



Australian Government

**Department of Health and Ageing
Office of the Gene Technology Regulator**

July 2010

APPLICATION FOR LICENCE FOR INTENTIONAL RELEASE OF GMOs INTO THE ENVIRONMENT: Application No. DIR 107

SUMMARY INFORMATION

Project Title:	Limited and controlled release of banana genetically modified for disease resistance ¹
Applicant:	Queensland University of Technology
Common name of the parent organism:	Banana
Scientific name of the parent organism:	<i>Musa acuminata</i> (Cavendish) and a <i>Musa acuminata</i> / <i>Musa balbisiana</i> hybrid (Lady Finger)
Modified trait(s):	Disease resistance and maker gene expression (antibiotic resistance and reporter genes)
Identity of the gene(s) responsible for the modified trait(s):	Specific pathogen resistance: <ul style="list-style-type: none">• <i>RGC2</i> and <i>RGC5</i> genes from <i>Musa acuminata ssp malaccensis</i> (banana) Disease resistance through inhibition of apoptosis: <ul style="list-style-type: none">• <i>Ced-9</i> gene from <i>Caenorhabditis elegans</i> (nematode)• <i>Sf-IAP</i> gene from <i>Spodoptera frugiperda</i> (fall armyworm)• <i>AtBag4</i> and <i>BI-1</i> genes from <i>Arabidopsis thaliana</i> (thale cress)• <i>OsBag4</i> from <i>Oryza sativa</i> (rice)• <i>Ps61</i> and <i>Ps65</i> genes from Citrus tristeza virus (plant virus)• <i>P35</i> gene from <i>Autographa californica nucleopolyhedrovirus</i> (insect virus) Antibiotic resistance selectable marker: <ul style="list-style-type: none">• <i>nptII</i> (neomycin phosphotransferase type II) gene from the bacterium <i>Escherichia coli</i> Reporter gene: <ul style="list-style-type: none">• <i>GUS</i> gene from the bacterium <i>E. coli</i>
Proposed Location(s):	One site in the Litchfield LGA (Northern Territory)
Proposed Release Size:	Up to 1.5 hectare
Proposed Release Dates:	November 2010 – November 2014

Introduction

The *Gene Technology Act 2000* (the Act) in conjunction with the Gene Technology Regulations 2001, an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, comprise Australia's nationally consistent regulatory system for gene technology. Its objective is to protect the health and safety of people, and the environment, by

¹ The title of the licence application submitted by QUT is 'Field Trial of Banana Genetically Modified for Fungal Resistance'.

identifying risks posed by or as a result of gene technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs).

The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer and make decisions under the legislation. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), an Australian Government regulatory agency located within the Health and Ageing portfolio.

The legislation sets out requirements for considering applications for licences for dealings with GMOs, including matters that the Regulator must take into account before deciding whether or not to issue a licence. The Regulator's *Risk Analysis Framework*² outlines the assessment process that will be followed.

The application and the proposed dealings

The Regulator has received an application from Queensland University of Technology (QUT) for a licence for dealings involving the intentional release of genetically modified (GM) banana, into the Australian environment on a limited scale under controlled conditions.

QUT is seeking the approval to conduct a field trial of banana genetically modified for disease resistance. The GM banana lines³ proposed for release would contain one or more genes that are expected to provide protection from certain disease-causing (pathogenic) micro-organisms, such as Fusarium wilt and Sigatoka leaf spot. Some of the GM banana lines would contain a reporter gene that enables visual indication of where successful transformation of plant tissues has occurred. The purpose of the proposed trial is to conduct proof of concept experiments to assess the disease response and/or developmental effects on the GM banana lines. The applicant proposes to limit the release to one site in the Litchfield Municipality LGA (NT) on a maximum area of 1.5 ha between November 2010 and November 2014. The GM bananas will not be used for human food or animal feed.

