Australian Government
Bureau of Rural Sciences

What's in the Pipeline?

Genetically modified crops under development in Australia

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Foreword

Knowing what GM crops are under development is needed to ensure a balanced debate and informed decision making on their adoption. Advanced notice is beneficial for crops that require monitoring or industry stewardship. It is also important to assess GM crops under development internationally because of their potential impacts on Australia's trade and competition, as well as the issues surrounding the unplanned presence of GM material in non-GM crop imports.

This report responds to a need to collate and review GM crops under development in Australia. Through a combination of research and consultation BRS scientists have identified a selection of GM crops in the pipeline, focussing on broad-acre crops, which may be ready for commercialisation within the next 15 years. This report also identifies and discusses significant barriers and bottlenecks along the path to commercialisation.

C. Samson

Dr Cliff Samson
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Executive summary

Modern biotechnology is being used to develop new varieties of plants…

Biotechnology, the use of living things to make products, has existed in various forms for over 8000 years, ever since yeast was first used for brewing beer and making bread. In 1953, the double helix structure of DNA was discovered, and modern biotechnology was born. From the development of the first genetically modified (GM) plant in the early 1980s, the use of modern biotechnology as a tool to develop new varieties of plants with beneficial traits has grown rapidly. This report is about GM plants, defined as plants that have been modified by the direct incorporation of genes to introduce new traits.

…including first, second or third generation genetically modified crops.

GM crops can be categorised as having either first, second or third generation traits:

- first generation traits provide benefits on the farm;
- second generation traits provide benefits to the producer and consumer; and
- third generation traits allow the plant to be used as a 'factory' to produce pharmaceuticals or industrial products

Most GM crops commercialised worldwide carry herbicide tolerance or insect resistance but a broader range of GM crops are in the pipeline.

The current generation of GM crops commercialised worldwide mostly have first generation traits, such as herbicide tolerance and insect resistance. GM versions of these two traits made up 99% of the 81 million hectares of GM crops commercialised globally in 2004. To date, most of the public discussion and published information on agricultural biotechnology has focused on these two traits, however there are many more first, second and third generation GM crops under development worldwide.

In Australia GM crops are regulated by the Gene Technology Regulator …

In Australia, GM organisms are regulated by the Gene Technology Regulator (the Regulator) supported by the Office of the Gene Technology Regulator (OGTR). The role of the Regulator is to protect human health and safety and the environment by identifying and managing risks posed by the use of gene technology. The Regulator liaises with other regulatory agencies, including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), and the Therapeutic Goods Administration (TGA) to coordinate the approval of GM products for use and sale.

…who has approved three crops for commercial release, however only two have made it to market, cotton and carnation.

There are currently only two GM crops grown commercially in Australia, cotton – which has been modified for herbicide tolerance, pest resistance, or a combination of the two; and carnations, modified to produce blue flowers. Herbicide tolerant canola was the next crop expected to be grown commercially in Australia, with licences being granted by the OGTR in 2003. However, the instigation of State and Territory bans on commercial plantings of GM canola has stalled its progression to market.

This report identifies a selection of traits being developed in Australia…

Using a combination of research and consultation, BRS has identified a selection of GM crops within 15 years of commercialisation in Australia. GM technologies have the potential to help address current and future challenges facing Australian agriculture by allowing the development of varieties better adapted to environmental stresses such as drought, frost, acid soils and salinity, or with reduced susceptibility to pest and diseases. GM crops can also be developed that may provide new, high value crops and niche markets.

…and discusses the factors considered when deciding to develop a new crop variety using either conventional or GM methods.

Decisions on whether to pursue a GM or conventional breeding route depend on many factors including the genetic complexity of the trait, the capacity of current crop varieties to respond to challenges, and the existing presence of these traits within the species or in closely related plants. These factors will differ greatly depending on the crop itself, the desired trait and the nature of the challenge.

For some second and third generation traits, such as nutrient enhancement, conventional breeding approaches are feasible in some crops. However, for the vast majority of second and third generation traits, GM is the only route to obtaining these novel traits.

With a few exceptions, most third generation traits are in the technology discovery phase. Before the majority of the products can reach commercialisation, many technical, production and regulatory issues need to be resolved. For example, the logistics of scaling-up production, distribution and segregation of these products needs to be developed.

Reasons why some of these crops may not make it through to commercialisation are explored.

Not all GM crops under development will reach commercialisation. There are exit points along the pipeline where decisions have to be made about the potential of a product to reach commercialisation, and the value that the product will hold, balanced against the costs or risks. There are also many barriers to commercialisation of a GM crop and these are explored in more detail in this report. They include;

- Technical barriers
- Freedom to operate barriers
- Regulatory barriers
- Value capture
- Marketing uncertainties

In conclusion…

The GM crops in the pipeline discussed in this report represent only a proportion of Australian research and development in this area. There is huge breadth of ongoing high quality research and development in Australia that could lead to GM crop outputs. However what this BRS review shows is that the current regulatory and marketing environment in Australia has stalled many crops in the pipeline. Unless this environment changes, the capacity of breeding programs to quickly develop GM varieties suitable for Australian agriculture is becoming questionable. The impact of Australia’s agricultural competitors adopting GM technology in other key export crops also needs to be considered.

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List of Abbreviations

ABA	Abscisic Acid
ABS	Australian Bureau of Statistics
AMV	Alfalfa Mosaic Virus
ANZECC	Australia New Zealand Environment Conservation Council
APHIS	Animal and Plant Health Inspection Service
APVMA	Australian Pesticides and Veterinary Medicines Authority
AWB	Australian Wheat Board
BIOS	Biological Innovation for Open Society
BRS	Bureau of Rural Sciences
BSES	Bureau of Sugar Experiment Stations Limited
Bt	*Bacillus thuringiensis*
BYDV	Barley Yellow Dwarf Virus
CAMBIA	Center for the Application of Molecular Biology to International Agriculture
CRC	Cooperative Research Centre
CRC SIIB	Cooperative Research Centre Sugar Industry Innovation through Biotechnology
CREB	Centre for Rural and Environmental Biotechnology
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo
DAFF	Department of Agriculture Fisheries & Forestry – Australia
DHA	Docosahexaenoic Acid
DIR	Direct Intentional Release
DNA	Deoxyribonucleic Acid
EPA	Eicosapentaenoic Acid
FSANZ	Food Standards Australia and New Zealand
FTO	Freedom to Operate
GA	Gibberellic Acid
GBA	Grain Biotech Australia
GM	Genetically Modified
GMO	Genetically Modified Organism
GRDC	Grains Research and Development Corporation
GTR	Gene Technology Regulator
IP	Intellectual Property
IRRI	International Rice Research Institute
ISAAA	International Service for the Acquisition of Agri-biotech Applications
mAbs	Monoclonal Antibodies
MPBCRC	Molecular Plant Breeding Cooperative Research Centre
MRST	Ministry of Research, Science & Technology – New Zealand
MUFA	Monounsaturated Fatty Acid
NHMRC	National Health & Medical Research Council
OGTR	Office of the Gene Technology Regulator
PUFA	Polyunsaturated Fatty Acid
PTGS	Post-transcriptional Gene Silencing
RDI	Recommended Daily Allowance
RNA	Ribonucleic Acid
RNAi	RNA interference
ROS	Reactive Oxygen Species
TGA	Therapeutic Goods Administration
TP	Tangible Property
USDA	United States Department of Agriculture
VIP	Vegetative Insecticidal Proteins
YCV	Yellow Clover Virus

Section 1: What's in the pipeline?

1.1. Chapter 1: Introduction

1.1.1. Rationale behind this report

Knowing what genetically modified crops are in the pipeline in Australia is important for regulators, policy makers, agricultural and related industries to make informed decisions. Advance notice is also beneficial for crops that may require monitoring or industry stewardship. For some GM crops, such as pharmaceutical or industrial crops, supply chain management issues will need to be addressed. What's in the pipeline overseas is important to assess well ahead of commercial release because of potential impacts on trade and competition, as well as the issues of unintended presence of GM material in imports.

1.1.2. Structure of this report

This report is the result of a study funded by DAFF that aims to enhance understanding of and encourage development of innovative applications of biotechnology in the agriculture and food sectors. Publications and seminars arising out of this work will help identify any potential benefits or impacts of proposed new biotechnology to Australian agriculture. This report is structured as follows:

- Section 1 – Introduction and discussion of the main issues
- Sections 2 – 4 – Classification of traits and brief explanation of some of the GM crops in the pipeline in Australia and internationally
- Appendices – Data tables and consultation questionnaire.

1.1.3. GM crops are only one component of plant biotechnology

The field of plant biotechnology encompasses diverse fields with technologies and outputs far broader that just GM crop outputs. Non-GM applications of plant biotechnology can be used to improve variety selection and screening strategies in conventional breeding programs, to identify and source new variations in land races and wild relatives and to better understand the genes and proteins controlling plant responses. The use of markers to track genes or groups of genes responsible for complex traits can increase the success and greatly reduce the time required for conventional breeding programs, giving greater flexibility, more precision and better varieties sooner (MRST, 2005). As these technologies do not result in a GM crop plant, they will not be discussed in detail in this report.

1.1.4. What is meant by ‘the pipeline’?

The ‘pipeline’ in the context of this report is referring to the product development processes for GM crops. Predictions of time taken to commercialisation depend on many factors, including the crop, the complexity of the trait, the type of product, business negotiations including access to intellectual property (IP), and the regulatory regime. For agricultural products in the United States the time span from initial discovery to commercialisation is generally 8 – 12 years (Gutterson and Zhang, 2004). This ‘pipeline’ has various stages as shown in Figure 1.1. These can also be loosely grouped as technology discovery, proof of concept, field trial and commercialisation (David Hudson, pers comm.).

1.1.5. First, second and third generation GM crops

GM crops in the pipeline are also commonly categorised as first, second or third generation traits. First generation crops are designed for easier production on the farm and are often referred to as input traits. These crops carry agronomic traits such as herbicide tolerance and insect resistance that allow for increased production, minimise environmental stresses, or reduce the impact of pests and diseases. Second generation traits are sometimes called output traits, and these crops aim to produce food and feed with increased nutrition, enhanced quality or better processing characteristics. Third generation crops use plants as production systems for pharmaceutical or industrial purposes. In this report environmental (abiotic) stress tolerances will be referred to as first generation traits as they are agronomic or input traits (Cockburn, 2004), although it is important to note that some reviewers class these traits as third generation due to the predicted time to delivery of product (MRST, 2005).

There has been much speculation that the development and commercialisation of second and third generation traits will have positive implications for the uptake of GM technology. Traits that offer obvious consumer benefits, or more widely understood environmental benefit[1], are predicted to reduce market resistance and in some cases may even drive adoption. In a recent Australian consumer response study, indications were that consumers would be more likely to buy GM products with health benefits, however this would depend on many factors, including the type of product, its price, labelling, and amount of negative publicity (Owen et al., 2005). Others have also stressed the complexity of technology acceptance, in particular the importance of risk perception and trust in regulators and risk communicators (Rowe, 2004).

Some consider that the agricultural biotechnology industry has been slow to deliver on the next generation of crops (Jaffe, 2005). Impediments or bottlenecks along the pathway from technology development to a commercial product entering the market formed a significant component of the consultations undertaken during this study and are discussed in more detail in Chapter 2.

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[1] It is important to note that some first generation traits hold significant environmental benefits. For example *Bt* crops reduce pesticide sprays, and herbicide tolerant crops reduce tillage and result in more efficient crop water use.

1.1.6. Setting the scene worldwide

To date, most of the public discussion and published information on agricultural biotechnology has focused on the release of genetically modified (GM) crops with production enhancing traits such as herbicide tolerance and insect resistance. These two traits, in four main crops, are the most common throughout the world, encompassing 99% of the global area of GM crops commercialised in 2004. GM crops with these two traits combined or 'stacked' are becoming more significant, comprising 8% of the total GM cropping area worldwide in 2004 (see Table 1.1).

Table 1.1 Countries where GM crops are commercially grown (James, 2002, 2003, 2004)

Country	2002 Area under GM crop (mHa)[2]	2003 Area under GM crop (mHa)[3]	2004 Area under GM crop (mHa)[4]	Commercialised Crops
USA	39.0	42.8	47.6	Bt[5] maize, HT[6] maize, HT soybean, cotton, canola
Argentina	13.5	13.9	16.2	Soybean, maize, cotton
Canada	3.5	4.4	5.4	Canola, maize, soybean
Brazil	0	3	5.0	HT soybean
China	2.1	2.8	3.7	Bt cotton
South Africa	0.3	0.4	0.5	Bt maize, soybean, cotton
Australia	0.1	N/A	0.2	Cotton, carnation
India	<0.1	N/A	0.5	Bt cotton
Romania	<0.1	N/A	0.1	Soybean
Spain	<0.1	N/A	0.1	Bt maize
Uruguay	<0.1	N/A	0.3	Ht soybean, maize
Mexico	<0.1	N/A	0.1	Soybean, Bt cotton
Bulgaria	<0.1	N/A	N/A	Ht maize
Indonesia	<0.1	N/A	N/A	Bt cotton
Colombia	<0.1	N/A	<0.05	Bt cotton
Honduras	<0.1	N/A	<0.05	Bt maize
Germany	<0.1	N/A	<0.05	Bt maize
Philippines	0	<0.1	0.1	Bt maize
Paraguay	-	-	1.2	Soybean

GM crops released worldwide have recently been reviewed (Runge and Ryan, 2004) and up-to-date data on field trial and commercial releases worldwide can be obtained from the web databases shown in Table 1.2.

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[2] ISAAA Brief No. 27 – 2002 Global status of commercialised transgenic crops: 2002.

[3] ISAAA Brief No. 30 – 2003 Global status of commercialised transgenic crops: 2003.

[4] ISAAA Brief No. 32 – 2004 Global status of commercialised biotech/GM crops: 2004.

[5] Bt – Containing gene/s from an insecticidal soil bacterium – *Bacillus thuringiensis*.

[6] HT- Containing genes which confer tolerance to particular herbicides.

Table 1.2 Some databases of GM crop releases worldwide

Database	Details	Maintained by	URL
Agbios	Global database of genetically modified plants	Canadian company	http://www.agbios.com/dbase.php?action=ShowForm
Biotechnology and GMOs	Database of European releases (commercial and field trials)	Joint Research Centre of the European Commission	http://gmoinfo.jrc.it/
United States Regulatory Agencies Unified Biotechnology Website	Information on genetically engineered crop plants intended for food or feed that have completed all recommended or required reviews for planting, food, or feed use in the US.	US Department of Agriculture, Environmental Protection Agency, US Department of Health and Human Services	http://usbiotechreg.nbii.gov/database_pub.asp
Biotechnology Industry Database	Biotechnology companies are providing information on commercial status of agricultural biotechnology products	Biotechnology Industry Organization (BIO)	http://www.biotradestatus.com/
Web based Information Services for Agricultural Research for Development	WISARD is a public domain information platform that provides searchable information on agricultural research	WIS International.	http://www.wisard.org/wisard/home.asp

A recent United States Department of Agriculture (USDA) report[7] identified the major drivers of the future of agriculture in the US, as well as key uncertainties that may push future outcomes one way or another. Many of these may also apply to Australian agriculture. The authors then used these drivers to create three different scenarios on how biotechnology may impact on agriculture. The three scenarios presented are as follows;

1. ‘Rosy future’, where by 2015 agricultural biotechnology has been adopted worldwide and offers substantial environmental and consumer benefits.
2. ‘Continental islands’ where the majority of biotechnology products are developed in countries such as US, Canada, Argentina, China, Brazil and South Africa, and in other countries such as Australia the slower regulatory processes have delayed commercialisation or investment in this area.
3. ‘Biotech goes niche’ where GM crops do not end up to be major components of world agriculture but do thrive in important niche markets.

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[7] http://w3.usda.gov/agencies/biotech/ac21/reports/scenarios-4-5-05final.pdf

Regardless of differing opinions on the likelihood and merits of each scenario, they are excellent starting points for discussion on how to prepare for uncertain futures. A similar scenario planning exercise in Australia, coupled with social, economic and environmental impact assessments, would be a useful tool for decision makers.

1.1.7. Significance of China as biotech investor

China is recognised as a significant player in the agricultural biotechnology field and is investing heavily in this technology. Government funding for biotechnology in China is second only to the US, with hundreds of millions of dollars being invested in the late 1990s and early 2000s. China has at least thirteen different biotech crops in field trials including chillies, cabbage, maize, cotton, peanuts, melon, papaya, soybeans, tobacco, sweet pepper and tomatoes (Runge and Ryan, 2004). Staple food crops like rice, potato and wheat are also being developed (MRST, 2005). Predictions of GM rice being commercialised in China range from within 6 months to 5 years time. How China's uptake of GM food crops will impact on world markets, and Australian agriculture in particular is an important area for future work.

1.1.8. Setting the scene in Australia

Continuing challenges face the Australian agriculture and food sector; namely, the declining terms of trade, protectionist international trading policies and in some areas significant land and water degradation. New challenges are emerging that will also have impacts on Australian agriculture. In March 2005, Minister Truss announced the appointment of a high level reference group to prepare a report on future directions for Australian agriculture. This reference group has published an issues paper outlining the questions that they aim to answer and an overview of the Australian agriculture and food sector[8]. Some issues of importance include:

- The increasing importance of consumer demand for markets
- Maintaining the competitiveness of Australian products in the international marketplace
- Increasing importance of efficient and well-linked supply chains
- Appropriate infrastructure, for example transport, communication, water and energy availability
- Higher demands on management skills and access to suitable skilled labour
- Sustainable resource management
- Encouragement of research and development in the agriculture and food sectors
- Impacts of climate variability and change

To help meet many of these future challenges, research into new crops and varieties is needed now, particularly because of the time taken to develop new varieties for commercialisation, both by conventional breeding and GM.

1.1.9. GM crops in Australia

Cotton is the only broadacre GM crop commercialised in Australia. Insect resistant cotton, Ingard®, was first grown in Australia in 1996 and allowed for large reductions

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[8] http://www.agfoodgroup.gov.au/publications/Issues_Paper.pdf

in pesticide sprays. Ingard® was recently been phased out of commercial production and replaced with Bollgard® II. Bollgard® II contains two different genes to allow for improved pest resistance and in the 2004-05 season, these varieties accounted for nearly 70% of planted cotton varieties in Australia. Herbicide tolerant canola was the next broadacre crop expected to be commercialised in Australia, with licences being granted by the Gene Technology Regulator in 2003, but the instigation of State and Territory moratoria on the commercial plantings of GM crops have stalled its progression to market (see Table 2.3). Regulatory barriers to GM crop commercialisation are discussed later in this report.

