellet Winery, Charles Krug Winery, Clos Du Val Wine Company, Clos Pegase Winery, Duckhorn Vineyards, Far Niente, Flora Springs, Grgich Hills Winery, Groth Vineyards, Heitz Wine Cellars, Joseph Phelps Vineyards, Madonna Estates, Markham Winery, Merryvale, Quintessa, Robert Mondavi, Rothschild/ Mondavi (Opus One), Rubicon Estate Winery, Shafer Vineyards, Silver Oak, Spring Mountain Vineyard, Sterling Vineyards, Trefethen Vineyards, Tres Sabores, V. Sattui Winery, Wlliam Hill Estate.

In Canada, a GM yeast has been developed which can be used in the production of red wine. The modification aims to reduce the concentration of the cancer causing substance, Ethyl carbamate. The GM yeast is permitted in Canada and recognized as safe in the USA (GRAS). Concerning the use of the GM yeast ML01, the organization Health Canada notified Dr. Hennie van Vuuren, University of British Columbia, that it had no objection to the use of the genetically modified wine yeast in winemaking, and of wines derived from the use of this yeast. The Department conducted a comprehensive assessment of wine yeast ML01 according to its *Guidelines for the Safety Assessment of Novel Foods*. These Guidelines are based upon internationally accepted principles for establishing the safety of foods with novel traits (www.hc-sc.gc.ca/fn-an/gmf-agm/appro/nf-an107decdoc-eng.php).

Mainland Canada is also one of the world's largest producers of GM canola. In 2005, the government of Prince Edward Island in Canada assessed a proposal to ban the production of GMOs in the province, however, the ban was not passed (CBC news, December 2nd 2005). Since then, the use of GM crops on the island has rapidly increased.

5.2.2 Australia and New Zealand

In 2003, several states of Australia placed bans on the planting of GM crops (www.parliament.nsw.gov.au), however, in late 2007 the states of New South Wales and Victoria lifted their bans. On 23rd December 2008, the Western Australia Minister for Agriculture and Food Media released a press statement that they were lifting their ban on the planting of GM crops, however, South Australia still continues its ban and Tasmania has extended its moratorium until November 2014. The state of Queensland has allowed the growing of GM crops since 1995 and has never had a GM ban.

Regardless of the state, before any GMOs (and GMO-derived products) can be used in food production or processing in Australia they are subjected to prescribed risk assessments, and considerable public consultation is required during this process. On November 23rd 2005, the Australian Wine Research Institute released a press statement saying that "The Australian wine industry's position on the application of gene technology in grape and wine production is that no genetically modified organisms are to be used in the production of Australian wine". The research institute stated that they are not against GM foods/ wine and agree that the technology has potential benefits, but rather that they acknowledge the importance of safety and public acceptance before adopting any new technology in wine production.

Australia's national regulatory system for gene technology includes the Gene Technology Act 2000 which took effect on 21st June 2001. This Act is supported by the Gene Technology Regulations 2001, which is an inter-governmental agreement and corresponding legislation that is being enacted in each state and territory. Its objective is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and managing those risks by regulating certain dealings with genetically modified organisms.

New Zealand has taken the standpoint that no genetically modified food is to be grown in the country, and that no medicines containing live genetically modified organisms have been approved for use.

5.3 Old World countries

The European Regulatory System is one of the strictest in the world and states that GMOs must receive authorization before they enter the market. This applies to GMOs used in food, feed and seeds for GM crops. In 2004, a new fundamentally revised legal system took effect in all EU member states. The essential foundations of this legal system are strict safety standards and freedom of choice for consumers and farmers (GMO Compass; www.GMOdatabase.mht). Approval for use of GMOs is granted only under certain conditions by the EU's main risk assessment organization: European Food Safety Authority (EFSA):

- Safety – the product must be safe and cannot pose a threat to human or animal health. It must also be safe for the environment.

- Freedom of choice – Consumers, farmers and businesses must be given the freedom to either use or to reject the products.

The EU has stringent regulatory processes, requirements and labeling and according to Regulation (EC) No 1830/2003, products containing more than 0.9 percent of genetically modified ingredients must be labeled with the following statement: “This product contains genetically modified organisms.” A summary of international GM labeling regulations can be found in table 5.1.

Commercial use of GM grapevines in the EU must have obtained authorization, and wine made out of these kinds of vines would have to be labeled as such. Imported wine from GM vines must also be authorized and labeled appropriately. In the EU, there are a number of directives with regards to GM foods and feed in connection with additives and processing aids. A processing aid is defined as a substance added during processing with a technological purpose and which is eliminated during the process. One EU directive (Regulation 1829/2003) states that: “when a GM micro-organism is used as a processing aid, the food and feed resulting from such production are considered as not falling under the scope of the Regulation”. The implication of such is that no labelling is required. For example, if a GM yeast was completely removed from the wine during winemaking and is thus no longer measurable, no declaration of the use of GM yeast has to be added on the wine label. This is because legally, yeast no longer has to be rated as an "ingredient for wine". A similar rule can be applied to imported wine, which neither has to be authorized nor labeled, in this case.

On 23 July 2003, the EU issued guidelines for the development of national strategies and best practices to ensure the coexistence of genetically modified crops with conventional and organic farming. The main objectives behind the recommendation were:

• No form of agriculture, be it conventional, organic or agriculture using genetically modified organisms (GMOs), should be excluded in the European Union.

• The European Commission considers that measures for coexistence should be developed and implemented by the Member States.

This recommendation introduced the concept of “coexistence” - that in principle, farmers should be able to cultivate the types of agricultural crops they choose. Measures for coexistence should be efficient, cost-effective and proportionate.

The EU member states have a certain degree of freedom when interpreting the EU's regulatory framework. They may accept EU regulations upon certain conditions, and each member state assigns its own national authorities to deal with GMOs. Below are examples of certain member states and their specific viewpoints as to GMOs.

5.3.1 France

France has very strict measures with regards to GM crops. Only one GMO has been authorized in France, Monsanto's MON810 corn, however this has since been banned by the French government on February 9th 2008.

5.3.2 Germany

The German public is generally opposed to the release of GM plants. While the government seeks to respect popular opinion, many current politicians support biotechnology, as they consider it an import factor for economic growth. GM maize has been commercially cultivated in Germany since 2004, and in 2005 it accounted for 0.1 percent of the country's total maize production.

