ESR

CURRENT AWARENESS OF ISSUES RELATED TO GENETICALLY MODIFIED FOOD AND FOOD FROM CLONED ANIMALS

July – December 2005

Prepared as part of a New Zealand Food Safety Authority
contract for scientific services

by

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Client Report FW0636

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SUMMARY

This report is one of a series intended to provide the New Zealand Food Safety Authority with an independent source of current information on issues related to genetically modified foods and foods from cloned animals. This report covers developments in the period July to December 2005 and it is noted that during this period:

Approvals, Legislative and General Issues

- Plantings of GM crops continue to increase, particularly within the EU.

- The legislative situation on GM foods within the EU continues to be influenced by Member State bans - both those deemed to be illegal, and the continued application for bans.

- Approvals of GM crops by the EU also continue to be hampered by the inability of member states of the EU Agricultural Council to reach agreement on approvals. Proposals are therefore continuing to have to be sent back to the EU Commission for a decision. This results in long time frames for approvals to be made.

- Approvals for GM crops being sort both within the EU and within other countries remain largely for lines of Roundup Ready corn and soy and for insect-protected corn.

- Japan continues to test for contamination of seed imports with unapproved GMOs; specifically Bt10 in corn and RT73 in rapeseed.

GM Crop Research

The remainder of this report details new crops in development and new techniques to develop GM crops.

- The literature contains much information related to new GM crops that have been developed or are currently in development. These include lines of rice and potatoes as well as other crops like brassicas, coconut and sugarcane. There is an increasing emphasis on engineering crops to resist biotic and abiotic stress, for example drought stress. A lot of this work is being done outside of the US, in India and Asia in particular. It is likely that we may see a flood of smaller GM crops entering the human food chain in the next few years as some of these lines are released commercially. The implications of this for food labelling and for compliance testing are significant as i) the number of different crops that may be GM increases, and ii) the range of GM events that these crops contain increases.

- There is also a lot of research being reported on ways to improve the generation of transgenic crops. Issues associated with the use of antibiotic resistant marker gene system are being addressed. Plant-derived antibiotic resistance genes have been identified as an alternative, as have marker systems that target plant physiological processes. Work is continuing to develop systems to transfer DNA into plants that rely solely on the use of plant-derived sequences, ie: intragenic vector systems. These new ways of producing

transgenic plants will eventually result in a larger range of types of GM DNA constructs present in the food chain. There are likely to be fewer crops containing the same elements than presently, where for example, most commercial GM crops contain the 35S promoter element. This will have implications for testing of GM crops and will increase the reliance of regulatory bodies on detailed information provided by crop producers on the GM constructs in particular plants.

Food from Cloned Animals

- There continues to be little development in regulatory issues associated with food from cloned animals. The US FDA has yet to release its final policy on safe use of foods from cloned animals. Codex, while recognising the issue, has decided not to address it in the short-term, but is focussing instead on food from transgenic animals. Overall the impression is that foods from transgenic animals possess more of a food safety concern than food from cloned animals. Where it has been assessed, food from cloned animals has been deemed to be as safe as food from conventional animals.

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1 INTRODUCTION

This project is intended to provide the New Zealand Food Safety Authority with an independent source of current information on genetically modified foods (GMFs) and foods from cloned animals. It is intended to include:

- scientific issues concerning safety, detection, and nutritional quality of genetically modified foods and foods from cloned animals;
- the legislative situation overseas.

The aim of the project is to condense this material into a useful form so that the Authority can respond to issues and enquiries from other government agencies, industry and the general public. The project also aims to provide information to support the enhancement of New Zealand's enforcement strategy on standards for genetically modified foods.

This is the first report for the 2005/2006 year and covers events from July to December 2005.

Wider issues concerned with environmental or social effects of genetic modification and genetically modified organisms (GMOs), biodiversity, gene transfer, insect resistance, etc., are only covered peripherally in this report. This reflects the division of responsibility for genetically modified material, between the New Zealand Food Safety Authority and Food Standards Australia New Zealand (FSANZ) for GMFs on one hand, and the Environmental Risk Management Authority (ERMA) for GMOs on the other.

For consistency, some alternative terms have been standardised in this report. "Corn" and "maize" are interchangeable; in this document "corn" is used throughout. Canola is a genetic variation of rapeseed (or oilseed rape) developed by traditional plant breeding to be low in both erucic acid and glucosinolates ("double low" variety). In this document "canola" is used for this "double low" variety of rapeseed.

Again, for consistency the names of genes appear in this document in lowercase italics and the names of proteins in uppercase. This may not reflect usage in the original referenced document.