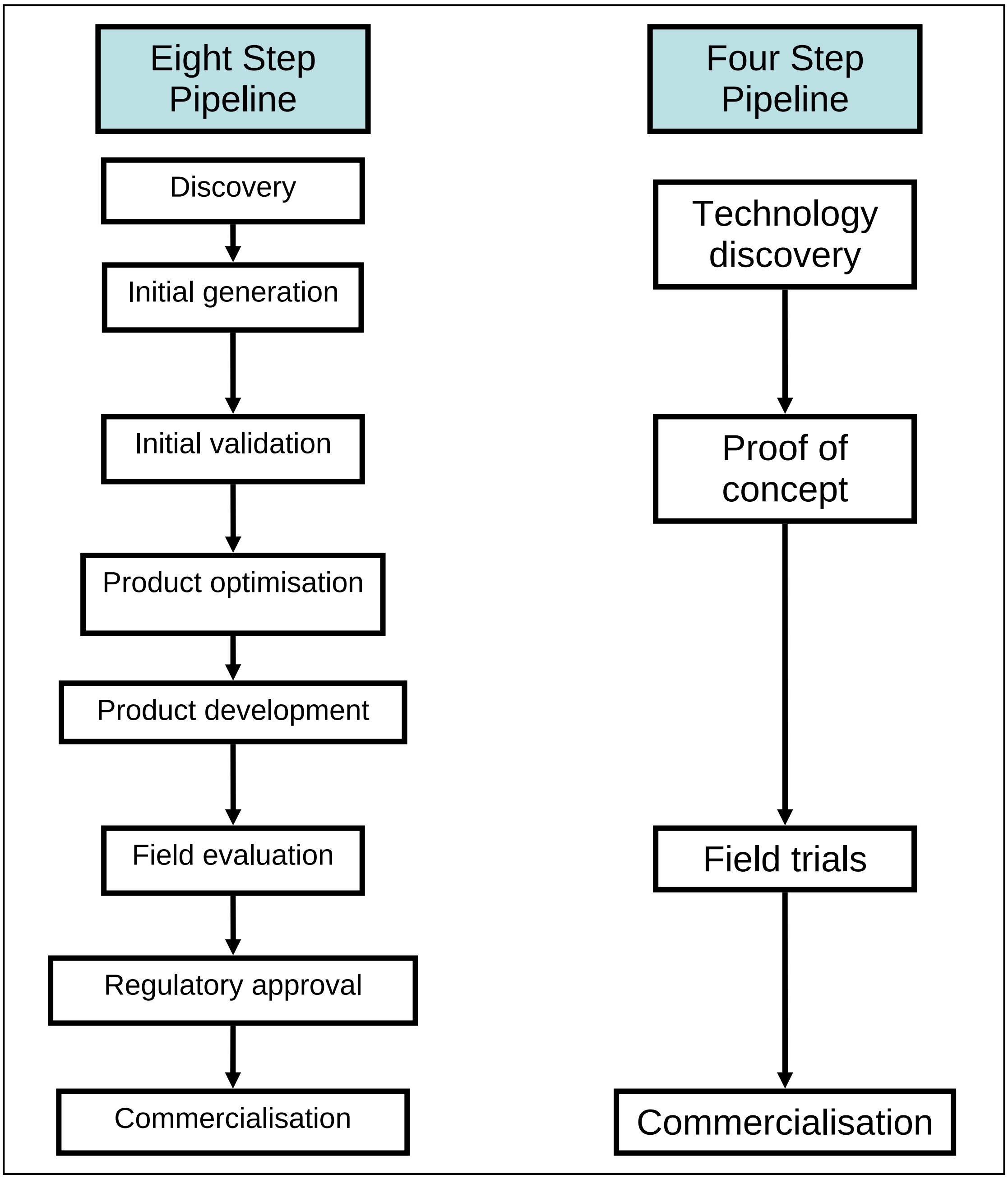

Figure 1.1 Typical development ‘pipelines’ for GM crops (adapted from Gutterson and Zhang, 2004; David Hudson, pers. comm.)

1.2. Chapter 2: Main results of this project

1.2.1. Methodology used

Predicting what's in the pipeline for GM crops is no small task. Other researchers have recently reviewed biotechnology research and commercialisation of GM crops worldwide (Lheureux et al., 2003; James, 2004; Runge and Ryan, 2004; MRST, 2005). All of these reports have a range of different focuses, from assessment of area grown to GM crops to future predictions and scenario planning. Data on Australia specifically is more limited, setting the scene for this work. A recent publication by Agrifood Awareness Australia[9] thoroughly reviews horticulture crops in the pipeline, so this industry sector will not be discussed in this report.

What this BRS research aims to do is to identify GM crops in the pipeline of particular interest for Australian agriculture in order to inform and prepare stakeholders and policy makers of important future issues. This requires a well-developed understanding not only of GM crops in the pipeline, but also of the major issues impacting on Australian agricultural industries that these crops may help address. The focus of this report is on traits that may be ready for commercialisation within the next 5 to 10 years, although because of the many steps in 'the pipeline' as shown in Figure 1.1, and the many factors impacting on progression through the steps, this is always difficult to predict.

For this study the pipeline was divided into four stages;

1. Technology discovery – referring to the research stage of the process, where a scientific principle or idea is explored, and in the context of this report, with a GM output in mind.
2. Proof-of-concept – where gene or genes are tested, usually in model plants, for those which show the most promise for application to crop plants.
3. Field trials – where genes are tested under field conditions experimentally to determine the likely success of the crop, and sometimes also their performance in elite varieties. Appendix B shows current and past Australian GM crop field trials approved by the Office of the Gene Technology Regulator. It is important to realise that not all field trials are an indication of imminent commercial release. For example the GM rice field trials being carried out by CSIRO are large-scale experiments, rather than a step towards commercialisation of a particular product. Likewise, GrainBiotech are carrying out GM salt tolerant wheat field trials in Western Australia, but have no short term plans to commercialise a product in Australia.
4. Commercialisation – where for successful traits, business plans have been launched, seeds are being bulked for sale and regulatory approvals have been obtained. After commercialisation the extent of market acceptance and widespread production could also be considered as a component of the pipeline. The 'Flavor Savor' tomato is an example of an early GM product that reached commercialisation in 1994 in the US, however is not currently in widespread production.

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[9] http://www.afaa.com.au/

Table 2.1 shows a classification of the main traits that are discussed in this report. It is important to note that this list, and the traits that are discussed in more detail in Sections 2-4, is not exhaustive. There are a significant number of traits in the pipeline worldwide that are not captured. Most of these are still in the technology discovery phase, but some, like herbicide tolerance, are widely commercialised. For example, other significant traits that were omitted include improved nutrient use, functional foods, longer post-harvest life and post-harvest pest protection. Additionally, traits for ornamental crops were not discussed, despite GM carnations being one of the two GM crops commercialised in Australia (the other being cotton).

Table 2.1 Classification of GM crop traits used in this report

Category	Specific traits	Also called
First generation	Environmental (abiotic) stress tolerances Improved pest and disease control Improved nutrient use	Input traits
Second generation	Enhanced nutrition Improved oil quality Longer post-harvest life Improved feed and pastures	Output traits
Third generation	Pharma crops Industrial crops Phytoremediation	Non-food industrial products and processes

The scope of this project was confined to those traits listed because they are either the most advanced or the most commonly represented traits in the literature surveyed. Similarly, GM crops listed in the summary tables in the Appendices are not intended as an exhaustive list, but rather as an indication of trends in GM research. For an exhaustive list, a survey approach as undertaken recently in Europe would be required (Lheureux et al., 2003).

Initially this project involved reviewing the published literature for advances in the development of particular GM traits. However, what quickly became clear was that the scientific literature has limitations for predicting what is in the pipeline. Commercial sensitivities often delay or prevent publications of key advances until a product is close to release. There is also often a tendency for researchers to make very optimistic, sometimes completely unrealistic, claims about the predicted progress of their work, simply to attract funding for this area.

For these reasons a significant component of this project involved structured consultations with scientists in the public and private sectors in Australia. Appendix D shows examples of the questions that were asked and Appendix C shows the list of organisations that were consulted. Although there was reluctance from a few individuals to speak with us, in general most were keen to take the opportunity to share their knowledge and views. From our point of view the consultations were an invaluable insight into the issues facing the agricultural biotechnology research and development sector in Australia.

1.2.2. Main outcomes of the consultations

There are exit points along the pipeline where decisions have to be made about the potential of a product to reach commercialisation, and the value and benefits that the product will deliver to end-users, balanced against the costs of product development and market entry. There are also many barriers to commercialisation of a GM crop and these issues were raised repeatedly during the consultation phase of this project.

1.2.3. Barriers or bottlenecks to commercialisation of a GM crop

1.2.3.1. Technical barriers

Crop physiology and its genetic control is very complicated and GM crops, just as varieties in conventional breeding programs, need field trial testing under diverse environmental conditions to assess their performance. There are many examples, both in Australia and overseas, of GM crops that have reached field trial stages but were not developed further because of technical barriers. GM disease resistant field peas are an Australian example of a GM crop in the pipeline that did not reach commercialisation, because, although the crop resisted disease in the field trials, it was not an effective stockfeed.

Another significant issue is the insertion and control of multiple genes, also known as ‘gene stacking’. Most of the traits that are discussed in later chapters of this report involve the expression of multiple genes in a crop plant. In Chapter 3, the complexities of the pathways involved in environmental stress regulation are explained, and engineering tolerance to most of these stresses will involve coordinated switching of multiple genes. Similarly the production of adequate levels of a biodegradable plastic will likely involve five or six genes (Slater et al., 1999). Although the number of commercialised stacked GM traits is increasing, for example 8% of the worldwide crops commercialised in 2004 contained stacked traits, expression and manipulation of multiple genes in plants is still a significant technical hurdle (MRST, 2005).

Strategies for addressing this issue are being employed and have been reviewed recently (Halpin, 2005). These include iterative strategies of either sequentially transforming a plant with multiple genes, or crossing plants containing separate transgenes. A significant disadvantage of these strategies is that the genes introduced by these methods will sit at different places in the plant’s genome and can segregate out in future generations. For retransformation different marker genes are needed and these may be limited by availability, public acceptance and regulatory approval. Other innovative strategies are becoming available with solutions to the issue of gene stacking, but it still remains a technical bottleneck for complex genetic traits (Halpin, 2005).

1.2.3.2. Freedom to operate (FTO) barriers

The global trend of increasing private sector investment in agricultural research has increased the importance of protecting both intellectual property[10] (IP) and tangible property[11] (TP) rights. When examining the development of a GM crop, from technology discovery to commercialisation, multiple property rights need to be considered. These include covering platform technologies such as the gene insertion process, marker genes and gene switches, to the specific traits involved. Ownership and origin of these processes and components must be tracked to ensure that once an end product is obtained, the freedom to operate has not been fettered. The IP/TP rights landscape should be evaluated at the commencement of any project to ensure any barriers are recognised early and can be negotiated or circumvented. For example, where the product will be produced and marketed will govern the laws, patents and licences that need to be navigated prior to commercialisation (Kowalski et al., 2002). The complexity of the IP/TP landscape will only increase with the advent of 'stacked' or multi-gene traits and hence early identification of the barriers to commercialisation will become increasingly significant.

Thus, the IP landscape for GM crops is akin to a legal obstacle course. Although the performance of a research organisation, be it public or private, is increasingly assessed by the number of patents that it holds, far more important for the successful commercialisation of any biotech product is the effective use of the IP it incorporates (Barrett, 2005). Knowledge of the freedom to operate, that is, does the invention infringe on the IP rights held by others, is essential for commercialisation of a biotech product (Graff et al., 2004). During consultations for this project, it was suggested many times that biotechnology projects in the pipeline in Australia have had to be shelved due to the inability to access the IP rights required to progress the project, in particular where the patent owner is unwilling to negotiate a licensing deal.

Although Australia has a solid reputation in basic research in many fields, including agricultural sciences, its US patenting rate is very low by international standards. Australia holds approximately 0.6-0.7% of world life science patents, thus the chance that any given Australian invention will have an unfettered freedom to bring a product to market is very low. There is consensus that development of an IP management strategy is essential to successful business outcomes. However, where differences occur is how early in the pipeline such a strategy should be developed. Commercially driven research organisations will tend to have a well-developed IP program early in the pipeline, whereas others will examine the IP landscape in detail only when close to commercialisation. Table 2.2 shows the pros and cons for the timing of an IP management strategy.

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[10] Examples include patent rights, plant variety protection certificates, and unpublished patent applications.

[11] Examples include computer software and germplasm.

Table 2.2 Options for timing of IP program development (adapted from Kowalski et al, 2002)

Timing	Strategy	Pros	Cons
Before research work is started	Search of relevant patent and scientific literature	Plan development and make decisions on how to proceed with FTO in mind Little investment so loss is minimised	May hold back promising lines of research May waste time and resources before a product is even possible
After proof of concept	Need to consider materials and methods in addition to products and processes	Know that product is possible If IP/TP constraints exist still have time to redesign product	If IP/TP constraints exist, redesigning product may be costly in time and resources
After the product has been developed	Must consider aspects of large-scale production as well as marketing/licencing opportunities	Know that the product has commercial potential and able to assess value	Significant resources already invested Risk of product not getting FTO Protracted licence negotiations may delay marketing
Product ready for commercialisation	Need to work through who owns what and options available for IP rights access	None	Owners of third party IP/TP in strong position to dictate terms of licence or even refuse access

There are three major parts to an IP program:

1. The acquisition process which ‘molds the output of inventors minds into legally protected assets’ (Barrett, 2005)
2. Understanding the patent landscape and risks of infringement which involves product deconstruction and clearance
3. The IP strategy which guides the process. It would consider who to negotiate with, who to bypass and who to beat.

A recent publication discusses how the International Rice Research Institute (IRRI) has been developing policies and procedures to deal with IP rights management in the context of an international agricultural research centre, and how well these policies are serving the international public good sharing mandates (Egelyng, 2005).

New initiatives are being developed to address this issue of complexity and restrictions to successful innovation. BIOS, the Biological Innovation for Open Society[12], has been developed by an Australian based research organisation, CAMBIA, that builds on models of open source access to information technology and applies these concepts to innovations in biology, including agricultural biotechnology. For example, CAMBIA has developed an alternative plant transformation procedure that is available to the international community ‘with no commercial restrictions other

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[12] www.bios.net

than covenants for sharing of improvements, relevant safety information and resultant data and for preserving the opportunity for others freely to improve and use the technology' (Broothaerts et al., 2005). Another aim of BIOS is to restore transparency of the patent landscape, and as such the BIOS patent lens, a cost free patent search engine has been developed.

Although Australia is only a very small player in the international field of agricultural biotechnology development, it offers significant advantages for investment. The high quality of basic plant research in Australia has already been mentioned. Also significant is the strength of the IP/TP systems in place, giving higher protection for inventions and more chances for recuperation of investment than if products were developed in developing countries, where the systems are often unreliable (Kowalski et al., 2002). Australia thus could be positioned as an agricultural biotechnology gateway for Asia. This advantage was emphasised during the consultation process. However, any advantage may be lost due to the regulatory barriers to bringing a GM product to market that exist in Australia and are discussed in more detail below.

1.2.3.3. Regulatory barriers

In Australia, GM organisms are regulated by the Gene Technology Regulator (the Regulator) supported by the Office of the Gene Technology Regulator (OGTR) under the *Gene Technology Act 2000 (Cth*). The role of the Regulator is to protect human health and safety and the environment by identifying and managing risks posed by the use of this technology. The OGTR has developed a risk analysis framework which describes how the Regulator approaches risk assessment and risk management for GMOs[13].

The Regulator liaises with other regulatory agencies, including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), and the Therapeutic Goods Administration (TGA) to coordinate the approval of GM products for use and sale.

There are currently only two GM crops grown commercially in Australia: cotton, which has been modified for herbicide tolerance, pest resistance, or a combination of the two (Box 1); and carnations, with modified flower colour. Herbicide tolerant canola was the next crop expected to be grown commercially in Australia, with licences being granted by the Regulator in 2003. However, the instigation of State and Territory moratorium legislation have prevented commercial plantings of GM canola crops and stalled its progression to market (Table 2.3).

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[13] http://www.ogtr.gov.au/pubform/riskassessments.htm

Table 2.3. Status of Australian Commonwealth, State and Territory GM Legislation in relation to GM food crops

Jurisdiction	Details
Commonwealth	No ban on GM crops
New South Wales*	Ban on commercial cultivation of all GM food crops (including GM canola) until March 2008. Exemptions permitted for field trials
Victoria	Ban on commercial cultivation of GM canola until February 2008. Exemptions permitted for field trials
South Australia	Ban on commercial cultivation of GM food crops to be reviewed by April 2007. Exemptions permitted for field trials
Tasmania	Ban on commercial cultivation of all GM crops (including GM canola) until June 2008. Exemptions permitted for field trials of non-food crops.
Western Australia	Ban on commercial cultivation of all GM crops (including GM canola) to be reviewed by December 2009. Exemptions permitted for small scale field trials.
Australian Capital Territory	Ban on the commercial cultivation of GM canola until July 2006. Exemptions permitted for field trials.
Queensland*	No ban on GM crops
Northern Territory	No ban on GM crops

* Large scale growth of GM cotton in these States.

A common thread of the consultations was the negative impact of the State moratoria on research and development of GM crops in Australia. Investors in research, including public sector organisations, are responding to the lack of a clear and consistent path to market for a GM food crop (AusBiotech, 2004). The overwhelming message was that the vast majority of GM crops in the pipeline in Australia are stalled, waiting for changes in the regulatory and marketing climate. For various reasons the capacity of these crops to be developed further and commercialised when moratoria were lifted is questionable. Even disregarding the State moratoria, Australia’s gene technology regulatory system was considered by most researchers interviewed to be overly stringent and not commensurate with risk (BRS consultations).

1.2.3.4. Value capture

Another significant element in the pathway to the commercialisation of a GM product is the development of business cases which identify how to capture value from research and development. Although not typically part of a research scientist’s training, in the increasingly competitive research environment applying for funding grants or looking for private sector investment partners requires these skills. Being able to define a problem in economic terms, and then describe the scientific solution and how value may be captured, particularly by investment returns, is increasingly important. Some research organisations have responded to these pressures by employing staff with experience in this area to liaise between researchers and potential commercial partners.

Although value capture is also important for the adoption of input traits, for output traits the value chain is more complex. A typical value chain for input traits involves the researcher or product developer, the seed producer and the farmer [14]. For output traits there may be as many as four or more additional participants in the value chain. Value capture for GM feed traits has been discussed in detail recently (Williams, 2003), with the conclusion that insufficient value within a trait may limit the adoption of the technology. In this report the three generations of GM crops are discussed, with detailed examples of each included.

Viewing economic value capture as the only driver of research and development has many limitations and the risks of only following short-term, commercially driven research are widely recognised by Australian scientists. Other types of value may be important, such as social and environmental advantages. Public sector organisations are well aware of the strategic, long term, advantages of the triple bottom line approach.

1.2.3.5. Marketing uncertainties

Marketing uncertainties continue to be a key barrier to the progression of GM crops through the pipeline to commercialisation. The risk of developing a GM product with restricted markets or where the path to market is unclear, has had a huge impact on research and development investment decisions in Australia. Concerns have been expressed that the commercialisation of GM grains in Australia could pose market access problems for exports. However, trade data shows that Australian grains currently compete in export markets in which GM crops have a significant market share. In the case of some meat and dairy industries, certain markets reportedly require assurance that animals were raised on non-GM feed. For individual industries, monitoring of GM status of export markets and key competitors in these markets provides vital knowledge.

In the United States (US) and Canada, where GM crop uptake has been extensive, commercialisation of GM herbicide tolerant wheat was put on hold by the major developers in part because of concerns for domestic and export markets such as the European Union (EU). Significantly, however, research and development of first and second generation GM wheat characteristics is continuing in laboratories and glasshouses worldwide, including in the US, Canada, the EU and Australia.

Some markets may require separate supply chains for GM and non-GM products, increasing the importance of segregation and identity preservation systems. The extra value of second and third generation crops would be a driver for implementing such systems and justifying the extra costs involved.

Whether marketing barriers to GM crops are due to consumer or importer preferences, or whether they are actually being used as barriers to trade is questionable. Many studies and surveys in Australia and overseas have explored consumer attitudes towards GM foods and crops and the results are often contradictory. This represents not only global differences in the attitude, design and objectives of individual studies but also the complexity of consumer opinion.