5.3.3 Spain

In Spain, GM maize has been commercially grown since 1998, thus, it is the EU country with the longest practical experience in cultivating GMOs. In 2006, GM maize accounted for 60,000 hectares of farmland. There have also been many field trials, most being conducted with varieties of wheat and barley. Despite this, coexistence regulations have not yet been enacted. In July 2006, however, the Ministry of Agriculture presented a second draft of the Royal law on coexistence.

5.3.4 United Kingdom

The UK government is not fundamentally opposed to cultivating GM crops, but it has opted for a cautious approach. It has therefore restricted commercial production of biotech crops until coexistence rules with traditional crops are in place. In the meantime, however, the UK has been the site for the world's largest ever field study on GMOs.

5.3.5 Austria

The Austrian public is strongly opposed to genetically modified crops. With small average field sizes, few farmers believe that coexistence between organic farming, conventional agriculture, and GM crops can occur. The Austrian government's stance on the issue is in line with popular opinion and has banned several GMOs assessed as safe at the European level. There has been no commercial cultivation of GM plants in Austria to date.

The EU as a whole does not cultivate significant quantities of GM-crops, and consequently it depends heavily on imported GM-crops, particularly soybean for animal feed, from North and South America. Due to the EU having possibly the most stringent GMO regulations in the world as mentioned above (GMO safety, GMO thresholds, GMO labeling, GMO detection, and coexistence), these regulations are difficult to reconcile with its dependence on GMO imports. During the summer of 2008, the EU had serious discussions as to whether to raise the threshold level of imported GM-crops which had been authorized in other countries. A positive decision would have had a dramatic effect on EU imports but, eventually, the EU decided to maintain zero tolerance. The fact that Europe has become a risky destination for food exporters may lead to higher food prices.

Table 5.1. International GM labeling trends

Area	Labelling	Threshold
USA	Voluntary	5% GM material permitted
Canada	Voluntary	5%
Brazil	Mandatory	1%
China	Mandatory	1%
Japan	Mandatory	5% (3 main ingredients in product)
Thailand	Mandatory	5%
Taiwan	Mandatory	5%
EU	Mandatory	0.9% (authorized)

Data adapted from Viljoen *et al*. (2006), EU Joint research commission, Gruere and Rao (2007)

The International Organization of Wine and Vine (OIV) has been developed which is an inter-governmental organization aiming to contribute to the international harmonization of existing practices and standards concerning vines, wine, wine-based beverages, table grapes, raisins and other vine-based products (www.oiv.int\uk). The organization prepares new international standards in order to improve the conditions for producing and marketing vine and wine products, and helps ensure that the interests of consumers are taken into account. The OIV does not however, have authority to instigate laws regarding production practices in the individual member countries and is still in the process of developing a GMO policy (OIV Resolution VITI 1/2006). Membership to the OIV is voluntary and its recommendations are viewed as guidelines only. South Africa is a member of the OIV, whereas, the USA and Canada, where the GM yeasts have been approved for commercial use, are not members. The OIV currently supports research activities involving GM organisms, but not the commercial use thereof in wine making.

CHAPTER 6

OPINIONS ON THE USE OF GMOs IN THE SOUTH AFRICAN WINE INDUSTRY

6. Opinions on the use of GMOs in the South African wine industry

6.1 Consumer

A questionnaire was sent out by email to a number of wine consumers (Appendix 1a). A total of 25 responses were obtained and will be discussed in more detail. 92% of responders were aware of what a genetically modified organism is, 4% believed GMOs to be harmful organisms caused by tampering with nature, and 4% were unsure as to the proper definition of a GMO. In question two, 56% of consumers were not aware of any GMOs being utilized in the wine industry, 32% of consumers thought that GMOs were being utilized/ available, and 12% were unsure. Of those responses where the use of GMOs was known, the following GMOs were identified:

- "Various yeasts – fermentation under specific conditions".
- "GM yeasts, GM Malolactic bacteria, GM vines for increased resistance to disease. Improved efficiency and flavour development under alcoholic fermentation. Combined alcoholic and malolactic fermentation".
- "Yeasts – cells have been modified so that they can withstand higher concentrations of alcohol".
- "Transgenic grapevines – to try and breed fungal/ viral resistant vines".

In question four, 52% of consumers believed that the use of GMOs in wine would be beneficial to the **wine industry**, 12% thought they would be harmful, 32% felt there would be no significant impact, and 4% were unsure about the consequences. Reasons for GMOs being either beneficial or harmful to the wine industry are stated below:

- "Tampering with nature leads to disastrous results. Nature works best when left alone."

- “Better productivity and less disease problems in the wine industry”.

- “To increase sales to persons who previously would not buy due to allergies (e.g. sulphur)”.

- “Will allow winemakers greater control over winemaking process by providing predictable outcomes that are under the direct control of the winemaker. More consistent quality”.

- “To ensure that vineyards are not susceptible to viruses, disease or erratic climate. Modification can create more resilient vineyards. Fewer chemicals will be used”.

- “Streamlining the process, as the modified yeast is able to complete the fermentation step without additional bacteria required”.

- “Positive (improved success in wine production) and negative aspects (may put consumers off). Ultimately, I think these will balance each other out in terms of wine sales, and success of a wine estate”.

- “Science always helps, embrace the future”.

- “Would likely increase costs due to R&D, but supply/ demand of consumers could balance this out. Not concerned with GMO “scare stories” in media, as I believe GMOs are commonly misunderstood”.

- “Provided that correct and regulated procedures are put into place, can be incredibly beneficial for the wine industry in terms of flavour enhancement, longevity and added benefits such as antioxidants, fragrance etc”.

- “As long as the taste is not affected most people don’t care less”.

In question six, the same percentages relating to the wine industry were found to relate to the effects of GMOs in wine on the **consumer** (52% beneficial, 12% harmful, 32% no difference, and 4% unsure). Reasons for GMOs being either beneficial or harmful to the consumer are stated below:

- “Wine has been made for centuries without interference and should stay that way”.

- “I believe the long term effects of many GMOs are unknown”.

- “I don’t think the use of GMOs will affect the consumer. If GMO yeast is considered to optimize fermentation, it should not interfere with the end product”.

- “Some people may refuse to buy GM wine, which could hurt sales”.

- “An increase in the enjoyment of wine, flavour, fragrance”.

- “Larger quantities of wine should become available therefore prices could be reduced”.

- “Most consumers will be pleased with a uniform and predictable wine that can be made using GMO products”.

- “Fewer chemicals used on the grapes means less chance of toxins being carried across into the final wine product”.

- “When one hears “genetically modified”, it immediately raises concerns”.