The applicant has proposed a number of control measures to restrict the spread and persistence of the GM plants and their introduced genetic material, which will be considered in the assessment of this application, including:

- locating the proposed trial site on flat land 1.5 km away from the nearest natural waterway
- utilising a parent plant that has been cultivated to the extent that it is essentially female and male sterile (see Parent organism)
- applying bunch covers to emerged inflorescences to prevent access to the developing fruit by birds and mammals that may feed on the fruit
- removing the immature male bud (bell) of inflorescences or bagging the bell to prevent access by pollinators
- complying with State Government legislation for banana disease control that would also aid in containment of GM plants
- destroying all (GM and non-GM) plant materials from the field trial by herbicide treatment and surface decomposition of non-propagative material

² The *Risk Analysis Framework* and further information on the assessment of licence applications is available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au>>.

³ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

- analysing GM plant materials from the trial in a certified PC2 facility and then destroying the materials by autoclaving
- post harvest monitoring of the trial site for 12 months and destroying any volunteers
- transporting GM plant materials to and from the proposed trial site in accordance with Regulator's transportation guidelines
- not using the GM plant material for human food nor animal feed.

Parent organism

The parent organism is *Musa acuminata* (Cavendish) or the *Musa acuminata/Musa balbisiana* hybrid (Lady Finger), which are exotic to Australia. Bananas are grown commercially on the east coast of Australia from northern New South Wales to far north Queensland. They are also grown in Western Australia around Carnarvon, Kununurra and Broome and in the Northern Territory near Darwin. Cavendish accounts for approximately 95% of the bananas on the Australian market. Lady Finger comprises about 4% of the Australian market. Members of the Cavendish subgroup set seed so rarely that they can be regarded as female sterile and produce so little viable pollen that they are effectively male sterile. Lady Finger bananas are also essentially male sterile.

The genetic modifications and their effect

A total of up to 151 GM banana lines are proposed for release, comprising 18 GM Cavendish banana lines and 133 GM Lady Finger banana lines. Each line would contain one or two of the genes described below which are expected to confer disease resistance or the *GUS* reporter gene. All of the GM banana lines would also contain the antibiotic resistance marker gene.

Specific disease resistance genes

The *RGC2* and *RGC5* (resistance gene candidate) genes were isolated from a banana species that is resistant to Fusarium Tropical Race 4, and show sequence similarity to known Fusarium resistance genes.

Genes conferring disease resistance through inhibition of apoptosis

The following genes are all expected to confer disease resistance by preventing cells from undergoing programmed cell death (or apoptosis) in response to infection by certain pathogenic micro-organisms.

The *Ced-9* gene obtained from the nematode *Caenorhabditis elegans*, and the *mCed-9* gene, which is a synthetic version of *Ced-9* with sequence optimised for plant codon usage.

The *Sf-IAP* gene, from fall army worm, encodes a protein that blocks apoptosis by inhibiting an enzyme involved in an apoptosis pathway. The *AtBag4* and *BI-1* genes (from thale cress), and the *OsBag4* gene (from rice), which encode proteins that appear to be widely distributed in plants and also act through inhibiting apoptosis.

The *ps61* and *ps65* genes (also known as the *heat shock protein (Hsp) 90h* and *Hsp70h* genes, respectively) from the citrus virus *Citrus tristeza* are involved in virus particle assembly and cell to cell movement. The plant Hsp70h protein has also been implicated in protection against stress-induced apoptosis.

The *ps35* gene from *Autographa californica nucleopolyhedrovirus*, an insect Baculovirus, encodes a protein that inhibits virus-induced apoptosis in insect cells and has also been found to inhibit apoptosis in plants.

The GM Cavendish banana lines proposed for release comprise nine lines containing *RGC2* and nine lines containing the *Ced-9* gene.

The GM Lady Finger banana lines proposed for release comprise: 13 lines containing *Ced-9*; 10 lines containing *mCed-9*; 10 lines containing *RGC5*; 10 lines containing *Sf-IAP*; 10 lines containing *AtBag4*; 10 lines containing *OsBag4*; 10 lines containing *ps61*; 10 lines containing *ps65*; 10 lines contain both *AtBag4* and *ps65*; 10 lines containing *BI-1* and 10 lines containing *ps35*.