An important source of information for this project is the AgNet email newsletter produced by staff at the University of Guelph. Information and archives of the newsletter can be found at: http://www.plant.uoguelph.ca/safefood/

Abbreviations used throughout this document:

WTO: World Trade Organization
WHO: World Health Organization
FAO: Food and Agricultural Organization of the United Nations
FDA: Food and Drug Administration (US)
FSANZ: Food Standards Australia New Zealand

2 PART A: FOODS FROM GENETICALLY MODIFIED ORGANISMS

2.1 STATUS OF GM CROPS WORLDWIDE

2.1.1 GM Crop Planting in the EU

Indications are that planting of GM crops is continuing to increase in the EU. Farmers in five EU countries have begun growing GM crops. These are Spain, France, Germany, Portugal, and the Czech Republic. Insect-resistant Bt corn is the predominant crop being planted. Spain is still the largest grower of GM crops in the EU (planting thousands of hectares for animal feed), but France has significantly increased its plantings from just 17 hectares in 2004 to at least 500 hectares in 2005.
Source: Reuters, 23 September 2005, via International Herald Tribune
http://www.int.com

2.2 LEGISLATIVE POSITION OF OVERSEAS GOVERNMENTS REGARDING GENETICALLY MODIFIED FOODS

2.2.1 European Union – Member State Bans on GMOs

The EU legislative position on GMOs is still dogged by Member State bans. EU law provides that Member States can institute bans on GMOs if the governments can justify their prohibition. Currently bans are in place in five Member States – Austria, France, Greece, Germany and Luxembourg. GMO products affected by the bans include three lines of maize and two of rapeseed. The EU Scientific Committees and the European Food Safety Authority have reviewed justifications made by the Member States and have concluded that the bans are not justified, however, 22 Member States in the EU's Environment Council voted to uphold the national bans. This is seen by the EU biotech industry as another example of the inability of the EU regulatory system to "play by its own rule".
However, a bid by the Upper Austria Region to legally ban GMO crops and food has failed. Austria drafted a law in 2003 to ban all GMOs in that region for three years. The ban could not legally be enforced as the draft law needed approval from the Commission, which ruled in September 2005 that no new scientific evidence justified such a ban. An appeal against this ruling was made to the Court of First Instance (CFI), the EU's second highest court, who rejected it in October 2005.
Source: CropBiotech Update, 1 July 2005. www.isaaa.org/kc
Checkbiotech, 6 October 2005. www.checkbiotech.org

2.2.2 Swiss Vote on Five Year GMO Farming Ban

On 27 November 2005 Swiss voters approved a referendum to place a 5-year ban on the use of GM plants and animals in farming. Fifty five percent of voters were in favour of the ban. This will force the Swiss government to put in place some of the toughest legislations on GMOs in Europe. Switzerland is not a member of the EU and so does not come under EU regulations on GMOs. EU regulations apply restrictions to specific crops only and are temporary in nature, while legislation proposed in the Swiss referendum will place a blanket ban on all GM animals and crops for 5 years, with the exception of use in certain research and to produce medicines.
Source: AgBioView, 27 November 2005, via AgNet

2.2.3 Regulatory Decision on GMOs by Vietnam

In August 2005 the Vietnamese government released its decision on regulating genetically modified organisms. Under the decision GMOs must be labelled. Government permission is required to produce, trade in or put to use GMOs, as well as for the use of GMOs in research and development.
The decision can be viewed online at:
http://www.agbiotech.com.vn/en/?mnu=preview&key=349

2.2.4 Belarus Announces GM Food Labelling Requirements

The Belarus government has announced that labelling is now required of all GM food. Regulations also explicitly forbid the production or trade in children's food prepared using GM constituents.
Source: GM Watch, 20 December 2005, via AgNet

2.3 GMF APPROVALS

2.3.1 EU Authorizations

Proposals to place maize lines MON863 and GA21 on the market as food and food ingredients were submitted to the EU Agricultural Council in late July/early August 2005. The Council failed to reach a qualified majority decision in October 2005 and so the proposals were sent back to the EU Commission to make a decision.

2.3.1.1 Summary of Notifications to the EU

The online EU Community Register of GM Food and Feed can be found at:
http://europa.eu.int/comm/food/dyna/gm_register/index_en.cfm

Since 18 April 2004, Genetically Modified Food and Feed applications have been regulated in the European Community under Regulation (EC) 1829/2003, and it provides for a single

Community procedure for their authorization. The European Food Safety Authority (EFSA) is responsible for the scientific assessment of genetically modified food and feed. Information related to the applications submitted to EFSA under Regulation (EC) 1829/2003 on genetically modified food and feed can be found at:
http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html

2.3.2 GM Approvals in Spain

The Spanish Government has approved Monsanto's NK603 variety of Roundup Ready corn for planting in Spain.
Source: Reuters, 20 July 2005, via AgNet

2.3.3 GM Approvals in China

In July 2005 China approved the import of GM Roundup Ready corn variety NK603. China now has approval in place for eight varieties of GM corn, two of cotton, seven of canola and one variety of GM soybeans.
Source: USDA Press Release, 12 July 2005, via AgNet

2.3.4 GM Approvals in Argentina

Argentina is the world's second largest corn exporter after the US. In August 2005 Argentina approved the planting of GA21, a glyphosate-herbicide resistant corn variety made by Syngenta.
Source: Reuters, 23 August 2005, via AgNet

