



Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 076/2007**

**Limited and controlled release of banana genetically
modified for enhanced nutrition**

Applicant: Queensland University of Technology

April 2008

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the limited and controlled release of up to 1,290 banana lines modified for enhanced nutrition into the environment in respect of application DIR 076/2007 from Queensland University of Technology (QUT).

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a GMO. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public¹.

The application

QUT applied for a licence for dealings involving the intentional release of up to 1,290 genetically modified (GM) banana lines on a limited scale and under controlled conditions. The GM banana lines would be modified for increased levels of pro-vitamin A, vitamin E or iron, or to assess promoter specificity. The trial would take place at one site in the local government area of Cassowary Coast, Queensland on a maximum total area of 1.4 hectares between May 2008 and May 2012.

Up to 390 of the GM banana lines would contain one or two of three different genes that are involved in pro-vitamin A synthesis. These genes are derived from the plants banana and maize and a common soil bacterium *Erwinia uredovora*.

Up to 360 of the GM banana lines would contain one or two of five different genes that are involved in vitamin E synthesis. The genes are derived from the plants thale cress, maize and rice.

Up to 120 of the GM banana lines would contain one or more of three different genes that are involved in iron accumulation. These genes are derived from the plants wild soybean and thale cress.

Up to 420 of the GM banana lines would contain a marker/reporter gene derived from the common gut bacterium *Escherichia coli*. This 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.

All of the GM banana lines would contain an antibiotic resistance selectable marker gene, derived from *Escherichia coli*. This gene was used as a selective marker to identify transformed plants during initial development of GM plants in the laboratory.

The purpose of the trial is to conduct proof of concept research involving experiments with the GM banana lines to assess growth, fruit and yield characteristics and analyse the nutrient

¹ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/ir/process.htm>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at <<http://www.ogtr.gov.au/pubform/riskassessments.htm>>.

content of fruit and vegetative parts. A number of promoters are also being tested in order to identify those that achieve best expression of the introduced genes in the fruit. GM bananas produced during the trial will not be used for human food or animal feed.

QUT proposed a number of controls to restrict the dissemination or persistence of the GM banana lines into the environment that have been considered during the evaluation of the application.

Risk assessment

The risk assessment takes into account information in the application (including proposed containment measures), relevant previous approvals, current scientific knowledge, advice received from a wide range of experts, agencies and authorities consulted on the RARMP, and a submission from the public.

A **hazard** identification process was used to determine potential pathways that might lead to harm to people or the environment as a result of gene technology.

Nine events, including one identified in the consultation process, were considered whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the nine events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM banana lines into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. As none of the nine events characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is considered to be **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, a range of measures have been imposed to limit the release to the size, location and duration requested by the applicant, as these were an important part of establishing the context for assessing the risks.

The licence conditions require QUT to **limit** the duration of the release to between May 2008 to May 2012 on a maximum total area of 1.4 ha at one site. The **control** measures to restrict the spread and persistence of the GMOs include preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies;

transporting GM plant materials in accordance with OGTR transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed².

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 1,290 GM banana lines on a maximum total area of 1.4 ha over four years in the Queensland local government area of Cassowary Coast poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concludes that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

² The licence for DIR 076/2007 is available on the OGTR website (<http://www.ogtr.gov.au/gmorec/ir.htm#table>) via the link to DIR 076/2007

Table of Contents

EXECUTIVE SUMMARY	III
INTRODUCTION	III
THE APPLICATION	III
RISK ASSESSMENT.....	IV
RISK MANAGEMENT	IV
CONCLUSIONS OF THE RARMP	V
TABLE OF CONTENTS	VI
ABBREVIATIONS	VIII
TECHNICAL SUMMARY	1
INTRODUCTION	1
THE APPLICATION	1
RISK ASSESSMENT.....	2
RISK MANAGEMENT	3
LICENCE CONDITIONS TO MANAGE THIS LIMITED AND CONTROLLED RELEASE.....	4
OTHER REGULATORY CONSIDERATIONS	4
IDENTIFICATION OF ISSUES TO BE ADDRESSED FOR FUTURE RELEASES.....	4
SUITABILITY OF THE APPLICANT	5
CONCLUSIONS OF THE RARMP	5
CHAPTER 1 RISK ASSESSMENT CONTEXT	7
SECTION 1 BACKGROUND	7
SECTION 2 THE LEGISLATIVE REQUIREMENTS	8
SECTION 3 THE PROPOSED DEALINGS	8
3.1 The proposed activities	8
3.2 The proposed limits of the dealings (size, location and duration).....	9
3.3 Proposed controls to restrict the dissemination or persistence of the GMOs and their genetic material in the environment.....	9
SECTION 4 THE PARENT ORGANISM.....	10
SECTION 5 THE GMOs, NATURE AND EFFECT OF THE GENETIC MODIFICATION	10
5.1 Introduction to the GMOs	10
5.2 The introduced genes, encoded proteins and end products	12
5.3 The regulatory sequences.....	19
5.4 Method of genetic modification	20
5.5 Characterisation of the GMOs.....	21
SECTION 6 THE RECEIVING ENVIRONMENT.....	22
6.1 Relevant abiotic factors.....	22
6.2 Relevant biotic factors.....	22
6.3 Relevant agricultural practices.....	23
6.4 Presence of related plants in the receiving environment	24
6.5 Presence of the introduced genes or similar genes, encoded proteins and end products in the environment	24
SECTION 7 AUSTRALIAN AND INTERNATIONAL APPROVALS	25
7.1 Australian approvals of the GM banana lines	25
7.2 International approvals.....	26
CHAPTER 2 RISK ASSESSMENT	27
SECTION 1 INTRODUCTION	27
SECTION 2 HAZARD CHARACTERISATION AND THE IDENTIFICATION OF RISK	28
2.1 Production of a substance toxic/allergenic to people or toxic to other organisms.....	30
2.2 Spread and persistence (weediness) of the GM banana lines in the environment.....	31
2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	32
2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	33
2.5 Unintended changes in biochemistry, physiology or ecology.....	34
2.6 Unintended presence of <i>Agrobacterium tumefaciens</i> containing the introduced genes, during release	36

2.7	Unauthorised activities.....	37
SECTION 3	RISK ESTIMATE PROCESS AND ASSESSMENT OF SIGNIFICANT RISK	37
SECTION 4	UNCERTAINTY	38
CHAPTER 3	RISK MANAGEMENT.....	40
SECTION 1	BACKGROUND	40
SECTION 2	RESPONSIBILITIES OF OTHER AUSTRALIAN REGULATORS	40
SECTION 3	RISK TREATMENT MEASURES FOR IDENTIFIED RISKS.....	41
SECTION 4	GENERAL RISK MANAGEMENT	41
4.1	Proposed licence conditions.....	41
4.2	Other risk management considerations	43
SECTION 5	ISSUES TO BE ADDRESSED FOR FUTURE RELEASES	45
SECTION 6	CONCLUSIONS OF THE RARMP	45
REFERENCES	47
APPENDIX A	DEFINITIONS OF TERMS IN THE RISK ANALYSIS FRAMEWORK USED BY THE REGULATOR.....	55
APPENDIX B	SUMMARY OF ISSUES RAISED IN SUBMISSIONS RECEIVED FROM PRESCRIBED EXPERTS, AGENCIES AND AUTHORITIES ON THE CONSULTATION RARMP FOR DIR 076/2007.....	57
APPENDIX C	SUMMARY OF ISSUES RAISED IN SUBMISSIONS RECEIVED FROM THE PUBLIC ON THE CONSULTATION RARMP FOR DIR 076/2007	58

Abbreviations

(does not include gene and protein abbreviations)

the Act	<i>Gene Technology Act 2000</i>
AI	Average Intake
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
BBTV	Banana Bunchy Top Virus
CaMV	Cauliflower mosaic virus
2,4-D	2,4-dichlorophenoxyacetic acid
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
EC	European Commission
EFSA	European Food Safety Authority
EPA	(United States) Environmental Protection Agency
FAO	Food and Agricultural Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	hectare(s)
JECFA	Joint FAO/WHO Expert Committee on Food Additives
mRNA	Messenger Ribonucleic Acid
NH&MRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OGTR	Office of the Gene Technology Regulator
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
QDPI&F	Queensland Department of Primary Industries and Fisheries
QUT	Queensland University of Technology
RARMP	Risk Assessment and Management Plan
RE	Retinol Equivalents
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RDI	Recommended Daily Intake
RNA	Ribonucleic Acid
TaBV	Taro bacilliform badna virus
TGA	Therapeutic Goods Administration
UL	Upper Level of intake
WHO	World Health Organisation

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Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence (DIR 076/2007) to Queensland University of Technology (QUT) for dealings involving the intentional release of genetically modified (GM) banana lines into the Australian environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a GMO. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public³.

The application

QUT applied for a licence for dealings involving the intentional release of up to 1,290 lines⁴ of banana (*Musa acuminata* cv. Williams) on a limited scale and under controlled conditions. The GM banana lines would be genetically modified for increased levels of pro-vitamin A, vitamin E or iron, or to assess promoter specificity. The trial would take place at one site in the local government area of Cassowary Coast, Queensland on a maximum total area of 1.4 hectares between May 2008 and May 2012.

Up to 390 of the GM banana lines would contain one or two of three different genes that encode proteins involved in pro-vitamin A carotenoid⁵ synthesis. The *APsy2a* gene derived from the banana cultivar 'Asupina' encodes the protein phytoene synthase which has a primary role in the conversion of geranylgeranyl diphosphate to phytoene. The *PsyB73* gene derived from maize (*Zea mays*) also codes for phytoene synthase. The *CrtI* gene from the bacterium *Erwinia uredovora* encodes the protein carotene desaturase that converts phytoene into lycopene and substitutes for two plant desaturases (phytoene desaturase and ξ -carotene desaturase) as well as for carotene isomerase. The expression of these genes, either singly or in a combination, is expected to increase the levels of pro-vitamin A carotenoids in banana tissues.

Up to 360 of the GM banana lines would contain one or two of five different genes that are involved in vitamin E⁶ synthesis. 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

³ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/ir/process.htm>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at <<http://www.ogtr.gov.au/pubform/riskassessments.htm>>.

⁴ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

⁵ The three major pro-vitamin A carotenoids are α -carotene, β -carotene and β -cryptoxanthin but in this trial it is expected that β -carotene would be the major end product.

⁶ The term Vitamin E refers to a family of molecules comprising 4 tocopherols and 4 tocotrienols

gene **HGGT** from rice (*Oryza sativa*) encodes a protein (homogentisic acid geranylgeranyl transferase) that is essential for tocotrienol biosynthesis. Expression of these genes either 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 would 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 would 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 would contain the antibiotic resistance selectable marker gene, neomycin phosphotransferase type II (**nptII**). This gene, encoding for the enzyme neomycin phosphotransferase, was derived from *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 purpose of the trial is to conduct proof of concept research involving experiments with the GM banana lines to assess growth, fruit and yield characteristics and analyse the nutrient content of fruit and vegetative parts. A number of promoters are also being tested in order to identify those that achieve best expression of the introduced genes in the fruit. GM bananas produced during the trial will not be used for human food or animal feed.

QUT proposed a number of controls to restrict the dissemination or persistence of the GM banana lines and their genetic materials into the environment. These controls have been considered during the evaluation of the application.

Risk assessment

The risk assessment took into account information contained in the application, relevant previous approvals, current scientific knowledge, and advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP. The feedback received from prescribed experts, agencies and authorities (summarised in Appendix B of the RARMP) and the public (summarised in Appendix C of the RARMP) led to the identification of an additional hazard that was examined in the finalised RARMP (Event 8, Section 2.6, Chapter 2) but was not considered to give rise to an identified risk to human health and safety and/or the environment that required further analysis.

A reference document on the parent organism, *The Biology of Musa L. (banana)*, was produced to inform the risk assessment process for licence applications involving GM banana plants. The document is available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

The risk assessment begins with a hazard identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

Nine events, including one identified in the consultation process, were considered whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

The characterisation of the nine events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principle reasons for this include:

- ♦ limits on the size and duration of the release proposed by QUT;
- ♦ suitability of controls proposed by QUT to restrict the dissemination or persistence of the GM banana plants and their genetic material;
- ♦ limited capacity of the GM banana lines to spread and persist outside the areas proposed for release;
- ♦ limited ability and opportunity for the GM banana lines to transfer the introduced genes to commercial banana crops or other sexually related species;
- ♦ none of the GM plant materials or products will be used in human food or animal feed;
- ♦ widespread presence of the same or similar proteins encoded by, and end products produced as a result of the activity of, the introduced genes in the environment and lack of known toxicity or evidence of harm from them.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM banana lines into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment.

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. As none of the nine events characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk is considered to be **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, a range of measures have been imposed to limit the release to the size, location and duration requested by the applicant, as these were an important part of establishing the context for assessing the risks.

Licence conditions to manage this limited and controlled release

The Regulator has imposed a number of licence conditions including requirements to:

- ♦ conduct the release on a total area of up to 1.4 ha at one site in the local government area of Cassowary Coast (Queensland) between May 2008 and May 2012.
- ♦ remove and destroy all male/hermaphrodite flowers on the inflorescences unless they are required for experimental analysis;
- ♦ cover any male/hermaphrodite flowers left on the inflorescences;
- ♦ cover fruit bunches;
- ♦ remove and destroy all fruit not required for experimental analysis;
- ♦ destroy any plant waste containing meristematic tissue;
- ♦ clean all equipment used in cultivation practices;
- ♦ not permit any materials from the release to be used in human food or animal feed; and
- ♦ at the end of the trial, destroy all plant materials not required for further analysis.

The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs, July 2007; Policy on transport and supply of GMOs, July 2005*). Licence conditions based on these guidelines and policies have also been proposed to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including Food Standard Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)⁷.

FSANZ is responsible for human food safety assessment, including GM food. As the trial involves proof of concept research, the applicant does not intend any material from the GM banana lines proposed for release to be used in human food. Accordingly, the applicant has not applied to FSANZ to evaluate any of the GM banana lines. FSANZ approval would need to be obtained before they could be used in human food in Australia.

Identification of issues to be addressed for future releases

Additional information has been identified that may be required to assess an application for a large scale or commercial release of any of these GM banana lines that may be selected for further development, or to justify a reduction in containment conditions. This would include:

- ♦ characterisation of the introduced genetic material in the plants, including copy number and genotypic stability;

⁷ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/pubform/riskassessments.htm>>.

- ♦ additional data on the potential toxicity of plant materials from the GM banana lines;
- ♦ additional data on the allergenicity of proteins encoded by the introduced genes for enhanced nutrition; and
- ♦ characteristics indicative of weediness including measurement of altered reproductive capacity; tolerance to environmental stresses; and disease susceptibility.

Suitability of the applicant

The Regulator determined, at the commencement of the assessment process for this application, that QUT is suitable to hold a DIR licence under the requirements of section 58 of the Act. The Regulator is satisfied that QUT remains suitable as no relevant convictions have been recorded, no licences or permits have been cancelled or suspended under OGTR legislation relating to the health and safety of people or the environment, and the organisation has confirmed its ability to comply with the licence conditions.

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of up to 1,290 GM banana lines on a maximum total area of 1.4 ha over four years in the Queensland local government area of Cassowary Coast poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

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Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which risks that may be posed to the health and safety of people and the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation⁸, details of the intended dealings, the genetically modified organism(s) (GMO(s)) and parent organism(s), previous approvals and releases of the same or similar GMO(s) in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.

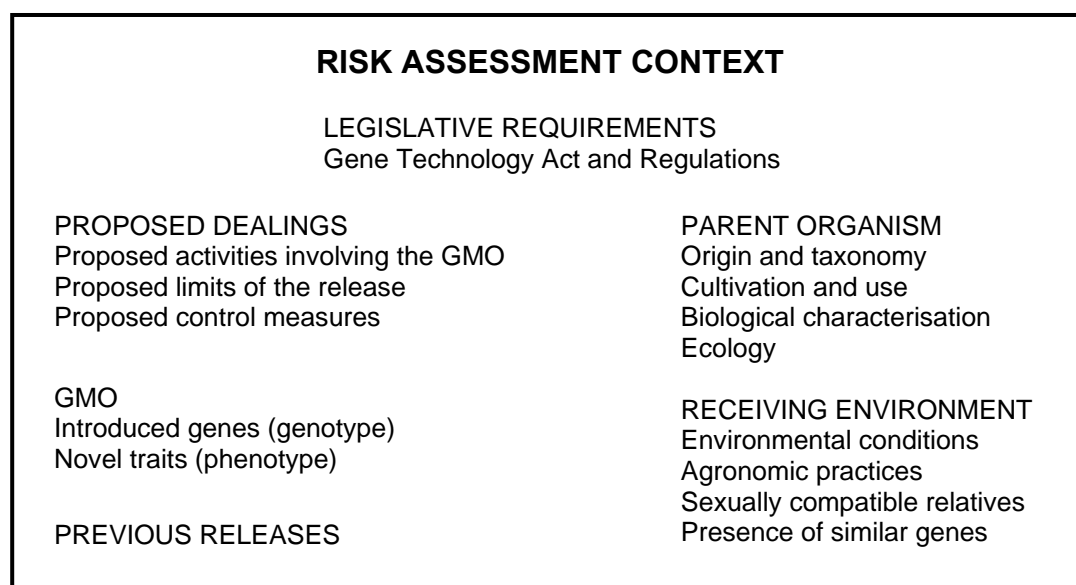


Figure 1 Components of the context considered during the preparation of the risk assessment

2. For this application, establishing the risk assessment context includes consideration of:
- ♦ the proposed dealings (Section 3.1)
 - ♦ the limits proposed by the applicant (Section 3.2)
 - ♦ the controls proposed by the applicant (Section 3.3)
 - ♦ characteristics of the parent organism (Section 4)
 - ♦ the nature and effect of the genetic modification (Section 5)
 - ♦ the environmental conditions in the location where the release would occur (Sections 6.1 and 6.2)
 - ♦ relevant agricultural practices (Section 6.3)
 - ♦ the presence of related plants in the environment (Section 6.4)
 - ♦ the presence of the introduced or similar genes in the environment (Section 6.5)
 - ♦ any previous releases of these or other GMOs relevant to this application (Section 7)

⁸ The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <<http://www.ogtr.gov.au/ir/process.htm>> and in the *Risk Analysis Framework* (OGTR 2007) <<http://www.ogtr.gov.au/pubform/riskassessments.htm>>.

