Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 146

Limited and controlled release of banana genetically modified for disease resistance

Applicant: Queensland University of Technology

December 2016

PAGE INTENTIONALLY LEFT BLANK

Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 146

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for the limited and controlled release (field trial) of a genetically modified organism (GMO) into the environment. A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with the requirements of the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that the field trial poses negligible risks to human health and safety and the environment and that any risks posed by the dealings can be managed by imposing conditions on the release.

The application

Application number	DIR 146
Applicant	Queensland University of Technology (QUT)
Project title	Limited and controlled release of banana genetically modified for disease resistance
Parent organism	Banana (*Musa acuminata* L. and *M. acuminata x M. balbisiana*)
Introduced genes and modified traits	Each GM banana line[1] would contain only one of the following ten genes conferring resistance to *Fusarium* wilt: • Eight genes putatively involved in providing resistance to *Fusarium* wilt disease, all derived from banana[2] • One stress tolerance gene derived from banana[3] • One anti-apoptotic gene derived from the nematode *Caenorhabditis elegans* The GM banana lines may also contain this selectable marker gene: • *nptII (*neomycin phosphotransferase type II) gene from bacterium *Escherichia coli* as a selectable marker that confers tolerance to antibiotics such as kanamycin and neomycin
Proposed location	One site in Litchfield Municipality, Northern Territory
Proposed release size	Up to 6 hectares (ha) in total
Proposed release dates	January 2017 – January 2022
Primary purpose	To evaluate the resistance to *Fusarium* wilt disease and agronomic performance of the GM banana lines under field conditions.

[1] The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.
[2] The identities of seven of these genes have been declared as Confidential Commercial Information (CCI).
[3] The identity of this gene has been declared as CCI.

Risk assessment

The risk assessment concludes that risks to the health and safety of people, or the environment, from the proposed release are negligible. The risk assessment process considers how the genetic modification and proposed activities conducted with the GMOs might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account current scientific/technical knowledge, information in the application (including proposed limits and controls) and relevant previous approvals. Both the short and long term impacts are considered.

Credible pathways to potential harm that were considered included exposure of people or animals to the GM plant material, increased potential for spread and persistence of the GMOs, and transfer of the introduced genetic material to sexually compatible plants. Potential harms associated with these pathways included toxicity or allergenicity to people, toxicity to other desirable organisms, and environmental harms due to weediness.

The principal reasons for the conclusion of negligible risks are that the GM plant material will not be used for human food or animal feed, the proposed limits and controls effectively contain the GMOs and their genetic material and minimise exposure; and the GM banana has limited ability to establish populations outside cultivation or transfer the introduced genetic material to other plants.

Risk management plan

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the licence includes limits on the size, location and duration of the release, as well as controls to prohibit the use of GM plant material in human food or animal feed, to minimise dispersal of the GMO or GM pollen from trial sites, to transport GMOs in accordance with the Regulator's guidelines, to destroy GMOs not required for testing or further planting, and to conduct post-harvest monitoring at trial sites to ensure all GMOs are destroyed.

Table of Contents

DECISION III
THE APPLICATION III
RISK ASSESSMENT IV
RISK MANAGEMENT PLAN IV

TABLE OF CONTENTS V

ABBREVIATIONS VII

CHAPTER 1 RISK ASSESSMENT CONTEXT 1

SECTION 1 BACKGROUND 1
SECTION 2 REGULATORY FRAMEWORK 1
SECTION 3 THE PROPOSED DEALINGS 2
3.1 The proposed limits of the dealings (duration, size, location and people) 2
3.2 The proposed controls to restrict the spread and persistence of the GMOs in the environment 3
SECTION 4 THE PARENT ORGANISM 3
SECTION 5 THE GMOS, NATURE AND EFFECT OF THE GENETIC MODIFICATION 4
5.1 Introduction to the GMOs 4
5.3 Introduction to plant-pathogen interactions 7
5.4 The introduced genes, encoded proteins and their associated effects 8
5.5 Toxicity/allergenicity of the proteins associated with the introduced genes 12
5.6 Characterisation of the GMOs 12
SECTION 6 THE RECEIVING ENVIRONMENT 13
6.1 Relevant agronomic practices 13
6.2 Relevant abiotic factors 15
6.3 Relevant biotic factors 15
6.4 Presence of similar genes and encoded proteins in the environment 16
SECTION 7 RELEVANT AUSTRALIAN AND INTERNATIONAL APPROVALS 16
7.1 Australian approvals 16
7.2 International approvals 17