2.4 SURVEILLANCE AND POST-MARKET MONITORING

2.4.1 Japan to Test for Unapproved Rapeseed RT73

Japan has announced plans to test rapeseed imports from Canada for the GM rapeseed line RT73. This is an herbicide-resistant line of *Brassica rapa* developed by Monsanto and is approved for food and feed in Canada but not approved for human consumption in Japan. The GMO line was actually taken off the list of seeds for commercial production in Canada in 2003, at the request of Monsanto, but the Canada government has confirmed that a small amount of rapeseed line RT73 was planted in 2004 and 2005. The Canadian government maintains that farmers who planted the line in 2005 have been identified and rapeseed from those farmers consumed domestically; however, some seed harvested in 2004 may have been mixed with conventional seed lines and exported.
Source: Reuters, 22 December 2005, via AgNet

2.4.2 Bt10 Contamination of Corn

Japan continues to find US feed grain cargos tainted with Bt-10 corn. At least nine shipments have been identified. Importers are notified to either destroy tainted shipments or ship them back to the US.
Source: Reuters 23 August 2005 via AgNet

2.4.3 Unapproved GM Rice Found in China

During the reporting period there have been several more reports of GE rice availability in China. China has not yet authorised commercialisation of GE rice, however, Greenpeace maintains it has found Bt-rice available in supermarkets in central China and that GE rice has been grown illegally in the Hubei province for more than three years. GeneScan, a global tester of GMOs in food, has echoed the claims made by Greenpeace and maintains that they have tested rice found in Wuhan as well as in the southern province of Guangdong and have positively identified GE Bt-rice.
Sources: AgNet, 2 August 2005; Reuters, 11 August 2005, via AgNet.

2.4.4 More GM Papaya Contamination in Thai Crops

Thailand's Human Rights Commission has claimed that GM seeds have contaminated one third of papaya orchards examined in July 2005. Eleven out of 31 samples collected in eastern and northeastern regions tested positive for GM contamination. The Commission has urged the Thai government to destroy the contaminated fields and compensate farmers for their loss. They also want the government to tighten the laws banning GMO field trials.
Source: Agence France Presse, 6 September 2005, via AgNet.

2.5 BIOTECH RESEARCH

2.5.1 New Crops in Development

2.5.1.1 Novel genes providing high-level tolerance to glyphosate

In October 2005 the American biotechnology company Athenix Corp. announced that they had completed successful field trials on a number of transgenic plants containing a new class of genes that provide high-level tolerance to the herbicide glyphosate. The novel genes are proprietary to Athenix, who say they will combine them with nematode and insect resistant technologies to provide corn with advanced stacked traits.
Source: Athenix Corp, 6 October 2005, via AgNet. www.athenixcorp.com

2.5.1.2 GM crop trials in India

India's Department of Biotechnology released a document in October 2005 outlining fourteen transgenic food crops approved for contained and limited field trials. Target traits include insect tolerance, herbicide tolerance, viral and fungal resistance and stress tolerance. Modified food crops include brinjal (aubergine) varieties, cauliflower, cabbage, chickpeas, groundnuts, pigeon peas, mustard, potato and rice.
Source: Checkbiotech, 17 October 2005. http://www.checkbiotech.org

2.5.1.3 Development of GM potatoes

- Researchers at the Agricultural Research Council Institute at Roodeplast are testing a potato engineered for insect-resistance. Researchers at Michigan State University, originally developed the potato variety, called eco-spunta, using a gene obtained from Syngenta. Latest varieties of the line have been developed by the researchers in South Africa and have already undergone four years of field testing – two years focussing on the efficacy of the introduced gene construct in the plant and two years on impacts of the plant on the environment. It is now planned to perform human safety tests over two years before considering the release of the variety.
  Source: Creamer Media, 16 August 2005. http://www.engineeringnews.co.za

- Potatoes with increased levels of calcium have been developed by introduction of the gene for the calcium transporter CAX1. Transgenic tubers were shown to have up to 3 times the calcium of conventional tubers. Calcium deficiency is an increasingly important health issue world wide and increased calcium levels in potatoes have also been shown to protect the plant from pathogen attack. US researchers are hoping that the development of the high calcium potatoes will therefore provide farmers with a better crop and consumers with a nutritious product.
  Source: Hirschi, et al. *J. Agric and Food Chem*., 30 September 2005, via AgNet.

- Over-expression of the proline metabolic enzyme pyrroline-5-carboxylate synthetase (P5CS) in transgenic potato plants has been shown to confer increased salt tolerance. Introduction of the *Arabidopsis* gene for P5CS has previously been shown to increase tolerance to salt stress in rice, wheat, and carrot plants, suggesting a general mechanism to confer salt tolerance to a range of crops.
  Source: Hmida-Sayari *et al*. *J Plant Sci*., 2 September 2005, via AgNet.

- Canadian researchers have introduced a frog toxin gene into potatoes to confer resistance to a range of bacterial and fungal infections of tubers. Known as B1 the toxin belongs to the dermaseptin class of chemicals and is produced in the skin of particular tropical rain-forest tree frogs and protects them from bacteria and other pathogens that live in the same hot, humid climate. A synthetic version of the toxin gene was introduced into potatoes and shown to confer resistance to a broad range of fungi that cause disease in potato tubers as well as bacterial storage rots. As the potatoes were able to resist a large range of pathogenic organisms the researchers believe that the gene for B1 could be used to protect other crops from disease. Ongoing work is directed at testing the safety of the toxin to humans if consumed.
  Source: SciDev Net, 15 August 2005, via AgNet.