- “Wine undergoes many clarification processes so that most debris, including cells, are removed from wine before bottling. One potential cause for concern is the formation of harmful/undesirable byproducts by one of these organisms. That said, one would assume that these undergo stringent testing before they are released for use in the production of beverages and food. I think that consumers may be put off by a label that says that a wine has been made using GMOs”.

- “GMO’s already don’t have a good effect on the food industry to the consumer. There are not enough studies on the long term affects of ingesting GMO’s, and the short term effects thus far are not good”.

For question eight, 68% of consumers said they would indeed purchase or consume a wine which was made using GMOs, 16% would not, and 16% were unsure. Despite this acceptance of GMOs, the vast majority of consumers were adamant that they would like the product to be labeled as having been made using GMOs (88%), 8% were not bothered about the labeling, and 4% were unsure. In question ten, it was asked whether the consumer would pay extra for a non-GM wine. 56% would not pay any extra, 12% were prepared to pay extra for a non-GM wine, and 32% were unsure.

6.2 Wine Producer

A questionnaire was sent out by email to 186 wine farms within South Africa (Appendix 1b). A total of 25 responses were obtained and the results thereof are tabulated in table 6.1. 87.5% of responses were aware of what a genetically modified organism is, 8% thought it to be a harmful organism, whereas 4% were unsure. For question 2, the vast majority of responses were not aware of any GMOs being utilized within the wine industry (75%), 12.5% of responses knew of some sort of GMO in the wine industry, whereas, 12.5% were unsure. Of those responses where the use of GMOs was known, the following GMOs were identified:

- “Genetically modified yeast allowing for a lower alcohol conversion rate, and the ability to metabolize malic acid”.

- “Nat Pure – a genetically modified yeast able to allow fermentation to occur at higher alcohol levels”.

- “Yeast that produce gram positive organisms that eliminate gram negative ones, such as lactobacillus, pediococcus and streptococci. There are also yeasts that enhance classic flavours, such as the French pong”.

The majority of responders believed that GMOs could be beneficial to the wine industry (44%), whereas 27% felt that GMOs would not add benefit, and 29% were unsure. Characteristics which wine producers believed would add benefit included:

- “Lower alcohol conversion rate of yeast”.
- “Non-sulphur producing yeast, non-histamine producing yeast”.
- “Yeasts and the vineyard itself to be resistant against diseases”.
- “Eradicate TCA from cork”.
- “Produce a vine that has full fruit flavours at a low degree balling”.
- “Vines that are resistant to mildew infections”.
- “Altering the vine to be resistant to GLR aV-3 virus”.
- “Vineyards to be more disease resistant, yeast to produce less alcohol, vineyard to reach ripeness without high sugar – leading to lower alcohol, yeast to do alcohol and malo fermentation at the same time”.
- “High alcohol fermenter, producing lower alcohol wines even if high sugar. Tolerance to VA, even the fermentation of VA”.
- “Malic acid degradation”.

Interestingly, even though 44% of responders believed GMOs to be of benefit to the wine industry, it was strongly felt by 72% that GMOs would also be harmful to the wine industry, 12% of responders did not believe that GMOs would produce any harm, whereas, 16% were unsure. Reasons for GMOs possibly being harmful to the wine industry which were mentioned in the survey are listed below:

- “Wine is a natural product. It is in my interest to keep it as natural as possible”.

- “GMOs are seen to be negative in all other food industries such as maize production, so I think wines which contain GMOs will be boycotted by the consumer if aware of their use”.

- “Cannot form part of sustainable or organic winemaking practices”.

- “If there is not enough scientific data collected on certain issues, and it is not properly tested, it could harm people’s health”.

- “From a marketing point of view it would be suicide, going against the “Variety is in our nature” focus, as well as against the new “Sustainability” drive”.

- “Winemaking should still be regarded as Art and with GMOs we are taking the skill away. Wine is also not a necessity but a luxury, when we talk about food production it might be more valuable to use GMOs”.

- “Perception by consumers, especially in the first world, is definitely negative. Also, it poses a problem to the image of wine as a natural product”.

- “In the vineyard, could cause mass extinction if all vines are the same GMO and one specific disease kills everything, like the phylloxera situation. Be careful to keep a balance with nature. The same with yeast as well, do not want one GMO that takes over the world of yeast”.

- “Marketing is the biggest hurdle in the use of GMOs as the consumer has been brainwashed against their use, regardless of any potential production benefits”.

- “It removes terroir from the picture. Weak wine producing areas can then also make good wine. It can have a huge impact on the sales, perception and personal values. Overall, I think the bacteria GMOs are good, such as better MLF, lower VA producers and so on, but the yeasts are curveball in a way that the world of wine will turn into a world of chemist, and not passionate winemakers”.

- "Unnatural and perfect conditions (terroir) become less important. This could result in harmful effects. Negative effect on image of wine as a natural produce. Marketing nightmare – resistance to producers influenced by GMOs. Negative environmental impact".

Concerning question 8, all responders were aware that the use of GMOs in wine production in South Africa is currently illegal, and all denied the use of any GMOs in their wine production. Should the use of GMOs become legal, only 25% of wine producers claimed they would use GMOs in wine production in the future. In contrast, 58% would still not use GMOs, and 17% of responders were unsure. 56% of responders claimed they would not purchase/consume a wine which contained GMOs, whereas, 28% of responders would, and 16% were unsure.

Table 6.1: Results from Wine Producer Questionnaire:

Wine producer	Q1	Q2	Q4	Q6	Q8	Q9	Q10
1	b	b	a	a	b	b	b
2	a	b	b	a	b	b	b
3	c	c	c	c	b	c	c
4	b	a	a	b	b	a	a
5	b	b	b	a	b	b	b
6	b	b	a	a	b	a	a
7				a			b
8	b	c	a	b	b	a	a
9	b	b	c	c	b	c	c
10	b	a	b	a	b	b	b
11	b	b	a/b	a	b	b	b
12	b	b	a	a	b	b	b
13	b	a	c	a	b	b	b
14	b	b	a	a	b	a	b
15	b	c	b	a	b	b	a
16	b	b	a	b	b	a	a
17	b	b	b	a	b	b	b
18	b	b	c	a	b	b	b
19	b	b	a	a	b	a	a
20	b	b	c	c	b	c	c
21	b	b	b	a	b	b	b

22	b	b	a	a	b	b	a
23	b	b	c	c	b	b	b
24	b	b	a	a	b	c	c
25	a	b	c	a	b	b	b

6.3 Additional South African viewpoints

The following views were expressed by the Cape Wine Makers Guild (CWG) in a media release from December 2006:

"In view of the recent wine industry applications for both genetically modified yeast and field trials of genetically modified vines, the 37 members of the Cape Winemakers Guild (CWG) wish to fully support the decision taken by the SA Wine Industry Council, chaired by Professor Kader Asmal, not to allow the commercial use of GM organisms in any South African wine".