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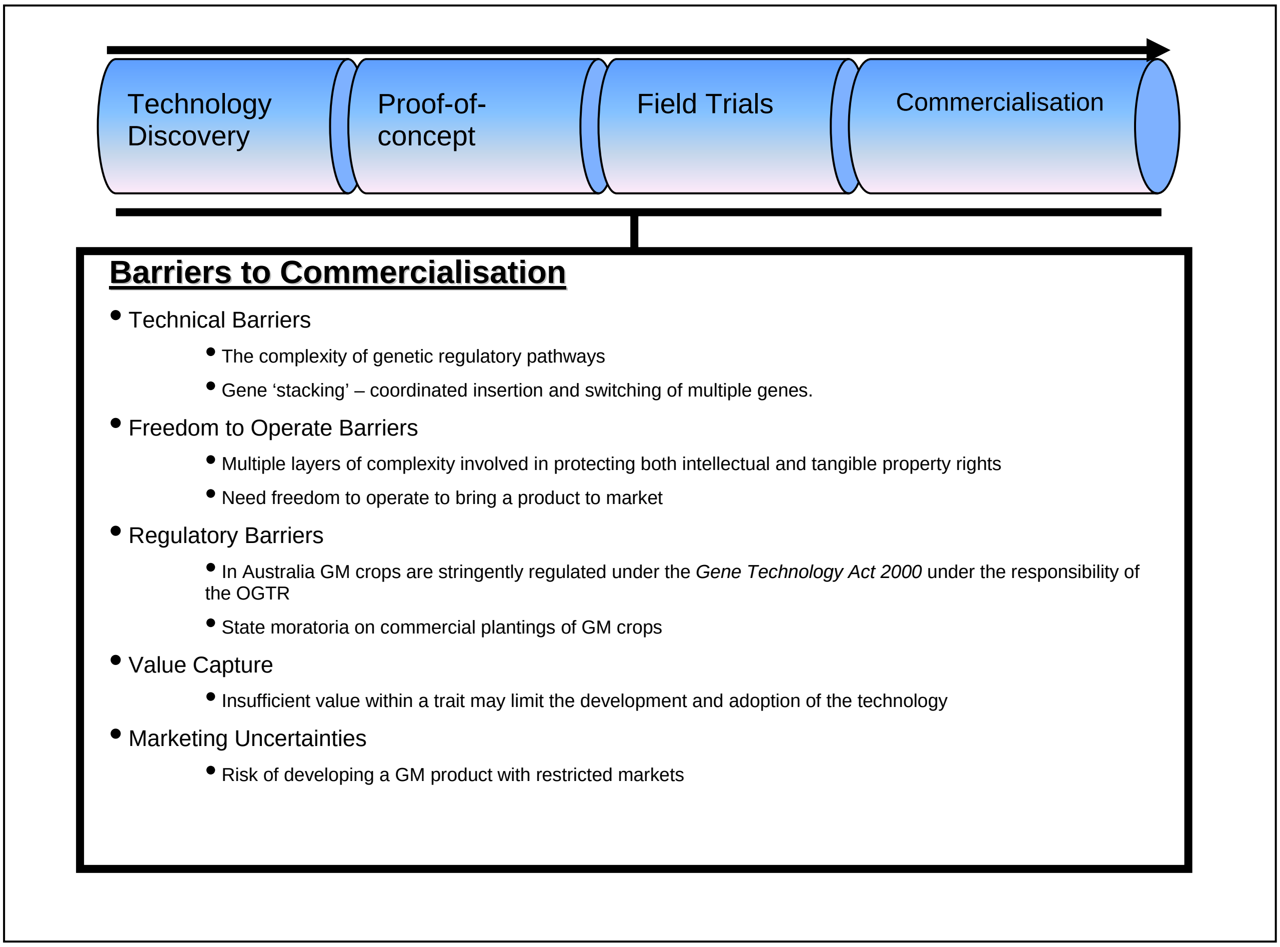
[14] For some input traits such as herbicide tolerant traits, agricultural chemical developers and suppliers would also be a part of the chain.

Trade in GM products has been the subject of discussion in the World Trade Organization (WTO). WTO agreements on sanitary and phytosanitary issues, technical barriers to trade and trade-related aspects of intellectual property rights are all relevant to trade in GM crops, as with any other commodity. The United States, Canada and Argentina have mounted a WTO challenge asserting that the moratorium applied by the EU since October 1998 on the approval of biotech products has restricted imports of agricultural and food products (disputes DS291-3). Australia is a third party to these challenges. The WTO is expected to issue its final report on the GMO case in 2006.

1.2.3.6. Other barriers to commercialisation

Funding limitations are an issue often discussed in the context of barriers to commercialisation. This barrier would intersect with regulatory issues, marketing issues, and also lack of well-constructed business cases. For example, if the regulatory process requires extensive time and resources, if there is no obvious immediate return on investment, or no obvious way of capturing benefits, then a GM project is unlikely to be funded. The barriers to commercialisation of a GM crop in Australia are summarised in Figure 2.1.



Barriers to Commercialisation

- Technical Barriers
    - The complexity of genetic regulatory pathways
    - Gene 'stacking' – coordinated insertion and switching of multiple genes.
- Freedom to Operate Barriers
    - Multiple layers of complexity involved in protecting both intellectual and tangible property rights
    - Need freedom to operate to bring a product to market
- Regulatory Barriers
    - In Australia GM crops are stringently regulated under the *Gene Technology Act 2000* under the responsibility of the OGTR
    - State moratoria on commercial plantings of GM crops
- Value Capture
    - Insufficient value within a trait may limit the development and adoption of the technology
- Marketing Uncertainties
    - Risk of developing a GM product with restricted markets

Figure 2.1. Barriers to commercialisation of a GM crop in Australia.

Section 2: First Generation Traits

2.1. Chapter 3: Environmental Stress Tolerance in Crop Plants

2.1.1. The Problem

Environmental (abiotic) stresses, caused by salinity, drought, acid soils or temperature extremes pose serious threats to Australian crop plants. They result in a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang et al., 2003).

2.1.2. Plant Strategies to Combat the Problem

In contrast to animals, plants are sessile (non-moving) organisms, hence, to tolerate or adapt to unfavourable environmental conditions they must be able to moderate their response to stress. This is achieved by the activation and regulation of suites of stress responsive genes (Wang et al., 2003). Of those named above, salt, drought and temperature stress are often interconnected and may induce similar damage to the cell. Salt and drought stress manifest themselves primarily as osmotic stress[15], resulting in the disruption of homeostasis[16] and ion distribution in the cell (Wang et al., 2003). They may also result in the production and accumulation of reactive oxygen species (ROS), leading to oxidative stress[17]. Oxidative stress in turn can cause damage to cell membranes and proteins and ultimately loss of yield in the plant.

There are three interconnected aspects of plant activity that are needed to achieve tolerance to abiotic stress (Zhu, 2001); namely, damage must be prevented or alleviated, homeostatic conditions must be re-established, and growth must resume.

The complete molecular control mechanism(s) plants use to combat abiotic stress is unclear. There are, however, specific stress related genes which have been identified, and the development of GM stress-tolerant crop plants is based on their modification. These genes fall into three categories: (i) those that are based on transcriptional control; (ii) those that function directly in the protection of membranes and proteins; and (iii) those that are involved in water and ion uptake and transport (Wang et al., 2003).

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[15] **Osmotic stress** is the stress caused by an ionic imbalance within the cell.

[16] **Homeostasis** is the tendency of a biological system to resist change and to maintain itself in a state of stable equilibrium.

[17] **Oxidative stress** is the stress caused by the production and accumulation within the cell of reactive oxygen species (ROS) such as hydroxy radicals, singlet oxygen and hydrogen peroxide.

2.1.3. Conventional Breeding Solutions

It is possible to create new varieties of crop plants which exhibit abiotic stress tolerant traits by using traditional breeding methods combined with the use of scientific gene selection criteria. For example, Graingene, a joint venture between AWB Ltd, the Grains Research and Development Corporation (GRDC), Syngenta and CSIRO Plant Industry have in recent years released new wheat varieties bred specifically for dry conditions, two of which are ‘Drysdale’ and ‘Rees’. The scientists developed a way of making these plants use available water more efficiently. These varieties have an advantage over comparable wheats in dry years, being able to produce more grain, despite receiving less rainfall (CSIRO, 2004a, b).

In recent years, traditional breeders have linked up with biotechnologists to develop ‘molecular markers’. Molecular markers enable breeders quickly and accurately to keep track of traits in a breeding program. Molecular markers are short fragments of DNA already present in an organism that can be used by breeders to quickly and accurately identify and track the inheritance of a desired characteristic in a breeding programme. Molecular markers allow better control over which genes are retained during plant breeding and can be used to facilitate traditional breeding programs without necessarily resulting in a GM product (GRDC, 2003).

2.1.4. Limitations with the Conventional Approach

Tester and Bacic (2005) outline three contributing factors as to why traditional approaches to breeding crop plants with improved abiotic stress tolerance have so far had only limited success – (i) the focus has been on yield rather than on specific traits; (ii) there are difficulties in breeding for tolerance traits; and (iii) desired traits can only be introduced from closely related species. The overall trait (of stress tolerance) is determined by a number of sub-traits, each of which may be determined by numerous genetic pathways (Flowers, 2004). In other words, the difficulty of traditional breeding for abiotic stress tolerance in crops is that it is not just a matter of conferring a single trait, but rather a combination of quality, disease resistance, yield, *and* the trait of interest is required. Biotechnology allows manipulation to take place in an existing desirable background, although this is not always possible. For example, the elite cultivars of wheat and barley cannot be transformed using current technology, so any traits enhanced by GM must be backcrossed into these lines by conventional breeding methods.

Additionally, it is argued that as conventional breeding methods transfer many genes between crossed lines, in addition to those of interest, it is less precise than GM technology, in which only the gene(s) of interest are transferred.

2.1.5. Applications of Biotechnology

The first generation of commercialised GM crop plants focused on the addition of single gene traits such as herbicide resistance and pest tolerance. In contrast, it may prove more difficult to regulate the plant's genetically complex response to abiotic stress (Wang et al., 2003).

An underlying problem for both conventional breeders and biotechnologists is that the majority of research into the genetic control of abiotic stress tolerance has been performed in the model plant *Arabidopsis thaliana*, a dicotyledon. The majority of crop plants (cereals and grasses) are monocotyledons, and there are fundamental differences in development and anatomy between the two. Many of the mechanisms of abiotic stress tolerance may differ between the two plant groups, and hence knowledge gained from *Arabidopsis* may not be transferable to the major crop species (Tester and Bacic, 2005).

As suggested by Wang et al (2003), it is necessary that a GM crop possess a tightly-regulated stress-response capability in order that it does not affect crop performance or yield in the absence of stress. They believe that it is in this respect that conventional breeding and selection techniques will continue to have an impact. Some researchers believe that the impact of abiotic stress tolerant GM crops at the whole farm level when all environmental factors are taken into account may be as small as a 2-3% advantage (BRS consultations).

Engineering of stress tolerant crops is not simply a matter of creating plants which can survive stress. To be of use to agriculture, a worthwhile stress tolerant plant must be able to produce yields comparable to its non-stressed counterparts, or be more cost-effective than traditionally bred varieties under the same stresses.

There are currently a large number of crops exhibiting abiotic stress tolerant traits in the pipeline which are being bred using the assistance of molecular marker technology. However, this project has revealed there are comparatively few in the pipeline which are being developed using GM technology.

There are five steps which need to be taken to commercialise a novel trait in a crop plant; (i) identify the problem; (ii) identify useful genetic variation; (iii) incorporate useful genetic variation into suitable background; (iv) validate selected lines in the field; and (v), if results are positive, release the selected lines if they meet all release criteria (BRS consultation).

2.1.6. Salt Tolerance – Why is it Necessary?

It is estimated that about 30 million hectares of mainland Australia are underlain by salt (Atwell et al., 1999). Western farm management practices since European settlement are often said to have contributed to the estimated 5.7 million hectares of land in Australia with a high potential of developing dryland salinity (Australian Dryland Salinity Assessment 2000[18]). This figure could reach 17 million hectares by

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[18] http://audit.ea.gov.au/ANRA/land/docs/national/Salinity_Contents.html

the year 2050. Western Australia currently contains 80% of the national total, and the proportion of its agricultural land which is affected by salinity is expected to be 30% by 2050, as compared to 15% of agricultural land nationally (Pannell et al.). Globally, increased salinisation of agricultural land is expected to result in a 30% loss of land in the next 25 years and up to 50% by 2050 (Wang et al., 2003).

In Australia most dryland salinity can be found in the wheat growing and sheep-grazing zones of Western and South Australia. The extensive land clearing in these areas, together with cropping and grazing has resulted in a net increase of rain going into saline groundwater, raising the water table and bringing salt to the surface (Atwell et al., 1999).

Salt inhibits plant growth in two ways – by reducing water uptake, and by becoming toxic to the plant when present in excessive quantities.

Biotechnology currently provides two options to combat saline landscapes. Plants can be engineered to be tolerant to saline soils. For example, over-expression of the *Arabidopsis* gene *AtNHX1* in *Brassica napus* (canola) plants resulted in the *Brassica* lines being able to grow, flower and produce seed in the presence of high levels of sodium chloride (Zhang et al., 2001). Alternatively, plants that ‘recharge’ or ‘remediate’ salt damaged land can be designed. For example, lucerne is currently used to recharge saline land. Lucerne has deep roots and is grown in 2-3 year cycles to lower the water table, reducing salt levels in the topsoil and allowing the subsequent propagation of crops. However, lucerne is highly sensitive to acid soils, a feature of many saline areas. As discussed below, there exists the potential to use biotechnology to develop acid tolerant lucerne lines.

With conventional breeding techniques it may be possible eventually to confer salt tolerant traits to crop species; however, salinisation of arable land is occurring more rapidly than crop breeders can produce saline tolerant lines. The use of biotechnology allows for the introduction of salt tolerant traits into crop species in a single generation, as opposed to the several that would be required using conventional techniques. However, this statement is subject to the caveat discussed earlier that elite grain cultivars often cannot be transformed, so any GM traits would have to be first introduced into a ‘non-elite’ cultivar, before backcrossing into an elite line.

In general, wheat’s roots are very effective at excluding salt. It is likely that GM would only make small improvements in this area. In contrast, the roots of barley and canola are very poor at excluding salt and this is a possible target for GM technology. Barley and canola have alternative methods of protecting their cells from salt toxicity (BRS consultation).

For example, barley is better than wheat at producing osmoprotectants[19] to maintain ionic balance within salt affected cells. Wheat is particularly poor at doing this, a reason why a gene for the osmoprotectant amino acid proline has been inserted into wheat lines by Grain Biotech Australia in an attempt to generate salt tolerant lines, described in more detail below.

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[19] **Osmoprotectants** are compounds which reduce the damage that may be caused by osmotic stress.

However, there are doubts over whether the overexpression of osmoprotectants will work in the field (BRS consultation). This is because an increase in salt tolerance is often associated with slow growth, stunting and low yields. There are no crop species yet which have evolved to be truly effective at both salt tolerance *and* yield. Most plants which can be observed growing in salt affected areas are stunted, straggly and very slow growing.

An important factor to take into account when thinking about salinity is the variability of the landscape. For example, it can be shown that where salinity is patchy, as it usually is, increasing yield potential by 10% across the field is more effective than doubling the yield in the very salty areas, since the most yield comes from the least salt-affected areas (BRS consultation).

It has been suggested that GM may not be the best approach for generating salt tolerant wheat and barley (BRS consultation). In wheat, there is already a large amount of natural diversity in the various wheat ancestors, and the ability to exclude salt has already been found in some land races. There has never been the selection pressure for salt tolerance in commercial cultivars. Barley, too, has scope in its germplasm as it has some wild relatives that evolved in saline areas (BRS consultation). In contrast, rice is a crop where genetic modification for salt tolerance could be useful as its germplasm has been extensively screened for salt tolerance with little success (BRS consultation).

2.1.7. Salt Tolerance – What's in the Pipeline?

A review of the literature reveals numerous research papers and reviews about the engineering of salt tolerance in plants. The majority of published research has been in the dicotyledon *Arabidopsis*, a model plant for GM research, and it is as yet uncertain whether this knowledge can be transferred directly to the monocotyledon crop species. It is often unclear from these papers as to whether or not the research is targeted towards commercialisation.

It is likely that manipulating the expression of a single gene may not be sufficient to confer adequate tolerance in the field. This is due mainly to the complexity of abiotic stress tolerance, discussed previously. Plants growing in saline environments often suffer from a combination of ionic, osmotic and oxidative stress. Combating these stresses requires the manipulation of whole suites of genes, the technology to do this is currently either unwieldy or unavailable.

The OGTR has recently approved an application by Grain Biotech Australia (GBA) to undertake a small, limited and controlled release of GM wheat in Western Australia. The aim of the trial is to test how lines of wheat engineered to have increased salt tolerant properties perform in saline conditions in comparison with conventional wheat and other cereal crops.

GBA have engineered a line of wheat with increased production of the amino acid proline. Increasing proline levels should allow the plants to grow in the presence of increased salt levels in soil (OGTR Licence DIR 053/2004). Laboratory testing revealed that the GM wheat produced 3 times the yield of non-GM salt tolerant wheat when grown in water that was one third the saltiness of sea water.

Scientists introduced a gene from the model plant *Arabidopsis*. The new gene is an enzyme known to be involved in the production of proline. However, proline is very nitrogen rich and extracts a high cost on the plant to produce. Therefore, it has been suggested that high levels of proline synthesis within the cell may draw nitrogen away from where it is normally needed for cell growth and metabolism (BRS Consultation).

The West Australian newspaper has reported that if successful, it is likely that GBA's salt tolerant wheat would be commercially released in Mexico, a loss for Western Australian farmers (The West Australian, 11/5/05).

Scientists at CSIRO Plant Industry have identified two sodium-excluding loci in durum wheat, the expression of which results in sodium exclusion that is 5-10 times better than current cultivars. If one or both of these loci are due to a single defined and clonable gene, they may be able to transfer this gene to other species that lack the ability to exclude sodium (BRS consultation).

2.1.8. Drought Tolerance – Why is it necessary?

In Australia, the efficient use of scarce water resources is a major natural resource management issue. Critical to sustaining the crop industry is the development of plants which use water more efficiently (see the examples of 'Drysdale' and 'Rees' wheat lines above) and produce good yields even during dry conditions.

The Australian Bureau of Statistics (ABS) estimates that the direct effect of the recent drought on Australian agricultural production in 2002-03 was equivalent to just over $6.5 billion dollars (National Accounts Article – Impact of the drought on Australian production in 2002-03[20]).

As is the case for salt stress, drought stress is very complex and can manifest itself in the plant in many ways. Plants have evolved complex signalling pathways to recognise and respond to drought stress. Again, there is unlikely to be a 'drought tolerant gene', it is more likely that plants which display tolerance to desiccation do so by regulating whole suites of genes, some of which may be the same as those involved in the salt stress response. It is likely that genes which confer an ability to survive cell desiccation will not confer a better ability to accumulate biomass under limited water conditions. It has been observed that most transformed plants with increased desiccation tolerance exhibit reduced growth even in well-watered conditions (Tardieu, 2005).

There are three ways to improve water-use efficiency; namely, (i) select for deeper roots to access ground water; (ii) select for early vigour to reduce evaporation from the soil; and (iii) improve water-use efficiency at the leaf level (BRS consultation). Both (i) and (ii) are traits which are likely to involve the regulation of multiple genes, making them potential candidates for selection by conventional breeding assisted by molecular marker technology.

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[20] http://www.abs.gov.au/Ausstats/abs@.nsf/Lookup/191429634F16BC4DCA256C8400825F82

It is suggested that what matters most when dealing with a limited water supply is resource economics. Namely, given a limited water supply, what is the best (economically optimal) yield that can be produced (BRS consultation)? The best approach may be to put research dollars into improving ‘water productivity’ by selecting for appropriate behavioural plant characteristics, as opposed to searching for a single ‘drought tolerant’ gene using functional genomic approaches. For example, as little as an extra 20mm of water extracted by the roots from the subsoil late in the growing cycle can then produce an extra tonne/ha of yield. This may be a good target for research towards making crop plants viable when grown under conditions of limited water availability (BRS consultation).

2.1.9. Drought Tolerance – What’s in the Pipeline?

In the Australian grain belts most of the rain falls during the growing season. Hence the question is: how best to capture this water? One way is to select for early plant vigour so that when the rain hits the soil there is plenty of shade to prevent direct evaporation. It has been shown that when there is only sparse vegetative cover up to 50% of rainwater is lost from soil through evaporation alone. Improving the vigour of a crop can reduce the amount water lost to evaporation to only 25% (BRS consultation). This is an example of drought tolerance being conferred by modification of a seemingly unrelated trait, in this case plant vigour.