2.5.1.4 New traits introduced into rice

During the reporting period there were a number of reports of novel traits being introduced into rice. These include:

- Japanese researchers have conferred tolerance to several herbicides by introducing the human *Cyp2B6* gene into rice.
  Source: Kawahigashi *et al*. *J. Agric. Food Chem*., 20 October 2005, via AgNet.

- The *Rxo1* gene from maize, that confers resistance to bacterial streak disease, has been transferred to rice. This is an example of a resistance gene from one plant being used to successfully confer resistance in a distantly related plant. It is suggested that non-host resistance genes, like *Rxo1*, may be able to be used to control disease in a wide variety of crops.
  Source: Hulbert *et al*. *PNAS (USA*), 19 October 2005, via AgNet.

- The Philippine Rice Research Institute is beginning development of a multinutrient rice line with increased amounts of beta-carotene, vitamin E, iron and protein. It is planned to incorporate some traits into the new line by using conventional breeding systems, while others will involve the use of genetic transformation.
  Source: CropBiotech Update, 14 October 2005, via AgNet.

- Improving phosphorus uptake in plants:
  Phosphorus is an essential macronutrient for plant growth and development. Phosphorus is usually available to plants either as inorganic phosphate (Pi) in soil or as organic phosphate from fertilizers. However, the ability of plants to utilise Pi is low in most soils and P-fertilizer application is unsustainable and causes soil and water pollution. Researchers in China have identified a transcription factor (OsPTF1) from rice that confers a tolerance to Pi-deficiency conditions. This factor was introduced into another rice cultivar susceptible to Pi deficiency. When over-expressed in the system the introduced factor conferred tolerance to Pi-deficiency in both solution culture and soil systems. This study provides evidence that modification of a key regulator involved in a nutrient signalling pathway may be able to be used to improve the utilization of nutrients by plants.
  Source: ISB News Report, 8 September 2005. www.isb.vt.edu/
  Yi, K. *et al*. (2005). OsPTF1, a novel transcription factor involved in tolerance to phosphate starvation in rice (*Oryza sativa* L.). *Plant Physiol* online

- GM rice with potential to treat allergies:
  Japanese scientists have successfully modified rice so that it reduces allergic responses in mice. The rice was engineered to produce certain portions of the allergenic protein from cedar pollen. Mice fed the transgenic rice showed fewer allergic symptoms when exposed to cedar pollen than their counterparts not receiving therapy. The researchers reported their results in the prestigious journal Proceedings of the National Academy of Sciences (USA), and believe that putting allergy 'vaccines' into food will provide relief more efficiently and with less risk of anaphylactic shock than current allergen injection therapies.

Source: Takagi, H., Hiroio, T., Yang, L., Tada, Y., Yuki, Y., Takamura, K., Ishimitsu, R., Kawauchi, H., Kiyono, H. and Takaiwa, F. (2005). A rice-based edible vaccine expressing multiple T cell epitopes induces oral tolerance for inhibition of Th2-mediated IgE responses. *PNAS (USA)* 102(48): 17525-17530

2.5.1.5 Virus resistant sugarcane

Researchers at the University of Florida have performed field trials on sugarcane engineered for resistance to sugarcane mosaic virus (SCMV). SCMV is a lethal pathogen of sugarcane and due to the plant's high ploidy level it is difficult to breed new traits into sugarcane using traditional methods. Sugarcane modified by introduction of the SCMV strain E coat protein showed increased productivity and lower SCMV incidence than non-transformed lines.
Source: CropBiotech Net, 26 September 2005. www.isaaa.org/kc/

2.5.1.6 High lauric acid coconut

The Phillipines is reported to be developing a GM coconut with increased lauric acid content. Coconut oil is the Phillipines' largest export and is currently meeting 65% of the world's need for vegetable oil. However, the recent development of a GM line of high lauric acid canola to support the international market's increased desire for high lauric acid oils means that conventional coconut oil is becoming non-competitive in the world market.
Source: AgNet, 21 August 2005.

2.5.1.7 Genetically modified wine yeast

The world's first genetically modified wine yeast was released into the North American market in late 2005. The yeast was modified in France and is expected to enable faster and more reliable fermentation. In the US system this yeast is considered to be essentially the same as any other yeast and the company that has commercialised it is not required to label the yeast as GM when they sell it to winemakers. This could have labelling implications for US wines imported into Australia and New Zealand.
Source: ABC News Online, 15 November 2005, via AgNet.

2.5.1.8 Production of isoflavones in transgenic alfalfa

Isoflavonoids are a group of compounds produced mostly by legumes in response to plant stress. These compounds have also been shown to act as hormone mimics and to have potential for therapeutic benefits in the treatment and prevention of some hormone-dependant cancers, cardiovascular disease, osteoporosis and menopausal symptoms. Engineering production of isoflavonoids in plants therefore has the potential to provide a good source of these compounds for dietary supplements, as well as providing plants with extra defense mechanisms. Researchers at the Samuel Roberts Noble Foundation in the US have engineered alfalfa leaves to produce the isoflavonoid compound genistein glucoside. Genistein is a well known isoflavonoid found in soybean but is not normally produced by alfalfa. Production of the novel compound in alfalfa did not affect the regular growth of the plant or expression of other alfalfa genes. This is an example of the increasing ability to engineer plant secondary metabolite biosynthetic pathways.