Section 2 The legislative requirements

3. Sections 50, 50A and 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Regulator must take into account, and with whom she must consult, in preparing the RARMPs that form the basis of her decisions on licence applications. In addition, the Gene Technology Regulations 2001 (the Regulations) outline matters the Regulator must consider when preparing a RARMP.

4. In accordance with section 50A of the Act, the Regulator has considered information provided in the application and is satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits on the size, location and duration of the release and controls have been proposed by the applicant to restrict the dissemination or persistence of the GMO and its genetic material in the environment. Those limits and controls are such that the Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application qualifies as a limited and controlled release and the Regulator has prepared a RARMP for this application.

5. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Gene Technology Regulations (the Regulations), the Minister for the Environment, local council(s) where the release is proposed to take place, and the public. The advice from the prescribed experts, agencies and authorities and where it was taken into account, is summarised in Appendix B. One submission was received from a member of the public, and its consideration is summarised in Appendix C.

6. As a result of advice received from GTTAC, an additional hazard relating to unintended presence in the environment of *Agrobacterium tumefaciens* containing the introduced genes was included in the risk assessment (Event 8, Section 2.6, Chapter 2) but was not considered to give rise to an identified risk to human health and safety and/or the environment that required further analysis.

7. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a 'significant risk' to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d).

Section 3 The proposed dealings

8. Queensland University of Technology (QUT) proposes to release up to 1,290 banana lines⁹ that have been genetically modified for increased nutrient levels (pro-vitamin A carotenoid, vitamin E or iron), or to assess promoter specificity. The release would be conducted under limited and controlled conditions.

3.1 The proposed activities

9. The applicant has stated that the principal purpose of the proposed release is to conduct proof of concept research involving experiments with the GM banana lines to assess growth, fruit and yield characteristics and analyse the nutrient content of fruit and vegetative parts. A number of promoters are also being tested in order to identify those that achieve best expression of the genes in the fruit.

⁹ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

10. GM bananas produced during the trial will not be used for human food or animal feed

3.2 The proposed limits of the dealings (size, location and duration)

11. The release is proposed to take place at one site within the State Government Research Station (Queensland Department of Primary Industries & Fisheries – QDPI&F) at South Johnstone in the local government area of Cassowary Coast in Queensland. Within the site there is a field location and a shadehouse (to be used for hardening off tissue-cultured plants for approximately 4 months before they are transferred to the field location) that together will occupy a maximum total area of 1.4 ha. The release is proposed to occur between May 2008 and May 2012.

3.3 Proposed controls to restrict the dissemination or persistence of the GMOs and their genetic material in the environment

9. The field location does not directly abut any public roads. The shadehouse is a permanent, glass-panelled, lockable structure located some 200 m from the field location. The closest population centre (South Johnstone, population 300) is 1 km from the site; the next largest centre is Innisfail (population 15,000), 12 km from the site. Only trained and authorised staff will be permitted access to the proposed locations.

10. The applicant has proposed a number of controls to restrict the dissemination or persistence of the GM banana lines in the environment including:

- ♦ locating the proposed field location 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;
- ♦ applying bunch covers 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 these to prevent access by pollinators;
- ♦ complying with Queensland State Government legislation for banana disease control that would also aid in containment of GM plants;
- ♦ destroying (GM and non-GM) plant materials from the field trial by:
 - herbicide treatment and surface decomposition (material containing vegetative meristems),
 - surface decomposition (non-propagative vegetative parts),
 - decomposition in covered containers (fruits and male flowers not required for experimental analysis).
- ♦ analysing GM plant materials from the trial in an OGTR-certified PC2 facility and then destroying the materials by autoclaving;
- ♦ post harvest monitoring of the field location for 12 months and destroying any volunteer GM banana suckers; and
- ♦ transporting GM plant materials to and from the proposed site and within the site in accordance with OGTR transportation guidelines.

11. These controls, and the limits outlined in Section 3.2, have been taken into account in establishing the risk assessment context (this chapter), and their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.

Section 4 The parent organism

12. The parent organism is 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. Further detailed information about the parent organism is contained in a reference document, *The Biology of Musa L.(banana)* (the *Musa* Biology document), that was produced to inform the risk assessment process for licence applications involving GM banana plants (OGTR 2008). The document is available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

Section 5 The GMOs, nature and effect of the genetic modification

5.1 Introduction to the GMOs

13. The GM banana lines would contain one or more of 11 genes encoding proteins involved in the biosynthesis of pro-vitamin A carotenoids or vitamin E (tocochromanol), or in the uptake/storage of iron. In addition, all of the banana lines would contain a selectable marker gene and some of the lines would contain a reporter gene (Table 1).

Table 1 The genes used to genetically modify banana

Gene	Accession No (GenBank)	Protein produced	Protein involved in	Source	Intended purpose
<i>APsy2a</i>	Not Available	phytoene synthase	Carotenoid synthesis	Fe'i Banana ¹	Increase β -carotene
<i>PsyB73</i>	U32636	phytoene synthase	Carotenoid synthesis	Maize	Increase β -carotene
<i>CrtI</i>	D90087	carotene desaturase	Carotenoid synthesis	<i>Erwinia uredovora</i>	Increase β -carotene
<i>vte1</i>	NM_119430	tocopherol cyclase	Tocochromanol synthesis	Arabidopsis	Increase vit E
<i>vte2.1</i>	Not Available	homogentisic acid phytyltransferase	Tocochromanol synthesis	Maize	Increase vit E
<i>vte3</i>	NM_116206	2 methyl-6 phytyl-1, 4-benzoquinone methyltransferase	Tocochromanol synthesis	Arabidopsis	Increase vit E
<i>vte4</i>	NM_10571	γ – tocopherol methyltransferase	Tocochromanol synthesis	Arabidopsis	Increase vit E
<i>HGGT</i>	NM_001064737	homogentisic acid geranylgeranyl transferase	Tocochromanol synthesis	Rice	Increase vit E
<i>Ferritin</i>	EF524082	chloroplast ferritin	Storage of iron	Wild soybean	Increase iron
<i>IRT1</i>	NM_118089	iron-regulated transporter 1	Iron uptake	Arabidopsis	Increase iron
<i>FRO2</i>	NM_100040	ferric-chelate reductase	Iron uptake	Arabidopsis	Increase iron
<i>nptII</i>	AAF65403	neomycin phosphotransferase	Kanamycin resistance	<i>Escherichia coli</i>	Selectable marker

Gene	Accession No (GenBank)	Protein produced	Protein involved in	Source	Intended purpose
<i>uidA</i>	AF23416	β -glucuronidase (GUS)		<i>Escherichia coli</i>	Reporter

¹ The Fe'i bananas are widely distributed throughout the Pacific islands, from the Moluccas to Hawaii and Tahiti and their domestication is thought to have occurred independently of other bananas and plantains (Sharrock 2000).

14. Up to 1,290 GM banana lines are proposed for release, with each line generated using 1 of 43 different transformations involving 41 constructs (see Table 2). Each transformation would be used to generate up to 30 GM banana lines. Up to 30 non-GM plants would be used as controls.

Table 2. Gene constructs used to generate the GM banana lines proposed for release

Identity of construct(s) associated with each experiment	Promoter	Signal Peptide	Gene of interest	Terminator	Max lines per construct	Total no. of lines	
pBin-Ubi-GUSCat	Ubi	-	<i>uidA</i> -Cat	Nos	30	420	
pBin-Nos-GUSCat	Nos	-	<i>uidA</i> -Cat	Nos	30		
pBin-35S-GUSCat	35S	-	<i>uidA</i> -Cat	Nos	30		
pBin-BT1-GUSCat	BT1	-	<i>uidA</i> -Cat	Nos	30		
pBin-BT4Act-GUSCat	BT4 -Act	-	<i>uidA</i> -Cat	Nos	30		
pBin-BT5Act-GUSCat	BT5 -Act	-	<i>uidA</i> -Cat	Nos	30		
pBin-BT6Act-GUSCat	BT6 -Act	-	<i>uidA</i> -Cat	Nos	30		
pBin-TaBV-GUSCat	TaBV	-	<i>uidA</i> -Cat	Nos	30		
pBin-Exp-GUSCat	Exp	-	<i>uidA</i> -Cat	Nos	30		
pBin-ExpAct-GUSCat	Exp-Act	-	<i>uidA</i> -Cat	Nos	30		
pBin-APsy2a-GUSCat	APSY2a	-	<i>uidA</i> -Cat	Nos	30		
pBin-ACO-GUSCat	ACO	-	<i>uidA</i> -Cat	Nos	30		
pBin-ACS-GUSCat	ACS	-	<i>uidA</i> -Cat	Nos	30		
pBin-SPS-GUSCat	SPS	-	<i>uidA</i> -Cat	Nos	30		
pCam-Ubi-APsy2a	Ubi	-	<i>APsy2a</i>	Nos	30	390	
pCam-Exp-APsy2a	Exp	-	<i>APsy2a</i>	Nos	30		
pCam-ACO-APsy2a	ACO	-	<i>APsy2a</i>	Nos	30		
pCam-Ubi-PsyB73	Ubi	-	<i>PsyB73</i>	Nos	30		
pCam-Exp-PsyB73	Exp	-	<i>PsyB73</i>	Nos	30		
pCam-BT4Act-PsyB73	BT4 -Act	-	<i>PsyB73</i>	Nos	30		
pCam-ACO-PsyB73	ACO	-	<i>PsyB73</i>	Nos	30		
pCam-ACS-PsyB73	ACS	-	<i>PsyB73</i>	Nos	30		
pCam-Ubi-APsy2a + pBin-Ubi-Crt1	Ubi	CMSSP	<i>APsy2a</i> <i>Crt1</i>	Nos	30		
pCam-Exp-APsy2a + pBin-Exp-Crt1	Exp	CMSSP	<i>APsy2a</i> <i>Crt1</i>	Nos	30		
pCam-Ubi-PsyB73 + pBin-Ubi-Crt1	Ubi	CMSSP	<i>PsyB73</i> <i>Crt1</i>	Nos	30		
pCam-Exp-PsyB73 + pBin-Exp-Crt1	Exp	CMSSP	<i>PsyB73</i> <i>Crt1</i>	Nos	30		
pCam-BT4Act-PsyB73 + pBin-BT4Act-Crt1	BT4 -Act BT4 -Act	CMSSP	<i>PsyB73</i> <i>Crt1</i>	Nos	30		
pCam-Ubi-vte1	Ubi	-	<i>vte1</i>	Nos	30		360
pCam-Exp-vte1	Exp	-	<i>vte1</i>	Nos	30		
pCam-Ubi-HGGT	Ubi	-	<i>HGGT</i>	Nos	30		
pCam-Exp-HGGT	Exp	-	<i>HGGT</i>	Nos	30		
pCam-Ubi-vte2.1	Ubi	-	<i>vte2.1</i>	Nos	30		
pCam-Exp-vte2.1	Exp	-	<i>vte2.1</i>	Nos	30		
pCam-Ubi-vte3	Ubi	-	<i>vte3</i>	Nos	30		
pCam-Exp-vte3	Exp	-	<i>vte3</i>	Nos	30		
pCam-Ubi-vte4	Ubi	-	<i>vte4</i>	Nos	30		
pCam-Exp-vte4	Exp	-	<i>vte4</i>	Nos	30		
pCam-Exp-vte3 + pCam-Exp-vte2.1	Exp	-	<i>vte3</i> <i>vte2.1</i>	Nos	30		

Identity of construct(s) associated with each experiment	Promoter	Signal Peptide	Gene of interest	Terminator	Max lines per construct	Total no. of lines
pCam-Exp-HGGT + pCam-Exp-vte3	Exp Exp	-	<i>HGGT</i> <i>vte3</i>	Nos	30	
pBin-Ubi-Ferritin	Ubi	-	<i>Ferritin</i>	Nos	30	120
pCam-Exp-Ferritin	Exp	-	<i>Ferritin</i>	Nos	30	
pBin-Ubi-Ferritin + pCam-35S-IRT1 + pCam-35S-FRO2	Ubi 35S 35S	- - -	<i>Ferritin</i> <i>IRT1</i> <i>FRO2</i>	Nos	30	
pCam-Exp-Ferritin + pCam-Ubi-IRT1 + pCam-Ubi-FRO2	Exp Ubi Ubi	- - -	<i>Ferritin</i> <i>IRT1</i> <i>FRO2</i>	Nos	30	

5.2 The introduced genes, encoded proteins and end products

5.2.1 Genes expected to enhance pro-vitamin A carotenoid synthesis, and their encoded proteins

15. Up to 390 of the GM banana lines would contain one or two of three different genes that encode proteins involved in pro-vitamin A carotenoid synthesis (see Figure 2). None of these introduced genes has previously been heterologously expressed in banana. The expression of these genes, either singly or in a combination, is expected to increase the levels of pro-vitamin A carotenoids in banana tissues.

16. The *APsy2a* gene derived from the banana cultivar 'Asupina' encodes the protein phytoene synthase which has a primary role in the conversion of geranylgeranyl diphosphate to phytoene. The *APsy2a* gene has not been previously characterised or used for genetic modification of plants.

17. The *PsyB73* gene derived from maize (*Zea mays*) also codes for phytoene synthase. Heterologous expression of *PsyB73* has been reported in GM rice where it resulted in a marked increase in carotenoid biosynthesis compared with *PSy* from daffodil (Paine et al. 2005).

18. The *CrtI* gene from the common soil bacterium *Erwinia uredovora* encodes the protein carotene desaturase that converts phytoene into lycopene and substitutes for two plant desaturases (phytoene desaturase and ξ -carotene desaturase) as well as for carotene isomerase (Figure 2). Heterologous expression of *CrtI* has been reported in GM rice (Paine et al. 2005).

19. Non-GM Cavendish banana plants contain the biosynthetic pathway for carotenoids (Figure 2). Therefore, a homologue of the *APsy2a* and *PsyB73* genes occurs in non-GM Cavendish banana and a phytoene synthase protein would be produced. While non-GM plants would not produce the carotene desaturase protein encoded by *CrtI*, they would produce the three enzymes normally associated with carotenoid synthesis in plants (Figure 2).

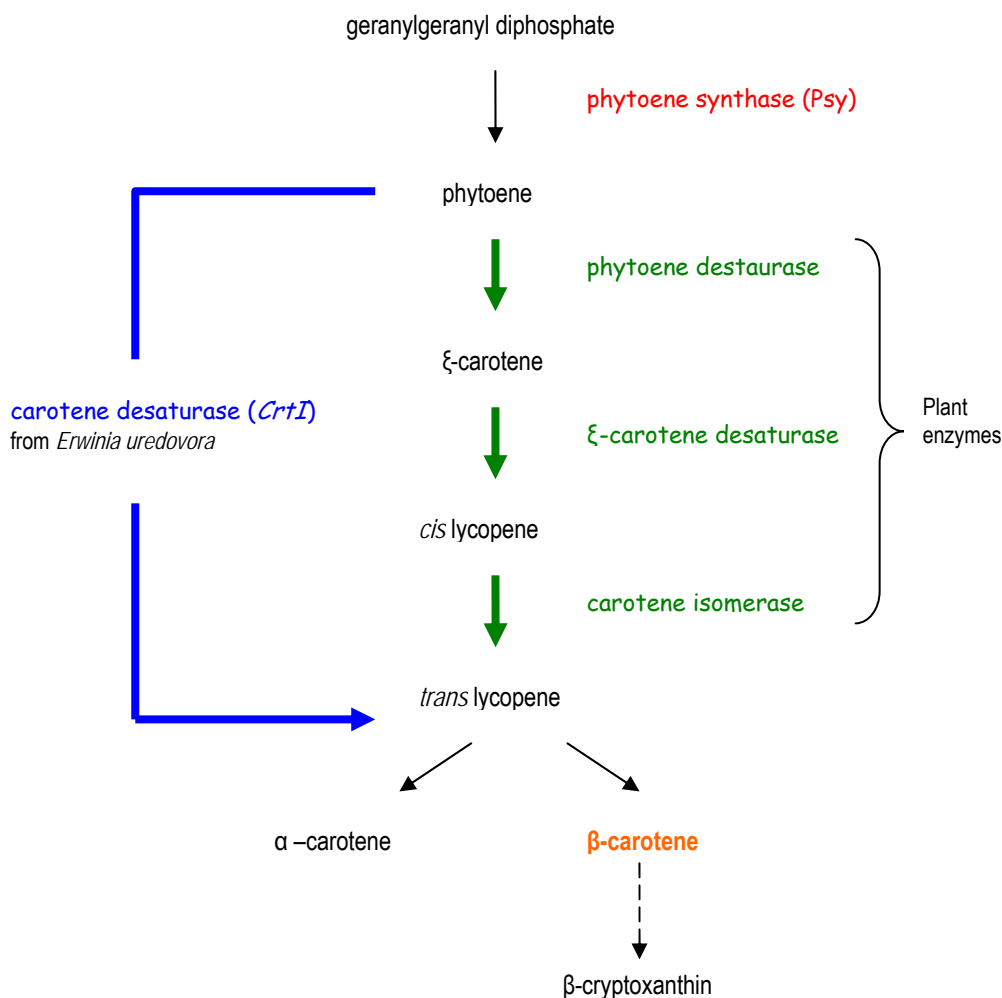


Figure 2. Simplified biosynthetic pathway for the pro-vitamin A carotenoids [adapted from DellaPenna & Pogson (2006) and Al-Babili et al. (2006)]

5.2.2 Genes expected to enhanced vitamin E synthesis, and their encoded proteins

20. Up to 360 of the GM banana lines would contain one or two of five different genes that encode proteins involved in vitamin E synthesis (see Figure 3). None of the introduced genes has previously been heterologously expressed in banana. A detailed discussion of the enzymes produced by the proteins can be found in DellaPenna & Pogson (2006). Expression of these genes either singly or in a combination is expected to lead to an increased accumulation of vitamin E in banana tissues.