"South African wine is completely GM free," says outgoing Guild Chairman, Gary Jordan. "The CWG represents the premium sector of the industry, and the introduction of GM into our cellars and vineyards would compromise not only the future of the CWG, but the future of the South African wine industry as a whole, so much so that it would be doubtful whether it could survive."

The guild acknowledges that laboratory based GM techniques are an invaluable complement to traditional procedures, and that in the research laboratory, genetic modification is a very powerful tool to gain information about fundamental aspects of the grapevine and wine yeast. They believe that this helps to unravel complex issues and test hypotheses, but that scientists should then try and solve these problems via a non-GM strategy.

South Africa has in the recent past built a positive international image around environmentally-friendly procedures, and in 1998 introduced the "Integrated Production of Wine" (IPW) principles. The South African wine and spirit board has recently launched a seal to certify those producers complying with its IPW certification system (Fig 6.1) and concentrates strongly on “Sustainability”. The seal provides the consumer with a guarantee that grape production was undertaken with consideration of the environment, was produced in an environmentally responsible manner, and is safe for the consumer. In light of these advances, many may feel that research into genetically modified grapevines goes against the IPW principles, however, in an article written by Cathy van Zyl in August 2009 entitled “Are genetically modified grapevines sustainable?”, reference is made to a publication from OgilvyEarth stating that consumers have learnt that “green is a very different idea- about the environment, not people. They are interested in the sustainable economy, not the green economy.” It is debated that “green” or environmentally responsible is not the same thing as “sustainable”.

Figure 6.1. Integrated Production of Wine certification seal.

6.4 General South African viewpoints on GMOs

In 2004, the PUB (Public Understanding of Biotechnology) together with the HSRC (Health Science Research Council) performed a survey to determine the consumers' opinions as to truthful sources of information and as to labeling, with regards to general GM products. Fig 6.2 demonstrates consumers' opinions as to most trusted sources of information with regards to biotechnology (www.pub.ac.za):

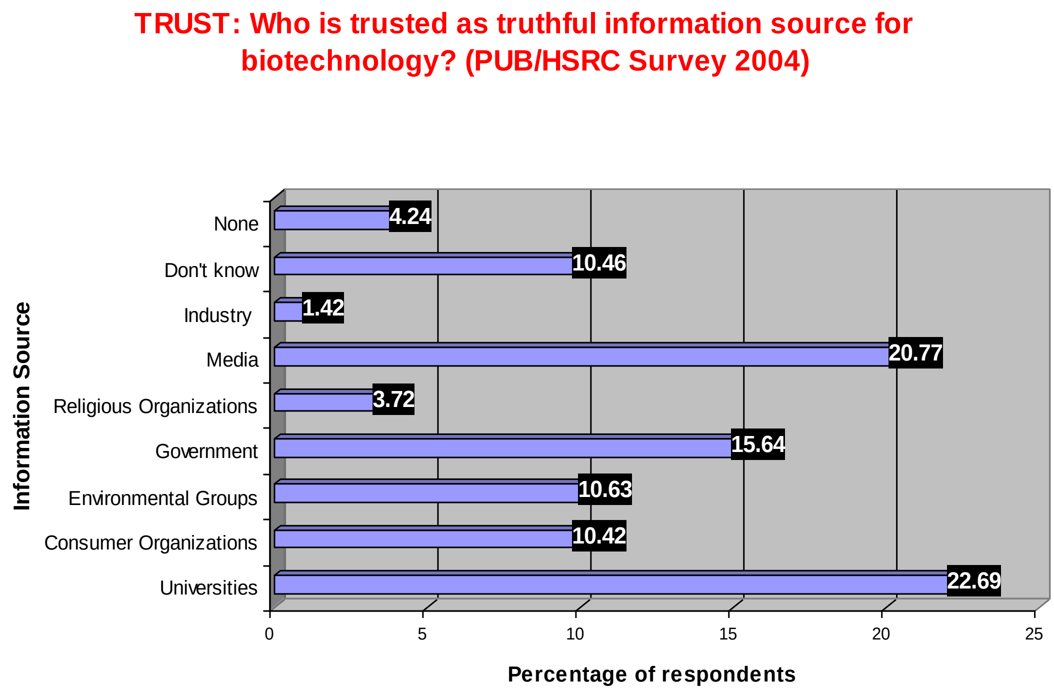

Figure 6.2. Consumers' opinions as to most trusted information sources with regards to biotechnology.

Concerning labeling of food products, 51% of responders stated that they never read food labels, whereas, 23% of responders always read food labels. Of those responders interested in what was stated on a food label, the following criteria were of importance (Fig. 6.3):

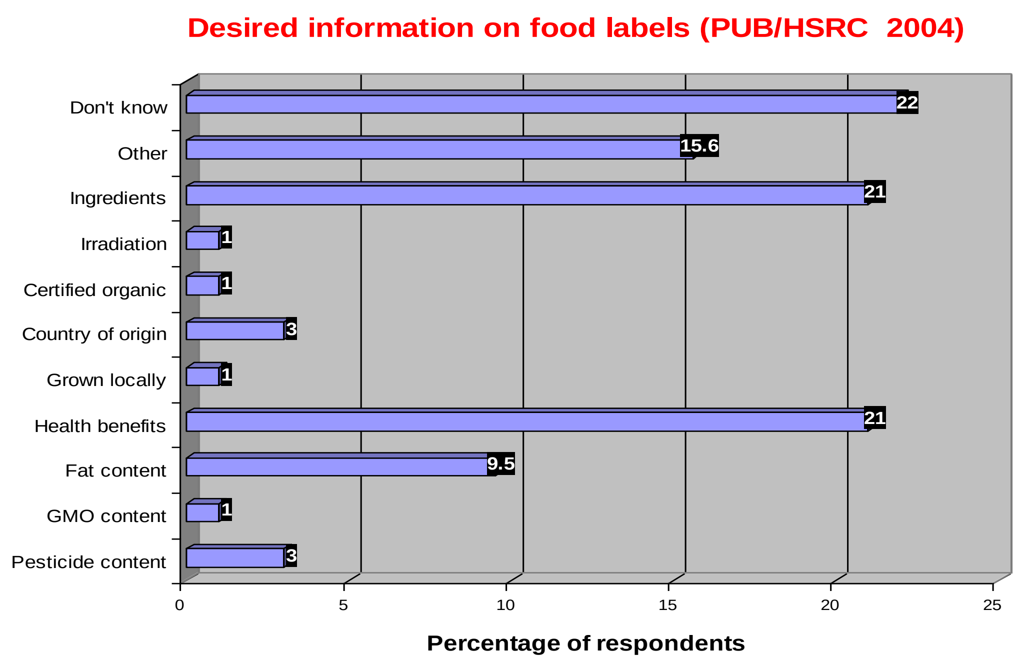

Figure 6.3. Consumers' opinions as to important information required on food labels.