CSIRO Plant Industry has developed delta-carbon technology, a means to assess the water use efficiency of plants. This was the technology used to select for the ‘Drysdale’ and ‘Rees’ drought tolerant wheat lines mentioned previously. In 2003, independent field trials showed that Drysdale yielded 23% more under dry conditions than the widely cultivated Diamondbird variety (CSIRO, 2004a, b)

Genes of interest to emerge from functional genomic approaches are the DREB/CBF family, implicated in desiccation tolerance. These genes have been expressed in wheat and the resulting lines field trialled by CIMMYT in Mexico. Desiccation is rare in viable agriculture; recovery from desiccation would not be useful to rain-fed annual crops as the growing season is limited. However, it might be a useful adaptation for perennial crops (BRS consultation). Chinese scientists have tried expressing the same gene in rice, the results are as yet unknown (BRS consultation). In separate work, Chinese scientists are trying to express in rice a suite of ten genes which have been identified as possible candidates for drought resistance, again, the results are unknown (BRS consultation).

Also of promise is the genetic modification of plants to protect their floral tissue against short, sharp drought events at the time of flowering, which can otherwise dramatically reduce the number of seeds set and hence yield, especially in rice and maize, which are very sensitive to such drought impacts (BRS consultation). This is a complex trait but it may yet prove amenable to manipulation. It is known that sugar metabolism is disrupted resulting in a lack of supply to developing embryos. Induction of senescence genes is also involved, which may commit the plant to abort many embryos (BRS consultation).

Wheat often has the opposite problem, setting too many seeds despite drought during grain filling. The result is a large proportion of ‘screenings’; namely, grain that is too small and the price per tonne paid for the harvested grain is reduced accordingly. However, the biochemistry and physiology of seed-set in wheat is not yet well enough understood to gauge the prospects of GM approaches (BRS consultation).

Some scientists view that, apart from herbicide tolerance in crops, which helps reduce competition for water by weeds, there are no genetic transformations in the pipeline that are likely greatly to improve yield in conditions where water supply is scarce (Passioura, 2004). A search of the BIOS Patent Lens full life science patent database using the keywords ‘drought AND tolerance’ yields 4048 results[21]. However, Passioura (2004) believes that it is hard to discern any of these as likely to have practical influence at field level. This is because a quick scan reveals that most are concerned with metabolic or stress-inducible genes which have doubtful functional significance at the level of a field crop grown in water limited conditions (Passioura, 2004).

In the short term, conventional breeding techniques, with the aid of advanced breeding technologies, are generally considered more likely than GM to result in significant yield improvements under dry conditions due to the complex nature of multiple-gene related traits. It is likely that future work will concentrate on developing genotypes which are appropriate for limited water conditions rather than in finding a unique genetic solution to drought resistance (Tardieu, 2005).

2.1.10. Acid Tolerance – Why is it Necessary?

It is estimated that acid soils cover 40% of the world’s arable land (Delhaize et al., 2004). Australia has naturally acid soils which have been made more acidic over time by farming, pasture improvement and nitrogen fertilisation of crops. Except for South Australia, most of the Australian grain belts suffer from acid soils. When acidity increases to the point where toxic elements such as aluminium and manganese dissolve in the soil, major production losses occur (CSIRO, 2004c). Aluminium becomes solubilised by the acidity to form toxic Al^{3+} cations. Similar toxicity occurs when manganese is dissolved in the soil. These toxic cations inhibit crop root growth and prevent the uptake of nutrients.

The National Land and Water Audit has identified soil acidity as the most serious land degradation issue for Australian agriculture, costing $1 billion in lost production each year (CSIRO, 2004c). Currently, 33 million hectares of Australia’s farming land has highly acidic soils, and a further 55 million hectares of moderately acidic soils are also at risk of extreme degradation (CSIRO, 2004c). Although not as publicised as salinity since it lacks the visual impact, in terms of both area affected and cost to the economy, acid soils pose a more serious problem.

2.1.11. Acid Tolerance – What’s in the Pipeline?

Tolerance to aluminium and manganese toxicity is slightly different to that required for salt, drought and cold stress. The protection for plants growing in acid soils is required at the growing tip of the root apex. Plants combat acid soils by excreting

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[21] http://www.bios.net/cgi-bin/cipr/TT3_bios/simple.cgi (4048 results obtained when searched using the keywords ‘drought AND tolerance’ as at 9 June 2005)

organic acids such as citrate, oxalate and malate from their root tips. It is thought that the secreted organic acid binds the toxic cations into a non-toxic form and protects the root apex from damage (Delhaize et al., 2004). It is possible that Al- and Mn-tolerance may be conferred by only one or a few genes, and considerable research has gone into identifying potential genes of interest.

Crop production on acid soils can be maintained by neutralising the acidity with lime and by growing acid-tolerant species. However, lime can take decades to correct the soil, particularly at depth, and many crop species lack sufficient genetic diversity within their germplasm to allow effective breeding for this trait (Delhaize et al., 2004). The use of GM technology provides a means to enhance the acid-tolerance of sensitive crop species through either the overexpression of endogenous[22] genes or the expression of acid tolerance genes from other species (Delhaize et al., 2004).

For example, barley is an economically important crop in Australia, yet it is extremely sensitive to aluminium toxicity. Recently, Delhaize et al. (2004) have shown that expression of the wheat gene *ALMT1* in barley conferred an aluminium activated efflux of malate which resulted in increased aluminium tolerance in both hydroponic culture and acid soils.

When malate is released in the soil it binds with aluminium to make it non-toxic. Isolation of the gene which controls the release of malate will provide the means to engineer crop and pasture plants with aluminium tolerant traits. This work is interesting as it demonstrates the ability to confer Al-tolerance by the expression of a single foreign gene, distinct from the complexity of gene expression usually required for salt, drought and cold tolerance. Previously, a family of genes in *Arabidopsis* responsible for conferring manganese tolerance were identified (Delhaize et al., 2003). These tolerance genes can be used to develop both GM plants and as molecular markers for traditional breeding.

CSIRO Plant Industry research into aluminium tolerance in barley is at the proof-of-concept stage, awaiting funding for field trials. This is a leading example of where GM has successfully been used to combat an abiotic stress. Wheat will not be targeted for GM aluminium tolerance as it already carries the Al-tolerance gene in its germplasm. Instead, the Al-tolerance gene will be used as a molecular marker in breeding programs (BRS consultation).

Investigation is continuing into putting the Al-tolerance gene into acid sensitive pasture species (such as lucerne) and some interesting results have been achieved (BRS consultation) This work is still at the technology discovery stage.

The availability of acid tolerant lines would give farmers an additional option to improve yields on acid soils. They will not replace the need to apply lime to treat the soil. The best option for farmers who have acid soils will be a combination of lime treatment and acid tolerant genotypes (BRS consultation).

Lucerne is being feted as a practical tool for recharge control of salt damaged soils. However, it displays a distinct lack of tolerance to acid soils, a factor severely limiting

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[22] An **endogenous gene** is a gene which originates from the organism in question.

its further use as recharge crop. As mentioned previously, saline soils are often highly acidic.

Australian scientists are using a traditional breeding approach to develop lucerne varieties that will survive acidic soils. A team from the CRC for Plant-Based Management of Dryland Salinity (CRC Salinity) have used three inter-related approaches to select for lucerne which will grow on acidic soils (Munday, 2005). Their approach combines traditional breeding with modern techniques for selecting heritable traits. Firstly, they are developing a screening method for selecting lucerne plants with a heritable tolerance for acidic soils. They are then selecting for rhizobia (root nodulating bacteria) that can both survive in acid soils and still fix nitrogen. Finally, they are screening lucerne populations to select individuals for enhanced nodulation and nitrogen fixation in acid soils (Munday, 2005).

South American countries are very interested in the work on acid tolerance. In general, these countries are more accepting of biotechnology and it is possible that the Australian research may be developed for overseas markets, resulting in the loss of a possible advantage for Australian farmers (BRS consultation).

2.1.12. Cold Tolerance – Why is it Necessary?

Spring frosts are responsible for significant economic losses throughout the southern and western wheat belts. Frost events close to flowering can cause sterility, significantly reducing the yield. In Victoria and South Australia, the annual cost of frost damage to the barley industry is $9.2 million from direct yield losses, $22.5 million from indirect yield losses and $1.9 million from quality downgrading (Reading, 2004). In WA, the average financial cost of lost grain yield from frost is estimated at $4.98 million annually (Knell and Povey, 2002).

In general, very little work has been done towards frost tolerance and there is a lack of a targeted response to frost tolerance in crops. Frost affects grains the most at the time of pollen production, rendering the pollen sterile and useless. Crop plants that are still in the vegetative state have been shown to survive below freezing temperatures without any ill effect (BRS consultation). It is suggested that the problem with frost is its randomness. For example, whilst it possible to delay sowing wheat to avoid spring frosts, this is estimated to cost one percent of yield for each day’s delay. The yield penalty can be exasperating if there is not a frost that particular season (Olsen, 2005).

Sterility due to cold is the main yield affecting factor for rice grown in Australia and it is problematic in all temperate rice growing regions worldwide. A temperature of less than 18°C during flowering can result in a yield loss of 40-60%. This translates to economic losses of $15-29 million (CSIRO website). This has now occurred in Australian rice growing regions for the past two years.

2.1.13. Cold Tolerance – What's in the Pipeline?

Researchers at CSIRO Plant Industry are currently investigating the molecular basis of cold-induced pollen sterility in rice. They have identified and partially characterised a number of genes of interest. From their research it appears that cold and drought stress act on the same genes.

In addition to preventing yield loss due to cold, it is hoped that this research will result in the reduction of the amount of water needed to cultivate rice. Rice paddies are currently flooded to provide a form of insulation against cold damage. Cold-tolerant rice varieties would reduce the amount of water used to grow rice. Ideally, to reduce water use even more, some researchers are of the opinion that rice breeders in Australia should consider breeding dryland rice. However, this will require a major shift in farming practices and it may take up to ten years before suitable varieties are obtained (BRS consultation).

The knowledge from this CSIRO project will most likely be used to find molecular markers for breeders. It is unlikely to be used to make GM rice unless a market acceptance becomes apparent.

The CSIRO scientists have found that there is an inverse correlation between cold tolerance and semi-dwarfism traits that resulted from the 'Green Revolution'. The Green Revolution during the 1960s and 1970s selected for semi-dwarf crops which carried a mutation in the pathway of a hormone, gibberellin. Green Revolution traits increased yields but as a trade-off they may have decreased tolerances to abiotic stresses.

Non-domesticated (wild) cold tolerant rice varieties have extremely poor grain quality and yield potential. If crossed with existing commercial lines it is currently very hard to select for cold tolerance in the field as 'cold-snaps' generally only occur every 3-4 years. Once molecular markers for cold tolerance have been identified they will rapidly speed up the breeding process.

It has been found that there is a difference in the abscisic acid (ABA) levels in cold tolerant varieties, with ABA levels being much lower in cold tolerant lines when compared to sensitive lines. The hormones ABA and gibberellic acid (GA) act antagonistically and it is this hormone balance that may be disturbed in favour of ABA in the semi-dwarf cultivars.

This research will be carried out as proof-of-concept, not in field trials. The only place where GM rice could possibly be field trialled in Australia is just outside the rice growing area at Charles Sturt University in Wagga Wagga, NSW. This is the only place where the climate is similar enough to the rice growing region and sufficiently removed. Field trials must be sufficiently removed from commercial rice growing regions to minimise the potential of outcrossing caused by pollen flow.

However, CRC funding for this research is coming to an end and more resources for generating molecular markers are needed. Research is required into how the genes involved in cold tolerance are regulated and especially what differences there are between tolerant and sensitive varieties.

This research has shown that classical breeding may have reached its limitations and acceptance of different technologies may be required. Eighty-five percent of rice in Australia is exported so any GM rice would require overseas acceptance. In Australia we need to keep ahead of our competition and Australian researchers are concerned that once American scientists become informed about this research, there is nothing to stop them heading down the same track and impinging on the traditional markets of Australian farmers.

Researchers at the Centre for Rural and Environmental Biotechnology (CREB) at the University of Southern Queensland are exploring novel ways introducing frost tolerance into wheat plants. They have introduced new genes into wheat plants, including one which produces a so-called ‘antifreeze’ protein which is active in the transformed plants (Olsen, 2005). This research is at the proof-of-concept stage and a greater market acceptance of GM plants is required before it would be taken any further (Olsen, 2005).

2.1.14. Conclusion

As the above review has demonstrated, environmental stresses may result in the loss of billions of dollars to crop growers annually. Breeding for abiotic stress tolerant crop plants, whether by advanced conventional breeding methods or the use of biotechnology, will continue to be a research priority in Australia.

2.2. Chapter 4: Pest and Disease Resistance in Crop Plants

2.2.1. Introduction

Successful agriculture has always been subject to the limitations imposed by pests and disease (Prins, 2003; Ferry et al., 2004; Gurr and Rushton, 2005; Ritzenthaler, 2005). Pest and disease infestations of cropping areas may result in enormous unpredictable losses in yield. Over the last 10 years, biotechnology has emerged as a tool for protecting crop plants against insect and nematode pests, in addition to viral, bacterial and fungal disease.

Traditional breeding approaches are still being used to exploit natural variations in the germplasm which confer resistance to pests and disease. However, selecting for such resistance traits using conventional methods is slow, expensive, and may often be associated with a yield drag. In the United States, transgenic plants engineered to contain genes for pest protection have been field tested since 1988 and grown commercially since 1995 (NRC, 2000).

2.2.2. Protection Against Insect Pests – Why is it Necessary?

It is estimated that insect herbivores are responsible for a 10-20% loss of yield in major crops worldwide (Ferry et al., 2004), and far more in developing countries. For example, *Helicoverpa* caterpillars have the potential completely to destroy a cotton crop if not properly managed (CSIRO, 2003). Hence, a future goal of biotechnology is to engineer a durable multimechanistic resistance to insect pests. This has been partially achieved by the addition of single or multiple insecticidal[23] δ-endotoxin[24] *cry* genes from the bacteria *Bacillus thuringiensis* (*B. thuringiensis* or *Bt*). Over 10 million hectares worldwide are currently planted to crops genetically modified with *Bt* genes, mainly expressing endotoxins effective against lepidopteran (butterflies and moths) pests, although toxins effective against other insects have also been identified (Ferry et al., 2004). The *Bt* endotoxin in a crystallised form is also used to control pests in certified organic agriculture, but not in a GM manner.

2.2.3. The Advantage of Natural Pesticides over Manufactured Chemicals

Natural pesticides such as the *Bt* toxins have the following advantages over factory-produced chemicals; firstly, they are harmless to most non-target species; secondly, they do not contaminate the environment; and thirdly, because they can be synthesised inside the plant, do not require spraying applications[25]. *Bt* protected plants were developed as a means to reduce the significant amount of chemical pesticides that were required to protect cotton crops from insect herbivores.

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[23] **Insecticidal** means that it kills insects.

[24] **Endotoxin** is the term used to describe a toxin present inside a bacterial cell.

[25] http://www.csiro.au/pubgensite/research/cotton/fitt_bt_final1_long.htm

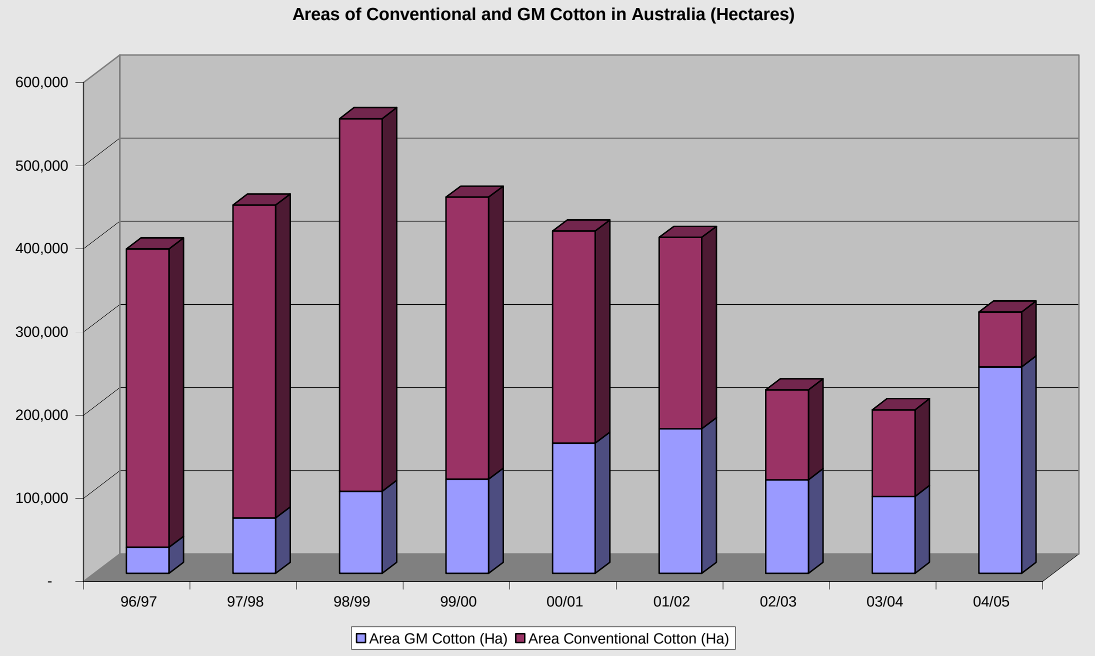
Box 4.1 – GM cotton in Australian agriculture

In 1996, Australia's first GM cotton – Ingard® – was commercially released. Ingard® cotton contained a single insecticidal gene from the bacterium *Bacillus thuringiensis* (*Bt*). On average Ingard® cotton reduced overall pesticide use on the crop by 50% and the use of toxic chemicals such as endosulfan by up to 90%. Ingard® cotton has recently been phased out of commercial production and replaced with Bollgard® II cotton, a line containing two different *Bt* genes to provide improved pest resistance. The advantage of having two different *Bt* genes in the Bollgard® II cotton varieties is the reduction in the threat of development of insect resistance to the toxin as it is less likely that an insect would have resistance to both proteins. In the future, inserting a third *Bt* gene would offer even more protection for cotton crops. Interestingly, Australian cotton farmers already had experience managing the application of *Bt* toxins on their crops. This is because preceding their use in GM plants, *Bt* toxins in a crystallised form were used as a spray-on pesticide to control insect pests.

In addition to pest resistance, cotton has also been genetically modified to be tolerant to the herbicides glufosinate ammonium (i.e. Liberty ®) or glyphosate (i.e. Roundup®) and varieties have been developed which combine both the pest resistance and herbicide tolerance characteristics.

Uptake of GM cotton in Australia has been rapid, as indicated by Chart 2 below, which shows the estimated area planted to both conventional and GM cotton since the 1996/97 growing season.

Chart 2 – Area of conventional and GM cotton grown in Australia since the 1996/97 growing season[26]



Inter-annual variations in area under cotton are influenced by environmental factors such as the drought in 2002/03 and 2003/04 growing seasons.

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[26] For further information about cotton research see the Cotton Research and Development Corporation website - http://www.crdc.com.au/

2.2.4. Delaying the Emergence of Resistance to *Bt* Genes

There is a risk that pests will develop resistance to the *Bt* toxins currently employed. One strategy to avoid this resistance is by 'stacking' the plant with different *Bt* genes, as described above. However, a key strategy for delaying the emergence of pest resistance is to grow special crops that provide safe havens, or 'refugia', close to the *Bt* protected fields. The rationale of this genetic refuge strategy is that susceptible strains of the pests will survive in these refugia and breed with any that managed to endure the *Bt* toxin in the nearby *Bt*-protected crops. Since resistant strains will only comprise a minority of the overall breeding population, it is hypothesised that resistance genes will be constantly swamped by wild-type genes, making the population as a whole susceptible to *Bt* toxins[27].

2.2.5. Future Strategies for Controlling Pest Resistance

In addition to the strategies of adding multiple genes encoding *Bt* toxins and the provision of refugia discussed above, other strategies for engineering pest resistance are being developed. These include the expression of toxins produced by foreign genes from plant and animal sources, including insects, exploiting the plants' endogenous defence mechanisms, deciphering the signals which regulate herbivore-responsive gene expression, identifying insect specific elicitors, and exploring the role of indirect defence through the production of plant 'volatiles' (Ferry et al., 2004).