21. 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; $\gamma\gamma$ – tocopherol methyltransferase, respectively) that has a role in tocopherol biosynthesis.

22. The *vte2.1* gene is from maize (*Zea mays*) and its protein product, homogentisic acid phytyltransferase is also important in tocopherol biosynthesis.

23. The *HGGT* gene from rice (*Oryza sativa*) encodes a protein (homogentisic acid geranylgeranyl transferase) involved in tocotrienol biosynthesis.

24. Non-GM Cavendish banana plants contain the biosynthetic pathway for vitamin E (Figure 3). While tocopherols have been found in many different plant species, tocotrienols are much less widespread and *Musa* spp. may not contain significant amounts of tocotrienols (Horvath et al. 2006). Therefore, homologues of the *vte1*, *vte2.1*, *vte3* and *vte4* genes occur in non-GM Cavendish banana and the corresponding proteins would be produced. HGGT may not be produced in non-GM Cavendish banana.

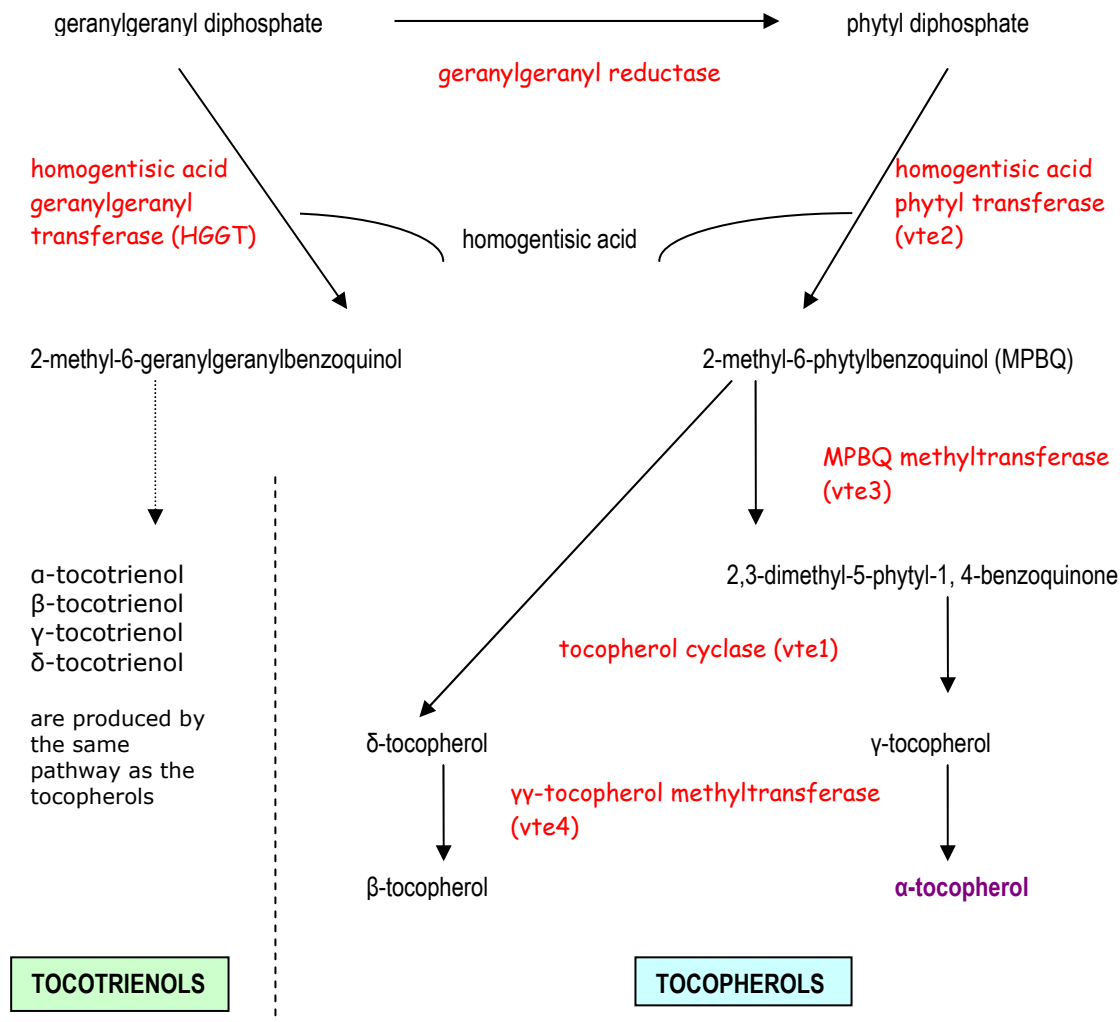


Figure 3. Simplified biosynthetic pathway for the tocochromanols [adapted from DellaPenna & Pogson (2006) and Cahoon et al. (2003)]

5.2.3 Genes expected to enhance iron levels, and their encoded proteins

25. Up to 120 of the GM banana lines would contain one or more of three different genes encoding proteins that are involved in iron accumulation. None of the introduced genes has previously been heterologously expressed in banana. The expression of these genes, either singly or in a combination, is expected to lead to enhanced iron levels in banana tissues.

26. The **Ferritin** gene from wild soybean (*Glycine soja*) encodes an iron-binding protein (chloroplast ferritin) that has a role in the storage of iron. Ferritin is the iron storage protein found in animals, plants and bacteria. It is particularly abundant in legume seeds and is

consumed as part of the normal human diet (Theil & Briat 2004). Use of genetically modified plants overexpressing ferritin has been proposed as a safe mechanism for supplementing iron in human diets, particularly through cereal seeds (Theil et al. 1997; Lucca et al. 2002). Increases in iron content due to heterologous expression of soybean ferritin have been reported in maize seeds (Drakakaki et al. 2006), tobacco seedlings (Van Wuytswinkel et al. 1998) and tobacco leaves (Vansuyt et al. 2000).

27. Ferrous iron transporter proteins (such as **IRT1** from *Arabidopsis*) actively transport Fe(II) across the membrane of root epidermal cells and into the plant vascular system and are critical for iron uptake by plants (Vert et al. 2002). Iron transport proteins such as the IRT1 protein have a broad substrate range that also enables loading of other metals present in the soil, some of which are potentially toxic to humans (Korshunova et al. 1999; Vansuyt et al. 2000; Connolly et al. 2002).

28. The enzyme ferric-chelate reductase (e.g. **FRO2** from *Arabidopsis*) is required for most plants to acquire soluble iron (Robinson et al. 1999). It is found in root epidermis and acts via the reduction of Fe(III) to Fe(II), the latter being the substrate for ferrous iron transporter (Connolly et al. 2003).

29. On the basis of the widespread occurrence in plants of iron transport and storage genes, non-GM Cavendish banana are likely to contain homologues of all three of the introduced genes for enhancement of iron.

5.2.4 Toxicity/allergenicity of the proteins encoded by the introduced genes for nutrient enhancement

30. Homologues of all of the encoded proteins occur naturally in a range of organisms, including plants widely consumed by people and animals (see discussion in Section 6.5). On this basis, people and other organisms have a long history of exposure to the proteins involved in pro-vitamin A carotenoid and vitamin E synthesis, and iron uptake.

31. No toxicity/allergenicity tests have been performed on any of the purified encoded proteins as the proposed trial is still at proof of concept stage. Such tests would have to be conducted if approval was sought for the GMOs to be considered for human consumption in Australia (see discussion in Section 7.1.2).

32. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing (Goodman et al. 2008). The predicted amino acid sequences of the proteins encoded by each of the introduced genes for nutrient enhancement were compared to a database of known allergens. The results of this analysis did not indicate that any of the encoded proteins shared any significant sequence homology with any known allergens.

33. A comprehensive search of the scientific literature also yielded no information to suggest that any of the encoded proteins are toxic or allergenic to people, or toxic to other organisms.

5.2.5 The reporter gene (*uidA*) and the encoded protein

34. Up to 420 of the GM banana lines would contain the marker/reporter gene *uidA*, derived from the common gut bacterium *Escherichia coli*. It encodes an enzyme, β -glucuronidase (GUS), which enables visual identification of plant tissues in which this gene is expressed. GM banana plants (in both the laboratory and in the field) 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.

35. The *uidA* gene is the most widely used reporter gene in GM plants (Miki & McHugh 2004) as it allows GM tissues to be identified using a simple visual assay. The toxicity/allergenicity of the GUS protein has been comprehensively considered in previous RARMPs [see the RARMP for DIR 056/2004 (available at <<http://www.ogtr.gov.au/ir/dir056.htm>> or by contacting the OGTR) for the most detailed discussion] and it has been concluded that GUS has neither toxic nor allergenic properties. The United States Environmental Protection Agency (EPA) does not consider the GUS protein to be toxic and has approved its exemption from the requirements to establish tolerance levels (EPA 2001). Hence, the *uidA* gene will not be considered further in this assessment.

5.2.6 The selectable marker gene (*nptII*) and the encoded protein

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

37. The *nptII* gene is used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004). As discussed in previous DIR RARMPs, most recently in DIR 070/2006 (available at <<http://www.ogtr.gov.au/ir/dir070.htm>> or by contacting the OGTR), regulatory agencies in Australia and in other countries have assessed the use of the *nptII* gene in GMOs as not posing a risk to human or animal health or to the environment. The most recent international evaluation of *nptII* in terms of human safety was by the European Food Safety Authority (EFSA 2007). Hence the *nptII* gene will not be considered further in this assessment.

5.2.7 The end products associated with the introduced genes for nutrient enhancement

38. There are at least 49 nutrients that are essential for humans to meet their metabolic needs (Welch & Graham 2004). Micronutrients¹⁰ that include Vitamin A, vitamin E and iron make up three of these essential nutrients.

Pro-vitamin A carotenoids

39. Vitamin A is needed by humans for the normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction (FAO/WHO 2001).

40. Dietary needs of vitamin A are normally supplied by the consumption of pre-formed vitamin A (from foods derived from animals e.g. milk, glandular meats, liver and egg yolks) and pro-vitamin A carotenoids (from foods derived from fruits and vegetables e.g. green leafy vegetables and yellow/orange fruits such as pumpkin, carrot, mango and papaya). The three major pro-vitamin A carotenoids are β -carotene, α -carotene and β -cryptoxanthin but, in the human diet, β -carotene is the most important vitamin A precursor (Hess et al. 2005). Some examples of the levels of β -carotene contained in commonly consumed plant (or plant-derived) foods are given in Table 3. The recommended daily intake (RDI) of vitamin A as Retinol Equivalents (RE) for adult (19+ years) men is 900 $\mu\text{g}/\text{day}$ and for adult women is 700 $\mu\text{g}/\text{day}$ (NH&MRC 2006).

¹⁰ The term micronutrient is used in the human nutrition context to encompass both vitamins and nutrient elements.

Table 3 Levels of β -carotene, retinol equivalents (RE), vitamin E (α -tocopherol equivalents), and iron in commonly consumed plant-based foods (value per 100g)*

Food Source	β -carotene (μ g)	RE (μ g) ¹	Vitamin E: α -tocopherol equivalents (mg) ²	Iron (mg)
Banana - Cavendish (peeled fruit)	64	11	0.1	0.5
Blackberry - fresh	150	53	1.4	0.4
Bok Choy (stir-fried)	692	116	0.2	2.1
Breakfast cereal (mixed grain flakes), iron-fortified	3	0	0.4	8.1
Broccoli (boiled)	273	46	0.2	0.8
Canola Oil	0	0	23.6	0
Capsicum (red, fresh)	282	215	4.0	0.3
Carrot (fresh, mature)	5,996	1,316	0.4	0.3
Cashew	6	1	0.7	5.0
Kidney bean (canned)	0	0	0.1	2.1
Lettuce - Iceberg	120	23	0	0.6
Mango (fresh)	2,195	366	1.3	0.3
Olive Oil	13	2	14.2	0
Pasta (fresh, white flour)	0	0	0.2	0.7
Soybean (canned)	0	0	1.7	1.8
Spinach – English (boiled)	2,201	376	1.2	3.5
Walnut	21	4	2.6	2.5

* data taken from NUTTAB 2006 (FSANZ 2006)

1. Vitamin A intakes or requirements are expressed as Retinol Equivalents (RE) (NH&MRC 2006)

2. The standard unit for dietary intake of vitamin E is expressed as α -tocopherol equivalents (FSANZ 2006)

41. The Fe'i banana cultivar 'Asupina' that is consumed in Micronesia, and from which the *APsy2a* gene used in the proposed trial for DIR076/2007 has been sourced, contains 1,412 μ g β -carotene/100 g (259 RE) and meets the estimated vitamin A requirements for an adult woman within normal consumption patterns (Englberger et al. 2006b). Several other Fe'i cultivars also contain very high levels of β -carotene with 'Utin Iap', and 'Utimwas' containing approximately 8,000 μ g β -carotene/100 g and 'Karat' containing approximately 2,000 μ g β -carotene/100 g (Englberger et al. 2006a).

42. The use of the *CrtI* gene from *Erwinia* in conjunction with the *Psy* gene from maize (both genes are proposed to be introduced into the GM banana lines) in the genetic modification of rice resulted in the accumulation of up to 3,100 μ g β -carotene/100 g in the grain of one line (Paine et al. 2005). Based on this, it was estimated that 50% of a child's RDI could be delivered by consumption of 72 g (uncooked) of this rice (a normal child's portion would be 60 g uncooked rice).

43. The biosynthetic pathway for the major provitamin A carotenoids is given in Figure 2. Both pro-vitamin A carotenoids and tocochromanols (the group that collectively makes up vitamin E – see below) are synthesized in whole or in part from the isoprenoid biosynthetic pathway located in the plant chloroplasts (DellaPenna & Pogson 2006). Geranylgeranyl diphosphate is a key intermediate in the synthesis of both carotenoids and tocochromanols

Vitamin E

44. The major role of vitamin E in the human diet is to protect polyunsaturated fatty acids and other components of cell membranes and low-density lipoprotein from oxidation by free radicals.

45. Vitamin E comprises a family of eight, naturally occurring homologues (four tocopherols and four tocotrienols, collectively known as tocochromanols and designated as α -,

β -, γ -, and δ -) that are synthesised only by plants (Figure 3) (Dörmann 2007). From a human nutritional perspective, the most important form of vitamin E is α -tocopherol (FAO/WHO 2001). However, more than 95% of all studies on vitamin E deal specifically with α -tocopherol and the other tocopherols remain poorly understood; there is some evidence that, in fact, each member of the vitamin E family is functionally unique (Sen et al. 2007).

46. Vegetable oils, nuts, and green leafy vegetables are the main dietary sources of vitamin E. Some plant dietary sources of vitamin E are given in Table 3. The Fe'i banana cultivar 'Karat' consumed in Micronesia contains levels of α -tocopherol over ten times higher (1.55 mg α -tocopherol /100 g) than the Cavendish banana (Englberger et al. 2006a).

47. The recommended average intake (AI) for adult (19 years +) men is 10 mg/day (as α -tocopherol equivalents) and for adult women is 7 mg/day, while the upper level of intake (UL)¹¹ is 300 mg/day (NH&MRC 2006).

Iron

48. Iron has several vital functions in the body, serving as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues (FAO/WHO 2001).

49. HEME iron is found only in meat, fish and poultry and is absorbed much more easily than NON-HEME iron, which is found primarily in fruits, vegetables, dried beans, nuts and grain products (NH&MRC 2006). Some plant dietary sources of iron are given in Table 3. By comparison with these plant sources, the level of iron in lean red meat such as lamb or beef, which is regarded as the best dietary source of iron, varies from 2.2 – 3.3 mg/100g (FSANZ 2006).