CHAPTER 7

CONCLUSIONS

7. Conclusions

Whether the consumer, producer, environmentalist, researcher or government are pro the use of GMOs within the wine industry or not, it is clear that biotechnology has become the way of the future and that vast amounts of research are being conducted for improvement of processes within the wine industry, both locally and internationally. By not embracing these technological advances, it may be detrimental in that a country may get “left behind” in the drive for more cost effective, sustainable and efficiently processed products. South Africa has world class researchers studying the various aspects of plant, bacterial and yeast improvement for the wine industry with cutting edge technology, and who have potential to be world leaders in this field. As has been discussed in this dissertation, there are already 2 genetically modified yeasts available on the market for use in wine processing, and there is no doubt that many more will become available in the future. Besides the possible scientific and commercial benefits of GM wines, the wine industry stands to gain in image and international appreciation as being innovative and forward-looking. One such research program initiated by the IWBT in South Africa entitled “Functional Wineomics” will continue to develop the tools and expertise required to be at the frontier of research for wine biotechnology.

Environmental lobby groups would however disagree, and have in the past objected to field trials for GM grapevines on the basis that there is no “need” for GM wine. Understandably, wine is a luxury item, not a commodity for feeding the poverty-stricken masses, for which genetic engineering would be a more apt solution for plant improvement. However, as has been discussed in previous chapters, the damaging use of herbicide and insecticide chemicals applies to all plant types and thus the search for a more environmentally-friendly biological solution is greatly needed, and could possibly be overcome by the use of GMOs. This is just one example of how GMOs could benefit the wine industry. There are many other examples including increased levels of healthy substances such as Resveratrol, and reduced levels of unwanted carcinogens such as Ethyl carbamate.

A number of obstacles exist for the commercialization of GMOs, several of which have been previously discussed. Public perception is a huge concern for wine producers due to the fact that wine has a “traditional” and “natural” image which could be negatively influenced by the implementation of GMOs in the wine industry. More so than most other food and beverage industries, the wine industry has deep cultural roots with proud local traditions. As a consequence, the industry is generally less receptive to changes brought about by new technologies. The negative perception of GMOs has also been largely fuelled by the media referring to "Frakenfoods" and "unnatural" genetic interference with nature. In many cases, regulatory authorities appear more willing to approve the use of GMOs than the public is to use or accept them. In the case of the wine industry, human genetic-intervention was already carried out a century and a half ago when *Phylloxera* almost wiped out the world's vineyards. Cuttings of European *Vitis vinifera* were grafted onto phylloxera-resistant American native vines. It is debatable as to whether this could have been seen as tampering with nature and as rather "Frankensteinian", however, no such negative media hype resulted from such action. A further example of previous genetic "meddling" involves the development of new cultivars such as our very own Pinotage, by Professor Perold, who crossed the noble variety Pinot noir with Cinsaut in 1925. Again, no negative perception has resulted from such action.

With regards to public perception in South Africa, results from the survey conducted in this study showed the majority of consumers being willing to purchase or consume a wine made using GMOs, and believing that GMOs would be beneficial to the wine industry. Whether this is a true reflection of the South African public's perception of GMOs is unclear, and a larger survey with an increased number of respondents could be useful. It was interesting to note that the majority of wine producers believed the use of GMOs to be harmful to the wine industry, mostly due to a presumed negative perception by the public.

It is also feared that genetic modification of grapevines could lead to a tendency to standardize wines to satisfy large supermarket chains and the average consumer, leading to a loss of identity, variety and uniqueness, and where terroir would no longer have the all important impact that is does today. Long term environmental and health effects of GMOs also pose a large concern, and possibly, there is not enough data yet to provide evidence to support, or to be against, this. However, GM crops are at least required to undergo extensive biosafety assessments before government approval and commercial release, which does not happen for conventional or even organic farming.

Consumer education is thus imperative in order to remove this fear of the unknown. Scientists need to play their part by being open and clear about experiments and research being conducted, and governments need to reassure the consumer of the implementation of biosafety legislation, and ensure that strict risk assessments are being performed.

The marketing of GM wines to the public must also be carefully considered. Marketing of wine often lies heavily upon the wine's label and what is stated thereon. The varietal name, together with the origin of production and the vintage form the cornerstones of the information that is presented on the label, and generally influences the consumer's choice when purchasing. Thus, it is of utmost importance that genetically improved grapevines do not interfere with the established varietal names and wine styles. Currently, there is a debate as to the description and naming of transgenic grapevines which entails several factors such as the source of genes introduced into a particular grapevine, the "true to typeness" of the transgenic vine when compared with the original cultivar, and the organoleptic and sensory qualities of the resulting wine (Vivier and Pretorius, 2000). It is uncertain as to whether transgenic grapevines with altered fruit qualities such as improved colour and flavor compound composition would have to be assigned a new varietal name or just a new clonal number. However, given the immense marketing value contained in varietal names, there is an urgent need for consensus that GM grapevines would be little different from grapevine clonal selections, and as thus, could be named the same (Kikkert *et. al.,* 2001). In the survey carried out in this study, it was of utmost importance to the majority of responders that a wine made using GMOs be labeled as such. In chapter 5, it was shown that labeling criteria for GM products differs from country to country, ranging from strict labeling regulations in the EU, to no obligatory GMO labeling in the USA.

Guidelines for the approval of genetically modified products and the release of genetically modified organisms have been discussed in chapter 5, and usually require several obvious guarantees. These are broadly similar in most countries and include a complete definition of the DNA sequence introduced in order to produce the genetically modified organism. They also include determination that there is no selective advantage conferred on the transgenic organism that could allow it to become dominant in natural habitats. It must pose no danger to human health and/or the environment, and should have a clear advantage to both the producer and the consumer (Pretorius, 2000). The concept of "substantial equivalence" is widely used in the USA for the determination of safety by comparison with analogous conventional food and beverage products, such that the initial problems with approval for the use of genetically engineered plants and organisms in the agro-industry are now being slowly dissolved. When substantial equivalence can be demonstrated, no further safety considerations are usually necessary.