Source: CropBiotech Update, 2 September 2005, via AgNet.
Deavours, B.E. and Dixon, R.A. (2005). Metabolic Engineering of Isoflavonoid Biosynthesis in Alfalfa. *Plant Physiol*. 138: 2245-2259.

2.5.2 New Genes Identified for Engineering Resistance to Abiotic and Biotic Stress

2.5.2.1 Engineering plants for tolerance to low temperature

Researchers at Duke University, North Carolina, have shown that the gene for mitochondrial alternative oxidase (AOX) can confer low temperature tolerance on transformed plants. Transgenic *Arabidopsis thaliana* containing the *Aox1a* gene were shown to have better survival and growth features at low temperatures than non-transformed plants.
Source: Fiorani, F., Umbach, A.L. and Siedow, J.N. (2005). The Alternative Oxidase of Plant Mitochondria is Involved in the Acclimation of Shoot Growth at Low Temperature. A Study of *Arabidopsis AOX1a* Transgenic Plants. *Plant Physiology* 139: 1795-1805.

2.5.2.2 Drought resistant tomatoes

Over-expression of the vacuolar H^+-pyrophosphatase (H^+-PPase) *Avp1* gene has previously been shown to confer drought resistance on transgenic *Arabidopsis thaliana* plants. Researchers at the University of Connecticut and Texas A&M have now over-expressed the *Avp1* gene in a commercial tomato cultivar and shown enhanced plant performance under conditions of soil water deficit. The *Avp1* gene plays a role in root development through the facilitation of auxin fluxes. Auxin is a natural plant growth regulator. Over-expression of *Avp1* in transgenic tomato plants resulted in increased root biomass and an enhanced ability of transformed plants to withstand and recover from an episode of soil water deficit. This study is the first example of *Avp1*-induced drought resistance in a commercial crop species and is described as documenting a general strategy for improving drought tolerance of crops.
Source: Park, S., Li, J., Pittman, J.K., Berkowitz, G.A., Yang, H., Undurraga, S., Morris, J., Hirschi, K.D. and Gaxiola, R.A. (2005). Up-regulation of a H^{+-}pyrophosphatase (H^+-PPase) as a Strategy to Engineer Drought-resistant Crop Plants. *PNAS (USA)* 102(52): 18830-18835.

2.5.2.3 Drought resistant Chinese Cabbage

Japanese researchers have successfully conferred tolerance to salinity and drought on Chinese cabbage by the introduction of a gene from another brassica, *Brassica napus*. The *Lea* gene from *B. napus* is expressed during late stages of seed development but may also be expressed when plants are exposed to environmental stresses. Chinese cabbage is an important vegetable crop in Asia.
Source: CropBiotech Update, 2 September 2005, via AgNet.

2.5.2.4 Use of transcriptional co-activators for resistance to a range of stresses

Acclimation of plants to abiotic conditions such as drought, high salinity or heat and to biotic stresses like pathogen attack is known to be mediated by a complex network of transcription factors and other regulatory genes that control multiple metabolic pathways. Associated with the activity of different transcription factors are transcriptional coactivators that enhance the binding of the transcription factor to the transcription 'machinery'. Researchers in the US have recently engineered *Arabidopsis thaliana* to constitutively express the stress-response coactivator multiprotein bridging factor 1c (MBF1c). Transgenic plants were shown to have enhanced tolerance to bacterial infection, heat and osmotic stress. Tolerance to heat and osmotic stress was also maintained when the two factors were used in combination. It is suggested that co-activator genes like the genes for MBF1 could be used to enhance the tolerances of plants to a ranges of different stresses.

Source: Suzuki, N., Rizhsky, L., Liang, H., Shuman, J., Shulaev, V. and Mittler, R. (2005). Enhanced Tolerance to Environmental Stress in Transgenic Plants Expressing the Transcriptional Coactivator Multiprotein Bridging Factor 1c. *Plant Physiol*. 139: 1313-1322.

2.5.2.5 Engineering plants with resistance to parasitic weeds

Parasitic plants, such as species of *Orobanche*, present a particularly intractable weed problem in crops in many parts of the world. These plants generally attack the root systems of plants, taking nutrients, water and photosynthetic products from the host and compromising yields of crop plants. Due to their close association with the tissue of the host they are particularly difficult to control using conventional weed control methods.

Researchers from Virginia and Israel have demonstrated a mechanism to engineer resistance to *Orobanche aegyptiaca* (Egyptian broom rape) based on expression of the gene for sarcotoxin 1A in transgenic tobacco. Sarcotoxin 1A was originally isolated from the flesh fly *Sarcophaga peregrine* and is a small (40 residue) peptide with antibiotic activity and known toxicity against several plant pathogenic bacteria and fungi. Tobacco plants expressing the sarcotoxin 1A peptide showed enhanced resistance to the parasitic plant as seen by abnormal parasite development and higher parasite mortality compared to non-transformed plants. Transformed plants also showed an increase in accumulated biomass in the presence of the parasite compared to non-transformed plants. Ongoing research is testing whether sarcotoxin 1A can confer resistance to other types of parasitic weeds.

