Australian Government
Department of Health and Ageing
Office of the Gene Technology Regulator

APPLICATION FOR LICENCE FOR INTENTIONAL RELEASE OF GMOs INTO THE ENVIRONMENT: Application No. DIR 076/2007

SUMMARY INFORMATION

Project Title:	Limited and controlled release of banana genetically modified for enhanced nutrition [1]
Applicant:	Queensland University of Technology
Common name of the parent organism:	Banana
Scientific name of the parent organism:	*Musa acuminata* Colla cv. Williams
Modified trait(s):	Increased levels of pro-vitamin A, vitamin E and iron
Identity of the gene(s) responsible for the modified trait(s):	• ***APsy2a*** (phytoene synthase), from banana cv. Asupina (enhanced pro-vitamin A) • ***PsyB73*** (phytoene synthase) from *Zea mays* [maize] (enhanced pro-vitamin A) • ***Crt1*** (carotene desaturase) from the bacterium *Erwinia uredovora* (enhancd pro-vitamin A) • ***vte1*** (tocopherol cyclase) from *Arabidopsis thaliana* (enhanced vitamin E) • ***vte2.1*** (homogentisic acid phytyltransferase) from *Zea mays* [maize] (enhanced vitamin E) • ***vte3*** (2 methyl-6 phytyl-1, 4-benzoquinone methyltransferase) from *Arabidopsis thaliana* (enhanced vitamin E) • ***vte4*** (γ-tocopherol methyltransferase) from *Arabidopsis thaliana* (enhanced vitamin E) • ***HGGT*** (homogentisic acid geranylgeranyl transferase) from *Oryza sativa* [rice] (enhanced vitamin E) • ***Ferritin*** (chloroplast ferritin) from *Glycine soja* [wild soybean] (enhanced iron) • ***IRT1*** (iron regulated transporter) from *Arabidopsis thaliana* (enhanced iron) • ***FRO2*** (ferric chelate reductase) from *Arabidopsis thaliana* (enhanced iron) • ***nptII*** (neomycin phosphotransferase type II) from the bacterium *Escherichia coli* (antibiotic resistance selectable marker) • ***uidA*** (β-glucuronidase or GUS) from the bacterium *Escherichia coli* (reporter gene)
Proposed Location(s)	One site in the shire of Johnstone (QLD)

[1] The title of the licence application submitted by QUT is *Limited and controlled release of GM nutrient enhanced banana*

Proposed Release Size: Up to 1.4 ha

Proposed Release Dates: May 2008 to May 2012

Introduction

The *Gene Technology Act 2000* (the Act) took effect on 21 June 2001. The Act, supported by the *Gene Technology Regulations 2001*, an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, underpins Australia's nationally consistent regulatory system for gene technology. Its objective is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs).

The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation 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 the requirements for considering applications for licences for dealings with GMOs and the matters that the Regulator must take into account before deciding whether, or not, to issue a licence. The Regulator's *Risk Analysis Framework* outlines the assessment process that will be followed[2].

The application and the proposed dealings

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

The GM banana lines proposed for release contain genes from a number of sources that are expected to increase the concentration of pro-vitamin A, vitamin E or iron in the banana fruit. The purpose of the proposed release is to conduct proof of concept experiments with up to 1,290 GM banana lines[3] to analyse altered nutrient content of fruit and vegetative parts and assess growth, fruit and yield characteristics. A number of promoters are also being tested in order to identify those that achieve best expression of the genes in the fruit. The GM bananas will not be used for human food or animal feed.

The applicant proposes to limit the release to one site in the shire of Johnstone, Queensland (QLD) on a total area of up to 1.4 ha between May 2008 and May 2012.

The applicant has also proposed a number of controls to restrict the dissemination or persistence of the GM plants and their introduced genetic material that will be considered in the assessment of this application, including:

- locating the proposed trial site on flat land 250 m away from, and 18 m, above the nearest natural waterway (South Johnstone River).
- utilising a parent plant that has been cultivated to the extent that it is essentially female and male sterile (see Parent organism).

[2] More 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/ir/process.htm>.

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

- applying bunch covers to emerged inflorescences to prevent access to the developing fruits 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 parts.
- analysing GM plant materials from the trial in a certified PC2 facility and then destroying the materials by autoclaving.
- post harvest monitoring of trial site for 12 months and destroying any volunteer GM banana suckers.
- transporting GM plant materials to and from the proposed trial site in accordance with OGTR transportation guidelines

Parent organism

The parent organism is a sweet banana (*Musa acuminata* Colla cv. Williams), which is 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. The 'Williams' cultivar is one of several cultivars in the sub-group Cavendish that accounts for approximately 95% of the bananas on 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.

The genetic modifications and their effect

Up to 1,290 banana lines are proposed for release. The lines contain either genes to enhance nutrient levels or a marker gene that is used to investigate gene expression.