In addition to the obstacles for commercialization of GMOs mentioned above, a number of patents have been taken out which cover DNA sequences, gene markers and transformation protocols commonly used in genetic engineering, and which allow for little freedom to operate. It is thus imperative that formal agreements be taken out, to address any intellectual property issues. If ownership of a transgenic grapevine is in dispute, the release of such genetically improved grapevines may cause serious impediment to the commercialization process. 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 (Pretorius 2000).

The application of recombinant DNA technology in the wine industry is thus a fairly controversial topic, with many unanswered questions. To ensure any future possibility of the use of GMOs in the wine industry, there needs to be assurance that existing desirable characteristics will not be damaged, that the requirements of beverage legislation are met, that the engineered cultivar will be stable in practice, and that there will be no adverse human health effects. A holistic approach towards wine science is thus needed, which 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.

"*No conceptual distinction exists between genetic modification of plants and microorganisms by classical methods or by molecular techniques that modify DNA and transfer genes."*

National Research Council, USA

"We have recently advanced our knowledge of genetics to a point where we can manipulate life in a way never intended by nature. We must proceed with the utmost caution in the application of this new-found knowledge."

Luther Burbank

"Biotechnology is a train on a track that will never be stopped. If the biotechnologists can convince us that the use of GMO's is safe for human beings, and increase the quality of the product, such products can only benefit the world going forward".

Gary Baumgarten, General Manager, Graham Beck Wines

CHAPTER 8

REFERENCES

8. References

Aitchinson, P A; Macleod, A J; Yeoman, M M. (1977). Growth patterns in tissue (callus) cultures. In: Plant tissue and cell culture. Edited by: Street, H E, Blackwell Scientific, Oxford, pp. 267-306.

Amoako, K. (2003). GM technology to counter world starvation? *Asia Pacific Biotech News* **7** (25): 1614-1620.

Anslow, M. (2008). Going organic: 10 reasons why (and how) organics can feed the world. *CCPA Monitor* **15** (6): 21-24.

Bakshi, A. (2003). Potential adverse health effects of genetically modified crops. J. Toxicol. Environ. Health B Crit. Rev. 6(3): 211-25.

Barre, P; Vezinhet, F; Dequin, S; Blondin, B (1992). Genetic improvement of wine yeasts. In: Fleet, G (Ed). Wine Microbiology and Biotechnology. pp: 265-287. Harwood Academic Publishers.

Bauer, F F; Dequin, S; Pretorius, I S; Shoeman, H; Wolfhaardt, G; Schroeder, M B; Grossmann, M K. (2004). The assessment of the environmental impact of genetically modified wine yeast strains. Bulletin de l'IOV 77: 515-528.

Becker, J V W; Armstrong, G O; van der Merwe, M J; Lambrechts, M G. (2003). Metabolic engineering of *Saccharomyces cerevisiae* for the synthesis of the wine related antioxidant resveratrol. FEMS Yeast Res. 4:79-85.

Bengochea, T and Dodds, J H. (1986). Plant protoplasts: A biotechnological tool for plant improvement. Chapman and Hall, London.

Berg, P; Baltimore, D; Brenner, S; Roblin III, R O; Singer, M F. (1975). Summary statement of the Asilomar conference on recombinant DNA molecules. Proc. Natl. Acad. Sci. USA. 72(6): 1981-4.

Blackhall, N W; Davey, M R; Power, J B. (1994). Isolation, culture and regeneration of protoplasts. In: Plant Cell Culture. A Practical Approach, 2nd Edition. Edited by: Dixon, R A; Gonzalez, R A, Oxford University Press, Oxford. Pp. 27-39.

Bryant, J; Baxter, L; Cave, C.B.; Milne, R. (2007). Recombinant growth hormone for idiopathic short stature in children and adolescents. Cochrane Database Syst rev (3): CD004440.

Caboche, M. (1990). Liposome-mediated transfer of nucleic acids into plant cells. Physiology of the Plant 79: 173-176.

Carstens, M; Vivier, M A; Pretorius, I S. (2003). The Saccharomyces cerevisiae chitinase, encoded by the CTS1-2 gene, confers antifungal activity to transgenic tobacco. Transgen. Res. 12: 497-508.

Christou, P and Ford, T L. (1996). Parameters influencing stable transformation of rice immature embryos and recovery of transgenic plants using electric discharge particle acceleration. Annals of Botany 75: 401-413.

Cocking, E C. (1960). A method for the isolation of plant protoplasts and vacuoles. Nature 187: 962-963.

Cohen, S N; Chang, A C Y; Boyer, H W; Helling, R B. (1973). Construction of biologically functional bacterial plasmids in vitro. Proc. Natl. Acad. Sci. USA. 70(11): 3240-3244.

Constabel, F (1984). Callus culture: Induction and maintenance. In: Cell Culture and somatic cell genetics of plants, Vol. 1. Edited by: Vasil, I., Academic Press, N.Y., pp. 27-35.

Cornejo, M J; Luth, D; Blankenship, K M; Andersonn, O D; Blechl, A E. (1993). Activity of a maize ubiquitin promoter in transgenic rice. Plant Molecular Biology 23: 567-581.

Crossway, A; Oakes, J V; Irvine, J M; Ward, B; Knauf, V; Shewmaker, C K. (1986). Integration of foreign DNA following microinjection of tobacco mesophyll protoplasts. Molecular and General Genetics 202: 179-185.

DeBeer, A; Vivier, M A. (2008). Vv-Amp1, a ripening induced peptide from *Vitis vinifera* shows strong antifungal activity. BMC Plant Biol. 8: 75.

Dequin, s; Baptista, E; Barre, P. (1999). Acidification of grape musts by *Saccharomyces cerevisiae* wine yeast strains genetically engineered to produce lactic acid. American Journal of Enology and Viticulture 50: 45-50.

Dodds, J H and Roberts, L W. (1985). Experiments in plant tissue culture, 2nd Ed, Cambridge University Press, London.

Duan, Y-P; Powell, C A; Webb, S E; Purcifull, D E; Hiebert, E. (1997). Geminivirus resistance in transgenic tobacco expressing mutated BC1 protein. Molecular Plant Microbe Interactions 10(5): 617-623.