50. The RDI of iron from a mixed diet in adult (19+ years) men and women over 50 is 8 mg/day and is 18 mg/day in women aged 19 – 50 years. The UL for adult men and women is set at 45 mg/day (NH&MRC 2006).

5.2.8 Toxicity of the end products associated with the introduced genes for nutrient enhancement

51. Pro-vitamin A carotenoids, vitamin E and iron are essential micronutrients required by people and other animals. The levels of these compounds generated in the GM banana plants are not expected to exceed the levels found naturally in edible plants.

52. The mammalian toxicity of β -carotene, vitamin E and iron has been independently assessed by regulatory agencies or health advisory bodies in Australia and overseas for the purposes of determining safe levels of intake via food or supplements. Assessments of these compounds has been conducted by the National Health & Medical Research Council (NH&MRC 2006), the UK's Expert Group on Vitamins and Minerals (Expert Group on Vitamins and Minerals 2003), the European Commission (EC 2000; EC 2003) and the Joint Food and Agricultural Organisation of the United Nations/World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA 1974; JECFA 1975; JECFA 1983; JECFA 1987; JECFA 1993). These assessments have examined both animal and human data.

¹¹ The UL is the highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases.

53. A comprehensive search of the scientific literature found no information or data to suggest that pro-vitamin A carotenoids (such as β -carotene) or vitamin E have any toxicity potential in other organisms. While soluble iron salts present in soils can be toxic to plants, there is no indication that the forms and levels of iron found naturally in plant or animal material are toxic.

54. Pro-vitamin A carotenoids (such as β -carotene), vitamin E and iron occur naturally in the environment by virtue of their widespread presence in plant and/or animal material. In addition, carotene forms of vitamin A, vitamin E and iron are all permitted food additives in Australia (FSANZ 2007). On this basis, people and other organisms have a long history of exposure to these end products.

5.3 The regulatory sequences

5.3.1 Regulatory sequences for the nutrient enhancement genes

55. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Information on the 13 promoters used in the proposed release is given in Table 4. Part of the purpose of the proposed trial is to determine whether any of these promoters provide good expression of the introduced genes in the banana fruit.

56. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. The mRNA termination region for the introduced genes in all of the constructs is derived from the nopaline synthase gene (*Nos*) of *Agrobacterium tumefaciens*. This sequence is widely used in constructs for plant genetic modification (Reiting et al. 2007).

Table 4 Details of the promoters

Promoter abbrev.	Source/Full name	GenBank Accession No.	Commentary	Reference
Ubi	Maize/polyubiquitin	S94466	Constitutive promoter widely used in plant genetic modification	(Christensen et al. 1992)
Nos	Agrobacterium/nopaline synthase	X01077	Constitutive promoter widely used in plant genetic modification	(Depicker et al. 1982)
35S	Cauliflower mosaic virus (CaMV)/35S	AF234316	Constitutive promoter widely used in plant genetic modification	(Odell et al. 1985)
BT-1	Banana bunchy top virus (BBTV)/DNA 1	NC_003479	Promoter associated with the DNA-1 gene that encodes a replication initiation protein	(Dugdale et al. 1998)
BT-4	BBTV/DNA 4	NC_003474	Promoter associated with the BBTV DNA-4 gene (function unknown)	(Dugdale et al. 1998)
BT-5	BBTV/DNA 5	NC_003477	Promoter associated with the BBTV DNA-5 gene (function unknown)	(Dugdale et al. 1998)
BT-6	BBTV/DNA 6	NC_003476	Promoter associated with the BBTV DNA-6 gene (function unknown)	(Dugdale et al. 1998)
TaBV	Taro bacilliform badna virus (TaBV)	NC_004450	Constitutive promoter that has driven strong expression in transgenic banana and tobacco plants	(Yang et al. 2003)
Exp	Banana/expansin	AF539640	Drives fruit-specific expression of proteins	(Trivedi & Nath 2004)
APSY2a	Banana/phytoene synthase	Not available	Predicted to drive fruit-specific expression of proteins	No published reference

Promoter abbrev.	Source/Full name	GenBank Accession No.	Commentary	Reference
ACO	Banana/1-aminocyclopropane 1-carboxylate oxidase	AF221107	Predicted to drive fruit-specific expression of proteins	No published reference
ACS	Banana/1-aminocyclopropane 1-carboxylate synthase	AF119096	Predicted to drive fruit-specific expression of proteins	No published reference
SPS	Banana/sucrose phosphate synthase	AY850374	Predicted to drive fruit-specific expression of proteins	No published reference

57. Two introns have been used in the constructs. The intron from the rice actin gene (Act) has been placed with several of the promoter elements (see Table 2) for the purpose of enhancing expression of the gene of interest (Dugdale et al. 2001). The *uidA* gene has been linked to an intron known as Cat, derived from *Ricinus communis*, that prevents expression in *Agrobacterium tumefaciens*. This ensures that any expression of glucuronidase in the GMOs is occurring in eukaryotic cells rather than in residual *Agrobacterium* (Ohta et al. 1990).

58. The targeting signal of the *Chrysanthemum morifolium* rubisco small-subunit protein (CMSSP) contains a natural intron from the Rubisco (*RbcS*) gene that is found in the chloroplasts of all plants and directs protein product into the chloroplast stroma (Wong et al. 1992). The heterologous protein is fused to the first 11 amino acids of the mature Rubisco protein in order to allow proper processing of the signal peptide (Wong et al. 1992). This signal peptide has been included in constructs containing the carotene desaturase gene (*CrtI*) with the aim of directing the formation of lycopene into the chloroplasts, where another precursor in the carotenoid synthesis pathway, phytoene, is also formed (Ye et al. 2000).

59. While some of the regulatory sequences are derived from plant pathogens (*Agrobacterium tumefaciens*, BBTV, CaMV, TaBV) the sequences are not pathogenic in themselves nor do they cause any disease symptoms in the GM plants. Those regulatory sequences derived from plants that are associated with allergenic or toxic responses in humans (*Musa* spp., *Chrysanthemum morifolium*, *Oryza sativa*, *Ricinus communis*, *Zea mays*) are not in themselves allergenic or toxic.

5.3.2 Regulatory sequences for the expression of the *nptII* gene

60. All of the constructs (see Table 2) have been based on either the pBIN-Plus vector (accession #DQ320121) or the pCAMBIA 2300 vector (accession # AF234315).

61. In the pBIN-Plus vector, the bacterial *nptII* gene has been modified by the addition of the *Nos* transcription initiation region and the *Nos* transcription termination region from *A. tumefaciens*. Although *A. tumefaciens* is a plant pathogen, the regulatory sequences comprise only a small part of the total genome, and are not capable of causing disease.

62. In the pCAMBIA 2300 vector, the bacterial *nptII* gene has been modified by the addition of the 35S RNA transcription initiation region and the 35S RNA transcription termination region from CaMV. The regulatory sequences comprise only a small part of the total genome, and are not capable of causing disease.

5.4 Method of genetic modification

63. All of the GM banana lines will be generated by *Agrobacterium tumefaciens*-mediated transformation. This method of transformation is used extensively to genetically modify plants (Valentine 2003) and has been discussed in previous RARMPs [most comprehensively

for DIR 060/2005 (available at <<http://www.ogtr.gov.au/ir/dir060.htm>> or by contacting the OGTR)].

64. Disarmed binary plasmid vectors containing each of the constructs (see Table 2) will be introduced into *Agrobacterium tumefaciens* strain AGL1 (ATCC® Number: BAA-101™) through electroporation. Strain AGL1 carries the hypervirulent, attenuated tumor-inducing plasmid pTiBo542 (Lazo et al. 1991) and shows high rates of T-DNA transfer when used with banana suspension cultures (Khanna et al. 2004).

65. For each transformation, embryogenic cell suspensions that have been derived from immature male flowers of *Musa acuminata* cv. Williams are co-cultivated with the *A. tumefaciens* using a centrifugation-assisted procedure (Khanna et al. 2004). Following transformation, the banana cells are cultured on medium containing kanamycin to select for those that have been transformed. Somatic embryos are developed on a regeneration medium and are then grown on into plantlets that are transferred to soil after hardening off in a shadehouse (see paragraph 80). PCR testing by the applicant, using primers specific to regions outside the T-DNA has demonstrated that the *A. tumefaciens* does not persist in GM banana plants for longer than approximately 3 weeks after removal from the *in vitro* environment. The applicant intends to confirm this by testing samples of plants prior to release.

66. The 1,290 GM banana lines would be generated from independent transformation events.

5.5 Characterisation of the GMOs

5.5.1 Stability and molecular characterisation

67. All genes, promoters and terminators have been sequenced to confirm the nucleotide sequence of each segment to be used for vector construction.

68. The GM banana lines are at a very early development stage and have not been tested for genotypic stability.

69. The exact location of the inserted genes within the banana genome of the lines is not known. *Agrobacterium tumefaciens* inserts introduced genes into plant genomic DNA via illegitimate recombination¹² and the banana genome is poorly characterised. The combination of these two factors suggests that meaningful data on the location of transgenes cannot be determined at this stage.

70. According to the applicant, *Agrobacterium tumefaciens*-mediated transformation of banana routinely results in the integration of between one and three copies of the introduced gene into the banana genome. Representative samples of the GM banana lines will be analysed by Southern hybridisation to confirm this. PCR will be performed on all GM plants to verify the presence of the introduced gene(s).

5.5.2 Characterisation of the phenotype of the GMOs

71. The purpose of the proposed trial is to conduct proof of concept research involving experiments with the GM banana lines to assess growth, fruit and yield characteristics and analyse the nutrient content of fruit and vegetative parts. To date, no GM banana plants of sufficient size have been obtained from any of the lines to provide any meaningful phenotypic data. Phenotypic data will be collected during the proposed trial.

¹² Illegitimate recombination is a term used to describe the recombination that occurs between DNA sequences that contain no or very little homology. It results in the random insertion of foreign DNA into the host genome.

72. While there may be changes in the levels of products produced as a result of the activity of the encoded proteins, no new products should be produced by expression of the introduced genes. There may, however, be unintended effects due to random insertion of the introduced genes (see Chapter 2, Event 6).

Section 6 The receiving environment

73. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the size, location(s) and duration of the dealings, any relevant biotic/abiotic properties of the geographic regions where the release would occur; intended agronomic practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2007).

6.1 Relevant abiotic factors

74. The cultivation of bananas occurs throughout the tropics and sub-tropics of Asia, America, Africa and Australia.

75. The abiotic factors relevant to the growth and distribution of commercial bananas in Australia are discussed in the *Musa* Biology document (OGTR 2008).

76. The release is proposed to take place in the north Queensland local government area of Cassowary Coast. This region is in the tropical rainforest climatic type (as defined by the Koeppen Classification system used by the Australian Bureau of Meteorology) in which 70% of Australia's banana production occurs (Biosecurity Australia 2007). The rainfall and temperature statistics for South Johnstone, in which the proposed site is located, are given in Table 5.

Table 5 Monthly temperature and rainfall statistics for South Johnstone – Northern Queensland (Tropical rainforest)*

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Mean max temp (°C)	31.1	30.6	29.8	28.1	26.2	24.3	23.8	25.1	27.0	29.0	30.5	31.2	28.1
Mean min temp (°C)	22.5	22.7	21.9	20.4	18.3	15.8	15.0	15.4	16.6	18.8	20.5	21.8	19.1
Mean rainfall (mm)	522.8	580.6	615.4	393.8	270.9	154.9	109.4	90.1	85.3	89.1	143.0	252.0	3305.5

*data taken from the Australian Bureau of Meteorology website (<http://www.bom.gov.au/index.shtml>) Temperature data are an average of 54 years of records; rainfall data are an average of 78 years of records

77. The proposed field location is 7 m above the highest flood level recorded for the nearest waterway (South Johnstone River) on flat land that is not prone to heavy runoff or landslip and is approximately 250 m from the nearest waterway. There is no recorded incident of a storm or cyclone uprooting mature banana plants in the immediate area and dispersing vegetative propagative material.

6.2 Relevant biotic factors

78. The biotic factors pertaining to the growth and distribution of commercial bananas in Australia are discussed in the *Musa* Biology document (OGTR 2008). Of relevance to this proposed release are the following points:

- ♦ the Research Station, in which the proposed site is located, occurs within a major banana growing region.
- ♦ the applicant states that the nearest observed population of native bananas is approximately 10 km from the site (see paragraph 87).
- ♦ there are some 200 *Musa* spp. accessions growing as part of a germplasm collection at the Research Station in which the proposed site is located. Under Queensland State legislation these are not permitted to fruit or produce male flowers.
- ♦ invertebrates, vertebrates and microorganisms would all be exposed to the introduced genes, their encoded proteins and end products. In particular:
 - native vertebrates including nectar feeding birds, nectar feeding marsupials and flying foxes that are attracted to the flowers or fruit of banana plants may visit the field location.
 - feral pigs have access to the site but, while they have been observed knocking down and eating small plants, there is no evidence that they uproot plants, detach suckers or eat fruit that is still on the plant. The applicant states that trapping of the pigs commenced at the Research Station in January 2008, as a control measure. There are no domestic livestock at the Research Station on which the proposed site is located.

6.3 Relevant agricultural practices

79. The size, location and duration of the proposed limited and controlled release of the GM banana lines are outlined in Section 3.2 of this Chapter.

80. The applicant intends to transport the planting material (tissue cultured plants) to the site from a PC2 facility and to harden off the plants in a shadehouse for up to 4 months before transferring them to the field location.

81. Planting of bananas in north Queensland is possible all year round. The cultivation practices used for planting and managing the proposed trial will follow the standard practices used for commercial (non-GM) banana. These are outlined in Section 2.3.3 of the *Musa* Biology document (OGTR 2008) and detailed in Broadley et al. (2004) and include compliance with Queensland State Government legislation for banana disease control (Queensland Government 1999) – see next paragraph.

82. Several of the objectives of the Plant Protection (Banana Pest Quarantine) Regulation 1999 (Queensland Government 1999) complement containment measures that could be applied to GM plants and include:

- ♦ requirement for an inspector's approval to plant bananas
- ♦ provision of power to an inspector to remove/eradicate those plants that do not have an inspector's approval to be grown
- ♦ restriction of the movement of banana plants/plant parts/soil on which a banana plant has been growing into and within banana quarantine areas
- ♦ obligation to eradicate volunteer plants
- ♦ obligation of growers to control weed growth

83. It is standard practice to remove the male bell from inflorescences as this allows transport of assimilates to the developing fruit (Broadley et al. 2004). The applicant proposes

to remove the male bells from most inflorescences but wishes to observe phenotypes in some bells. In this case, the bells will be bagged.

84. Fruit will be obtained from the plant crop¹³, which will then be ratooned and grown to fruiting before the proposed trial is concluded. In a commercial situation, bunches from the plant crop are usually harvested about 16 to 18 months after planting, but this can be as early as 12 months. Subsequent (ratoon) crops are harvested 6 -12 months after sucker set (Morton 1987). Under the conditions at the proposed field location it is expected that the plant crop will start to produce bunches after 12 months and the ratoon crop will be harvested after 9 – 11 months. Fruit will be harvested while still green (standard commercial practice).

85. Non-propagative detached plant material at the field location (generated, for example, by desuckering, leaf thinning, cutting down of pseudostems) would be left on the ground to decompose. Any excess material potentially containing meristems would be treated with distillate or kerosene or other methods approved in writing by the Regulator. Excess fruit (not required for experimental analysis) would be decomposed in a secure container to prevent access by fruit-eating animals before being returned to the site. Plant waste from the shadehouse would also be decomposed on the ground at the field location, with meristematic tissue firstly receiving appropriate herbicide treatment

6.4 Presence of related plants in the receiving environment

86. Commercial sweet banana cultivars are grown in the vicinity of the proposed field location. These all show parthenocarpy and male sterility.

87. There are two recognised *Musa* species that are native to Australia (Ross 1987):

- ♦ *M. acuminata* subsp. *banksii*, a fertile diploid, is the most common and can be found along the tip of Cape York and northern Queensland. The applicant states that the nearest observed naturally occurring population of this species is approximately 10 km from the proposed site at South Johnstone but that native vegetation consistent with the type of community in which *M. acuminata* subsp. *banksii* is found occurs much closer to the site. Plantings of the species are also present in a field collection at the Research Station on which the site is located but under compliance with State legislation for banana disease control (Queensland Government 1999), are not permitted to fruit or produce male flowers. *M. acuminata* subsp. *banksii* has the potential to cross with cultivated triploid and tetraploid cultivars with a *M. acuminata* background (such as the cultivar ‘Williams’). However, the applicant has stated that seed has not been found in commercial banana plantations growing in northern Queensland where the native species is in close proximity.
- ♦ *M. jackeyi* has been found in Bellenden Ker (approximately 50 km north of South Johnstone) and Cooktown (approximately 350 km north of South Johnstone).