Up to 390 of the GM banana lines contain one or two of three different genes that are involved in pro-vitamin A[4] synthesis. The ***APsy2a*** gene derived from the banana cultivar 'Asupina'encodes the protein phytoene synthase which has a role in an early stage of the pathway that leads to the formation of α- and β-carotene. The ***PsyB73*** gene derived from maize (*Zea mays*) also codes for phytoene synthase and is similar in function to *APsy21*. The ***Crt1*** gene from the bacterium *Erwinia uredovora* encodes the protein carotene desaturase which also has a role in carotenoid synthesis. The expression of these genes, either singly or in a combination, is expected to increase the levels of the pro-vitamin A in banana cells.

Up to 360 of the GM banana lines contain one or two of five different genes that are involved in vitamin E synthesis[5]. The ***vte1***, ***vte3*** and ***vte4*** genes are derived from the plant *Arabidopsis thaliana* and each produces a protein (tocopherol cyclase; 2 methyl-6 phytyl-1, 4-benzoquinone methyltransferase; γ – tocopherol methyltransferase respectively) that has a role in tocopherol biosynthesis. The ***vte2.1*** gene is from maize (*Zea mays*) and its protein product, homogentisic acid phytyltransferase is also important in tocopherol biosynthesis. The gene ***HGGT*** from rice (*Oryza sativa*) encodes a protein (homogentisic acid geranylgeranyl transferase) that is essential for tocotrienol biosynthesis. Expression of these genes either

[4]Pro-vitamin A is a member of a group of molecules known as carotenoids, which includes both α- and β-carotene amongst others.
[5] The term Vitamin E refers to a family of molecules comprising 4 tocopherols and 4 tocotrienols

singly or in a combination is expected to lead to an increased accumulation of vitamin E in banana tissues.

Up to 120 of the GM banana lines contain one or more of three different genes that are involved in iron accumulation. The ***Ferritin*** gene from wild soybean (*Glycine soja*) encodes an iron-binding protein (chloroplast ferritin) that has a role in the storage of iron. The ***IRT1*** gene from the plant *Arabidopsis thaliana* encodes a protein (iron-regulated transporter 1) that affects the uptake of iron from the soil by roots. The ***FRO2*** gene, also from *Arabidopsis thaliana*, encodes a protein (ferric-chelate reductase) that also has a role in iron uptake by plant roots. The expression of these genes, either singly or in a combination, is expected to lead to enhanced iron levels in banana tissues.

Up to 420 of the GM banana lines contain the marker/reporter gene ***uidA***, derived from the common gut bacterium *Escherichia coli*. It encodes an enzyme β-glucuronidase (GUS) that enables visual identification of plant tissues in which this gene is expressed. GM banana plants containing the *uidA* gene will be used to investigate the level of activity of introduced promoters (regulatory sequences that control the expression of genes) to optimise gene expression in banana fruit.

In addition, all of the GM banana lines contain the antibiotic resistance selectable marker gene, neomycin phosphotransferase type II (***nptII***). This gene, encoding the enzyme neomycin phosphotransferase, was derived from the common gut bacterium *Escherichia coli*, and confers kanamycin or neomycin resistance on the GM plant. The *nptII* gene was used as a selective marker to identify transformed plants during initial development of GM plants in the laboratory.

The GM banana lines also contain short regulatory sequences that control expression of the introduced genes. These are derived from plants (*Musa* spp. - banana; *Zea mays* – maize; *Chrysanthemum morifolium*; *Oryza sativa* – rice; *Ricinus communis* – castor bean), a soil bacterium (*Agrobacterium tumefaciens)* and three plant viruses (banana bunchy top virus; taro bacilliform badna virus; and cauliflower mosaic virus). Although *A.tumefaciens,* banana bunchy top virus, taro bacilliform badna virus 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 GM banana lines were produced by introducing gene constructs, containing the gene(s) of interest along with the *nptII* gene and regulatory sequences, into cells from the commercial banana cultivar 'Williams'. The gene constructs were carried by *Agrobacterium tumefaciens,* which mediated the transformation of banana cells. These vectors are 'disarmed' since they lack the genes that encode the tumour-inducing functions of *A. tumefaciens*.

Transformed banana plants were grown in tissue culture in the laboratory and molecular analysis will be used to confirm the presence of the gene(s) of interest. Each of the 1,290 GM banana lines proposed for release is the result of a separate genetic modification event.

Previous releases of the GMOs

There have been no previous releases of these GM banana lines or any other GM bananas.

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 relevant convictions, revocation of a licence or permit relating to the health and safety of people, and capacity to meet the conditions of the licence.

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, in accordance with Section 50A of the Act. As its primary purpose is to enable the conduct of experiments, and the applicant has proposed limits on the size and duration of the release and controls to restrict the dissemination and persistence of both the GMO and its 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 seek comment on the assessment of this application until after a Risk Assessment and Risk Management Plan (RARMP) has been prepared. In the interim, copies of the application are available on request from the OGTR. Please quote application number DIR 076/2007.

After preparing the consultation RARMP, the Regulator will seek input 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 and Water Resources, and relevant local councils. 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 her decision whether or not to issue a licence.

At this stage, **the RARMP is expected to be released for comment in late February 2008.** 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, please contact the OGTR at:

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