Du Toit, M; Pretorius, I S. (2000). Microbial spoilage and preservation of wine: using weapons for nature's own arsenal. S. Afr. J. Enol. Viticult. 21: 74-96.

Guan, H. (2003). GM technology to counter world starvation? Asia Pacific Biotech News 7 (25): 1614-1620.

He, D G; Mouradov, A; Yang, Y M; Mouradova, E; Scott, K J. (1994). Transformation of wheat (*Triticum aestivum*) through electroporation of protoplasts. Plant Cell reports 14: 192-196.

Hinchee, M A W; Connor-ward, D V; Newell, C A; McDonnel, R E; Sato, S J; Gasser, C S; Fischoff, D A; Re, D B; Fraley, R T; Horsch, R B. (1988). Production of transgenic soybean plants using *Agrobacterium*-mediated DNA transfer. Biotechnology 6: 915-922.

Horsch, R B; Fry, J E; Hoffmann, N L; Eichholtz, D; Rogers, S G; Fraley, R T. (1985). A simple and general method for transferring genes into plants. Science 227: 1229-1231.

Jackson, R S. (1994). Grapevine species and varieties. In Wine Science: Principles and Application. Pp. 11-31. Academic Press.

Jaillon, O; Aury, J M; Noel, B; Policriti, A. (2007). French-Italian public consortium for grapevine genome characterization. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449: 463-467.

James, C. (2008). Executive Summary, Global status of commercialized biotech/GM crops: 2008. The first thirteen years, 1996 to 2008, ISAAA briefs, 39. The International Service for the Acquisition of Agri-biotech Applications (ISAAA).

Johnston, S A; Tang, D C. (1994). Gene gun transfection of animal cells and genetic immunization. Methods in Cell Biology 43 pt A: 353-365.

Joubert, D A; Slaughter, A R; Kemp, G; Bergmann, C. (2006). The grapevine polygalacturonase-inhibiting protein (*VvPGIP1*) reduces *Botrytis cinerea* susceptibility in transgenic tobacco and differentially inhibits fungal polygalacturonases. Transgen. Res. 15: 687-702.

Key, S; Ma, J K; Drake, P M. (2008). Genetically modified plants and human health. J. R. Soc. Med. 101(6): 290-8.

Kikkert, J.R, *et. al*. (2001). Grapevine genetic engineering. In Molecular Biology and Biotechnology of the Grapevine. (Roubelakis-Angelakis ed) 393-463.

King, P J and Street, H E. (1977). Growth patterns in cell cultures. In: Plant tissue culture and cell culture. Edited by: Street, H E, Blackwell Scientific, Oxford, pp. 307-387.

König, A; Cockburn, A; Crevel, R W. (2004). Assessment of the safety of foods derived from genetically modified (GM) crops. Food Chem. Toxicol. 42(7): 1047-88.

Kota, M; Daniell, H; Varmas, S; Garczynski, S F; Gould, F; Moar, W J. (1999). Overexpression of Bacillus thuringiensis (Bt) Cry2Aa2 protein in chloroplasts confers resistance to plants against susceptible and Bt-resistant insects. Proc. Natl. Acad. Sci. USA. 96(5): 1840-1845.

Lambrechts, M G; Pretorius, I S. (2000). Yeast and its importance to wine aroma. S. Afr. J. Enol. Viticult. 21: 97-129.

Leader, B; Baca, Q; Golan, D. (2008). Protein therapeutics: a summary and pharmacological classification. Nat. Rev. Drug Discov. 7(1): 21-39.

Lehrer, S B; Horner, W E; Reese, G. (1996). Why are some proteins allergenic? Implications for Biotechnology. Critical Reviews in Food Science and Nutrition 36:553-654.

Louw, C; La Grange, D; Pretorius, I.S; Van Rensburg, P. (2004). Improving wine processing with polysaccharide degrading wine yeast strains. Food Micro. 19th International ICFMH symposium pp251.

Malherbe, D F; Du Toit, M; Cordero Otero, R R; van Rensburg, P; Pretorius, I S. (2003). Expression of the *Aspergillus niger* glucose oxidase gene in *Saccharomyces cerevisiae* and its potential applications in wine production. Appl. Micribiol. Biotechnol. 61: 502-511.

May, G D; Afza, R; Mason, H S; Wiecko, A; Novak, F J; Arntzen, C J. (1995). Generation of transgenic banana (*Musa acuminate*) plants via *Agrobacterium*-mediated transformation. Biotechnology 13: 486-492.

McCabe, D E; Swan, W F; Martinell, B J; Christou, P. (1988). Stable transformation of soybean (*Glycine max*) by particle acceleration. Biotechnology 6: 923-926.

McCormick, S; Niedermeyer, J; Fry, J; Barnason, A; Horsch, R; Fraley, R. (1986). Leaf disc transformation of cultivated tomato (*L. esculentum*) using *Agrobacterium tumefaciens*. Plant Cell reports 5: 81-84.

Mills, D A; Rawsthorne, H; Parker, T; Tamir, D; Makarove, K. (2005). Genomic analysis of *Oenococcus oeni* PSU-1 and its relevance to winemaking. FEMS. Microbiol. Revs 29: 465-475.

Molvig, L; Tabe, L M; Eggum, B O; Moore, A E; Craig, S; Spencer, D; Higgins, T J. (1997). Enhanced methionine levels and increased nutritive value of seeds of transgenic Lupins (Lupinus angustifolius L.) expressing a sunflower seed albumin gene. Proc. Natl. Acad. Sci. USA. 94(10): 8393-8398.

Moore, J P; Benoit, D; Young, P R; Nieuwoudt, H L; Ramburan, V; du Toit, M; Bauer, F F; Vivier, M A. (2008). Wine Biotechnology in South Africa: Towards a system approach to wine science. Biotechnol. J. 3: 1355-1367.

Mullins, M G. (1992). In: Biology of the grapevine. pp 203-229. Cambridge University Press.

Neuhaus, G; Spangenburg, G; Nittlestensheid, O; Schweiger, H G. (1987). Transgenic rapeseed plants obtained by the microinjection of DNA into microspore-derived embryoids. Theories of Applied Genetics 75: 30-36.

Neuhaus, G and Spangenburg, G. (1990). Plant transformation by microinjection techniques. Physiology of the Plant 79: 213-217.

Otsuki, Y; Takebe, I; Honda, Y; Kajitas, C; Matsui, C. (1974). Infection of tobacco mesophyll protoplast by Potato Virus X. Journal of General Virology 22: 375-385.