CHAPTER 2 RISK ASSESSMENT 18

SECTION 1 INTRODUCTION 18
SECTION 2 RISK IDENTIFICATION 19
2.1 Risk source 19
2.1.1 The introduced genes for Fusarium wilt resistance 19
2.1.2 The reporter and selectable marker genes 19
2.1.3 The regulatory sequences 20
2.1.4 Unintended effects resulting from the process of genetic modification 20
2.2 Causal pathway 20
2.2.1 Tolerance to abiotic and biotic factors 20
2.2.2 Gene transfer to sexually compatible relatives 21
2.2.3 Horizontal gene transfer 21
2.2.4 Unauthorised activities 21
2.3 Potential harm 21
2.3.1 Production of a substance toxic or allergenic to people or toxic to other organisms 21
2.4 Postulated risk scenarios 22
SECTION 3 UNCERTAINTY 26
SECTION 4 RISK EVALUATION 27

CHAPTER 3 RISK MANAGEMENT PLAN 28

SECTION 1 BACKGROUND 28
SECTION 2 RISK TREATMENT MEASURES FOR SUBSTANTIVE RISKS 28
SECTION 3 GENERAL RISK MANAGEMENT 28
3.1 Licence conditions to limit and control the release 28
3.1.1 Consideration of limits and controls proposed by QUT 28
3.1.2 Summary of draft licence conditions to be implemented to limit and control the release 30

3.2 Other risk management considerations 31
3.2.1 Applicant suitability 31
3.2.2 Contingency plan 31
3.2.3 Identification of the persons or classes of persons covered by the licence 31
3.2.4 Reporting requirements 32
3.2.5 Monitoring for compliance 32
SECTION 4 ISSUES TO BE ADDRESSED FOR FUTURE RELEASES 32
SECTION 5 CONCLUSIONS OF THE CONSULTATION RARMP 32
REFERENCES 33
APPENDIX A SUMMARY OF SUBMISSIONS FROM PRESCRIBED EXPERTS, AGENCIES AND AUTHORITIES 38
APPENDIX B SUMMARY OF SUBMISSIONS FROM THE PUBLIC 41

Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
Avr	avirulence
bp	Base pair
CaMV	Cauliflower mosaic virus
CCI	Confidential Commercial Information
ced-9	Cell death abnormality gene-9
DBFC	Darwin Banana Farming Company
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
Foc	*Fusarium oxysporum* forma specialis (f. sp) *cubense*
Foc TR4	*Fusarium oxysporum* forma specialis (f. sp) *cubense* (Foc) tropical race 4 (TR4)
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
ha	Hectare
HR	Hypersensitive response
km	Kilometres
LGA	Local Government Area
m	Metres
NBS-LRR	nucleotide binding site-leucine rich repeat
NLRD	Notifiable Low Risk Dealing
nptII	Neomycin phospotransferase II gene
NSW	New South Wales
NT	Northern Territory
OGTR	Office of the Gene Technology Regulator
PCD	Programmed cell death
PC2	Physical Containment level 2
QLD	Queensland
QUT	Queensland University of Technology
R gene	Gene conferring resistance to a particular pathogen
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RGA2	NBS-LRR type resistance gene
TEV	Tobacco etch virus
the Act	The *Gene Technology Act 2000*
TR4	Tropical race 4
WA	Western Australia

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.