6.5 Presence of the introduced genes or similar genes, encoded proteins and end products in the environment

88. All of the introduced genes are isolated from naturally occurring organisms that are already widespread and prevalent in the environment

89. All of the introduced genes for nutrient enhancement in the GM banana lines, except *Crt1*, are derived from plant species widely occurring in the environment. Two of these species are common crop plants (*O. sativa*, and *Z. mays*); *Glycine soja* is closely related to

¹³ The term ‘plant crop’ is routinely used in the banana industry to designate the fruit-bearing plant that develops from the propagative material first planted in the ground. Subsequent fruit develops from a ‘ratoon crop’.

cultivated soybean (*Glycine max*) and *Arabidopsis thaliana* is a well characterised common laboratory plant used in experimental studies.

90. The biosynthetic pathways for pro-vitamin A carotenoids and vitamin E are ubiquitous in photosynthetic organisms and most plants contain genes involved in iron uptake and transport. Thus the introduced genes for enhanced nutrition (except the *CrtI* gene) are most likely orthologous to genes in all other plants. Therefore, it is expected that humans, herbivores/omnivores and microorganisms routinely encounter the introduced genes and their gene products, or their homologues, through contact with plants and food derived from plants. This information forms the baseline data for assessing the risks from exposure to these enzymes as a result of the trial of the GM banana lines. As shown in Table 3, the levels of pro-vitamin A, vitamin E and iron that occur in a range of plant parts and plant-derived products can be high; these plant parts are consumed by humans and animals without harm.

91. Humans, herbivores and microorganisms would encounter the *CrtI* gene from *Erwinia uredovora* by consumption of fruits and vegetables infected with the bacterium and also, as the bacterium exists in soil, from exposure to soil/soil water splashes. As the gene codes for an enzyme that is involved in pro-vitamin A carotenoid synthesis, the biosynthetic products of the action of the enzyme would be essentially similar to those produced in plant pro-vitamin A carotenoid synthesis and hence would be routinely encountered by humans, herbivores and microorganisms.

92. The *nptII* gene is derived from *E. coli*, which is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997). As such, it is expected that humans, animals and microorganisms routinely encounter the encoded protein.

93. The *uidA* gene, which encodes the GUS enzyme, is also derived from *E. coli*. GUS enzyme activity has been detected in numerous microbial, plant and animal species (Flavell et al. 1992; Gilissen et al. 1998). The GUS protein used in GM plants is 99.8% homologous to the *E. coli* GUS protein. GUS is recognised as commonly present on fresh food.

Section 7 Australian and international approvals

7.1 Australian approvals of the GM banana lines

7.1.1 Previous releases approved by the Gene Technology Regulator or authorised by the Genetic Manipulation Advisory Committee

94. There has been no release of these GM banana lines, or any other GM banana lines, in Australia.

7.1.2 Approvals by other Australian government agencies

95. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. Other government regulatory requirements may also have to be met in respect of release of GMOs, including those of the Australian Quarantine and Inspection Service (AQIS) and Food Standards Australia New Zealand (FSANZ). This is discussed further in Chapter 3.

96. FSANZ is responsible for human food safety assessment and food labelling, including GM food. The applicant does not intend to use materials from the GM banana lines in human food, accordingly an application to FSANZ has not been submitted. FSANZ approval would need to be obtained before materials from these GM banana lines could be used in food.

7.2 International approvals

97. There has been no release of these GM banana lines in any other country. The applicant states that at least three GM banana field trials using other introduced genes have been conducted overseas by private companies. Detailed data have not been published but the genetic modifications have been concerned with nematode and disease resistance.

Chapter 2 Risk assessment

Section 1 Introduction

98. Risk assessment is the overall process of identifying the sources of potential harm (hazards) and determining both the seriousness and the likelihood of any adverse outcome that may arise. The risk assessment (summarised in Figure 4) considers risks from the proposed dealings with the GMOs that could result in harm to the health and safety of people or the environment posed by, or as a result of, gene technology. It takes into account information in the application, relevant previous approvals and current scientific knowledge.

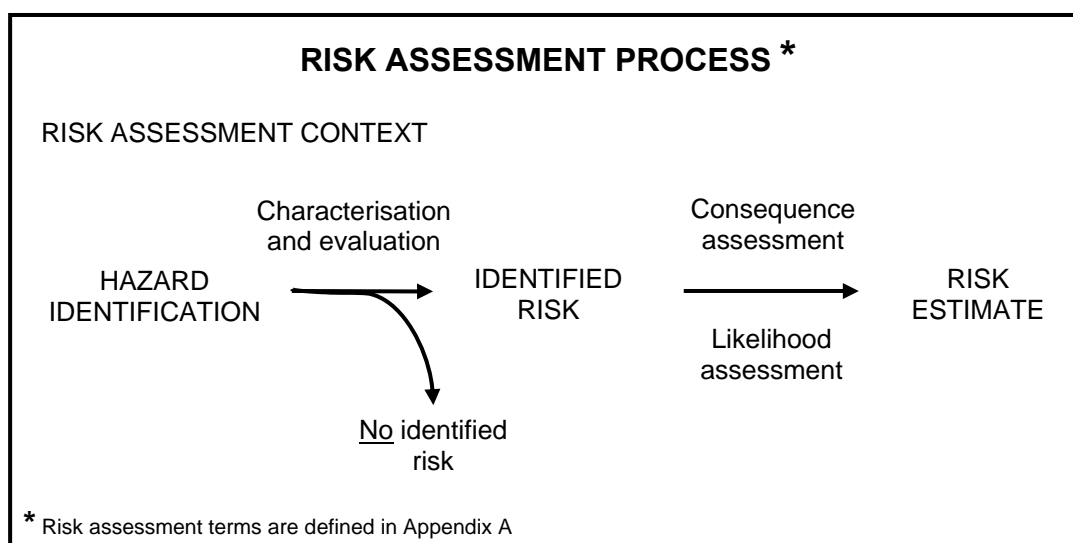


Figure 4 The risk assessment process

99. Once the risk assessment context has been established (see Chapter 1) the next step is hazard identification to examine what harm could arise and how it could happen during a release of these GMOs into the environment.

100. It is important to note that the word 'hazard' is used in a technical rather than a colloquial sense in this document. The hazard is a source of *potential* harm. There is no implication that the hazard will *necessarily* lead to harm. A hazard may be an event, a substance or an organism (OGTR 2007).

101. Hazard identification involves consideration of events (including causal pathways) that may lead to harm. These events are particular sets of circumstances that might occur through interactions between the GMOs and the receiving environment as a result of the proposed dealings. They include the circumstances by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products, the introduced genes, or products of the introduced genes.

102. A number of hazard identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2007). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

103. The hazard identification process results in the compilation of a list of events. Some of these events lead to more than one adverse outcome and each adverse outcome can result from more than one event.

Section 2 Hazard characterisation and the identification of risk

104. Each event compiled during hazard identification is characterised to determine which events represent a risk to the health and safety of people or the environment posed by, or as a result of, gene technology.

105. The criteria used by the Regulator to determine harm are described in Chapter 3 of the *Risk Analysis Framework* (OGTR 2007). Harm is assessed in comparison to the parent organism and in the context of the proposed dealings and the receiving environment. Wherever possible, the risk assessment focuses on measurable criteria for determining harm.

106. The following factors are taken into account during the analysis of events that may give rise to harm:

- ♦ the proposed dealings, which may be for the purpose of experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMOs
- ♦ the proposed limits
- ♦ the proposed controls
- ♦ characteristics of the non-GM parent
- ♦ routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- ♦ potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs¹⁴
- ♦ potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- ♦ the biotic and abiotic factors at the site of release
- ♦ agronomic management practices for the GMOs.

107. The nine events that were characterised are discussed in detail later in this Section. They are summarised in Table 6 where events that share a number of common features are grouped together in broader hazard categories. None were considered to lead to an identified risk that required further assessment.

¹⁴ As discussed in Sections 5.2.5 and 5.2.6, the *uidA* and *nptII* genes and their products have already been considered in detail in previous RARMPs and by other regulators. They have not been found to pose risks to either people or the environment and will not be considered further.

Table 6 Summary of events that may give rise to an adverse outcome through the expression of the introduced genes for enhanced nutrition.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Ingestion of, contact with, or inhalation of GM plant material containing proteins encoded by the introduced genes, or their end products.	Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> The encoded proteins and their end products are widespread in the environment and are unlikely to be toxic/allergenic to people or toxic to other organisms. The proposed release is limited and controlled, further reducing exposure of people and other organisms to products of the introduced genes.
Section 2.2 Spread and persistence (weediness) of the GM banana lines in the environment	2. Expression of the introduced genes improving the survival of GM banana plants.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> Non-GM, commercial banana does not possess weedy characteristics. The genetic modifications are not expected to affect the survival of the GM lines. The limits and controls proposed for the release would minimise persistence
	3. Dispersal of viable GM plant materials through various means, including animals and extreme weather conditions.	Weediness	No	<ul style="list-style-type: none"> Opportunities for dispersal are limited by the very low level of seed production and lack of easily dislodged vegetative propagules. The proposed limits and controls would minimize dispersal
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced genes or regulatory sequences in commercial, non-GM sweet banana plants or in other sexually compatible plants	Weediness Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> The very low fertility of non-GM, commercial banana is not expected to be altered by the introduced genes. Thus it is highly unlikely that crossing with sexually compatible plants would occur. The applicant proposes to remove or cover male flowers, further reducing the likelihood of gene flow.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	5. Presence of the introduced genes, or regulatory sequences, in unrelated organisms as a result of gene transfer.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> The introduced genes or similar genes and the introduced regulatory sequences are already present in the environment and are available for transfer via natural mechanisms. Events 1 – 3 did not identify any risks to people or the environment associated with expression of the introduced genes.
Section 2.5 Unintended changes in biochemistry, physiology or ecology	6. Changes to biochemistry (including innate toxic or allergenic compounds), physiology or ecology of the GM banana lines resulting from altered expression or random insertion of the introduced genes.	Weediness Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> Unintended, adverse effects, if any, would be minimized by the proposed limits and controls. Unexpected alterations are likely to be detected and eliminated during the selection process
	7. Presence of altered levels of metals as a result of expression of the introduced iron enhancement genes	Toxicity in people or other organisms	No	<ul style="list-style-type: none"> The proposed limits and controls would minimise any adverse effects

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.6 Unintended presence in the environment of <i>Agrobacterium tumefaciens</i> containing the introduced genes	8. Transfer of the introduced genes from <i>A. tumefaciens</i> to other organisms	Weediness Allergic reactions in people or toxicity in people or other organisms	No	<ul style="list-style-type: none"> There is very low likelihood that the <i>A. tumefaciens</i> could conjugate with other <i>A. tumefaciens</i> strains or other bacteria naturally present at the site. It is highly unlikely that the <i>A. tumefaciens</i> would infect other plants at the site Events 1 – 3 and 5 did not identify any risks to people or the environment associated with expression of the introduced genes
Section 2.7 Unauthorised activities	9. Use of the GMOs outside the proposed licence conditions.	Potential adverse outcomes mentioned in Sections 2.1 to 2.5	No	<ul style="list-style-type: none"> The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.

2.1 Production of a substance toxic/allergenic to people or toxic to other organisms

108. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000).

109. Allergenicity is the potential of a protein to elicit an immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al. 2006).

110. A range of organisms may be exposed directly or indirectly to the proteins (and end products) encoded by the introduced genes for enhanced nutrition. Workers cultivating the bananas would be exposed to all plant parts. Organisms may be exposed directly to the proteins through biotic interactions with GM banana plants (vertebrates, insects, symbiotic microorganisms and/or pathogenic fungi) or through contact with root exudates or dead plant material (soil biota). Indirect exposure would include organisms that feed on organisms that feed on GM banana plant parts or degrade them (vertebrates, insects, fungi and/or bacteria).

Event 1: *Ingestion of, contact with, or inhalation of GM plant materials containing proteins encoded by the introduced genes, or their end products.*

111. There are no known toxic properties of non-GM banana (OGTR 2008). Allergic reactions as a result of ingesting the fruit can take the form of either fruit-latex allergy or oral allergy syndrome (OGTR 2008).

112. It is not expected that any novel products would be produced as a result of the expression of the introduced genes because the introduced genes are likely to be orthologous to genes in all other plants (see Chapter 1, Section 6.5).

113. No information was found (Chapter 1, Section 5.2.4) to suggest that the proteins encoded by the introduced genes are toxic or allergenic to people or to other organisms.

114. The end products, β -carotene, vitamin E and iron can all induce adverse effects if consumed by humans or other animals at high enough levels. There are no data on the levels of these end products in the proposed GM banana lines. However, several Fe'i banana cultivars contain significantly higher levels of β -carotene and α -tocopherol than the

commercial sweet banana cultivars (see paragraphs 41 and 46). These Fe'i bananas are grown and consumed in Micronesia without any adverse effect on humans or animals. The level of β -carotene currently obtained in a GM rice line (using two of the genes proposed for introduction in banana) would, if consumed as normal dietary pattern, still be well below the RDI for vitamin A (see paragraph 42). With regard to iron, products such as breakfast cereals that are fortified with iron (see Table 3) contain significantly higher levels than any naturally-occurring plant sources; red meat also generally contains higher levels of available iron than plant sources. It is unlikely that the levels in the GM banana lines will exceed those normally found in food.

115. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would all minimize the likelihood of exposure of people and other organisms to GM plant materials. Contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff associated with the field trial. Potential for exposure of the public to plant materials via ingestion, skin contact or inhalation is very limited as no plant material will be used as food, animal feed or plant products and the public will not have access to the site. Fruit from the trial will be decomposed in a waste bin so as to avoid consumption by frugivores.

116. The introduced (or similar) genes and encoded proteins are naturally present in the environment and therefore humans, other vertebrates, invertebrates and microorganisms are already exposed to them.

117. **Conclusion:** The potential for allergic reaction in people, or toxicity in people or other organisms as a result of ingestion of, contact with, or inhalation of, GM plant materials containing proteins encoded by the introduced genes, or their end products as a result of the genetic modification, is **not an identified risk** and will not be assessed further.

2.2 Spread and persistence (weediness) of the GM banana lines in the environment

118. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM banana plants in particular, is provided in the *Musa* Biology document (OGTR 2008). In summary, commercial banana cultivars do not possess characteristics that are usually associated with weediness and such cultivars do not pose a weed problem in Australia because their low fertility limits sexual dissemination and the integrity of the underground plant structure limits vegetative spread. Additionally, State legislation ensures that there are obligations for growers to destroy volunteers that may arise.

119. Scenarios that could lead to increased spread and persistence of the GM banana lines include expression of the introduced genes conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials. These events could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the encoded proteins and their end products.

Event 2: Expression of the introduced genes improving the survival of the GM banana plants

120. Expression of heterologous plant genes for pro-vitamin A carotenoids and vitamin E synthesis and for iron transport/storage have not previously been reported in banana and therefore the impact of the genetic modifications on survival of the GM banana lines is not known. Based on the known functions of proteins expressed from the introduced genes (Zeiger & Zhu 1998; Vansuyt et al. 2000; American Society of Plant Physiologists 2000; Vert et al. 2002; Munné-Bosch & Alegre 2002; Dörmann 2007), none are expected to alter characteristics that limit survival of the GM banana lines.

121. One of the main purposes of the proposed release is to conduct proof of concept experiments with the GM banana lines to assess growth, fruit and yield characteristics. Thus, any characteristics that may impact on the survivability of the GM plants will be closely monitored during the proposed trial. Further, control measures proposed to limit the size, location and duration of the trial would restrict the spread and persistence of the GM banana lines.

122. **Conclusion:** The potential for the increased survival of the GM banana lines as a result of the expression of the introduced genes for enhanced nutrition is **not an identified risk** and will not be assessed further.

Event 3: *Dispersal of viable GM plant materials through various means, including, animals, and extreme weather conditions*

123. The lack of seed production that is a characteristic of commercial non-GM banana cultivars is not expected to be altered in the GM lines.

124. The limits and controls on the proposed release (outlined in Chapter 1, Sections 3.2 and 3.3) will ensure that dispersal of plant material is minimized. In addition, the fruits will be harvested while still green (a standard practice in the cultivation of commercial banana) so that they are less appealing to frugivores than fully ripe fruit and will be less likely to detach from a bunch and be inadvertently dispersed into the environment.

125. A number of native and feral animals such as kangaroos, flying foxes and feral pigs would have access to the proposed field location. They would be unable to reach the fruit either because of its height above ground or the fact that the applicant has proposed to place bunch covers over the fruit. Furthermore, even if fruit were dispersed by animals, it is highly unlikely to contain viable seed. Feral pigs, in particular could damage plants at ground level, but the applicant states that they have not been recorded uprooting plants or detaching suckers on the land at the site.

126. Information in paragraph 77 indicates that extremes of weather are unlikely to cause dispersal of plant parts.

127. In the unlikely event that material is dispersed away from the proposed locations, there is a Queensland State legislation requirement to destroy any volunteers that may arise as a result. This would limit the persistence of any dispersed materials in the environment.

128. **Conclusion:** The potential for dispersal of reproductive plant materials from the GM banana lines by various means is **not an identified risk** and will not be assessed further.

2.3 Vertical transfer of genes or genetic elements to sexually compatible plants

129. Vertical gene flow is the transfer of genetic information from an individual organism to its progeny by conventional heredity mechanisms, both asexual and sexual. In flowering plants, pollen dispersal is the main mode of gene flow (Waines & Hedge 2003). For GM crops, vertical gene flow could therefore occur via successful crosspollination between the crop and neighbouring crops, related weeds or native plants (Glover 2002).