Perl, A and Eshdat, Y. (1998). DNA transfer and gene expression in transgenic grapes. In: Biotechnology and genetic engineering reviews. Edited by: Tombs, M P. pp: 365-389. Intercept Ltd.

Pipe, S.W. (2008). Recombinant clotting factors. Thromb. Haemost. 99(5): 840-850.

Plahuta, P. and Raspor, P. (2007). Comparison of hazards: Current vs GMO wine. Food Control 18: 492-502.

Potrykus, I; Saul, M W; Petruska, J; Paszkowski, J; Shillito, R D. (1985). Direct gene transfer to cells of a graminaceous monocot. Molecular and General Genetics 199: 183-188.

Pretorius, I S. (2000). Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast 16: 675-729.

Pretorius, I S and Bauer, F F. (2002). Meeting the consumer challenge through genetically-customized wine yeast strains. Trends in Biotechnology 20 (10): 426-432.

Pua, E C; Mahra-Palta, A; Nagy, F; Chua, N H. (1987). Transgenic plants of *Brassica napus* L. Biotechnology 5: 815-817.

Register, J C; Peterson, D J; Bell, P J; Bulock, W P; Evan, I J; Frame, B; Greenland, A J; Higgs, N S; Jepson, I; Jiao, S P; Lewnau, C J; Silick, J M; Wilson, H M. (1994). Structure and function of selectable and non-selectable transgenes in maize after introduction by particle bombardment. Plant Molecular Biology 25(6): 951-961.

Reich, T J; Iver, V N; Miki, B L. (1986). Efficient transformation of alfalfa protoplasts by the intranuclear microinjection of Ti plasmids. Biotechnology 4: 1001-1004.

Runge, C F. (2004). The global diffusion of plant biotechnology: International adoption and research in 2004.

Scorza, R; Cordts, J M; Ramming, D W; Emershad, R L. (1995). Transformation of grape (*Vitis vinifera* L.) zygotic-derived somatic embryos and regeneration of transgenic plants. Plant Cell Reports 14: 589-592.

Shimamoto, K; Terada, R; Izawa, T; Fujimoto, H. (1989). Fertile rice plants regenerated from transformed protoplasts. Nature 338: 274-276.

Spörlein, B and Koop, H U. (1991). Lipofection: direct gene transfer to higher plants using cationic liposomes. Theories of Applied Genetics 83:1-55.

Stafford, A and Warren, G (1991). Plant cell and Tissue Culture, Open University, London.

Vasil, V; Castillo, A M; Fromm, M E; Vasil, I K. (1992). Herbicide resistant fertile transgenic wheat plants obtained by particle bombardment of regenerable callus. Biotechnology 10:667-674.

Vasil, I. (1995). Cellular and molecular improvement of cereals. In: Current Issues in Plant Molecular and Cellular Biology. Proceedings of the VIIIth International Congress on Plant Tissue and Cell Culture. Edited by: Terzi, M., Italy.

Vigne, E; Komar, V; Fuchs, M. (2004). Field safety assessment of recombination with transgenic grapevines expressing the coat protein gene of GFLV. Transgenic Research 13: 165-179.

Vivier, M A and Pretorius, I S. (2000). Genetic improvement of grapevine: tailoring grape varieties for the 3rd millennium. S. Afr. J. Enol. Vitic. 21: 5-26.

Vivier, M A and Pretorius, I S. (2002). Genetically tailored grapevines for the wine industry. Trends in Biotechnology 20(11): 472-478.

Walsh, G. (2005). Therapeutic insulins and their large-scale manufacture. Appl. Microbiol. Biotechnol. 67(2): 151-159.

Weber, G; Monajemboshi, S; Wolfrum, J; Greulich, K O. (1990). Genetic changes induced in higher plant cells by a laser microbeam. Physiology of the Plant 79: 190-193.

Wieczorek, A and Sanfaçon, H. (1995). An improved method for the generation and transfection of protoplasts from *Cucumis sativa* cotyledons. Plant Cell Reports 14: 603-610.

9. Appendices

APPENDIX 1- Wine Consumer Questionnaire

GENETICALLY MODIFIED ORGANISMS IN THE WINE INDUSTRY

QUESTIONNAIRE – Wine Consumer

1. What is a Genetically Modified Organism (GMO)?
   a. It is an organism produced by scientists who are tampering with nature and could cause harmful effects
   b. It is an organism whose genetic material has been altered by combining it with DNA molecules from a different source to produce beneficial results
   c. I don't know

2. Are you aware of any GMOs utilized in the wine industry?
   a. Yes
   b. No
   c. Unsure

3. If yes, please state the name of the GMO and it's purpose
   Name: ____
   Purpose: ____

4. I believe that the use of GMOs in wine will have the following effect on the wine industry:
   a. Beneficial
   b. Harmful
   c. No difference

5. Please state reason/s why

6. I believe that the use of GMOs in wine will have the following effect on the wine consumer:
    a. Beneficial
    b. Harmful
    c. No difference

7. Please state the reason/s why

8. Would you purchase/consume a wine which contains GMOs?
    a. Yes
    b. No
    c. Unsure

9. Would you prefer it if a wine containing GMOs be labeled as such?
    a. Yes
    b. No
    c. Unsure

10. Would you pay extra for a non-GM wine?
    a. Yes
    b. No
    c. Unsure

APPENDIX 2 – Wine producer Questionnaire

GENETICALLY MODIFIED ORGANISMS IN THE WINE INDUSTRY

QUESTIONNAIRE – Wine Producer

1. What is a Genetically Modified Organism (GMO)?
    a. It is an organism produced by scientists who are tampering with nature and could cause harmful effects
    b. It is an organism whose genetic material has been altered by combining it with DNA molecules from a different source to produce beneficial results
    c. I don't know

2. Are you aware of any GMOs utilized in the wine industry?
    a. Yes
    b. No
    c. Unsure

3. If yes, please state the name of the GMO and it's purpose
    Name: ____
    Purpose: ____

4. Do you believe that GMOs could be beneficial to the wine industry?
    a. Yes
    b. No
    c. Unsure

5. If yes, which characteristic would you choose to modify to gain benefit?

____

____

____

6. Do you believe that GMOs could be harmful to the wine industry?
    a. Yes
    b. No
    c. Unsure

7. If yes, please state the reason/s why.

8. Do you use any GMOs in the production of your wine?
    a. Yes
    b. No
    c. Unsure

9. In the future, would you use any GMOs in the production of your wine?
    a. Yes
    b. No
    c. Unsure

10. Would you purchase/consume a wine which contains GMOs?
    a. Yes
    b. No
    c. Unsure