Marker gene antibiotic resistance gene and regulatory elements

The marker/reporter gene *GUS*, derived from the common gut bacterium *E. coli* encodes an enzyme β -glucuronidase (GUS) that enables visual identification of plant tissues in which it is expressed. GM banana plants containing the *GUS* gene will be used to verify that any anti-apoptotic effects observed are due to the introduced apoptotic genes and not the transformation process. Up to 20 GM Lady Finger banana lines will contain the *GUS* gene.

In addition, all of the GM banana lines will contain the antibiotic resistance selectable marker gene *neomycin phosphotransferase type II (nptII)* from the common gut bacterium *E. coli*. This gene, encoding the enzyme neomycin phosphotransferase, confers kanamycin or neomycin resistance on GM plant cells. The *nptII* gene was used during initial development of the GM plants in the laboratory to select plant cells containing the introduced genes.

The expression of the introduced genes in the GM banana lines is under the control of short regulatory sequences. These are derived from plants (*Zea mays* – maize, *Ricinus communis* – castor bean and *Musa acuminata ssp. malaccensis* – banana), a soil bacterium (*Agrobacterium tumefaciens*) and a plant virus (Cauliflower mosaic virus). Although *A. tumefaciens* and Cauliflower mosaic virus are plant pathogens, the regulatory sequences comprise only a small part of their respective total genomes, and are not in themselves capable of causing disease.

Method of genetic modification

The genes of interest were introduced into Cavendish or Lady Finger bananas by *Agrobacterium* mediated transformation. This method has been widely used in Australia and elsewhere for the generation of GM plants.

Transformed banana plants were grown in tissue culture in the laboratory and molecular analysis will be used to confirm the presence of the genes of interest. Each of the 151 GM banana lines proposed for release is the result of a separate genetic modification event.

Previous releases of the same or similar GMOs

Other GM banana lines containing the *ced-9* gene have previously been released in Australia on a limited scale under controlled conditions, under the licence for DIR 079/2007. Bananas genetically modified for enhanced nutrition have been released in Australia on a limited scale under controlled conditions, under the licence for DIR 076/2007.

Suitability of Applicant

Section 43(2)(f) of the Act requires the Regulator to be satisfied regarding the suitability of the applicant to hold a licence as a pre-requisite for considering DIR applications. The matters to be considered are outlined in section 58 of the Act and include capacity to meet the conditions of a

licence, relevant convictions and revocation of a licence or permit held under law relating to the health and safety of people or the environment.

The Regulator has determined that QUT currently meets the suitability requirements and will verify this continues to be the case prior to making any decision regarding the issuing of a licence.

Consultation process for this DIR application

The Regulator has made an assessment of whether the application should be considered as a limited and controlled release, under section 50A of the Act. As its principal purpose is to enable the conduct of experiments, and the applicant has proposed limits on the size, location and duration of the release and controls to restrict the spread and persistence of both the GMOs and their genetic material in the environment, **the Regulator has decided that the application qualifies as a limited and controlled release.**

This means that the Regulator is not required to consult on the assessment of this application until after a RARMP has been prepared in accordance with section 51 of the Act. In the interim, copies of the application are available on request from the OGTR. Please quote application number DIR 107.

The Regulator will seek comment on the consultation RARMP from the public as well as a wide range of experts, agencies and authorities including the Gene Technology Technical Advisory Committee, State and Territory Governments, Australian Government agencies and the Minister for the Environment. The RARMP will then be finalised, taking into account matters raised relating to risks to human health and safety and the environment, and form the basis of his decision whether or not to issue a licence.

At this stage, **the RARMP is expected to be released for comment in October 2010.** The public will be invited to provide submissions on the RARMP via advertisements in the media and direct mail to anyone registered on the OGTR mailing list. The RARMP and other related documents will be available on the OGTR website, or in hard copy from the OGTR.

If you have any questions about the application or the assessment process, or wish to register on the mailing list, please contact the OGTR at:

The Office of the Gene Technology Regulator, MDP 54 GPO Box 9848 Canberra ACT 2601

Telephone: 1800 181 030 Facsimile: 02 6271 4202 E-mail: ogtr@health.gov.au

Website <http://www.ogtr.gov.au>