130. Baseline information on vertical gene transfer associated with non-GM banana plants is provided in the *Musa* Biology document (OGTR 2008). In summary, commercial banana cultivars are effectively sterile and therefore the chances of natural hybridisation either within the same species or between different *Musa* species are remote where a cultivated variety is one of the parents.

Event 4: Expression of the introduced genes and regulatory sequences in commercial, non-GM sweet banana plants or in other sexually compatible plants

131. Cavendish bananas are effectively male sterile and the introduced genes are not expected to alter this trait. Therefore, gene transfer occurring through pollination is highly unlikely.

132. The commercial, non-GM sweet banana varieties growing in proximity to the GM lines are effectively female sterile so even if pollen were to be produced by the GM lines, fertilization of non-GM plants would be highly unlikely.

133. The applicant proposes to remove most male flowers from the GM plants. Those flowers that would remain (for experimental analysis) will be bagged to prevent loss of pollen (in the unlikely event that pollen is produced).

134. Other *Musa* species growing as part of a germplasm collection at the site are not permitted to flower.

135. While it is unlikely that the GMOs will differ in their sexual reproduction characteristics from the parent organism, the likelihood of vertical gene transfer occurring will be further reduced by the close monitoring of the GM banana plants during the proposed trial. In addition, the control measures proposed to restrict size, location and duration of the trial would minimise the likelihood of vertical gene transfer occurring.

136. The nearest sexually compatible native *Musa* species is 10 km from the proposed release. It should be noted that blossom foraging bats of *Pteropus* spp., which could feed on the flowers of *Musa* spp., could carry pollen that becomes trapped in their head fur as far as 50 km (Eby 1995). However, the likelihood of this occurrence posing a risk with regard to gene flow involving pollen from GM banana flowers is very low because of the low viability of the pollen produced by Cavendish flowers (Fortescue & Turner 2004) and because of the proposal by the applicant to remove or bag any male flowers produced on the inflorescences.

137. All of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to banana plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. However, it is highly unlikely that a regulatory element would be transferred and, even if it did occur, the chance of an adverse effect to people or the environment is highly unlikely.

138. A number of regulatory sequences are derived from plant or pathogenic species that are associated with mild allergic reactions in susceptible individuals, a toxic response in humans and animals (in the case of *Ricinus communis*), or disease in plants or humans. However, the regulatory elements represent only a small proportion of the parent organism genetic material and have not been reported to cause any adverse effects.

139. **Conclusion:** The potential for the expression of the introduced genes and regulatory sequences in commercial, non-GM sweet banana plants or other sexually compatible plant species as a result of gene transfer is **not an identified risk** and will not be assessed further.

2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms

140. Horizontal gene transfer is the movement of genetic information (DNA) between sexually unrelated organisms (Thomson 2001). In the context of genetic modification, a major concern has been whether DNA introduced into crops could transfer into bacteria in the soil or into the cells of organisms that may eat the crops. Horizontal gene transfer has been considered in previous RARMPs (including in detail in DIR 057/2004 available at

<<http://www.ogtr.gov.au/ir/dir057.htm>> or by contacting the OGTR). These assessments have concluded that horizontal gene transfer from plants to sexually incompatible organisms occurs rarely and usually only on evolutionary timescales. There are no more recent data that alter this conclusion.

Event 5: Presence of the introduced genes, or regulatory sequences, in unrelated organisms as a result of gene transfer

141. The probability of transferring introduced genes contained in the GM banana plants is no greater than that of transferring any of the native genes. Non-GM bananas contain homologues of all of the introduced genes, with the exception of *CrtI*, and therefore these genes are already available for transfer via demonstrated natural mechanisms. In addition, homologues of the genes occur in all plant species and thus are widespread in the environment.

142. While the carotene desaturase protein (or a homologue) encoded by *CrtI* is not present in non-GM banana plants, the fact that *CrtI* replaces three genes that are normally present in all plants (see Chapter 1, paragraph 18) means that the product of the action of carotene desaturase (*trans* lycopene – see Figure 2) is the same product that would normally be produced in the pro-vitamin A carotenoid biosynthetic pathway of plants and thus occurs widely in the environment.

143. The introduced regulatory sequences are already present in the environment and are available for horizontal transfer.

144. Reports of horizontal gene transfer from plants to bacteria occurring during laboratory experiments have relied not only on the use of highly similar sequences to allow homologous recombination to occur, but also on conditions designed to enhance the selective advantage of gene transfer events (Mercer et al. 1999; Gebhard & Smalla 1998; Nielsen et al. 2000; Nielsen 1998; De Vries et al. 2001). This suggests that the likelihood of natural horizontal gene transfer is remote (see also discussion in Event 8).

145. The safety of the protein product(s) resulting from the expression of the introduced gene(s), rather than horizontal gene transfer *per se*, is a key consideration in the risk assessment process (Thomson 2001). If the protein products are not associated with any risk to humans, animals or the environment then, even in the unlikely event of horizontal transfer occurring, they should still not pose any such risk. Events 1 – 3 did not identify a risk associated with the introduced proteins or end products.

146. **Conclusion:** The potential for an adverse outcome as a result of horizontal gene transfer is **not an identified risk** and will not be assessed further.

2.5 Unintended changes in biochemistry, physiology or ecology

147. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy¹⁵ (Haslberger 2003). Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such effects may include:

- ♦ altered expression of an unrelated gene at the site of insertion

¹⁵ Pleiotropy is the effect of one particular gene on the expression of other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

- ♦ altered expression of an unrelated gene distant to the site of insertion, for example, due to the encoded protein of the introduced gene changing chromatin structure, affecting methylation patterns, or regulating signal transduction and transcription
- ♦ increased metabolic burden associated with high level expression of the introduced gene
- ♦ novel traits arising from interactions of the protein encoded by the introduced gene product with endogenous non-target molecules
- ♦ secondary effects arising from altered substrate or product levels in the biochemical pathway incorporating the protein encoded by the introduced gene.

148. Such unintended pleiotropic effects might result in adverse outcomes such as toxicity or allergenicity; weediness, altered pest or disease burden; or reduced nutritional value as compared to the parent organism. However, accumulated experience with genetic modification of plants indicates that, as for conventional (non-GM) breeding programs, the process has little potential for unexpected outcomes that are not detected and eliminated during the early stage of selecting plants with new properties (Bradford et al. 2005).

Event 6: *Changes to biochemistry, physiology or ecology of the GM banana lines resulting from altered expression or random insertion of the introduced genes*

149. Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced genes, have already been discussed in Events 1 -3 and no risk is identified.

150. Due to the occurrence of various end products, produced by expression of the introduced genes for enhancement of pro-vitamin A carotenoid and vitamin E, in a number of biochemical pathways there may be effects on the functioning of interrelated pathways.

151. Because of the large size, growing space and soil requirements of mature banana plants it has not been possible to conduct preliminary characterisation of the effects of the introduced genes for nutrient enhancement under contained conditions (such as found in a glasshouse). The proposed field trial thus represents the first opportunity for such characterisation. The likelihood of any pleiotropic effects causing adverse effects is minimized by the limits and controls outlined in Chapter 1, Sections 3.2 and 3.3.

152. **Conclusion:** The potential for an adverse outcome as a result of changes to biochemistry, physiology or ecology is **not an identified risk** and will not be assessed further.

Event 7: *Presence of altered levels of metals as a result of expression of the introduced iron enhancement genes*

153. If metals such as manganese, zinc and cadmium are already present in the soil at the field trial location, the expression of the introduced genes for enhancement of iron may result in elevated levels of these metals in banana tissues if the iron transport proteins are not specific (see Chapter 1, paragraph 27). Levels of copper, zinc, cobalt, magnesium, nickel, lead, arsenic, mercury, cadmium, chromium and aluminium in banana tissues will be measured as part of the data collected from the trial.

154. Adverse effects, if any, as a result of increased levels of metals in GM plant materials will be minimized by the limits and controls (Chapter 1, Sections 3.2 and 3.3) proposed for the release.

155. **Conclusion:** The potential for an adverse outcome resulting from the activity of the introduced genes for iron enhancement is **not an identified risk** and will not be assessed further.

2.6 Unintended presence of *Agrobacterium tumefaciens* containing the introduced genes, during release

156. *Agrobacterium tumefaciens* is a soil-borne, Gram-negative bacterium that, in nature, causes crown gall on plants. The bacterium enters wounded somatic cells of the host and causes surrounding host cells to proliferate irregularly and form a gall. The bacterium is confined to the cells of the gall. Eventually, degradation of the gall releases the *A. tumefaciens* back into the soil where it can live saprophytically for several years (Krimi et al. 2002; Escobar & Dandekar 2003).

157. For genetic modification, 'disarmed' strains of *A. tumefaciens* that cannot cause crown gall are used to transfer DNA to plant cells under controlled, optimized laboratory conditions. The strains used for genetic modification may also contain hypervirulent, attenuated tumour-inducing plasmids to increase cell transformation rates. *Agrobacterium tumefaciens* has been shown to be persistent in *in vitro* plant tissues and shoots. Broad spectrum antibacterial compounds tend to have a bacteriostatic effect, suppressing, but not eliminating bacterial growth and when removed the bacteria may resume growth. In particular, Gram-negative bacteria (such as *A. tumefaciens*) are considered to be difficult to eradicate completely from *in vitro* cultures (Barrett et al. 1997; Leifert & Cassells 2001), although persistence of *A. tumefaciens* in some transgenic plants has not been detected (Charity & Klimaszewska 2005).

158. During *Agrobacterium*-mediated transformation of plant cells, the *A. tumefaciens* attaches to plant cell walls and a virulence system is activated in the bacterium, ultimately allowing the transfer and integration of bacterial DNA into the plant DNA (de la Riva et al. 1998). As with most bacterial endophytes, disarmed strains of *A. tumefaciens* would be expected to inhabit the intercellular spaces and xylem vessels of plant tissue (Rosenblueth & Martínez-Romero 2006) via the formation of surface-associated biofilms (Danhorn et al. 2008). This means it is highly unlikely that *A. tumefaciens* would be incorporated into plant reproductive cells. For this reason, *A. tumefaciens* may persist in vegetatively propagated GM plants (such as banana) since there would be no opportunity for elimination of the *A. tumefaciens* in sexually produced generations.

159. The possibility of persistence of *A. tumefaciens* in GM plant tissue has led to suggestions for *Agrobacterium*-mediated transformation to include protocols for the detection and elimination of the bacterium (Cubero & López 2004). Suggested detection methods include PCR-based methods employing primers that amplify parts of the bacterial genome present in the Ti plasmid but are not transferred to the plant genome after transformation (Cubero & López 2004)

160. The transfer of GM banana plants, carrying *A. tumefaciens*, into the environment could result in the transfer of genes to non-target plants or other microorganisms (Leifert 2000). Possible risks associated with the use of *A. tumefaciens* for genetic modification of plants under laboratory conditions have also been considered in a previous RARMP concerning GM rose (see DIR 060/2005 - available at <<http://www.ogtr.gov.au/ir/dir060.htm>> or by contacting the OGTR). In this instance, the GM rose plants were grown hydroponically in pots above the soil and no risk was identified as a result of the unintended presence of *A. tumefaciens*.

Event 8: Transfer of the introduced genes from *A. tumefaciens* to other organisms

161. The GM banana lines proposed for release were generated by *Agrobacterium tumefaciens*-mediated genetic modification (Section 5.4, Chapter 1). The applicant has shown, through PCR testing of GM banana plant material, using primers specific to regions outside the T-DNA (see paragraph 65), that the *A. tumefaciens* does not persist in the GM banana plant material for longer than approximately 3 weeks after removal from the *in vitro* environment. As plants will be hardened off for several months in a shadehouse (see paragraph 80) it is unlikely that plants transferred to the field would be carrying residual *A. tumefaciens*.

162. If *A. tumefaciens* containing an introduced gene construct were present in the cells of GM banana plants it could transfer the introduced genes via conjugation with a wild type strain or other bacteria and yeast naturally present in the soil at the site (Hammerschlag et al. 2000). This general possibility of horizontal gene transfer has already been discussed in Event 5 and was not considered to be an identified risk.

163. If the *A. tumefaciens* were present in GM banana tissue it could also genetically modify cells of other plants. Although the conditions for *A. tumefaciens* infection and gene transfer to plants would exist in nature, the creation of a GM plant would be highly unlikely because: (1) it would be unlikely that the *A. tumefaciens* would genetically modify a cell or cells that would give rise to a new organism, (2) it is unlikely that conditions in nature would exist that would select for the survival of the infected GM plant cells, and (3) not all the GM plant cells would have expression of the introduced genes (eg due to position effect, genetic re-arrangements, silencing due to multiple copies). Should a new GM plant arise it is unlikely that it would have a selective advantage. In addition, homologues of the introduced genes are already widespread in plants.

164. **Conclusion:** The potential for an adverse outcome resulting from the persistence in the environment of *A. tumefaciens* containing the introduced genes is **not an identified risk** and will not be assessed further.

2.7 Unauthorised activities**Event 9: Use of GMOs outside the proposed licence conditions (non-compliance)**

165. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the GM banana lines outside of the proposed release areas. The adverse outcomes that this event could cause are discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

166. **Conclusion:** The potential for an adverse outcome as a result of unauthorised activities is **not an identified risk** and will not be assessed further.

Section 3 Risk estimate process and assessment of significant risk

167. The risk assessment begins with a hazard identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

168. Nine events were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the

introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

169. A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

170. The characterisation of the nine events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principle reasons for this include:

- ♦ limits on the size and duration of the release proposed by QUT;
- ♦ suitability of controls proposed by QUT to restrict the dissemination or persistence of the GM banana plants and their genetic material;
- ♦ limited capacity of the GM banana lines to spread and persist outside the areas proposed for release;
- ♦ limited ability and opportunity for the GM banana lines to transfer the introduced genes to commercial banana crops or other sexually related species;
- ♦ none of the GM plant materials or products will be used in human food or animal feed;
- ♦ widespread presence of the same or similar proteins encoded by, and end products produced as a result of the activity of, the introduced genes in the environment and lack of known toxicity or evidence of harm from them.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM banana lines into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment.

Section 4 Uncertainty

171. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. Both dimensions of risk (i.e. consequence and likelihood) are always uncertain to some degree.

172. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹⁶. For field trials some knowledge gaps are inevitable because they involve the conduct of research. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, rather than necessarily to treat an identified risk.

173. For DIR 076/2007 which involves proof of concept research, uncertainty exists in relation to the characterisation of:

- ♦ Event 1, regarding potential increases in allergenicity or toxicity through contact with plant material containing proteins encoded by the introduced genes or their end products;

¹⁶ A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2007) available at <<http://www.ogtr.gov.au/pubform/riskassessments.htm>> or via Free call 1800 181 030.

- ◆ Event 2, associated with a potential for increased survival of the GMOs;
- ◆ Event 6, regarding the consequences of the end products of the introduced genes being involved in a number of biochemical pathways; and
- ◆ Event 7, associated with the possibility of the expression of the introduced genes also causing accumulation of undesirable toxic metals.

174. Additional data including information to address these uncertainties, would be required to assess possible future applications for a larger scale trial, reduced containment conditions, or the commercial release of any of these GM banana lines that may be selected for further development.

175. Section 5, Chapter 3 discusses the additional data that may be required for future releases.

Chapter 3 Risk management

176. Risk management includes evaluation of risks identified in Chapter 2 to determine whether or not specific treatments are required to mitigate harm to human health and safety, or the environment, that may arise from the proposed release. Other risk management considerations required under the Act are also addressed in this chapter. Together, these risk management measures are used to inform the decision-making process and determine licence conditions that may be imposed by the Regulator under the Act. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

Section 1 Background

177. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment. All licences are required to be subject to three conditions prescribed in the Act.

178. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. Other mandatory statutory conditions contemplate the Regulator maintaining oversight of licensed dealings. For example, section 64 requires the licence holder to provide access to premises to OGTR monitors, and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

179. It is a further requirement that the licence be subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Responsibilities of other Australian regulators

180. Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Other agencies that also regulate GMOs or GM products include FSANZ, Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies¹⁷.

181. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR applications. *The Gene Technology (Consequential Amendments) Act 2000* requires the agencies to consult the Regulator for the purpose of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

¹⁷ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <<http://www.ogtr.gov.au/pubform/riskassessments.htm>>

182. FSANZ is responsible for human food safety assessment, including GM food. As the trial involves proof of concept research, the applicant does not intend any material from these GM banana lines to be used in human food. Accordingly the applicant has not applied to FSANZ for evaluation of any of the GM banana lines for use in human food. FSANZ approval would need to be obtained before they could be used in food.

183. No other approvals are required.

Section 3 Risk treatment measures for identified risks

184. The risk assessment of events listed in Chapter 2 concluded that there are **negligible** risks to people or the environment from the proposed trial of GM banana. The *Risk Analysis Framework* (OGTR 2007), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

185. These events were considered in the context of the scale of the proposed release [a maximum total area of 1.4 hectares over four years (May 2008 – May 2012) on a single site in the Queensland local government area of Cassowary Coast], the containment measures (Chapter 1, Section 3), and the receiving environment (see Chapter 1, Section 6).