2. The Act in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation in States and Territories, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

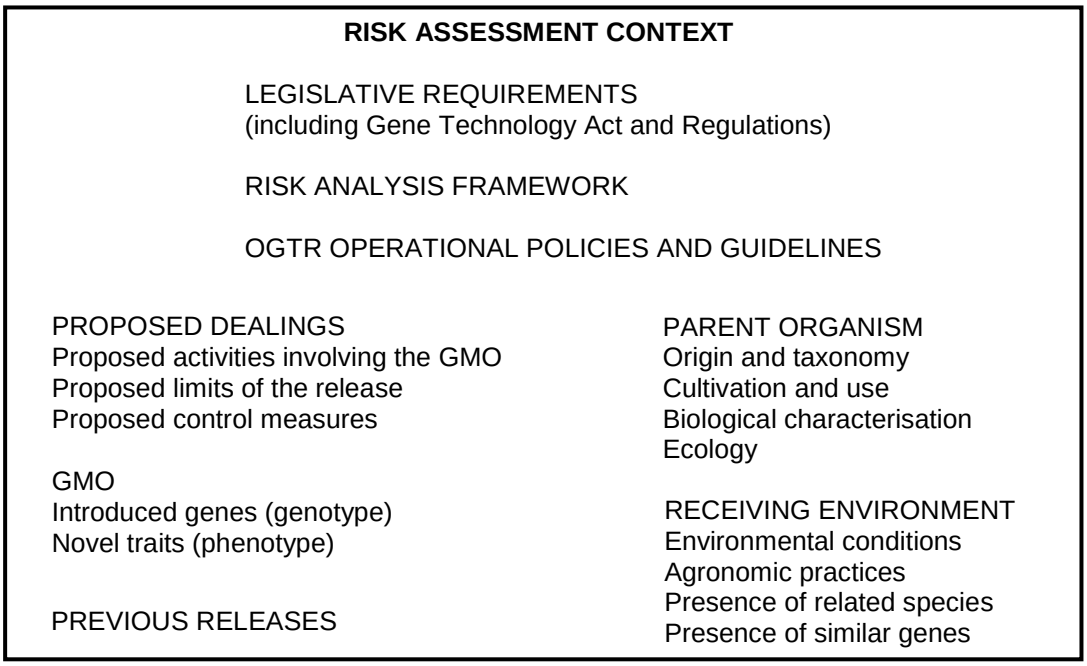

Figure 1. Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and who must be consulted, when preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.

5. In accordance with section 50A of the Act, this application is considered to be a limited and controlled release application, as its principal purpose is to enable the applicant to conduct experiments and the applicant has proposed limits on the size, location and duration of the release, as well as controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

6. 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, Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, relevant local council(s), and the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. Five public submissions were received and their considerations are summarised in Appendix B.

7. The *Risk Analysis Framework* (OGTR 2013b) explains the Regulator's approach to the preparation of RARMPs in accordance with the legislative requirements. Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the OGTR website.

8. Any dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration and the Department of Agriculture and Water Resources. These dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Section 3 The proposed dealings

9. Queensland University of Technology (QUT) proposes to release banana lines genetically modified for resistance to *Fusarium* wilt disease into the environment under limited and controlled conditions. The purpose of the release is to evaluate the level of resistance to the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* which causes *Fusarium* wilt disease and the agronomic performance of the GM banana lines under Australian field conditions. The applicant specifically wants to assess resistance to *Fusarium oxysporum* f. sp. *cubense* (Foc) tropical race 4 (TR4), hereafter referred to as Foc TR4.

10. Some of the gene source organisms and descriptions (i.e. gene identity, accession numbers, associated regulatory elements and relevant references) have been declared Confidential Commercial Information (CCI). In this document, CCI gene identities have been replaced with non-CCI identifiers. The remaining CCI has been removed and in its place 'CCI' is printed in red font. All relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

11. The dealings involved in the proposed intentional release are:

- conducting experiments with the GMOs
- propagating the GMOs
- growing the GMOs
- transporting the GMOs
- disposing of the GMOs and
- possession, supply or use of the GMOs for any of the purposes above.

These dealings are detailed further below.

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

12. The release is proposed to take place on one site at the Darwin Banana Farming Company (DBFC) located in Lambells Lagoon, near Humpty Doo, in the Litchfield Municipality, Northern Territory, on up to 6 hectares over a five year period from January 2017 to January 2022.