Source: Hamamouch, N., Westwood, J.H., Banner, I., Cramer, C.L., Gepstein, S. and Aly, R. (2005). A Peptide from Insects Protects Transgenic Tobacco from a Parasitic Weed. *Transgenic Research* 14(3): 227-236.

2.5.3 New Systems for Generating Transgenic Plants

2.5.3.1 Use of nanofibres to effect gene transfer into plant cells

Researchers led by Timothy McKnight at the Oak Ridge National Laboratory, US Department of Energy, are developing a system for introducing DNA into plant cells using carbon nanofibres. The system is designed to introduce DNA into millions of cells at once. Millions of carbon nanofibres are grown sticking out of a silicon chip and have strands of synthetic DNA attached to them. Living plant cells are then 'thrown' at the fibres in a process described as much like throwing a bunch of baseballs against a bed of nails. The carbon nanofibres pierce the living cells, injecting DNA into them in the process. A key advantage to this method of introducing DNA into plant cells is that, in theory, the DNA remains attached to the carbon nanofibres and is not integrated into the plant genome. The immediate cell can be regenerated into a plant that will express the introduced trait but this trait should not be passed on to subsequent progeny. The implication is that it should be possible to reprogramme cells for one generation only, thereby avoiding the gene flow issues seen by some as a concern associated with traditional methods of generating transgenic plants. A disadvantage of the system would be the necessity to continually generate modified plants in the laboratory without the potential for farmers to collect seed from such superior crops. This may limit the technology to particular niche plants. For example, the researchers at Oak Ridge are targeting loblolly pine, a primary source of pulpwood in the US market.
Source: Azonano.com, 15 August 2005, via AgNet
www.ornl.gov/sci/ment/personnel/mcknight.htm

2.5.3.2 Gene targeting systems in plants

Gene targeting (GT) refers to precision engineering of eukaryotic organisms and requires efficient techniques for homology-based replacement of an endogenous gene with an introduced gene. Foreign DNA is preferentially integrated into plants and animals by non-homologous recombination, so special strategies are needed to increase the efficiency and facilitate the detection of GT in these systems. An Israeli research team, led by Avaraham Levy, have developed a system for high-frequency gene targeting in *Arabidopsis* plants. Traditional strategies for GT in plants have efficiencies in the order of 10^{-3} to 10^{-4} targeted events per transformed plant. This low frequency seems to be the result of a natural barrier to integration of homologous sequences in higher plants. Levy *et al*. report that expression in *Arabidopsis* of the yeast *Rad54* gene enhanced gene-targeting frequency by one to two orders of magnitude (to give 10^{-2} to 10^{-1}). The *Rad54* gene is a member of a yeast chromatin remodelling gene family. Chromatin describes the material that makes up eukaryotic chromosomes and consists of DNA and proteins, arranges in a complex structure. The ability of *Rad54* to increase the frequency of gene-targeting in a plant system suggests that chromatin structure is a barrier to homologous recombination in plants, and that chromatin-remodelling is rate-limiting for gene targeting in plant. The findings improve the prospects for using gene targeting for the precise modification of plant genomes. This work was published in August 2005 in the prestigious journal Proceedings of the National Academy of Sciences (USA).

Source: Shaked, H., Melamed-Bessudo, C. and Levy, A.A. (2005). High-frequency gene targeting in *Arabidopsis* plants expressing the yeast *RAD54* gene. *PNAS (USA)* 102(34): 12265-12269.

2.5.3.3 Grafting as a method to obtain non-GM produce from GM plants

A combination of genetic modification and traditional plant grafting techniques has been used to help watermelon crops resist a potent plant virus without introducing foreign genes into the fruit. A team of Korean biotechnologists modified the rootstock to which commercial watermelon varieties are grafted. The plants were resistant to the rootstock virus while fruit from aerial grafted tissue remained non-transformed. Genes conferring resistance to the virus do not exist in plants in nature, so traditional breeding practices cannot be used to introduce resistance into watermelon. A viral gene was inserted into the watermelon rootstock, conferring resistance to one in ten modified rootstocks. The method could be applied to other crops that the virus can also damage, such as cucumber and melon, and so avoid some of the controversial issues associated with GM crops destined for the human food market.

Source: Park, S.M., Lee, J.S., Jegal. S., Jeon, B.Y., Jung, M., Park, Y.S., Han, S.L., Shin, Y.S., Her, N.H., Lee, J.H., Lee, M.Y., Ryu, K.H., Yang, S.G. and Harn, C.H. (2005). Transgenic watermelon rootstock resistant to CGMMV (cucumber green mottle mosaic virus) infection. *Plant Cell Reports* 24(6): 350-356.