Section 4 General risk management

186. Licence conditions have been proposed to control the dissemination and persistence of the GMOs and their genetic material in the environment and limit the release to the size, location and duration requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are detailed in Chapter 4 and summarised in Sections 4.1.2 and 4.1.3

4.1 Proposed licence conditions

4.1.1 Consideration of limits and controls proposed by QUT

187. Sections 3.2 and 3.3 of Chapter 1 provide details of the limits and controls proposed by QUT in their application, and discussed in the characterisation of events in Chapter 2. The appropriateness of these limits and controls is considered further below.

188. The proposed release would be confined to one site, which occurs within a State Government Research Station and is staffed by personnel who will receive appropriate training in practices relevant to the handling and disposal of GMOs. This minimizes the potential for the GM banana plants to disperse into the environment (Event 3) or come into contact with the public (Event 1). The fact that the field location is on level land reduces the likelihood of plant material being inadvertently moved by landslip or erosion and the placement of the field location well away from, and above the maximum recorded flood height of, the nearest waterway minimizes the chance of plant material being washed away from the site (Event 3). Limiting the duration of the release to a four year period would also restrict the opportunity for GM plants to establish outside the site.

189. The applicant's proposal to remove and dispose of male flowers, or bag any male flowers remaining on the plant, will minimize the chances of pollen dispersing in the unlikely event that pollen is produced (Event 4). Since Cavendish bananas also produce hermaphrodite flowers that can contain pollen, it would be appropriate to also remove or bag hermaphrodite flowers.

190. The covering of bunches on plants is a standard practice that is used in commercial, non-GM banana cultivation to protect the developing fruit from being eaten or damaged by frugivores as well as optimising ripening conditions (Broadley et al. 2004). This practice as applied to GM fruit would minimise the likelihood of adverse effects on frugivores (e.g. toxicity) (Event 1) and fruit/seed being dispersed, in the unlikely event that any seed is produced (Event 3).

191. During commercial cultivation of banana plants, it is necessary to undertake desuckering and removal of dead leaves for both disease management and encouragement of plant vigour. Desuckering is usually done by either cutting off suckers at ground level, gouging out the centre and then pouring in kerosene or distillate, or by using a gouging tool. (Broadley et al. 2004). The leaf material is usually cut from the plant and left as trash on the ground (Broadley et al. 2004). The applicant proposes to desucker plants at the field location using the kerosene/distillate method and to leave all waste on the ground at the field location to decompose. It is proposed that waste from the shadehouse used for hardening-off would be transported to the field location and left to decompose on the ground. Whole plants (that would not be more than approximately 4 months old and therefore still immature) would additionally be sprayed with herbicide, so as to destroy the corm, before their transfer to the ground at the field location. As the waste from both locations will be non-propagative and unlikely to contain products that are harmful to any organisms that may eat it (see Section 2.1 of this Chapter), the practice is considered to be appropriate for preventing dispersal (Event 3).

192. The applicant proposes destroying fruit that is not required for experimental analysis by removing it from the plant and allowing it to decompose in lockable commercial waste bins located at the field location. The addition of agricultural lime would hasten decomposition. Once the material has fully decomposed (i.e. is odourless and would not be of interest to frugivores) it will be removed from the bins and left on the ground at the field location. This practice would ensure that the fruit will not be consumed by frugivores (Events 1 & 3).

193. Similarly, it has been proposed that male flowers not required for analysis will be removed from the plants prior to the opening of the floral bracts which enclose the flowers, and allowed to decompose in waste bins before the decomposed material is left on the ground at the field location. This practice would ensure that any pollen that may be produced will not be viable or dispersed into the environment.

194. The applicant has stated that any plant material taken off-site for experimental analysis will be transported according to the OGTR *Guidelines for the transport of GMOs, July 2007* (<http://www.ogtr.gov.au/pubform/handbook.htm#guidelines>) and will be destroyed by autoclaving immediately after analysis. These are standard protocols for the handling of GMOs to minimize exposure of the GMO to human and other organisms (Event 1), dispersal into the environment (Event 3), and gene flow/transfer (Events 4 & 5)

195. The large-scale eradication of plants in banana plantations on level land can be achieved by track rolling (the rolling and squashing of plants) or knocking over the plants and then discing the row to destroy the leaves, stem and corm (Lindsay et al. 2003; Broadley et al. 2004). Destruction of plants is also routinely achieved through the application of systemic herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) amine or glyphosate (Lindsay et al. 2003; Broadley et al. 2004). This method involves injecting each stem (including attached suckers) with the herbicide. The systemic nature of the herbicide means that the whole plant, including the corm, starts to die and decay rapidly and virtually no regrowth occurs (Lindsay et al. 2003). Plants treated in this way and left over the summer and wet season period are in an advanced state of decay by the end of the wet season. The applicant's proposal to destroy

plants at the end of the trial using 2,4-D or glyphosate, to leave the plant material on the ground to decompose and to subsequently monitor the field location for a period of 12 months (and destroy any volunteer suckers) would prevent spread and dissemination (Event 3).

196. In addition to the above points, Queensland State Government legislation targeted to the restriction of non-GM banana growing (see Chapter 1, paragraph 82) will also apply to the release of GM bananas and will act as an effective adjunct to the proposed containment measures.

4.1.2 Summary of measures imposed by the Regulator to limit and control the proposed release

197. A number of licence conditions have been imposed to limit and control the proposed release, which are described in detail in Chapter 4. These include requirements to:

- ♦ conduct the release on a total area of up to 1.4 ha at one site in the local government area of Cassowary Coast (Queensland) between May 2008 and May 2012
- ♦ remove and destroy all male/hermaphrodite flowers on the inflorescences unless they are required for experimental analysis;
- ♦ cover any male/hermaphrodite flowers left on the inflorescences;
- ♦ cover fruit bunches;
- ♦ remove and destroy all fruit not required for experimental analysis;
- ♦ destroy any plant waste containing meristematic tissue;
- ♦ clean all equipment used in cultivation practices;
- ♦ not permit any materials from the release to be used in human food or animal feed; and
- ♦ at the end of the trial, destroy all plant materials not required for further analysis.

4.1.3 Measures to control other activities associated with the trial

198. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs, July 2007; Policy on transport and supply of GMOs, July 2005*). Licence conditions based on these guidelines and policies have been proposed regarding transportation and storage, and to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

199. Conditions applying to the conduct of experimental analyses are also included in the licence conditions.

4.2 Other risk management considerations

200. All DIR licences issued by the Regulator contain a number of general conditions that relate to general risk management. These include, for example:

- ♦ applicant suitability
- ♦ contingency and compliance plans
- ♦ identification of the persons or classes of persons covered by the licence
- ♦ reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment

- ♦ a requirement that the applicant allows access to the site by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

201. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under section 58 of the Act matters that the Regulator must take into account include:

- ♦ any relevant convictions of the applicant (both individuals and the body corporate)
- ♦ any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- ♦ the applicant's history of compliance with previous approved dealings
- ♦ the capacity of the applicant to meet the conditions of the licence.

202. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers QUT suitable to hold a licence.

203. The licence conditions include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability or their capacity to meet the conditions of the licence.

204. QUT must continue to have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2.2 Compliance and contingency plans

205. Prior to planting the GM banana lines, QUT is required to submit a plan detailing how it intends to ensure compliance with the licence conditions and to document that compliance.

206. QUT is required to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan would detail measures to be undertaken in the event of any unintended presence of the GM banana lines outside of the permitted areas.

207. QUT is also required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic materials in a recipient organism. This instrument is required within 30 days of the issue date of the licence.

4.2.3 Identification of the persons or classes of persons covered by the licence

208. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence.

4.2.4 Reporting structures

209. The licence obliges the licence holder to immediately report any of the following to the Regulator:

- ♦ any additional information regarding risks to the health and safety of people or the environment associated with the trial
- ♦ any contraventions of the licence by persons covered by the licence
- ♦ any unintended effects of the trial.

210. The licence holder is also obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

211. A number of written notices are required under the licence that will assist the OGTR in designing and implementing a monitoring program for all licensed dealings. The notices include:

- ◆ expected and actual dates of planting
- ◆ expected and actual dates of final harvest
- ◆ expected and actual dates of destruction and cleaning after final harvest.

4.2.5 Monitoring for Compliance

212. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing. Post-release monitoring continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the site.

213. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

214. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. These include the provision for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to health and safety of people or the environment could result.

Section 5 Issues to be addressed for future releases

215. Additional information has been identified that may be required to assess an application for a large scale or commercial release of any of these GM banana lines that may be selected for further development, or to justify a reduction in containment conditions. This includes:

- ◆ characterisation of the introduced genetic material in the plants, including copy number and genotypic stability;
- ◆ additional data on the potential toxicity of plant materials from the GM banana lines;
- ◆ additional data on the allergenicity of proteins encoded by the introduced genes for enhanced nutrition; and
- ◆ characteristics indicative of weediness including measurement of altered reproductive capacity; tolerance to environmental stresses; and disease susceptibility.

Section 6 Conclusions of the RARMP

216. The risk assessment concludes that this proposed limited and controlled release of up to 1,290 GM banana lines on a maximum total area of 1.4 ha over four years in the Queensland local government area of Cassowary Coast poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

217. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. If a licence were to be issued, conditions are proposed to limit the proposed release to the size, location and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

References

Al-Babili, S., Hoa, T.T.C., Schaub, P. (2006). Exploring the potential of the bacterial carotene desaturase *Crt1* to increase the β -carotene content in Golden Rice. *Journal of Experimental Botany* **57**: 1007-1014.

American Society of Plant Physiologists (2000). *Biochemistry & Molecular Biology of Plants*. Buchanan, B.B., Gruissem, W., Jones, R.L. (eds). American Society of Plant Physiologists, Rockville, Maryland.

Arts, J., Mommers, C., de Heer, C. (2006). Dose-response relationships and threshold levels in skin and respiratory allergy. *Critical review in Toxicology* **36**: 219-251.

Barrett, C., Cobb, E., McNicol, R., Lyon, G. (1997). A risk assessment study of plant genetic transformation using *Agrobacterium* and implications for analysis of transgenic plants. *Plant Cell, Tissue and Organ Culture* **47**: 135-144.

Biosecurity Australia (2007). Revised Draft Import Risk Analysis Report for the Importation of Cavendish Bananas from the Philippines, Parts B & C. Commonwealth of Australia, Canberra, available online at http://www.daff.gov.au/_data/assets/pdf_file/0006/157965/2007-06b.pdf (Part B) and http://www.daff.gov.au/_data/assets/pdf_file/0007/157966/2007-06c.pdf (Part C).

Blattner, F.R., Plunkett, G., Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., Gregor, J., Davis, N.W., Kirkpatrick, H.A., Goeden, M.A., Rose, D.J., Mau, B., Shao, Y. (1997). The complete genome sequence of *Escherichia coli* K-12. *Science* **277**: 1453-1462.

Bradford, K.J., van Deynze, A., Gutterson, N., Parrot, W., Strauss, S.H. (2005). Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. *Nature Biotechnology* **23**[4], 439-444.

Broadley, R., Rigden, P., Chay-Prove, P., Daniells, J. (2004). *Subtropical Banana Grower's Handbook*. Queensland Department of Primary Industries, pp 1-206.

Cahoon, E.B., Hall, S.E., Ripp, K.G., Ganzke, T.S., Hitz, W.D., Coughlan, S.J. (2003). Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. *Nature Biotechnology* **21**: 1082-1087.

Charity, J.A., Klimaszewska, K. (2005). Persistence of *Agrobacterium tumefaciens* in transformed conifers. *Environmental and Biosafety Research* **4**: 167-177.

Christensen, A.H., Sharrock, R.A., Quail, P.H. (1992). Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. *Plant Molecular Biology* **18**: 675-689.

Connolly, E.L., Campbell, N.H., Grotz, N., Prichard, C.L., Guerinot, M.L. (2003). Overexpression of the FRO2 ferric chelate reductase confers tolerance to growth on low iron and uncovers posttranscriptional control. *Plant Physiology* **133**: 1102-1110.

- Connolly, E.L., Fett, J.P., Guerinot, M.L. (2002). Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. *The Plant Cell* **14**: 1347-1357.
- Cubero, J., López, M.M. (2004). Agrobacterium Persistence in Plant Tissues After Transformation. In: *Methods in Molecular Biology, Vol 286: Transgenic Plants: Methods and Protocols*. Humana Press Inc., Totowa, NJ.
- Danhorn, T., Merritt, P. M., Tomlinson, A. D., Hibbing, M. E., Fuqua, C. (2008). Sticking on a point: Agrobacterium adherence and biofilm formation. *Biofilms 2007*. Proceedings of 4th American Society of Microbiology Conference on Biofilms, 25 - 29 March 2007, Quebec, available online at <http://www.asm.org/ASM/files/ccLibraryFiles/Filename/000000002940/Program%20Abstract%20Book.pdf>.
- de la Riva, G.A., González-Cabrera, J., Vázquez-Padrón, R., Ayra-Pardo, C. (1998). *Agrobacterium tumefaciens*: a natural tool for plant transformation. *Electronic Journal of Biotechnology (on-line)* **1**: available online at <http://www.ejbiotechnology.info/content/vol1/issue3/full/1/>.
- De Vries, J., Meier, P., Wackernagel, W. (2001). The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. by transgenic plant DNA strictly depends on homologous sequences in the recipient cells. *FEMS Microbiology Letters* **195**: 211-215.
- DellaPenna, D., Pogson, B.J. (2006). Vitamin synthesis in plants: Carotenoids and tocopherols. *Annual Review of Plant Biology* **57**: 711-738.
- Depicker, A., Stachel, S., Dhaese, P., Zambryski, P., Goodman, H.M. (1982). Nopaline synthase: transcript mapping and DNA sequence. *Journal of Molecular and Applied Genetics* **1**: 561-573.
- Dörmann, P. (2007). Functional diversity of tocopherols in plants. *Planta* **225**: 269-276.
- Drakakaki, G., Marcel, S., Glahn, R.P., Lund, E.K., Pariagh, S., Fischer, R., Christou, P., Stoger, E. (2006). Endosperm-specific co-expression of recombinant soybean ferritin and *Aspergillus* phytase results in significant increases in the levels of bioavailable iron. *Plant Molecular Biology* **59**: 869-880.
- Dugdale, B., Becker, D.K., Harding, R.M., Dale, J.L. (2001). Intron-mediated enhancement of the banana bunchy top virus DNA-6 promoter in banana (*Musa* spp.) embryogenic cells and plants. *Plant Cell Reports* **20**: 220-226.
- Dugdale, B., Beetham, P.R., Becker, D.K., Harding, R.M., Dale, J.L. (1998). Promoter activity associated with the intergeneric regions of banana bunchy top virus DNA-1 to -6 in transgenic tobacco and banana cells. *Journal of General Virology* **79**: 2301-2311.
- Eby, P. (1995). The biology and management of flying foxes in NSW. Report No. Species management report number 18, NSW National Parks and Wildlife Service, Hurstville, NSW.
- EC (2000). Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Beta Carotene. Report No. SCF/CS/NUT/UPPLEV/37 Final, European Commission

Scientific Committee on Food, Brussels, available online at http://ec.europa.eu/food/fs/sc/scf/out80b_en.pdf.

EC (2003). Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Vitamin E. Report No. SCF/CS/NUT/UPPLEV/31 Final, European Commission Scientific Committee on Food, Brussels, available online at http://ec.europa.eu/food/fs/sc/scf/out195_en.pdf.

EFSA (2007). Statement of the Scientific Panel on Genetically Modified Organisms on the safe use of the *nptII* antibiotic resistance marker gene in genetically modified plants. Adopted on 22-23 March 2007. European Food Safety Authority, available online at http://www.efsa.europa.eu/EFSA/Statement/gmo_statement_nptII.pdf.

Englberger, L., Schierle, J., Aalbersberg, W., Hofmann, P., Humphries, J., Huang, A., Lorens, A., Levendusky, A., Daniells, J., Marks, G.C., Fitzgerald, M.H. (2006a). Carotenoid and vitamin content of *Karat* and other Micronesian banana cultivars. *International Journal of Food Sciences and Nutrition* **57**: 399-418.

Englberger, L., Wills, R.B.H., Blades, B., Dufficy, L., Daniells, J.W., Coyne, T. (2006b). Carotenoid content and flesh color of selected banana cultivars growing in Australia. *Food and Nutrition Bulletin* **27**: 281-291.

EPA (2001). β -D Glucuronidase from *E. coli* and the genetic material necessary for its production as a plant pesticide inert ingredient: exemption from the requirement of a tolerance. *Federal Register* **66**: 42957-42962.

Escobar, M.A., Dandekar, A.M. (2003). *Agrobacterium tumefaciens* as an agent of disease. *Trends in Plant Science* **8**: 380-386.

Expert Group on Vitamins and Minerals (2003). Safe Upper Levels for Vitamins and Minerals. available online at <http://www.food.gov.uk/multimedia/pdfs/vitmin2003.pdf>.