Scientists are exploring the use of different genes from *B. thuringiensis* which are not endotoxins and have a different mode of action to the traditionally used *cry* genes. A number of insecticidal proteins expressed during the vegetative growth phase of *B. thuringiensis* have recently been identified. These vegetative insecticidal proteins (VIPs) differ from the widely used δ-endotoxin *cry* genes, described previously. The VIPs have a broad insecticidal spectrum and display a high toxicity against lepidopteran insects (Lee et al., 2003). The search for a new family of insecticidal toxins with a different mode of action to endotoxins is another way to delay resistance in insect populations.

2.2.6. Protection Against Insect Pests – What's in the Pipeline?

There are a number of GM cotton varieties in the pipeline in Australia with protection against insect pests. These are highlighted below.

Deltapine Australia Pty Ltd has applied to the OGTR (DIR 58/2005) for a licence to carry out a small scale field trial of transgenic cotton plants expressing the *vip3A* gene from *B. thuringiensis*[28]. The *vip3A* gene encodes a protein (VIP) that is toxic to the lepidopteran caterpillar pests of cotton. The aim of the trial is to produce seed for future larger scale trials which would require a separate assessment and approval process. A number of cotton lines will be tested, some of which have been previously approved for field trials by the OGTR under separate licences, namely, DIR 17/2002 (site currently undergoing post-harvest monitoring) and DIR 25/2002 (licence

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[27] http://www.csiro.au/pubgensite/research/cotton/fitt_bt_final1_long.htm

[28] http://www.ogtr.gov.au/pdf/ir/dir058appsum.pdf

surrendered on 22/09/04) to CSIRO; DIR 34/2003 to Syngenta; and DIR 36/2003 to CSIRO. For both DIR 34/2003 and DIR 36/2003 no plantings have yet occurred.

Monsanto Australia Ltd has an OGTR Licence (DIR 55/2004) to field trial herbicide tolerant / insect resistant (Roundup Ready® Flex MON 88913 / Bollgard II®) cotton[29]. This is a continuation of the field trials for the previously approved OGTR Licence DIR 35/2003. The herbicide tolerance is conferred by the *cp4 epsps* gene from *Agrobacterium* sp., and the insect resistance is conferred by the *cry1Ac* and *cry2Ab* genes from *B. thuringiensis*. The aim of the trial is to transfer the herbicide tolerance trait into Australian cotton varieties; test the performance of the GM cottons; produce seed for future releases; set up demonstration sites; and collect data for future applications to the OGTR and other regulators.

Hexima Ltd has an OGTR Licence (DIR 28/2003) to field trial transgenic cotton expressing natural plant genes *NaPI* from tobacco and *PotI* from potato (encoding protease inhibitors) for insect control[30]. The aim of the trial is to evaluate the agronomic performance of the GM cotton and the efficacy of the introduced insecticidal proteins.

Dow AgroSciences Australia Pty Ltd has an OGTR Licence (DIR 44/2003) for agronomic assessment and seed increase of transgenic cottons (Widestrike™) expressing insecticidal genes (*cry1Ac* and *cry1Fa*) from *B. thuringiensis*[31]. Of the two *Bt* genes expressed in Widestrike™, one is the same and one is different to those expressed in the currently released Bollgard II®. This is a continuation of the field trials for the previously approved OGTR Licence DIR 40/2003. It is hoped that by making available cotton lines expressing different *Bt* genes, pest resistance to the toxins can be better managed.

CSIRO has an OGTR Licence (DIR 36/2003) for breeding and pre-commercial evaluation of transgenic cotton expressing the *vip3A* gene from *B. thuringiensis*[32]. However no plantings have yet occurred, and it is anticipated the lines from this trials will be planted by Deltapine under licence DIR 58/2005 if it is deemed acceptable by the OGTR.

Syngenta Seeds Pty Ltd has an OGTR Licence (DIR 34/2003) to evaluate transgenic cotton plants expressing the *vip3A* gene from *B. thuringiensis*[33]. As for DIR 36/2003 above, no plantings have occurred and it is anticipated the line will be tested by Deltapine under DIR 58/2005.

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[29] http://www.ogtr.gov.au/pdf/ir/dir055qanda.pdf

[30] http://www.ogtr.gov.au/pdf/ir/dir048.pdf

[31] http://www.ogtr.gov.au/pdf/ir/dir044.pdf

[32] http://www.ogtr.gov.au/pdf/ir/dir036.pdf

[33] http://www.ogtr.gov.au/pdf/ir/dir034.pdf

2.2.7. Disease Resistance – Why is it Necessary?

Pathogens and parasites of plants pose an increasing threat to crop production worldwide (Gurr and Rushton, 2005). Every day, plants are constantly challenged by viruses, bacteria, fungi and nematodes, however disease is comparatively rare. This is because plants have evolved different layers of defence to cope with pathogen attack. Defences range from structural barriers and pre-formed antimicrobials to adaptive defence mechanisms, a detailed explanation of which is beyond the scope of this report. Gurr and Rushton (2005) postulate two questions to be considered before engineering plants with increased and durable disease resistance using transgenic technologies, namely, 'what gene or genes do we want to express to improve disease resistance?', and 'how are we going to express those genes so that crop yields are actually increased?'

Modern agriculture relies on the cultivation of huge areas of genetically identical crops, within which protection from disease is dependant on a small number of in-bred disease resistance genes and the wide spread application of chemical pesticides. This has led to only a transient control of pathogens being achieved, as pathogens are often able to overcome resistance genes by a simple genetic mutation and/or by becoming resistant to pesticides (Gurr and Rushton, 2005).

Gurr and Rushton (2005) report that the development of crops with transgenic resistance to fungal and bacterial diseases has been relatively unsuccessful, not because of the nature of the transgene, but the way it was expressed. Transgenes against bacteria or fungi that were constitutively expressed appear adversely to affect plant fitness. This problem could be overcome by the use of a site-specific, pathogen-inducible promoter; however, these authors report that few have been successfully used.

The post-genomic era has led to an increase of knowledge about endogenous plant defence and the unveiling of more sophisticated transgenic approaches to enhancing resistance. Candidate genes can come from either the plant itself, from other plants, from a pathogen or could be synthetically manufactured. These genes can be manipulated by a suite of approaches, including over-expression, inducible expression, tissue-specific expression, stable knockout, or silencing by RNAi[34] (Gurr and Rushton, 2005). Table 4.1 on the following page is adapted from Gurr and Rushton (2005) and highlights GM strategies for increasing disease resistance.

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[34] RNAi is a method of 'knocking-down' (reducing) a gene's expression (Wang et al., 2000).

2.2.8. Disease Resistance – What's in the Pipeline?

The Department of Primary Industries in Victoria has an OGTR Licence (DIR 47/2003) to field trial GM white clover expressing the Alfalfa Mosaic Virus coat protein (*AMV CP*) gene intended to confer resistance to infection by Alfalfa Mosaic Virus (AMV)[35]. This virus has the ability to devastate production from white clover pastures, causing up to a 60% loss in clover production. White clover production is worth up to $267 million to the dairy industry (AusBiotech, August 2004). The aims of the trail are the evaluation of the efficacy of resistance to AMV and the production of GM seed for future trials, subject to further approvals.

At the Molecular Plant Breeding CRC, research is being performed to examine the genetic precursors for disease resistance in cereals. To date, most research has dealt with the genes that control the mechanisms that recognise a pathogen and trigger a protective response. This approach is exploring the actual physiological mechanisms of disease resistance. This research is still in the technology discovery stage.

Scientists at the Australian National University's Research School of Biological Science are trying to express a tomato Fusarium resistance gene in cotton. The rationale behind this work is that the resistance would be common and it would be possible to transfer the resistance gene across species barriers. The gene and its native promoter have been shown to be active in cotton in the greenhouse. This research is currently at proof-of-concept stage with field trials a minimum of two years away if desired results are obtained (BRS consultation).

Many research laboratories in Australia, both public and private, are carrying out research looking at preventing disease in crop plants. However, due to the lack of publication of data as discussed in the introduction, and the practical limitations to consulting every research group in the country, this part of the report has only been able to capture a snapshot of what is happening. The barriers to adoption of this technology are well documented however, and apply to all research groups in Australia.

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[35] http://www.ogtr.gov.au/pdf/ir/dir047.pdf

Approach	Examples	Advantages	Disadvantages
Constitutive expression (Expression of gene(s)-of-interest at all times in all cells of the plant)	Pyramiding *R* genes	Can build more durable resistance *R* genes successfully used by breeders	Requires knowledge of specificity Might come with a fitness penalty Overexpression might activate defence
	PR genes	Many reports of increased resistance Do not activate the whole defence response	Might only be effective against a few pathogens Overexpression might reduce yield and/or fitness
	Antimicrobial peptides	Can increase durability by ‘stacking’ Can target the pathogen by linking to antibodies	Requires a range of active peptides Might come with a fitness penalty
	Pre-formed barriers	Could lead to durable resistance	Altering the cell wall might reduce size and yield
Local expression (Expression of gene(s)-of-interest in specific cell at time of pathogen attack)	Master switch genes	Activate banks of genes Might confer resistance without activating all defence responses	Requires a pathogen inducible promoter
	Elicitor or *Avr* genes	Trigger to activate successful defence Could be enough to change susceptible to resistant	Requires a pathogen inducible promoter
	Toxic genes	Could stop pathogen growth and lead to resistance	Pathogen inducible promoter a necessity Public perception of ‘toxic’ gene product
RNAi (Method of targeting pathogens by reducing or ‘knocking-down’ gene expression)	Silencing of pathogen essential genes	Can potentially target all pathogens Targets specific pathogens Unlikely to have any fitness penalty Does not activate defences	
Gene knockouts	Knockouts or mutations of negative regulators of defence	Mutations in genes such as *Mlo* could provide durable resistance	Need to identify negative regulators of defence Might come with a fitness penalty

Table 4.1 GM strategies for increasing disease resistance (from Gurr and Rushton, 2005). *R* genes are resistance genes; *PR* genes are pathogen responsive genes; *Avr* genes are avirulence genes.

Box 4.2 Barley yellow dwarf virus

Barley yellow dwarf virus (BYDV) is the most serious and widespread virus of cereals worldwide. Natural resistance gives inadequate control so scientists at CSIRO Plant Industry attempted to use post-transcriptional gene silencing (PTGS) to generate barley with protection against BYDV (Wang et al., 2000). PTGS, also known as RNAi is a method of reducing gene expression (Smith et al., 2000; Wang et al., 2000; Kusaba, 2004). Plants were transformed with a construct designed to produce double-stranded RNA containing viral sequences. Transformed progeny showed extreme resistance to the virus, subsequently rated as immunity since the virus could not be detected in the challenged plants by a number of different methods (Wang et al., 2000). Results from this work indicated that immunity against the virus would be robust in the field and may be useful in reducing losses in cereal production caused by this virus worldwide. There are currently no plans to commercialise this technology in Australia due mainly to market and public perceptions of GM crops, and the resulting reluctance from potential funding bodies. Additionally, it has been suggested that the intellectual property landscape for the commercialisation of RNAi technology is very complex, and a freedom to operate may prove to be the largest hurdle to bringing this technology to market (BRS consultation).

2.2.9. Conclusion

Pests and diseases of crop plants are responsible for major crop losses worldwide. Research into unravelling the many genetic facets of conferring pest and disease resistance to crop plants continues in both public and private laboratories. In Australia, funding of research is dependent on the importance of the particular pest or disease to this country’s agricultural industries. From our consultations, it is clear that the biggest barrier to commercialisation in Australia of pest and disease resistant crop plants is not the lack of research and development, but rather the absence of a clear, consistent and predictable path to commercialisation.

Section 3: Second Generation Traits

3.1. Chapter 5: Improving food value

3.1.1. Introduction

Plants are the main source of food in every country, either directly or through feed crops for livestock industries that produce food. One of the categories of second generation GM traits of potentially direct relevance to consumers is the engineering of plants for food and feed with a high nutritional quality. Improving the nutritional quality of food is of significance especially in developing countries where nutritional sources are lacking, but market demand also exists for higher quality and fortified plant foods to improve human health and life expectancy in the developed world (Galili et al., 2002).

In some cases farming practices can be adapted to enhance nutrient levels in growing crop plants. This approach is currently only useful for some nutrients such as nitrogen and zinc, and is not always practical or economically feasible (Poletti et al., 2004). Fortification of food products is another method that has been very efficient in increasing nutrients of staple foods. Observant consumers will have noticed the trend towards fortification of various foodstuffs on supermarket shelves. The issue of fortification for human nutrition and its regulation is complex, with some regulators and markets seeing the trend towards foods being over-enriched. Those issues aside, fortification of some nutrients presents significant technical challenges, for example the high solubility of folate or the instability of iron, so parallel approaches of producing crop plants with higher nutrient levels are being developed. This is often called bio-fortification.

As for most of the other crop improvements discussed in this report, the primary approach for improving nutrition of plants for food and feed is by breeding better varieties using classical genetics. Although successful in some cases, for example in the production of high lysine maize that has been extensively grown in Brazil, traditional breeding is a relatively slow process. Technology has accelerated this process by the use of DNA marker-assisted breeding and genomics, but the availability of genes in close relatives is always a major limitation. The progress towards plant food and feed improvements by classical breeding, GM and other technologies has been extensively reviewed (Galili et al., 2002). Some examples of these value improvements are discussed below.

3.1.2. Increasing levels of essential amino acids

Animals[36], including humans, cannot synthesise 10 out of the 20 amino acid building blocks for proteins and therefore need these essential amino acids in their diets. Of these 10, lysine and methionine are the most important as they are limited in major

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[36] Ruminant animals such as cattle and sheep have microbes in their gut that produce essential amino acids although not always to required levels for agricultural production.

food and feed crops. Cereal grains are generally deficient in lysine, and legumes deficient in methionine.

Research is continuing worldwide to increase amino acids in grains and forage crops. Two main approaches are used for GM high lysine crops, insertion of bacterial genes that allow higher production of lysine in the plant, and insertion of plant genes encoding lysine rich proteins. A combination of these two approaches in maize increased the total lysine content in seed from 0.2 to 0.7% of seed dry weight (Galili et al., 2002). Providing these changes do not affect the agronomics of the plant, then this looks to be a promising approach.

Expression of methionine rich proteins in crop plants is also promising, although not without complications. Australian researchers successfully overexpressed a sulphur rich seed storage protein in rice, however this resulted in a redistribution of sulphur from other proteins in the plant (Hagan et al., 2003). Other, modified approaches are being used to enrich amino acids in plants that do not change the quality and quantity of other proteins in the plant (reviewed in Poletti et al, 2004).

3.1.3. Improving oil quality

Improving lipids and fatty acid composition and quality is also an important priority for food and feed production. Vegetable oil traits are discussed in more detail in Chapter 7, with one example shown below.

3.1.4. Essential fatty acids

Long chain polyunsaturated fatty acids belonging to the Omega-3 and Omega-6 classes are nutritionally beneficial oils usually sourced from fish consumption. Many diets have inadequate intake of these oils, particularly the Omega-3's eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Therefore, interest is increasing worldwide in developing crop plants as alternative sources of these long chain polyunsaturated fatty acids (Green, 2004).

Scientists at the CSIRO Food Futures Flagship have made significant progress in this area by successfully producing DHA in GM plants. Progress towards this goal was reported last year (Qi et al., 2004), has been reviewed recently (Singh et al., 2005) with updates in a recent press release. Although still some years away from commercialisation, GM crop plants producing healthy oils such as Omega-3 may improve consumer acceptance GM technology.

However, it is important to note that the maze of intellectual property protecting technologies needed to alter oilseed metabolism in plants may be a significant barrier for bringing a product to market. For example Bioriginal Food and Science Corp in Canada have recently patented discoveries in the EPA and DHA synthesis pathways (GRDC, 2005). Monsanto is also targeting research in this area, with commercialisation of plants producing long chain fatty acids predicted by 2011 – 2012.

3.1.5. Improving levels of essential minerals

Phosphorous is an important mineral for animal nutrition, and many researchers have aimed, initially by traditional breeding and screening of mutants, and more recently by genetic modification, to increase the availability of phosphorous in food and feed. The GM approach has been to express bacterial genes encoding for the enzyme phytase in plants. Phytase degrades complexes that bind phosphorous, and expressing this enzyme in plants releases more phosphorous for absorption in the diet. Phytase genes have been expressed in tobacco, alfalfa, soybean and wheat in addition to being used in poultry and pig feeding trials (Galili et al., 2002).

Iron is another essential mineral that is limited in most major crops. Even in crops that are rich in iron such as spinach and legumes, adsorption by humans is limited as the iron is bound to other compounds. As a large percentage of the world's population suffers from iron deficiency, increasing iron levels in plant foods is an important nutritional goal. Classical breeding strategies, especially when supplemented with new technologies such as molecular markers, hold potential for improvements in iron levels in plants such as rice and wheat, due to higher iron levels in traditional varieties (Poletti et al., 2004).

However, the fact remains that the overall iron levels in cereals, even in these traditional varieties, is still very low, therefore genes from other sources may be needed significantly to increase available iron levels in plants. The main approach for increasing iron levels in plants by genetic modification has been to transform rice with genes encoding ferritin, a protein containing iron, from soybean and other beans (Gura, 1999). Further studies are currently underway in these areas to assess the nutritional significance of these modifications to human and animal diets.

3.1.6. Improving vitamin content of plants

3.1.6.1. Folates

Animals and humans cannot synthesize folates and rely entirely on diet, usually plant foods, for adequate intake. Low folate intake is a worldwide problem, and is linked to many diseases such as cancer, cardiovascular disease and neural tube defects (Basset et al., 2005). Fortification of processed foods in various countries, including Australia, is attempting to address this issue, but has some limitations. Researchers are also focussing on producing plants with higher folate content (biofortification) by GM. There are three main approaches for increasing folate levels in plants, increasing folate synthesis, partitioning folates in parts of the plant where they can accumulate, and decreasing the catabolism, or breakdown of folate in the plant. Much progress towards these goals has been made and reviewed recently (Basset et al., 2005), however, it is not likely that a GM plant product would reach commercialisation in the near future.

3.1.6.2. Carotenoids and Vitamin A

Vitamin A deficiency is one of the leading causes of night blindness throughout the world. β-carotene is a precursor for vitamin A, and increasing levels in crops such as tomato has been a goal of traditional breeding, marker-assisted breeding and more recently, genetic engineering (Galili et al., 2002). Rice grains contain poor levels of β-carotene, and thus scientists, working with genes from bacteria and other plants such daffodils, have genetically modified rice plants to produce higher levels of β-carotene, resulting in the high profile 'Golden rice' (Ye et al., 2000).

The merits of Vitamin A fortified rice as a platform for delivery to deficient populations in developing countries has been widely discussed in the popular press. Biotech opponents have focussed on the fact that Golden Rice would only supplement diets by supplying 15-20% of the Recommended Daily Allowance (RDI). In April this year, the development of Golden Rice 2 was reported that could provide up to 50% of the RDI (Paine et al., 2005). The next stage is to cross this trait into the elite, high-yielding, disease resistant lines used in commercial production throughout the world. It is predicted that field trials of these varieties are imminent.

3.1.6.3. Vitamin E

Experiments are also underway to increase Vitamin E levels in crop plants. Vitamin E deficiency is a known cause of foetal death in humans, and is also implicated in many other disorders and diseases. Initially the main approach was to utilise germplasm variation in classical breeding programs, but more recently genomics and GM have opened up further possibilities (reviewed in Galili et al, 2002). Experiments transferring barley genes into maize resulted in sixfold accumulation of Vitamin E precursors (Cahoon, 2003).