2.5.3.4 Plant derived transfer DNA

Research to develop intragenic plants is continuing. Intragenic plants make use of plant-derived transfer DNAs and this method of transforming plants could be used as an alternative to *Agrobacterium*-mediated transformation. Benefits of this technology include the potential to transform plant species traditionally recalcitrant to *Agrobactium,* as well as avoidance of the controversial introduction of bacterial DNA sequences into transgenic plants. In an effort to develop all-native DNA transformation vectors researchers from the J.R. Simplot Company in the US have identified 50 putative right border alternatives from a range of plant sequences in addition to the model plant *Arabidopsis*; including potato, tomato, pepper, alfalfa, rice, barley and wheat. Efficacy tests in tobacco have shown 14 of these elements to be at least 50% as effective as conventional *Agrobacterium* transfer DNA in integrating DNA into the plant. New insights found in border region requirements from these experiments have enabled the construction of an all-native alfalfa transfer DNA vector that can be used for the production of intragenic plants.

Source: Rommens, C.M., Bougri, O., Yan, H., Humara, J.M., Owen, J., Swords, K. and Ye, J. (2005). Plant-derived Transfer DNAs. *Plant Physiol*. 139: 1338-1349.

2.5.3.5 Alternatives to bacterial antibiotic resistance marker selection systems

Marker genes are used in genetic engineering to enable the selection of plants successfully transformed with the introduced DNA construct. Traditionally antibiotic resistance genes derived from bacteria have been used as selectable markers for plant transformation – conferring on the plant the ability to grow in the presence of antibiotics in the culture media used for regeneration after insertion of the gene construct. Use of antibiotic resistance genes has become controversial. It has been suggested that resistance may be taken up by soil and/or gut microbes by horizontal gene transfer and result in increasingly antibiotic resistant populations of 'superbugs'. To this end most researchers avoid the use of antibiotic resistance marker genes for antibiotics commonly used in medicine.

- Recent research at the University of Tennessee has successfully utilized a plant gene to confer antibiotic resistance to a transgenic plant. The *Atwbc19* gene from *Arabidopsis thaliana* encodes the ATP binding cassette (ABC) transporter. When over-expressed in transgenic tobacco plants the *Atwbc19* gene conferred resistance to the antibiotic kanamycin. This mechanism of resistance is novel and unrelated to the bacterial neomycin phosphotransferase (*nptII*) gene mechanism that has traditionally been used to confer resistance to kanamycin in transgenic plants. Unlike the *nptII* gene, which confers resistance to several related antibiotics, the *Atwbc19* gene confers very specific resistance to kanamycin only. This is the first time a plant gene has been shown to confer resistance to an antibiotic. The *Atwbc19* gene may therefore become an effective alternative to the bacterial *nptII* gene to provide an antibiotic-resistance selectable marker system for transgenic plants. By harnessing a gene from a plant it should be possible to overcome many of the undesirable effects proposed to result from use of bacterial antibiotic resistance genes.

  Source: Mentewab, A.A. and Stewart, C.N. Jnr. (2005). Overexpression of an *Arabidopsis thaliana* ABC transporter confers kanamycin resistance to transgenic plants. *Nature Biotechnology* 23(9): 1177-1180.

There has also been a move towards developing marker genes that don't rely on antibiotic resistance, but instead elicit an easily detected physiological change in the successfully transformed plant. The green fluorescent protein (GFP) from jellyfish has been used and confers a fluorescent emission from transformed tissue when excited by specific light wavelengths. The disadvantage with this marker is that it emits fluorescence very similar to the endogenous fluorescence of some plant tissues, making its detection difficult in some cases. Herbicide resistance genes have been used as marker genes for transformation but are only a suitable choice if the desired outcome for engineering that specific plant is herbicide resistance. During the reporting period there have been several reports of the use of a selectable marker system that selects for transformed plants on the basis of their ability to utilize mannose as a sole carbon source.

- The enzyme phosphomannose isomerase (PMI) converts the sugar mannose to mannose-6-phosphate; a step that is essential to use mannose as a carbon source. PMI is not present in many plant species and plants that lack PMI are unable to metabolize this sugar and so cannot survive on media that contains mannose as the sole carbon source. A bacterial *pmi* gene has been shown to confer the ability to utilize mannose on transgenic plants and forms the basis for a successful marker gene system. Researchers in Hawaii have successfully used the PMI/Man system to select for transgenic papaya and Italian researchers have recently used the system in duram wheat. In both cases the PMI/Man system gave increased selection frequencies for transformed plants compared to the use of an antibiotic-resistance marker system. Advantages to the PMI/Man system, aside for its non-antibiotic nature, are that PMI is readily digested by mammals, has shown no adverse toxic effects in mice and generates no obvious biochemical changes in the mannose-associated metabolic pathway. Genes encoding PMI are widespread in nature and while not common in plants can be found in soybean and several other legumes, providing the potential to use a plant gene as the selectable marker if there is market opposition to the use of the bacterial gene.

The PMI/Man selectable marker system has been used in the insect-protected corn line MIR604, developed by Syngenta and recently submitted to FSANZ for approval as a GM food in Australia and New Zealand (see FSANZ Application A564).