FAO/WHO (2001). Human Vitamin and Mineral Requirements. Report of a Joint FAO/WHO Expert Consultation, Bangkok, Thailand, September 1998. Food and Agriculture Organization of the United Nations/World Health Organization, Food and Nutrition Division, FAO, Rome, available online at <ftp://ftp.fao.org/docrep/fao/004/y2809e/y2809e00.pdf>.

Felsot, A.S. (2000). Insecticidal genes. Part 2: Human health hoopla. *Agrichemical & Environmental News* **168**: 1-7.

Flavell, R.B., Dart, E., Fuchs, R.L., Fraley, R.T. (1992). Selectable marker genes: safe for plants? *Biotechnology (NY)* **10**: 141-144.

Fortescue, J.A., Turner, D.W. (2004). Pollen fertility in *Musa*: Viability in cultivars grown in Southern Australia. *Australian Journal of Agricultural Research* **55**: 1085-1091.

FSANZ (2006). NUTTAB 2006, Nutrient Data for Australian Foods. Food Standards Australia New Zealand, available online at http://www.foodstandards.gov.au/_srcfiles/Final%20NUTTAB%202006%20Food%20Composition%20Tables%20-%20May%202007.pdf.

FSANZ (2007). Standard 1.3.2 Vitamins and Minerals. Australia New Zealand Food Standards Code (incorporating amendments up to and including Amendment 95), Food Standards Australia New Zealand, Commonwealth of Australia, Canberra, available online at <http://www.foodstandards.gov.au/thecode/foodstandardscode.cfm>.

Gebhard, F., Smalla, K. (1998). Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Applied and Environmental Microbiology* **64**: 1550-1554.

Gilissen, L.J.W., Metz, P.L.J., Stiekema, W.J., Nap, J.P. (1998). Biosafety of *E.coli* β -glucuronidase (GUS) in plants. *Transgenic Research* **7**: 157-163.

Glover, J. (2002). Gene flow study: Implications for the release of genetically modified crops in Australia. Bureau of Rural Sciences, Australian Government Department of Agriculture, Fisheries and Forestry, Canberra.

Goodman, R.E., Vieths, S., Sampson, H.A., Hill, D., Ebisawa, M., Taylor, S.L., van Ree, R. (2008). Allergenicity assessment of genetically modified crops - what makes sense? *Nature Biotechnology* **26**: 73-81.

Hammerschlag, F.A., Liu, Q., Zimmerman, R.H., Gercheva, P. (2000). Generating Apple Transformants Free of *Agrobacterium tumefaciens* by Vacuum Infiltrating Explants with an Acidified Medium and with Antibiotics. [530], 103-111. *Acta Horticulturae. Proceedings of the International Symposium on Methods and Markers for Quality Assurance in Micropropagation*. Cassells, A. C., Doyle, B. M., and Curry, R. F.

Haslberger, A.G. (2003). Codex guidelines for GM foods include the analysis of unintended effects. *Nature Biotechnology* **21**: 739-741.

Hess, S.Y., Thurnham, D.I., and Hurrell, R.F. (2005). Influence of Provitamin A Carotenoids on Iron, Zinc, and Vitamin A Status. HarvestPlus Technical Monograph 6, International Food Policy Research Institute & International Center for Tropical Agriculture, available online at <http://www.harvestplus.org/pdfs/tech06.pdf>.

Horvath, G., Wessjohann, L., Bigirimana, J., Jansen, M., Guisez, Y., Caubergs, R., Horemans, N. (2006). Differential distribution of tocopherols and tocotrienols in photosynthetic and non-photosynthetic tissues. *Phytochemistry* **67**: 1185-1195.

JECFA (1974). Seventeenth Report of the Joint FAO/WHO Expert Committee on Food Additives: Alpha-Tocopherol and Mixed Tocopherols Concentrate. Report No. WHO Food Additive Series No. 5, Joint FAO/WHO Expert Committee on Food Additives, World Health Organization, Geneva, available online at <http://www.inchem.org/documents/jecfa/jecmono/v05je33.htm>.

JECFA (1975). Eighteenth Report of the Joint FAO/WHO Expert Committee on Food Additives: Beta-Carotene. Report No. WHO Food Additive Series 6, Joint FAO/WHO Expert Committee on Food Additives, World Health Organization, Geneva, available online at <http://www.inchem.org/documents/jecfa/jecmono/v06je15.htm>.

JECFA (1983). Iron. Report No. WHO Food Additive Series 18, Joint FAO/WHO Expert Committee on Food Additives, World Health Organization, Geneva, available online at <http://www.inchem.org/documents/jecfa/jecmono/v18je18.htm>.

- JECFA (1987). Alpha-Tocopherol. Report No. WHO Food Additive series 21, Joint FAO/WHO Expert Committee on Food Additives, World Health Organization, Geneva, available online at <http://www.inchem.org/documents/jecfa/jecmono/v21je05.htm>.
- JECFA (1993). Carotenes from Natural Sources (Algal and Vegetable). Joint FAO/WHO Expert Committee on Food Additives, World Health Organization, Geneva, available online at <http://www.inchem.org/documents/jecfa/jecmono/v32je07.htm>.
- Kahl, G. (2001). *The dictionary of gene technology: genomics, transcriptomics, proteomics*. Wiley-VCH, Weinheim, Germany. pp 1-941.
- Khanna, H., Becker, D., Kleidon, J., Dale, J. (2004). Centrifugation assisted *Agrobacterium tumefaciens*-mediated transformation (CAAT) of embryogenic cell suspensions of banana (*Musa* spp. Cavendish AAA and Lady Finger AAB). *Molecular Breeding* **14**: 239-252.
- Korshunova, Y.O., Eide, D., Clark, W.G., Guerinot, M.L., Pakrasi, H.B. (1999). The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range. *Plant Molecular Biology* **40**: 37-44.
- Krimi, Z., Petit, A., Mougel, C., Dessaux, Y., Nesme, X. (2002). Seasonal fluctuations and long-term persistence of pathogenic populations of *Agrobacterium* spp. in soils. *Applied and Environmental Microbiology* **68**: 3358-3365.
- Lazo, G.R., Stein, P.A., Ludwig, R.A. (1991). A DNA transformation-competent *Arabidopsis* genomic library in *Agrobacterium*. *Biotechnology* **9**: 963-967.
- Leifert, C. (2000). Quality Assurance Systems for Plant Cell and Tissue Culture; The Problem of Latent Persistence of Bacterial Pathogens and *Agrobacterium*-Based Transformation Vector Systems. [530], 87-91. Acta Horticulturae. Proceedings of the International Symposium on Methods and Markers for Quality Assurance in Micropropagation. Cassells, A. C., Doyle, B. M., and Curry, R. F.
- Leifert, C., Cassells, A.C. (2001). Microbial hazards in plant tissue and cell cultures. *In Vitro Cellular and Developmental Biology-Plant* **37**: 133-138.
- Lindsay, S., Pattison, T., and Murad, Z. (2003). Eradicating banana crops with herbicide injection for better IPM and environmental outcomes. Report No. 31, Bananatopics, Agency for Food & Fibre Sciences, DPI, South Johnstone, Queensland,
- Lucca, P., Hurrell, R., Potrykus, I. (2002). Fighting iron. *Journal of the American College of Nutrition* **21**: 184S-190S.
- Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, L.A., Flint, H.J. (1999). Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Applied and Environmental Microbiology* **65**: 6-10.
- Miki, B., McHugh, S. (2004). Selectable marker genes in transgenic plants: applications, alternatives and biosafety. *Journal of Biotechnology* **107**: 193-232.
- Morton, J.F. (1987). Banana *Musa x paradisiaca*. In: *Fruits of Warm Climates*. Julia F. Morton, Distributed by Creative Resource Systems, Inc., Miami, Florida, pp 29 - 46, available online at <http://www.hort.purdue.edu/newcrop/morton/banana.html>.

- Munné-Bosch, S., Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. *Critical Reviews in Plant Sciences* **21**: 31-57.
- NH&MRC (2006). Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes. National Health and Medical Research Council, Commonwealth of Australia, Canberra, available online at http://www.nhmrc.gov.au/publications/synopses/_files/n35.pdf.
- Nielsen, K.M. (1998). Barriers to horizontal gene transfer by natural transformation in soil bacteria. *Acta pathologica, microbiologica, et immunologica Scandinavica* **106**: 77-84.
- Nielsen, K.M., van Elsas, J.D., Smalla, K. (2000). Transformation of *Acinetobacter* sp strain BD413(pFG4 Delta nptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. *Applied and Environmental Microbiology* **66**: 1237-1242.
- Odell, J.T., Nagy, F., Chua, N.H. (1985). Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. *Nature* **313**: 810-812.
- OGTR (2007). Risk Analysis Framework. Report No. Version 2.2, Document produced by the Australian Government Office of the Gene Technology Regulator, available online from <http://www.ogtr.gov.au/>.
- OGTR (2008). The Biology of *Musa* L. (banana). Document prepared by the Australian Government Office of the Gene Technology Regulator, Canberra, available online at <http://www.ogtr.gov.au/ir/dir076.htm>.
- Ohta, S., Mita, S., Hattori, T., Nakamura, K. (1990). Construction and expression in tobacco of a beta glucuronidase (GUS) reporter gene containing an intron within the coding sequence. *Plant Cell Physiology* **31**: 805-813.
- Paine, J.A., Shipton, C.A., Chaggar, S., Howells, R.M., Kennedy, M.J., Vernon, G., Wright, S.Y., Hinchliffe, E., Adams, J.L., Silverstone, A.L., Drake, R. (2005). Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology advance online publication*: 1-6.
- Queensland Government (1999). Plant Protection (Banana Pest Quarantine) Regulation 1999. Queensland Government, available online at <http://www.legislation.qld.gov.au/LEGISLTN/SLS/1999/99SL310.pdf>.
- Reiting, R., Broll, H., Waiblinger, H.-U., Grohmann, L. (2007). Collaborative study of a T-nos real-time PCR method for screening of genetically modified organisms in food products. *Journal of Consumer Protection and Food Safety* **2**: 116-121.
- Robinson, N.J., Procter, C.M., Connolly, E.L., Guerinot, M.L. (1999). A ferric-chelate reductase for iron uptake from soils. *Nature* **397**: 694-697.
- Rosenblueth, M., Martínez-Romero, E. (2006). Bacterial endophytes and their interactions with hosts. *Molecular Plant-Microbe Interactions* **19**: 827-837, available online at <http://apsjournals.apsnet.org/doi/pdf/10.1094/MPMI-19-0827>.

- Ross, E.M. (1987). Musaceae. In: AS George, ed. *Flora of Australia Volume 45, Hydatellaceae to Liliaceae*. Australian Government Publishing Service, Canberra. pp 16-19.
- Sen, C.K., Khanna, S., Rink, C., Roy, S. (2007). Tocotrienols: the emerging face of natural vitamin E. *Vitamins and Hormones* **76**: 203-261.
- Sharrock, S. (2000). Diversity in the genus *Musa*: Focus on *Australimusa*. International Network for the Improvement of Banana and Plantain, Montpellier, France, available online at http://bananas.bioversityinternational.org/files/files/pdf/publications/an00_en.pdf.
- Theil, E.C. and Briat, J.-F. (2004). Plant Ferritin and Non-Heme Iron Nutrition in Humans. HarvestPlus Technical Monograph 1, International Food Policy Research Institute, Washington DC, available online at <http://www.harvestplus.org/pdfs/tech01.pdf>.
- Theil, E.C., Burton, J.W., Beard, J.L. (1997). A sustainable solution for dietary iron deficiency through plant biotechnology and breeding to increase seed ferritin control. *European Journal of Clinical Nutrition* **52 Suppl 4**: S28-S31.
- Thomson, J.A. (2001). Horizontal transfer of DNA from GM crops to bacteria and to mammalian cells. *Journal of Food Science* **66**: 188-193.
- Trivedi, P.K., Nath, P. (2004). *MaExp1*, an ethylene-induced expansin from ripening banana fruit. *Plant Science* **167**: 1351-1358.
- Valentine, L. (2003). *Agrobacterium tumefaciens* and the Plant: The David and Goliath of Modern Genetics. *Plant Physiology* **133**: 948-955.
- Van Wuytswinkel, O., Vansuyt, G., Grignon, N., Fourcroy, P., Briat, J.-F. (1998). Iron homeostasis alteration in transgenic tobacco overexpressing ferritin. *The Plant Journal* **17**: 93-97.
- Vansuyt, G., Mench, M., Briat, J.-F. (2000). Soil-dependent variability of leaf iron accumulation in transgenic tobacco overexpressing ferritin. *Plant Physiology and Biochemistry* **38**: 499-506.
- Vert, G., Grotz, N., Dédaldéchamp, F., Gaymard, F., Guerinot, M.L., Briat, J.-F., Curie, C. (2002). ITR1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. *The Plant Cell* **14**: 1223-1233.
- Waines, J.G., Hedge, S.G. (2003). Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. *Crop Science* **43**: 451-463.
- Welch, R.M., Graham, R.D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* **55**: 353-364.
- Wong, E.Y., Hironaka, C.M., Fischhoff, D.A. (1992). *Arabidopsis thaliana* small subunit leader and transit peptide enhance the expression of *Bacillus thuringiensis* proteins in transgenic plants. *Plant Molecular Biology* **20**: 81-93.
- Yang, I.C., Iommarini, J.P., Becker, D.K., Hafner, G.J., Dale, J.L., Harding, R.M. (2003). A promoter derived from taro bacilliform badnavirus drives strong expression in transgenic banana and tobacco plants. *Plant Cell Reports* **21**: 1199-1206.

Ye, X., Al-Babili, S., Klöti, A., Zhang, J., Lucca, P., Beyer, P., Potrykus, I. (2000). Engineering the provitamin A (β -carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* **287**: 303-305.

Zeiger, E., Zhu, J. (1998). Role of zeaxanthin in blue light photoreception and the modulation of light-CO₂ interactions in guard cells. *Journal of Experimental Botany* **49**: 433-442.

Appendix A Definitions of terms in the Risk Analysis Framework used by the Regulator

(* terms defined as in Australia New Zealand Risk Management Standard AS/NZS 4360:2004)

Consequence

outcome or impact of an adverse event

Marginal: there is minimal negative impact

Minor: there is some negative impact

Major: the negative impact is severe

Event*

occurrence of a particular set of circumstances

Hazard*

source of potential harm

Hazard identification

the process of analysing hazards and the events that may give rise to harm

Intermediate

the negative impact is substantial

Likelihood

chance of something happening

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Quality control

to check, audit, review and evaluate the progress of an activity, process or system on an ongoing basis to identify change from the performance level required or expected and opportunities for improvement

Risk

the chance of something happening that will have an undesired impact

Negligible: risk is insubstantial and there is no present need to invoke actions for mitigation

Low: risk is minimal but may invoke actions for mitigation beyond normal practices

Moderate: risk is of marked concern requiring mitigation actions demonstrated to be effective

High: risk is unacceptable unless actions for mitigation are highly feasible and effective

Risk analysis

the overall process of risk assessment, risk management and risk communication

Risk analysis framework

systematic application of legislation, policies, procedures and practices to analyse risks

Risk assessment

the overall process of hazard identification and risk estimation

Risk communication

the culture, processes and structures to communicate and consult with stakeholders about risks

Risk Context

parameters within which risk must be managed, including the scope and boundaries for the risk assessment and risk management process

Risk estimate

a measure of risk in terms of a combination of consequence and likelihood assessments

Risk evaluation

the process of determining risks that require treatment

Risk management

the overall process of risk evaluation, risk treatment and decision making to manage potential adverse impacts

Risk management plan

integrates risk evaluation and risk treatment with the decision making process

Risk treatment*

the process of selection and implementation of measures to reduce risk

Stakeholders*

those people and organisations who may affect, be affected by, or perceive themselves to be affected by a decision, activity or risk

States

includes all State governments, the Australian Capital Territory and the Northern Territory governments

Uncertainty

imperfect ability to assign a character state to a thing or process; a form or source of doubt

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities¹⁸ on the consultation RARMP for DIR 076/2007

The Regulator received several submissions from prescribed experts, agencies and authorities on the consultation RARMP. One of the submissions raised a concern relating to risks to the environment that had not been considered in the consultation RARMP and which is summarised below:

Summary of issues raised	Where addressed in the RARMP
Risk of adverse effects as a result of the presence in GM banana plant material of residual <i>Agrobacterium tumefaciens</i> containing the introduced genes.	Event 8, Section 2.6, Chapter 2 – no risk was identified

¹⁸ GTTAC, State and Territory governments, Australian Government agencies, the Minister for Environment, Heritage & the Arts and the Local council where the release may occur.

Appendix C Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 076/2007

The Regulator received one submission from the public on the consultation RARMP. This submission, summarised in the table below, raised an issue relating to human health and safety. This was considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Submission Type: I: Individual.

Issues raised: H: Human health and safety

Other abbreviations: GM: Genetically Modified; FSANZ: Food Standards Australia New Zealand

Sub. No:	Type	Summary of issues raised	Issues raised	Comment
1	I	There is a lack of published epidemiological research on the long-term effects of 'nutritionally enhanced' bananas on human health	H	As noted in Section 7, Chapter 1, there have been no previous releases of bananas genetically modified for enhanced nutrition and this trial involves proof of concept research. It is a condition of the licence that plant material from the GM bananas not be used as human food or animal feed. FSANZ approval would need to be obtained before GM bananas could be consumed by humans.