3.1.7. Starch modification

Starch is the major carbohydrate in the human diet, and modification of starch produced by staples such as cereals is an expanding area of research, with significant contributions by Australian researchers (Morell and Myers, 2005). Targets include designing starch molecules with improved health, nutritional or food processing benefits. Starch is important in the production of biofuels such as ethanol, and increasing the efficiency of conversion of starch into ethanol is another goal of researchers worldwide. Starch also has potential as a renewable raw material for production of biodegradable plastics. Core genes in the starch biosynthesis pathway have been identified and studied in a wide range of species and modern biotechnology techniques such as genetic modification will continue rapidly to advance this field. However, because of the complexity of the pathways, this research is, in the main, still in the technology discovery stages (Morell and Myers, 2005).

A licence for a field trial of GM wheat with altered grain starch was approved by the OGTR earlier this year (Appendix B). This research, carried out by the CSIRO, aims to produce and study wheat grains containing an increased amount of 'resistant' starch, which is thought to have human health benefits. This trial is a large scale gene and phenotype discovery experiment and is not a move towards commercialisation of GM wheat (BRS consultations). Indeed in this instance genetic modification is being used as a technology discovery tool, with the idea that, if the desired results are obtained, conventionally bred varieties of interest can then be identified more effectively.

3.1.8. Modified plant cell walls

Another target for plant researchers is to modify plant cell wall composition to improve digestibility for human and animal nutrition, produce better quality straw for animal feed, and produce plants with more efficient biofuel production capacity. Researchers at the University of Adelaide, funded by the GRDC and in alliance with the ACPFG, are working on improving the digestibility of wheat and barley. This research is at the proof of concept stage (BRS consultation).

3.1.9. Other modifications

Plant scientists are also using GM to increase production in crop plants of other health related compounds such as resveratrol and flavonoids. Increasing the flavour and aroma of foods is also a target. Another interesting class of modifications to crop plants are those designed to reduce anti-nutritional compounds, for example caffeine-free GM coffee plants. These are reviewed elsewhere (Galili et al., 2002) and will not be discussed in detail in this report.

HarvestPlus[37] is a new project funded by the Bill and Melinda Gates Foundation that aims to develop staple plants fortified in essential nutrients. The program involves a range of international research organisations, including the South Australian Waite Agricultural Research Institute. Natural variations in genes of staple crops like rice, wheat, maize, beans, cassava, and sweet potato will be used to breed varieties richer in iron, zinc and vitamin A.

3.1.10. Conclusion

With a few possible exceptions, GM crops with added nutrients for food and feed, or with other health benefits are still in the technology development or proof-of-concept stage. Most of the nutrient pathways in the plant are complex and involve multiple genes or precise expression patterns in the plant to be functional. In some fields of research, Australia is well placed to reap benefits with leading scientists in their fields carrying out this work in Australian laboratories.

Increasing the value of agricultural products by the use of new technologies is a major goal of industry. Industries (for example the grains and sugar industries) have already made strategic steps in this direction. CSIRO’s Food Futures Flagship[38], the CRC for Sugar Innovation through Biotechnology[39], the Grain Foods CRC[40] and the Grains Industry Single Vision[41] are just some of the organisations that consider pursuing high value crops as a priority. Competition from overseas is significant, with huge investment in this by food giants such as Unilever and Nestle, in addition to venture capitalists. The prediction is that larger companies will focus on large-scale products, and Australia could position itself in niche markets for supplying high value products.

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[37] http://www.harvestplus.org/

[38] http://www.csiro.au/proprietaryDocuments/FoodFuturesFlagshipBrochure.pdf

[39] http://www.crcsugar.com/

[40] http://www.grainfoodscrc.com.au

[41] http://www.singlevision.com.au

However, the barriers to bringing a GM crop to market as discussed in the consultations, and summarised in Chapter 2, together with other impediments present a challenge. The complexity of human nutrition combined with the high scrutiny and regulation of GM crops make it extremely unlikely that a GM-fortified food will be commercialised in the next few years. Thus the theory that such traits that supply an obvious consumer benefit will sway public opinion towards GM crops is unlikely to be tested in the near future.

Table 5.1 Some targets for improving value of crops by genetic modification

Trait	Plant species[42]	Main goal
Increased essential amino acids		
Lysine	Soybean, maize, canola, beans	Increased nutrition of food and feed
Methionine	Soybean, beans, lupins	
Modification of seed oil content and composition		
High Lauric Acid oil	Canola[43]	Important oil in the confectionary industry
Increasing total seed oil content	Canola	Added value trait
Increasing oleic acid levels	Soybean	More stable frying and cooking oils
Long chain polyunsaturated fatty acids	Model plants	Increased nutrition of food
Increasing essential minerals		
Phosphorous	Tobacco, alfalfa, soybean and wheat	Increased nutrition of feed
Iron	Rice	Increased nutrition of food
Increasing vitamin content		
Folates	Tomato	Increased nutrition of food
Vitamin A	Rice	
Vitamin E	Maize	
Starch modification	Potato, wheat, maize, barley	Increased nutrition of food[44]
Modification of plant cell walls	Pasture grasses, wheat and barley	Improve digestibility[45]

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[42] Not necessarily the commercial crop target.

[43] In commercial production in North America since 1995.

[44] Other targets include more efficient biofuel production, and raw material for biodegradable plastics

[45] Other targets include better quality straw for animal feed and more efficient production of biofuels.

3.2. Chapter 6: Improved Feed and Pastures

3.2.1. Introduction

In Chapter 5, GM approaches to increasing the nutritional value of food were discussed. Most of these applications would also be of interest for animal feed: indeed some have been developed with that end use primarily in mind. The growth potential of livestock is rapidly improving because of advances in animal breeding technologies, and with this comes an increasing daily requirement for energy and protein. Another factor is the expanding market for animal food products due to population expansion and increasing world-wide reliance on animal products as the primary protein source in human diets.

One way of looking at a crop plant is as a source of energy and protein, and this is often how commodities are traded worldwide. As population increases drive demand for human food crops higher, pressure will increase on the animal feed industry to compete and to be able to source plant supplies of energy and protein at realistic costs (Williams, 2003). Thus output traits that modify the nutrient content of plants to increase protein and energy content and availability for animal feed are being developed worldwide and some examples of traits in the pipeline are shown in Table 6.1.

Table 6.1 GM feed crop traits in the pipeline worldwide (adapted from Williams 2003, identified from patent applications)

Crop	Trait	Improvement
Lucerne	Lignin Amino acids	Improved digestibility and/or low lignin Increased amino acids (methionine and cysteine)
Chickpea	Amino acid	Increased amino acids (methionine and lysine)
Clover	Amino acid	Increased amino acids (methionine and lysine)
Maize	Amino acid Mycotoxin Oil Oil and/or amino acids Oil and/or P	High protein with balanced amino acids Fumosin detoxifying High oil content High oil with increased digestibility High oil with increased P availability
Canola	Oil Oil	Low saturates and/or high MUFA[46] and/or low PUFA[47] High oil
Lupin	Amino acids	Increased amino acids
Peas	Amino acids	Increased amino acids (methionine)
Soybean	Protein levels Anti-nut factor	Increased levels of proteins Low stachyose
Sorghum	Carotenoid	High carotene

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[46] Monounsaturated fatty acids

[47] Polyunsaturated fatty acids

Most of the work throughout the world on output traits for GM feed is being done in maize and soybean. Maize is a common source of animal feed worldwide, with approximately 75% of the global production of maize being used for this purpose. Soybean dominates the global oilseed market with annual production in excess of one hundred and fifty million tonnes, oil and livestock feed account for 97% of total production destination. Also, maize and soybean dominate the North American domestic stockfeed markets, and as these are the countries where GM crops have been most readily adopted, these are also the areas that are investing in research into GM feed output traits. For GM feed output traits to have broad appeal in the United States they must be expressed in maize and soybean, and be suitable for at least two end-user sectors, that is poultry, pig, beef, dairy or export (Williams, 2003).

Three main areas of research into GM soybeans output traits for feed use have been identified and are briefly discussed below and elsewhere in this report.

1. Improvement of protein concentration or amino acid profiles

Most soybean meal produced worldwide is used as a preferred source of protein for poultry and pigs, due to its amino acid profile and high digestible energy (Willis, 2003). Typically soybean meal is used to meet an animal's nutritional requirements for essential amino acids, complementing the limited profile of corn feed. However this can sometimes result in overfeeding of non-essential amino acids, with the excess metabolised into carbon dioxide and urea, or excreted as undigested protein (Kerley and Allee, 2003). Improving the amino acid profile of soybeans and improving the intestinal availability of these (that is the digestibility) would greatly increase the value of this crop for animal feed.

2. Reduction of anti-nutritional factors

A large proportion of nutritional compounds in seeds of plants are complexed with phytic acid, and this makes them unavailable to humans and livestock. Probably the most important mineral that is affected is phosphorous. As much as 50 to 80% of the total seed phosphorous is not used and excreted in animal manure. Supplemental phosphorous is required in animal diets, which increases the quantities excreted into the environment and is a significant pollution problem. Modifying soybeans to prevent or reduce the amounts of phytic acid would have substantial value for animal feed applications.

3. Improvement in the oil profile and composition

Modification of the oil profile of soybean seeds has significant potential to improve animal production. Soybean oil and some of the traits of interest are discussed in Chapter 7.

3.2.2. Impact of GM soybean and maize feed output traits on Australian agriculture

Australian production of soybeans is minor in comparison to major exporting nations in North and South America. Australian growers focus on soybean production for human consumption and organic markets. Significant amounts of soybean meal are imported into Australia for use as stockfeed (Figure 6.1), and the vast majority is sourced from the USA (Foster et al., 2003).

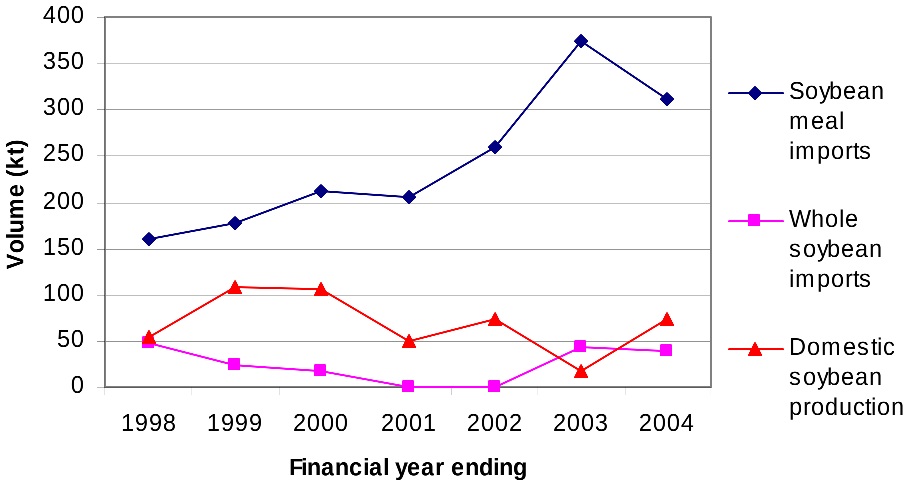

Figure 6.1 Trends in Australian soybean imports and production (ABARE, 2004)

Australian maize production averages 375,000 tonnes per annum with most being produced in NSW and QLD. Most of this maize is used domestically for human consumption through the breakfast cereal market or the domestic feed grain market (Cunningham et al., 2005). In times of drought maize is imported for use as livestock feed, although typically it is not a major stockfeed ingredient in Australia. Other cereals such as wheat, barley and triticale are far more significant.

Even though maize and soybean are not currently large markets in Australia, predictions are that developments in the maize and soybean markets overseas could have a direct or indirect impact on as much as two-thirds of Australian agriculture (Anderson and Jackson, 2005). Australia is a significant player in livestock and export production that compete with the grain-fed production of the northern hemisphere. High value GM feed output traits in soybean or maize could compete with domestic or export markets of Australian feed grains and oilseeds. Improved traits for animal production adopted elsewhere could also have impacts on markets for Australian livestock produce. Further research into this area is recommended, including economic analysis of possible impacts.

Australian researchers have GM high value traits for other feed crops and pastures in the pipeline. Researchers in Western Australia have developed GM high sulphur lupins with nutritive benefits for animal feed, however their OGTR licence for field trials has subsequently been withdrawn.

3.2.3. Improved Pasture Grasses and Clover

There is currently research in the pipeline in Australia which aims to produce superior pasture grasses with modified lignin biosynthesis, fructan metabolism and pollen allergens. It has been suggested that these qualities could not be achieved by conventional breeding (BRS consultation) and they are explained in more detail below.

Lignin is the part of the plant cell wall that is responsible for strength and rigidity. Consequently, high lignin content results in a stiffer and more unpalatable grass to livestock. The Molecular Plant Breeding CRC (MPBCRC) is trying to reduce lignin production and improve digestibility. They have reached the stage of isolating the key enzymes for lignin production in ryegrass and reduced their expression (BRS consultation).

Fructan is a naturally occurring carbohydrate (sugar) in pasture grasses. It is an excellent energy source, especially for cattle. Consequently, if the level of fructan expression can be increased, this will result in a concomitant increase in energy, live weight, milk production and possibly even fertility. Researchers at the MPBCRC have identified the genes involved in fructan metabolism and they are currently working to produce grasses with high levels of fructan production for the dairy industry (BRS consultation).

The majority of hayfever-causing pollen in Australia originates from ryegrass. Scientists at the MPBCRC are working to produce ryegrass whose pollen lacks the hayfever causing protein. They have identified the genes responsible and 'knocked-down' their expression using gene-silencing technology (BRS consultation). It is envisaged that this technology will not be commercialised for at least 10 years (Metcalfe, 2005).

The Australian dairy industry is also funding research into pastures, particularly clovers, which are resistant to Alfalfa Mosaic Virus (AMV) and Yellow Clover Virus (YCV) (see Chapter 4 on disease resistance for more information about the field trials of white clover resistant to AMV). It is estimated that disease in white clover costs the dairy industry up to $20 million annually; however, there is no commitment to commercialisation of virus resistant white clover at this stage (BRS consultation). The main driver for the adoption of these disease resistant traits would be increased efficiency in production, however it is suggested that this driver would not currently outweigh the perceived marketing risks of GM technology (BRS consultation).

To balance the risks to potential markets, a huge financial gain would need to be captured by a GM feed trait before a livestock industry might consider its adoption. As yet, there are no products close to commercialisation in Australia that would achieve this (BRS consultation). A major barrier to the adoption of this technology is the perceived marketing risks to Australia's meat and dairy export industries. For example New Zealand is our biggest competitor for dairy produce, and it is thought that there may be a risk of New Zealand capturing Australia's markets if GM feeds are widely sourced (BRS consultation).

Another major barrier to the commercialisation of GM improved pastures is one which is pasture specific; namely, pastures by their very nature are a mix of species, generally outcrossing persistent annuals and perennials. Hence, unlike in the grains industry where the segregation of GM and non-GM grain to realistic threshold levels is considered a possibility, if a GM pasture were released, segregation is unlikely to be a viable management strategy at the farm level. Consequently, this would require a major shift in the attitude of managing a GM crop, with adoption by the value chain as a whole (BRS consultation).

It is suggested that if desirable traits could be bred into pastures by non-GM methods, this would be an attractive approach with less marketing concerns. For that reason, a focus of research into pastures is looking at ways in which biotechnology can facilitate and improve conventional breeding programs (BRS consultation).

Section 4: Third Generation Traits

4.1. Chapter 7: Plant Molecular Farming

4.1.1. Potential for GM crops for industrial uses

Plant molecular farming refers to the production of industrial or pharmaceutical compounds using both GM crop and non-crop plants as a factory, otherwise called third generation traits. Such compounds could include pharmaceuticals such as hormones, antibodies, vaccines or enzymes, or industrial compounds such as biodegradable plastics, enzymes or lubricant oils. Plant molecular farming can also sometimes refer to functional foods or nutriceuticals, crop plants with added nutrients, however in this report they are discussed in a previous section under the category of second generation crops. There is scope for crop plants to be used as biofactories for a broad range of purposes, some examples are discussed below.

4.1.2. Vegetable oils

Oils produced by plants are an important source of chemicals for industry and can be used to produce soaps, detergents, lubricants, cosmetics, plastics and other chemicals. Most temperate climate oilseed plants produce the same five fatty acids, palmitate, stearate, oleate, linoeate and alpha-linolenate in different proportions, with different fatty acids desired for different industrial purposes. Table 7.1 shows some conventionally bred vegetable oils that are currently used for industrial use.

Table 7.1 Some examples of conventionally bred crops that produce industrial oils (adapted from McKeon, 2003)

Crop	Application	Desired fatty acid
Flax	Coatings, plasticizer	Alpha-linolenic acid
Castor	Lubricants, cosmetics, plastics	Ricinoleic acid
Coconut	Detergents	Lauric acid
Jojoba	Lubricants, cosmetics	Wax ester
Soybean	Inks, plasticizer, coatings	Linoleic acid

4.1.3. Potential for GM to produce new oilseeds

Three lines of research are opening up the use of GM crop plants to produce desired oilseeds.

1. Biochemical characterisation of most of the steps in fatty acid synthesis is very advanced
2. Genes controlling many of the key steps in the fatty acid biosynthesis pathway in plants have been identified
3. Research on biochemical and genetic pathways from plants that produce uncommon, industrially useful fatty acids is giving further genetic information

Plants can be modified to be enriched in a particular desirable industrial oil, and minimize purification steps, or modified to produce consistent oils of compositionally high quality, or modified to produce desired ratios of oils. Some examples of GM applications to different crops for oil production are shown in Table 7.2 and described in more detail below.

Table 7.2 Some examples of GM oilseed crops with modified oil content

Crop	Modification	Stage	Use	Reference
Canola	High laurate content	Commercial	Detergent	McKeon, 2003
Soybean	High oleate content	Commercial	Food, lubricants	Cahoon, 2003
Soybean	High linolenic	In development	Coatings	Cahoon, 2003
Canola	High stearate	Developed	Grease	McKeon, 2003
Canola	Petroselenate	In development	Food , monomers	McKeon, 2003
Soybean	Vernolate	In development	Plasticizer, coatings	McKeon, 2003
Cotton	Low-saturates	In development	Food uses	McKeon, 2003

4.1.4. Soybean oil

Soybean oil is the major oil crop of the United States. A driving force behind the development of new uses for soybean oil is its perennial unused surplus (McKeon, 2003). Although a significant amount of soybean oil is used for industrial purposes, mainly fatty acids, soaps and feed, this represents only 4% of total consumption of US soybean oil; the vast majority of oil is used in foods and food processing. Therefore the market for industrial use of soybean oil has considerable potential for expansion (Cahoon, 2003). Soybean is currently a minor crop in Australia, but an expansion of soybean markets may impact on Australian export markets for other competing oilseeds or stockfeed.

Conventional soybean oil contains oleic acid levels of around 25% of the total oil. Genetic modification has been used by researchers at DuPont to produce soybeans with high oleic acid, up to 80% of the total oil. The oils produced by these plants are considered to be healthier for food uses, and also to have a higher stability, making it better suited than conventional oils for industrial use in lubricants (McKeon, 2003). This GM crop is in commercial production in the United States and has approvals for food and feed in several other countries. Researchers overseas have also produced GM soybeans that produce oil high in linolenic acid, up to 50% of total oil content in contrast to 10% in conventional oil. This modification is a desirable for industrial oils that are used in paints, inks and varnishes (Cahoon, 2003).

Another avenue for research into GM soybean oil production is to use the plant as a factory to create desirable novel fatty acids. For example expression of genes from other plants in soybeans has been used to produce novel fatty acids such as calendic acid, 20-carbon monounsaturated fatty acids, epoxy and hydroxy fatty acids, which all have value for industrial uses (Cahoon, 2003). Again this research is being undertaken overseas, but expansion of soybean oil markets may impact on markets for Australian oil products.

4.1.5. Canola

Oilseed rape was first grown to provide industrial oil high in erucic acid. More recently varieties were bred with reduced levels of erucic acid and glucosinolates to be suitable for human consumption, and these edible oilseed rape varieties were named ‘canola’ by Canadian producers. Canola was first trialled in Australia in the early 1960s and first grown commercially in 1969, after the introduction of wheat delivery quotas. The industry suffered initial setbacks as the early varieties were susceptible to the fungal disease blackleg. In the 1970s breeding programs began and the industry steadily grew.