Source: Zhu, Y.J., Agbayani, R., McCafferty, H., Albert, H.H. and Moore, P.H. (2005). Effective selection of transgenic papaya plants with the PMI/Man selection system. *Plant Cell Rep*. 24: 426-432.

Gadaleta, A., Giancaspro, A. Blechl, A. and Blanco, A. (2006). Phosphomannose isomerase, pmi, as a selectable marker gene for duram wheat. *J. Cereal Sci*. 43(1): 31-37. Published online 2005 http://dx.doi.org/10.1016/j.jcs.2005.06.004

3 PART B: FOODS FROM CLONED ANIMALS

This section presents recent international information on the safety of foods from cloned animals. Issues associated with transgenic animals as foods are not covered in this report.

3.1 INTRODUCTION TO CLONING OF FOOD ANIMALS

Assisted reproductive technologies (ARTs) have been used in animal production systems for over a century. One of the more recent developments within this area is nuclear transfer technology (NTT), or more colloquially 'cloning'. Nuclear transfer techniques can be divided into two types:

- Embryonic nuclear transfer (ENT), where the nucleus from a very early embryo (blastocyte) is taken and transferred into the cytoplasm of a recipient cell that has had its own nucleus removed (e-nucleated host cell). The blastocyte stage from which the nucleus is taken is prior to morphologically distinct differentiation of cell types in the embryo and directs the recipient cell to develop into an embryo.

- Somatic cell nuclear transfer (SCNT), where a differentiated animal cell nucleus is transferred into an e-nucleated recipient cell. In this system the nucleus from the partially or terminally differentiated cell re-programmes the e-nucleus back to a de-differentiated state, which then directs development of the cell into an embryo.

Both of these systems have been used to successfully generate cloned animals, however, it should be noted that the vast majority of embryos reconstructed by nuclear transfer either die before birth or produce unhealthy offspring. This suggests that a normal developmental outcome is more of an exception than the rule. The technology however has potential to enhance agricultural practise, as elite animals are likely to be used as nuclear donors for the increased production of desirable characteristics.

3.2 SAFETY OF FOOD FROM CLONED ANIMALS

During the reporting period there has been little in the literature related to safety assessment of food from cloned animals nor any major legislative initiatives in this area.

3.2.1 Review of issues associated with safety assessment of foods from 'biotech' animals

A review of issues associated with the safety of food from transgenic and cloned animals has been published in the journal Rev. Sci. Tech. The author is L. Kelly from FSANZ. The review discusses the use of the comparative approach to safety assessment of foods, with particular emphasis on issues related to food from transgenic and cloned animals. The comparative approach is based on the idea that the safety of a food derived from biotechnology can be assessed, to a large extent, by comparison with a benchmark of commonly consumed food(s) already deemed to be safe. Overall it is suggested that there is less concern about food safety in relation to food products from cloned animals than there is to food products from transgenic animals. Some abnormalities (eg: large offspring syndrome) have been seen with cloning, however many of these are also seen with techniques such as *in vitro* fertilisation and other related assisted reproduction technologies. This suggests some of the observed effects may be related more to the use of *in vitro* embryo culture rather than cloning techniques *per se*. It is also suggested that it is more likely that the progeny of cloned animals will enter the food chain, at least initially, rather than the clones themselves. This is largely related to economics, with the high cost of cloning making cloned animals currently more valuable as breeding stock than as a food commodity in themselves.

Source: Kelly, L. (2005). The safety assessment of foods from transgenic and cloned animals using the comparative approach. *Rev. Sci. Tech. Off. int. Epiz.* 24(1): 61-74.

3.2.2 FDA Evaluation of Food from Cloned Animals

The US agricultural industry and livestock producers are still waiting for the FDA to release the final version of its policy on the use of products from cloned animals. In June 2005 the FDA announced its decision on whether meat and milk from cloned animals and their offspring are safe for human consumption. The FDA has been running a four-year evaluation and risk assessment. The results of this study were released at the annual conference of the Biotechnology Industry Organisation 2005 held in Philadelphia in June 2005, and concluded that cloned animals and their offspring are as safe for human consumption as their conventional counterparts. However, the FDA has still not released a formal policy on the use of products from cloned animals. In the meantime the US agricultural industry continues to observe a voluntary moratorium on using products from clones.

Source: Washington Post, 6 October 2005. www.washingtonpost.com

3.2.3 Codex Task Force Decision on Foods from Cloned Animals

Codex is currently determining the second-term scope of work for the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Modern Biotechnology. The first meeting was set for September 2005 and the issue of foods derived from cloned animals suggested as an area for discussion.

In the report from the session it was noted that some delegations proposed new work for the safety assessment of animals produced using cloning techniques, either as a separate work item or as part of the work on recombinant-DNA animals. It was recognised that animal cloning is often used complementary to the production of recombinant-DNA animals. Other delegations considered this work outside of the scope of the Task Force. The Task Force agreed that no new work would commence, at this stage, to address the food safety of cloned animals as such. It was, however, noted that the issue could be considered during the development of draft guidelines for the food safety assessment of recombinant-DNA animals.

Source: Codex Alimentarius Commission. www.codexalimentarius.net