Canola is grown primarily for its high oil-containing seeds. Oils extracted from canola seeds are used for cooking oil and margarine production. The remaining by-product, canola seed meal, is used as a high protein animal feed. Prior to the 2002-2003 drought, Australia was regularly exporting over 1.3 million tonnes of canola, the main markets being Japan, China, Pakistan and Bangladesh.

Canola is one of the cheapest edible oils on the global market. For this reason, interest has been high in modifications that may increase the value of the oils. In Canada canola varieties are being commercially produced which have been genetically modified to produce higher levels of lauric acid (Halford, 2004). These oils can then be used as a replacement for other lauric acid oils such as coconut and palm kernel oil, in confectionary coatings, spreads and commercial frying oils. Researchers in Australia have produced GM canola that is high in oleic acid, generating improved performance frying oil, however uncertainty about the path to market has stalled its progress towards commercialisation.

4.1.6. Cottonseed oil

Although cottonseed oil is not traditionally viewed as a food oil, it is commonly used in commercial kitchens for deep frying foods. Before the oil can be used in food applications, it needs to be processed to remove toxic and anti-nutritional compounds. Cottonseed oil also needs additional processing to obtain a higher stability oil which is more resistant to oxidation. This processing results in trans fatty acids that are less than ideal nutritionally because of the effect on cholesterol levels.

Researchers at CSIRO have genetically modified cotton to produce seed oils with higher proportions of oleic acids and decreased levels of saturated fatty acids (Liu et al., 2002). These oils have the potential to omit the extra processing step and to be used directly in frying or for margarines. A two hectare field trial of these varieties was planted in the 2003-04 season to conduct agronomic evaluation and test proof-of-concept under field conditions. Very positive results came from these field trials, however the cotton industry requested that this trait be combined with low palmitic acid variety before commercialisation. Work on this trait is still in the technology discovery phase (BRS Consultation).

4.1.7. Pharmaceuticals

Commercial production of proteins for pharmaceutical uses has traditionally depended on micro-organism production or isolation of products from animals or humans. Plants have potential to offer cheaper, safer and more efficient alternatives to these production systems. This plant-based production of pharmaceuticals is also referred to as ‘pharming’. Pharming on a large scale still has technical and regulatory limitations, but a small number of plant-derived products are approaching commercialisation (Fischer et al., 2004). Most major groups of human medicinal proteins have been produced successfully in a diverse array of crops including maize, rice, wheat, soybean, tomato, potato, mustard, canola, turnip, alfalfa, banana, tobacco and the model plant *Arabidopsis*.

Three broad categories of pharmaceuticals can be produced in plants; namely antibodies, vaccines and other therapeutics. These are discussed briefly below.

4.1.8. Antibodies

Monoclonal antibodies (mAbs) are an essential component of modern medicine, used extensively for therapeutic and diagnostic purposes. Traditionally mice were used to make these antibodies, and more recently yeast and other gene expression technologies have been used. Research into antibodies derived from plants or ‘plantibodies’ has been conducted for nearly twenty years. The use of GM plants to produce antibodies and other therapeutic proteins holds many advantages over other currently used production systems, and has been recently reviewed (Goldstein and Thomas, 2004). Plants hold potential for large scale, efficient, low cost production of therapeutics without the risk of contamination of human or animal pathogens that other production systems carry, and with improved delivery systems. Antibodies for various purposes have been made in tobacco, potato, soybean, alfalfa, rice, wheat and tomato. In the United States tomato plants expressing antibodies effective against dental caries have been commercialised.

4.1.9. Vaccines

There has been a great deal of interest in using plants to produce low cost, edible vaccines. The aim is to produce plant vaccines with increased safety, stability and efficacy. Plants offer advantages as a delivery system, as they could be grown where needed, eliminating the need for refrigeration and transport costs, and administered directly as a food product, eliminating purification costs. At least a dozen different vaccine antigens have been produced in many different crop plants, including tobacco, tomato, potato, alfalfa, lettuce, carrot and banana against many different disease agents including Hepatitis B, rabies, malaria and cholera. There are some significant limitations with the use of GM plants for vaccine production that need to be addressed, especially the high levels of protein expression which are required for total immunity. Further research and clinical trials will see refinement of this technology.

4.1.10. Other therapeutic agents

Research is continuing into the use of GM plants as factories for producing other pharmaceutical agents which have applications including for anaemia, blood plasma, wound repair, Hepatitis B and C treatment, liver cirrhosis, hypertension, cystic fibrosis, HIV and other diseases (see Table 4 and 5 of Goldstein and Thomas, 2004). For example hirudin is a blood anticoagulant originally isolated from leeches but that can now be effectively expressed in canola, tobacco and mustard.

4.1.11. Opium Poppies

Australia already has experience in producing a conventionally bred ‘pharma’ crop, the opium poppy. Alkaloids that are produced by commercial poppy crops are essential elements in analgesics. Tasmania grows over 40% of the world’s legal opiates and in 2001 the industry occupied almost 20 000 hectares (Napier et al., 2001).

Breeding targets for the poppy industry are to alter the alkaloid content or profile of the crop. This could involve increasing alkaloid synthesis, or altering poppy varieties so that they accumulate types of alkaloids that are normally only transiently produced. Indeed a conventionally bred variety of poppy that accumulates thebaine and oripavine, rather than converting them into morphine and codeine, has re-engineered the opioid industry in Tasmania (Millgate et al., 2004). These products and manufactured derivatives have uses as highly effective pain-killers and for the treatment of opioid addiction.

The use of genetic engineering to change alkaloid levels in poppies is also being explored worldwide. In Australia, a partnership of researchers from CSIRO, Tasmanian Alkaloids, and Johnson & Johnson Research recently reported the use of RNAi technology to produce poppies that accumulate a non-narcotic alkaloid reticuline (Allen et al., 2004). This research is at the proof of concept stage with no commitment to commercialisation mainly because of regulatory barriers (BRS Consultation).

4.1.12. GM crops for other industrial uses

Technology discovery in Australia and worldwide is being carried out on many other GM crops engineered to produce industrial products. Some examples include research into starch, fibre, forest products and natural polymers such as bioplastics. Biofuels are another potential area for investment in new technologies. At the Grains Week meeting in April 2005, the capacity of biofuels such as ethanol to offer a viable new demand stream for the Australian Grains Industry was discussed. The GRDC acknowledges that there is tremendous interest within the grains industry in alternative fuels because of rising oil prices.

Research and development organisations recognise the huge potential in these areas and are keen to position themselves quickly to access technologies and assess applications for Australia. For example the GRDC has a new initiative “crops as biofactories” that will focus resources on developing platform technologies for industrial crops, in particular monomer and polymer production from oilseeds[48].

As a further example, the Australian sugar industry has been suffering from low world sugar prices over the last decade and will need to diversify into other forms of value added products to regain former levels of profitability (Grice et al., 2003). New approaches in sugarcane include those which enable the sugar plant to improve production levels through improved sugar content and longer harvest seasons, storage

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[48] This initiative is described in the August/September 2005 edition of Ground Cover.

or alternative sugar products with wider markets, better water use efficiency, decreasing the dependence on chemicals for pest and disease control, and by the creation of precursors of biodegradable plastics (Grice et al., 2003). In Australia, research into future improvements of sugarcane is taking place at the CRC for Sugar Industry Innovation through Biotechnology and BSES Ltd (formerly Bureau of Sugar Experiment Stations).

There is potential for Australia to position itself in the area of industrial biomanufacturing of chemicals. Current products are petroleum based but opportunities are developing in the area of biofermentation (putting genes into biocatalysts and fermenting) and a long term goal is to produce these chemicals 'in planta' (BRS consultations).

4.1.13. General comments

Plant molecular farming holds great potential for the production of many different types of agents. With a few exceptions, these applications are still in the technology development stage. Before the majority of the products can reach commercialisation, many technical, production and regulatory issues need to be resolved. For example the logistics of scale-up of production, distribution and handling of this material need to be developed. Field testing of pharma crops has been taking place in the United States since the early 1990s, and the pace and number of these trials has accelerated in recent years. More than 352 field test permits issued were issued in the United States from 1991-2004 for pharmaceutical, industrial or novel traits (Table 7.3).

Table 7.3 Field testing permits issued by APHIS for pharma crops (taken from Elbehri 2005)

Plant	Industrial enzymes	Novel proteins	Pharma plants	Total
Maize	11	157	63	231
Soybean	10	4	16	30
Alfalfa	2	1	1	4
Barley	-	1	1	2
Rapeseed	2	-	1	3
Tobacco	-	1	14	15
Tomato	-	-	1	1
Rice	1	2	8	11
Safflower	1	2	-	3
Wheat	-	2	-	2
Sugarcane	-	-	1	1
Other	5	6	11	22

Choice of a crop as a target depends on many factors including the type of agent, and where it can be expressed most efficiently in the plant. Tobacco is often used because of its highly efficient leaf production, and well developed plant transformation systems. For protein or oil production, seed crops such as soybean and corn hold many advantages as they are highly productive and harvesting, processing and storage techniques are already available.

Debate continues about the advantages and disadvantages of producing pharmaceuticals and other industrial agents in food crops. The potential for these products to flow into the human food chain needs to be carefully addressed. Regulators in the United States have adopted a zero tolerance standard for the presence of pharma and industrial crops in the food system (Elbehri, 2005). This creates unique challenges for production systems and adoption of sophisticated risk management systems. A significant question is whether the economic benefits of growing pharma crops outweigh the costs associated with managing the risks of food system contamination.

Indeed there have already been a couple of incidents showing the type of risks facing product developers. In 2002, APHIS inspectors discovered pharma corn growing in a soybean field in Nebraska. Volunteers were left over from a trial by Prodigene in the previous season, testing a pig vaccine. In another incident, USDA forced Prodigene to burn 155 acres of conventional corn that may have cross-pollinated with some pharmaceutical plants. In both cases, Prodigene did not follow APHIS licence conditions. Prodigene was fined US$250,000 and was required to pay approximately US$3 million for clean up costs (Elbehri, 2005).

4.1.14. Predicted impact of biopharming on Australian agriculture

Although Australia is only a small player in this field, there is potential to capture niche markets in using crops as biofactories to produce ingredients for foods, pharmaceuticals, nutriceuticals and industrial products such as biofuels and plastics (Collis, 2004). The grains industry has recognised Australia's potential in this area and in March 2004 the launch of the Single Vision 20 year strategic plan of the grains industry emphasized the potential of high value crops such as pharma crops. Australia is geographically well-placed as a gateway to Asian markets and that may attract investment for research and development (Dennis and Byrne, 2004). The successful transformation of sectors of Australian agriculture into competitive and sustainable biopharms will depend on many factors including:

- capacity of supply chains to effectively segregate pharma products from the food chain
- freedom to operate and access to research tools
- investment in this area
- confidence of a clear path to market and consumer acceptance
- regulatory environment.

The clear message for Australia is that researchers, product developers, consumer groups and regulators need to be engaged in two-way communication on these issues early in the product development pipeline.

4.2. Chapter 8: Phytoremediation

4.2.1. Introduction

Phytoremediation is a general term used to describe various mechanisms by which living plants alter the chemical composition of the soil matrix in which they are growing. Essentially, it is defined as the use of green plants to clean-up contaminated soils, sediments, or water (Fiegl et al., 2005). In soil, the contaminants are often synthetic pollutants such as organic solvents, heavy metals, pesticides, or radionuclides. The presence of excess heavy metals in soil or water poses a major environmental and human health problem (Raskin et al., 1997).

4.2.2. Types of Phytoremediation

In a broad sense, phytoremediation consists of four different plant-based mechanisms (Chaney et al., 1997; Prasad and Freitas, 2003), each differing in how they clean up polluted environments. These include:

- Phytofiltration or Rhizofiltration – which involves the use of plants to clean aquatic environments;
- Phytostabilisation – where plants are used to stabilize rather than clean contaminated soil;
- Phytovolatilisation – which involves the use of plants to extract certain contaminants from the soil and then release them into the atmosphere as gases; and
- Phytoextraction – where plants absorb contaminants, generally heavy metals, from the soil via their roots and accumulate them in the shoots and leaves of the plant.

Although some plants show the ability to reduce organic pollutants, the greatest progress in phytoremediation has been the use of metal-accumulating plants, termed hyperaccumulators, to clean soil and water (Prasad and Freitas, 2003). Naturally occurring hyperaccumulator species can remove toxic metals from contaminated soil. Currently, there are over 400 species of known hyperaccumulators from at least 45 plant genus, the major of these being the *Brassica* (Robinson et al., 2003b).

4.2.3. Advantages and Limitations of Phytoremediation

Phytoremediation has a number of advantages over other remediation and metal extraction techniques,. The main advantage is in the reduction of costs, valuable metals can be extracted from the plants and recycled with the plants easily monitored to ensure proper growth. It is also one of the least environmentally destructive methods of remediation because it utilises natural organisms, thus preserving the natural state of the environment (Fiegl et al., 2005). Currently, basic plant physiology limits the scope of this remediation technique. The slow growth rate and the small biomass of most hyperaccumulators means there is a large time commitment involved, with a number of croppings and a limited use of mechanical harvesting techniques.

Leeching of contaminants into ground water cannot be fully prevented by plant based remediation systems and plant survival is at risk due to the toxicity of the contaminated soils as well as general soil condition. There is also the threat of bioaccumulation of heavy metals contaminants from primary to secondary consumers in the food chain (Fiegl et al., 2005). Despite these limitations, in cases where large surface areas of relatively immobile contaminants exist in the soil surface, phytoremediation may be the most appropriate remediation technique (Cunningham et al., 1995).

4.2.4. Economic Benefits of Phytoremediation

The current method for the clean-up of toxic heavy metal contaminated soils is the *ex situ* treatment or excavation and burial of the soil at a hazardous waste site and can cost around US$1,000,000 per hectare compared with an estimated US$60,000-US$100,000 per hectare for phytoremediation (Raskin et al., 1997; Robinson et al., 2003a). The costs associated with soil remediation are highly variable and dependent on the contaminant, site conditions, soil properties and the volume/area of material to be remediated. However, techniques that remediate soil *in situ*, such as phytoremediation, are generally less expensive and more cost effective than those that require conventional excavation methods (Cunningham et al., 1995). Some examples of the variation of costs between different methods of soil remediation in the United States in 1995 are shown in Table 8.1. Another possible economic role for phytoremediators has been reported in the mining industry through a process which has been coined 'phytomining' (Robinson et al., 2003a). This would involve generation of revenue by extracting saleable heavy metals from otherwise sub-economic ore bodies. No commercial phytomining operations have been conducted to date, although an American company, Viridian Environmental, has patents on the phytomining process (US Patent Nos 5711784 and 5944872) (Robinson et al., 2003a).

Table 8.1 Cost variation between *in situ* and *ex situ* soil remediation techniques (Cunningham et al, 1995)

Remediation Technique	Cost per m^3 (US$)
In situ remediation of volatile or water soluble contaminants – Phytoremediation	$10 - $100
Contaminated soil incineration (not including excavation cost)	$100
Landfilling or low-temperature thermal treatments	$60 - $300
Special landfill arrangements or high-temperature thermal treatments	$200 - $700
Intensive management techniques i.e. for radionuclides	$1000 - $3000

4.2.5. Natural phytoremediators and conventional breeding strategies

There are a number of limitations associated with most naturally occurring hyperaccumulating plants. They are often rare and grow in remote regions where their habitat is threatened by mining, development and other activities. Also, most hyperaccumulators grow slowly, have a small biomass, and often only accumulate a specific element. No single plant has yet been found that will remediate all contaminating elements of interest.

Limitations such as these have encouraged efforts into the development of traditional crop plants with ‘hyperaccumulator tendencies’. One possible strategy is to cross breed hyperaccumulators with high biomass relatives. However, no real success has been achieved to date, believed to be due to sexual incompatibility. *Arabidopsis* and pea mutants have been screened and lines identified which exhibit phytoremediative activity. The uptake and accumulation of heavy metals in existing phytoremediative plants has been enhanced by the use of chemical agents (Blaylock et al., 1997; Huang et al., 1997).

4.2.6. Biotechnology and Phytoremediation

The genetic control of metal hyperaccumulation in plants is not well understood. Although early work suggested that such control was complex, more recently it has been proposed that metal tolerance is regulated by a few major genes (Macnair, 1993; Lasat, 2002). Such improved understanding may facilitate the use of biotechnology to engineer plants with the ability to remediate metal-contaminated soils (Lasat, 2002).

Engineering plants that are fast growing and have a large, harvestable biomass, with greater metal tolerance and accumulating properties is the key to developing successful phytoremediators. Functional genomic approaches have made progress in this area, with some examples shown in Table 8.2.

Table 8.2 Some examples of phytoremediating GM plants

Agent	Plant	Reference
Methylmercury (neurotoxin)	Arabidopsis, tobacco and yellow poplar trees	(Rugh et al., 1996; Heaton et al., 1998; Rugh et al., 1998)
Arsenic	Arabidopsis	(Dhankher et al., 2002; McGrath and Zhao, 2003)
Selenium[49]	Indian mustard	(Pilon-Smits et al., 1999)
Lead, cadmium	Arabidopsis	(Song et al., 2003; Tong et al., 2004)

These examples illustrate the potential of biotechnology to enhance the effectiveness of phytoremediation. Most progress in phytoremediation research is taking place in the United States, with few field trials reported globally. To date there has been no commercialisation of phytoremediative crops anywhere in the world. The current status of phytoremediation field trials is summarised in Table 8.3.

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[49] This also has applications for cadmium, cobalt, copper, magnesium, nickel, lead and zinc contaminated soils.

Table 8.3 Current phytoremediation field trial status worldwide as of 9 May 2005

Crop	Status	Trait	Location	Hyperlink
Peanut *(Arachis hypogaea)*	Field test notification Pending 05/2005	Heavy metal phyto-remediation (Mercury)	USA	http://www.isb.vt.edu/cfdocs/fieldtests3.cfm?FIELDNAMES=NUM_VAL,LIST_AS,SELECT_ASCDESC,DB_CHOICE&num_val=05-119-01n&db_choice=com&list_as=detail&select_ascdesc=sort_date
Eastern cottonwood *(Populus deltoides)*	Field test permit 06/2004	Heavy metal phyto-remediation (Arsenic)	USA	http://www.isb.vt.edu/cfdocs/fieldtests3.cfm?FIELDNAMES=NUM_VAL,LIST_AS,SELECT_ASCDESC,DB_CHOICE&num_val=04-058-01r&db_choice=com&list_as=detail&select_ascdesc=sort_date
Eastern cottonwood *(Populus deltoides)*	Field test permit 06/2003	Heavy metal phyto-remediation (Mercury)	USA	http://www.isb.vt.edu/cfdocs/fieldtests3.cfm?FIELDNAMES=NUM_VAL,LIST_AS,SELECT_ASCDESC,DB_CHOICE&num_val=03-044-01r&db_choice=com&list_as=detail&select_ascdesc=sort_date
Poplar *(Populus deltoides)*	Field test permit 06/2004	Heavy metal phyto-remediation (Mercury)	USA	http://www.isb.vt.edu/cfdocs/fieldtests3.cfm?FIELDNAMES=NUM_VAL,LIST_AS,SELECT_ASCDESC,DB_CHOICE&num_val=04-090-02r&db_choice=com&list_as=detail&select_ascdesc=sort_date
Poplar *(Populus deltoides)*	Field test permit 06/2004	Heavy metal phyto-remediation (Mercury)	USA	http://www.isb.vt.edu/cfdocs/fieldtests3.cfm?FIELDNAMES=NUM_VAL,LIST_AS,SELECT_ASCDESC,DB_CHOICE&num_val=04-090-01r&db_choice=com&list_as=detail&select_ascdesc=sort_date
Poplar *(Populus deltoides)*	Field test permit 07/2003	Heavy metal phyto-remediation (Mercury)	USA	http://www.isb.vt.edu/cfdocs/fieldtests3.cfm?FIELDNAMES=NUM_VAL,LIST_AS,SELECT_ASCDESC,DB_CHOICE&num_val=03-099-01r&db_choice=com&list_as=detail&select_ascdesc=sort_date
Poplar *(Populus deltoides)*	Field test permit 09/2001	Heavy metal phyto-remediation	USA	http://www.isb.vt.edu/cfdocs/fieldtests3.cfm?FIELDNAMES=NUM_VAL,LIST_AS,SELECT_ASCDESC,DB_CHOICE&num_val=01-218-04n&db_choice=com&list_as=detail&select_ascdesc=sort_date

Rice *(Oryza sativa)*	Field test permit 07/2003	Heavy metal phyto-remediation (Mercury)	USA	http://www.isb.vt.edu/cfdocs/fieldtests3.cfm?FIELDNAMES=NUM_VAL,LIST_AS,SELECT_ASCDESC,DB_CHOICE&num_val=03-058-01r&db_choice=com&list_as=detail&select_ascdesc=sort_date
Brassica *(Brassica)*	Field test permit 03/2003	Heavy metal phyto-remediation	USA	http://www.isb.vt.edu/cfdocs/fieldtests3.cfm?FIELDNAMES=NUM_VAL,LIST_AS,SELECT_ASCDESC,DB_CHOICE&num_val=03-029-01r&db_choice=com&list_as=detail&select_ascdesc=sort_date
Tobacco *(Nicotiana tabacum)*	Field test notification Acknowledged	Heavy metal phyto-remediation (Mercury)	USA	http://www.isb.vt.edu/cfdocs/fieldtests3.cfm?FIELDNAMES=NUM_VAL,LIST_AS,SELECT_ASCDESC,DB_CHOICE&num_val=01-032-02n&db_choice=com&list_as=detail&select_ascdesc=sort_date
Poplar *(Populus deltoides)*	Field Trial 04/2003	Phyto-remediation of Soils (Heavy metals)	Germany	http://gmoinfo.jrc.it/gmp_report_onepag.asp
Poplar *(Populus deltoides)*	Field Trial pre-10/2002	Phyto-remediation of Soils (Heavy metals)	Germany	http://biotech.jrc.it/deliberate/doc/snifs.doc

4.2.8. Conclusion

Environmental pollution with metals is a global problem that has resulted from mining, industrial and agricultural practices. Therefore, the research and development of phytoremediative technologies for plant-based clean-up of contaminated soils is of significant interest. Although the avoidance of contamination should be the primary goal, if for various reasons this does not occur, cost effective, environmentally friendly remediation and detoxification technologies are needed. In the last 15 years, considerable research effort has been afforded to the development of phytoremediation as a possible low-cost environmentally friendly option for the clean-up of heavy metal contaminated soils and water.

Due to the limiting physiological features of natural metal hyperaccumulators, biotechnology has been at the forefront of research, to develop transgenic plants that are more tolerant, accumulate more metal, have a high biomass, and are easily harvestable. Currently, the development of such phytoremediators for environmental clean-up are only in the early stages of research and field trial testing due to the complexity of plant metal tolerance, hyperaccumulation mechanisms and metal soil interactions.

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Appendices

Appendix A: Some GM Crops being developed in Australia

Traits			**Crop**	**Stage in pipeline**
First generation traits	**Environmental stress tolerances**	Salt tolerance	Wheat	Proof of concept[50]
		Drought tolerance	Wheat	Proof of concept[51]
		Acid soil tolerance	Barley	Proof of concept
			Pasture species	Technology Discovery
		Frost tolerance	Wheat	Proof of concept
	Pest control	Insect pest protection – Bt/Ht	Cotton	Field trials
		Insect pest protection – Protease inhibitors	Cotton	Field trials
		Insect pest protection – Bt	Cotton	Field trials
		Insect pest protection – VIP	Cotton	Approved for field trials
		Resistance to canegrubs	Sugarcane	Proof of concept
	Disease control	Virus resistance	White clover	Field trial[52]
		Virus resistance	Barley	Proof of concept
Second generation traits	**Improved food, feed value and pastures**	Omega-3 oil production in plants	Oilseed	Technology discovery
		Starch modification	Wheat	Proof of concept[53]
		Improved digestibility	Wheat, barley	Proof of concept
		Improved oil quality	Cotton	Field trial[54]
		Modified lignin biosynthesis	Pasture species	Proof of concept
		Altered fructan metabolism	Pasture species	Proof of concept
		Reduction in hayfever-causing pollen	Ryegrass	Proof of concept
		Improved oil quality	Canola	Proof of concept
		Improved Sugar Content	Sugarcane	Proof of concept / Field trial
Third generation traits	**Plant molecular farming**	Alternative sugars for food ingredient and industrial applications – isomaltose	Sugarcane	Proof of concept / Field trial
		Alternative sugars for food ingredient and industrial applications – sorbitol	Sugarcane	Proof of concept[55]
		Production of precursors of biodegradable plastics	Sugarcane	Technology discovery/Proof of concept

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[50] Field trials are underway for this trait but they are proof-of-concept field trials examining performance under a natural salt gradient.

[51] Field trials carried out in Mexico – no plans to commercialise in Australia

[52] No short term plans to commercialise in Australia

[53] Proof of concept field trial underway – large scale experiment.

[54] Very positive results came from the field trials but the cotton industry requested combination with another trait before commercialisation. The other trait is still in the technology discovery phase.

[55] Currently deciding whether to take to field trials.

		Bioreactors producing pharmaceutical proteins	Tobacco	Proof of concept
		Alkaloid production	Poppy	Proof of concept

Appendix B: OGTR GM Crop Approvals and Applications

A. **Current, surrendered and withdrawn licences** (as of 7 November 2005)

Licence Number	**Organisation**	**Title of Project**	**Crop**	**Modified Trait**	**Issue Date**
DIR 58/2005	Deltapine Australia Pty Ltd	Small scale Field Trial of GM Insect Resistant (VIP) Cotton	Cotton	Insect resistance, antibiotic resistance	27 October 2005
DIR 57/2004	Bayer CropScience Pty Ltd	Intentional release of GM *Brassica juncea*	Indian Mustard	Herbicide tolerance and hybrid breeding system	2 June 2005
DIR 56/2004	Bayer CropScience Pty Ltd	Commercial release of herbicide tolerant cotton (LLCotton25)	Cotton	Herbicide tolerance	24 August 2005
DIR 55/2004	Monsanto Australia Ltd	Field trial of herbicide tolerant (Roundup Ready® Flex MON 88913) and herbicide tolerant/insect resistant (Roundup Ready® Flex MON 88913/Bollgard II®) cottons	Cotton	Prolonged herbicide tolerance, insecticidal action, antibiotic resistance, reporter gene expression	26 April 2005
DIR 54/2004	CSIRO	Field trial of genetically modified wheat with altered grain starch	Bread wheat	Altered grain starch and antibiotic resistance	13 April 2005
DIR 53/2004	Grain Biotech Australia Pty Ltd	Field trial of genetically modified salt tolerant wheat on saline land	Bread wheat	Salt tolerance, herbicide tolerance	21 April 2005
DIR 52/2004	CSIRO	Field trial of genetically modified rice – functional characterisation of the rice genome	Cultivated rice	Herbicide tolerance, antibiotic resistance and reporter genes have been randomly inserted into rice plants. Some plant growth traits may be modified by gene knockouts	18 February 2005
DIR 51/2004	The University of Queensland	Field trial of genetically modified sugarcane	Sugarcane	Altered sugar production and antibiotic resistance	11 February 2005

		expressing sucrose isomerase			
DIR 49/2004	CSIRO	GM cotton field trial – Evaluation under field conditions of the cotton rubisco small subunit promoter driving a reporter gene	Cotton	One reporter gene (enables detection and quantification of gene expression) linked to one of two promoters, and either one or two selectable marker genes)	21 October 2004
DIR 48/2003	Hexima Limited Ltd	Field trial to assess transgenic cotton expressing natural plant genes for insect control	Cotton	Insecticidal action, antibiotic resistance	30 July 2004
DIR 47/2003	Department of Primary Industries (Victoria)	Field evaluation of genetically modified white clover resistant to infection by *Alfalfa Mosaic Virus*	White clover	Viral disease resistance, antibiotic resistance	30 July 2004
DIR 44/2003	Dow AgroSciences Australia Pty Ltd	Agronomic assessment and seed increase of transgenic cottons expressing insecticidal genes (cry1Ac and cry1Fa) from *Bacillus thuringiensis*	Cotton	Insecticidal and herbicide tolerance	28 May 2004
DIR 43/2003	The University of Western Australia	Preliminary agronomic assessment of high sulphur lupin	Narrow leafed lupin	Increased level of sulphur in seed	Withdrawn
DIR 42/2003	CSIRO	Field evaluation of genetically-modified white clover resistant to infection by Alfalfa Mosaic Virus and Clover Yellow Vein Virus	White Clover	Virus resistance	Withdrawn
DIR 41/2003	Department of Primary Industries (Victoria)	Field evaluation of white clover resistant to infection by Alfalfa Mosaic Virus and Clover Yellow Vein Virus	White Clover	Virus resistance	Withdrawn
DIR 40/2003	Dow AgroSciences Australia Pty Ltd	Agronomic assessment and seed incr5ease of transgenic cotton expressing insect	Cotton	Insect resistance, herbicide tolerance	28 November 2003

		tolerance genes from *Bacillus thuringiensis*			
DIR 39/2003	CSIRO	Field evaluation of genetically modified high oleic cotton	Cotton	Modified fatty acid content in cottonseed oil	28 October 2003
DIR 38/2003	CSIRO	Field trial for breeding and pre-commercial evaluation of GM cotton expressing tolerance to the herbicide glufosinate ammonium	Cotton	Herbicide tolerance	3 November 2003
DIR 36/2003	CSIRO	Breeding and pre-commercial evaluation of transgenic cotton expressing a vegetative insecticidal protein (VIP) gene and a herbicide tolerance gene	Cotton	Insect resistance, herbicide tolerance, antibiotic resistance	31 October 2003
DIR 35/2003	Monsanto Australia Ltd	Field trials of herbicide tolerant (Roundup Ready® MON 88913) and herbicide tolerant/insect resistant (Roundup Ready® MON 88913/Bollgard II®) cotton	Cotton	Enhanced herbicide tolerance, insect resistance, antibiotic resistance, reporter gene expression	15 October 2003
DIR 34/2003	Syngenta Seeds Pty Ltd	The evaluation of transgenic cotton plants expressing the VIP gene	Cotton	Insect resistance, antibiotic resistance	15 October 2003
DIR 32/2002	Bayer CropScience Pty Ltd	Field trial – Seed increase and field evaluation of herbicide tolerant hybrid canola	Canola	Herbicide tolerant hybrid canola	10 March 2004
DIR 31/2002	CSIRO	Field trial of GM grapevines – evaluation of berry colour, sugar composition, flower and fruit development and gene flow study	Grapevines	Expression of modified colour, sugar composition, flowering and fruit development, expression of green fluorescent protein, antibiotic resistance	18 June 2003

DIR 30/2002	Florigene Ltd	Ongoing commercial release of colour modified carnations	Carnation	Modified flower colour	17 June 2003
DIR 28/2002	Department of Primary Industries (Queensland)	Field trial of pineapple plants modified for blackheart reduction and to delay flowering	Pineapple	Reduction of blackheart, delayed flowering, reporter gene expression	19 June 2003
DIR 27/2002	The University of Queensland	Field trial of pineapple plants modified to control flowering	Pineapple	Delayed flowering, herbicide resistance, reporter gene expression	19 June 2003
DIR 26/2002	The University of Queensland	Field trial for evaluation of GM papaya to delay fruit ripening and to test the expression of the introduced genes	Papaya	Delayed fruit ripening, reporter gene expression and antibiotic resistance	17 June 2003
DIR 25/2002 (Surrendered 22 September 2004)	CSIRO	Seed increase and efficacy studies in northern Australia of transgenic cotton expression a new insecticidal protein gene (vip3A)	Cotton	Insecticidal cotton	6 May 2003
DIR 23/2002	Monsanto Australia Ltd	Commercial release of herbicide tolerant (Roundup Ready ®) and herbicide tolerant/insect resistant (Roundup Ready ®/INGARD®) cotton	Cotton	Herbicide tolerant, insecticidal cotton	20 June 2003
DIR 22/2002	Monsanto Australia Ltd	Commercial release of insecticidal (INGARD®) cotton	Cotton	Insecticidal cotton	12 June 2003
DIR 21/2002	Bayer CropScience Pty Ltd	Commercial release of InVigor® hybrid canola (*Brassica napus*) for use in the Australian cropping system	Canola	Herbicide tolerance, hybrid breeding system	25 July 2003

DIR 20/2002	Monsanto Australia Ltd	General release of Roundup Ready® canola (*Brassica napus*) in Australia	Canola	Herbicide tolerant	19 December 2003
DIR 19/2002	Bureau of Sugar Experiment Stations	Agronomic assessment of transgenic sugarcane engineered with reporter genes	Sugarcane	Green fluorescent protein report gene	18 December 2002
DIR 18/2002 (Post-harvest monitoring)	CSIRO	Field trial of oilseed poppy in Tasmania to evaluate alkaloid production	Poppy	Altered alkaloid production pathway	6 November 2002
DIR 17/2002 (Post-harvest monitoring)	CSIRO	Field trials of insect resistant cotton	Cotton	Insect resistance	14 October 2002
DIR 16/2002 (Surrendered 22 September 2004)	CSIRO	Field trials of insect resistant of glufosinate tolerant cotton	Cotton	Insect resistance and herbicide tolerance	14 October 2002
DIR 15/2002 (Surrendered 22 September 2004)	CSIRO	Field trials of glufosinate ammonium tolerant cotton	Cotton	Herbicide tolerance	14 October 2002
DIR 12/2002	Monsanto Australia Ltd	Commercial release of Bollgard II® and Bollgard II®/Roundup Ready® cotton	Cotton	Insect resistance and herbicide tolerance	23 September 2002
DIR 11/2001 (Surrendered 2 June 2004)	Monsanto Australia Ltd	Field trials of Roundup Ready ® canola (*Brassica napus*) in Australia in 2002	Canola	Herbicide tolerance	22 August 2002
DIR 10/2001	Aventis CropScience Pty Ltd	Small and large scale trialling of InVigor® canola (*Brassica napus*) for development of the Australian cropping system	Canola	Hybrid breeding system and herbicide tolerance	30 July 2002
DIR 9/2001 (Post-harvest monitoring)	Department of Agriculture (WA)	Preliminary field evaluation of Bollgard II® cotton in the Kimberley region of WA	Cotton	Insect resistance	28 March 2002
DIR 8/2001	Department of	Integrated pest management	Cotton	Insect resistance	28 March 2002

(Post-harvest monitoring)	Agriculture (WA)	systems for INGARD® cotton			
DIR 7/2001 (Surrendered 23 July 2004)	Department of Agriculture (WA)	Improved alkaloid production in oilseed poppy (*Papaver somniferum*)	Cotton	Altered alkaloid production pathway	30 July 2002
DIR 6/2001 (Surrendered 4 November 2004)	CSIRO	Agronomic assessment and seed increase in northern Australia of transgenic cotton expressing *Cry1Ac* or *Cry1Ac* and *Cry2Ab* from *Bacillus thuringiensis*	Cotton	Insect resistance and herbicide tolerance	28 March 2002
DIR 5/2001 (Surrendered 1 November 2002)	Cotton Seed Distributors Ltd	Agronomic assessments and seed increase in eastern Australia of transgenic cotton expressing *Cry1Ac* and *Cry2Ab* from *Bacillus thuringiensis*	Cotton	Insect resistance and herbicide tolerance	18 January 2002

B. Applications for licences currently pending evaluation (as of 7 November 2005)

DIR Licence Application Number	**Organisation**	**Crop**	**Title of Project**	**Modified Trait**	**Early Bird Notification of receipt of application**	**Public calls for comment on RARMP open**
DIR 061/2005	Grain Biotech Australia	Wheat	Field testing of GM salt tolerant wheat on saline land	Salt tolerance, cyanamide resistance, antibiotic resistance	28 October 2005	
DIR 060/2005	Florigene Ltd	Rose	Propagation and trial of imported GM rose varieties	Altered flower colour, selectable marker	19 August 2005	
DIR 059/2005	Monsanto Australia Ltd	Cotton	Commercial release of herbicide tolerant (Roundup Ready Flex® MON 88913) and herbicide tolerant / insect resistant (Roundup Ready Flex® MON 88913 / Bollgard II®) cotton south of latitude 22° Southin Australia	Enhanced herbicide tolerance, insecticidal action, antibiotic resistance, reporter gene expression	29 July 2005	

Appendix C: List of Contacted Organisations

Organisation
Agrifood Awareness Australia (AFAA)
Australian Centre for Plant Functional Genomics (ACPFG)
Australian National University (ANU)
Bayer CropScience
BSES Ltd (formerly Bureau of Sugar Experiment Stations)
Center for the Application of Molecular Biology to International Agriculture (CAMBIA)
Cooperative Research Centre for Sugar Industry Innovation through Biotechnology
CSIRO
CSIRO Plant Industry
Australian Government Department of Agriculture, Fisheries and Forestry (DAFF)
Dairy Australia
Dow AgroSciences Australia Ltd
DuPont Australia
Grains Research and Development Corporation (GRDC)
Molecular Plant Breeding CRC
Monsanto Australia Ltd
New Zealand Ministry of Agriculture and Forestry
New Zealand Ministry of Research, Science and Technology
Office of the Gene Technology Regulator (OGTR)
Research School of Biological Sciences (RSBS) – ANU
SGA Solutions
University of Adelaide

Appendix D: Consultation Questionnaire

Consultation with R&D/Industry Bodies

The Bureau of Rural Sciences (BRS), with funding from the Department of Agriculture, Fisheries and Forestry (DAFF), is undertaking a project to identify potential genetically modified crop applications in the pipeline worldwide that are of relevance for Australian agriculture. A critical component of this project is consultation with relevant research, industry and regulatory organisations. This consultation will take the form of meetings (where possible) between BRS scientists and relevant experts. In order to focus discussion we have outlined questions below that will serve to structure the consultation process. Answers to these questions will help us in constructing our report and will not be attributed to individuals or organisations.

Consultation questions

1. What GM crop applications (traits), research, field trials, or commercialisation in the pipeline worldwide do you see as relevant to your industry/research organisation and Australian agriculture?

2. Can you give some examples of current research that you're aware of in Australia on these traits? (Status of GM crops in the pipeline in Australia – possible release?)

3. At what stage is this research?
    - Technology discovery
    - Proof of concept
    - Field trial
    - Commercialisation

4. Is this technology being developed for the Australian market or for overseas markets?

5. What do you see as the drivers of adoption of these GM traits? (For specific agricultural sectors or regions or specific crops in Australia)
    - For your industry
    - For other agricultural industries (cross-sector issues)

6. What do you see as potential impacts (positive and negative) of adoption of these GM traits?
    - For your industry (agricultural sectors or regions or specific crops)
    - For other agricultural industries (cross sector issues)

7. Can benefits or impacts be easily quantified? (i.e. economic advantage or other measures)

8. How would you assess the economic value of this trait?

9. How would you go about capturing this value?

10. What do you see as barriers to adoption of this technology?

11. Has the downstream supply chain been consulted about this trait/technology?

12. Are you aware of the impact of adoption that some of these traits have had overseas? If so, may they have any relevance to Australia?

13. What do you see as the average time span for the development of a GM crop to its potential commercial release? Specific examples? (particular trait/species)

14. Is it possible to breed these traits using non-GM techniques? If yes, estimates of time taken?

15. These are the main traits that BRS has identified as significant for Australian agriculture (to be discussed in the Pipeline report). In your opinion are there any traits that we have omitted or should not have included?

Classification	Trait
Input trait	Salt tolerance
	Drought tolerance
	Acid tolerance
	Frost tolerance
	Improved pest/disease control
Output trait	Enhanced nutrition/oil quality
	Post harvest life
	Improved feed and pastures
Other applications	Pharma crops
	Industrial applications
	Phyto remediation