13. Only trained and authorised staff would be permitted to deal with the GM bananas.

3.2 The proposed controls to restrict the spread and persistence of the GMOs in the environment

14. The applicant has proposed a number of controls to restrict the spread and persistence of the GM banana and the introduced genetic material in the environment. These include:

- not allowing GM plant material or products to be used for human food or animal feed
- locating the proposed trial site on flat land at least 1 km away from the nearest natural waterway
- restricting human and animal access by surrounding the farm and trial site each with a fence with lockable gates; only trained staff would be permitted access to the trial site
- any non-GM banana plant material grown on site would be treated as GM banana plant material
- fruit bunches will be assessed on site and then destroyed on site by shredding and decomposition
- prior to leaving the site, all machinery will be inspected for plant material, which will be removed and destroyed on site
- although the parent plants are essentially sterile, pollen flow will be further restricted by removal of the male inflorescence (de-belled) or bagged using bunch covers
- restricting access of birds and bats to flowers and fruit by de-belling and the use of bunch covers
- transporting and storing GM plant materials in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*
- complying with State Government legislation for banana disease control that would also aid in containment of GM plants
- destroying all plant materials from the field trial by herbicide and/or mechanical treatment.

Section 4 The parent organism

15. The parent organism is banana (*Musa ssp.* L.). Bananas are grown commercially on the east coast of Australia from northern NSW to far north QLD. They are also grown in WA around Carnarvon, Kununurra and Broome and in the NT near Darwin.

16. Most edible bananas are intraspecific or interspecific hybrids of *Musa acuminata* and *M. balbisiana*. Six cultivars were used to generate the GM bananas proposed for release: Cavendish, Williams, Grande Naine, Dwarf Cavendish, Gros Michel and Lady Finger. Cavendish, Williams, Grande Naine and Dwarf Cavendish are closely related cultivars and belong in the Cavendish subgroup of the triploid intraspecific hybrid of *M. acuminata* (AAA genome). Gros Michel belongs to the Gros Michel subgroup and is also a triploid intraspecific hybrid of *M. acuminata* (AAA genome).The Lady Finger cultivar is in the Pome subgroup of the interspecific hybrid of *M. acuminata* and *M. balbisiana* (AAB genome).

17. Cultivars from the subgroup Cavendish account for approximately 95% of the bananas on the Australian market. Lady Finger comprises about 4% of the Australian market. Edible banana plants have extremely low fertility. Members of the Cavendish subgroup set seed so rarely that they can be regarded as female sterile, and produce so little viable pollen that they are effectively male sterile. Lady Finger bananas also have poor fertility and produce very little or no viable pollen and no seeds.

18. Detailed information about the parent organism is contained in a reference document, *The Biology of* Musa *L. (banana)* (OGTR 2016*)* which was produced to inform the risk assessment process for licence applications involving GM banana plants. Baseline information from this document will be used and referred to throughout the RARMP.

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

5.1 Introduction to the GMOs

19. The applicant proposes the release of GM banana lines each containing one of ten candidate genes for resistance to Foc TR4 (Table 1). These candidate genes were derived from banana species (some of which have been declared CCI), with the exception of *ced-9* which was derived from a nematode, *Caenorhabditis elegans* (Table 1). The candidate genes function either by providing resistance to the fungal pathogen, enhancing plant stress tolerance or by protecting plant cells post-fungal infection.

20. Initially all of the GM banana lines would contain the antibiotic resistance selectable marker gene *neomycin phosphotransferase type II* (*nptII*) from the common gut bacterium *Escherichia coli* (Table 1). This gene, encoding the enzyme neomycin phosphotransferase, confers kanamycin or neomycin resistance on GM plant cells. The *nptII* gene was used during initial development of the GM plants in the laboratory to select plant cells containing the introduced genes.

21. However, in some GM banana lines, the *nptII* gene will be excised using an inducible recombinase system (see Section 5.4.3 for further detail). As the excision of the *nptII* gene occurs in the laboratory prior to release of the GM lines into the field trial, the genetic elements involved have not been included in Tables 1 and 2.

22. Short regulatory sequences which control the expression of the introduced genes will also be introduced into the GM banana plants. These sequences are derived from plants (maize and banana), a soil bacterium (*Agrobacterium tumefaciens*) and the plant viruses Cauliflower mosaic virus (CaMV) and Tobacco etch virus (TEV) (see Table 2 and Section 5.5).

23. The applicant has provided brief descriptions for each of the candidate genes (some of which have been declared CCI). The applicant intends to gather more information on the effects of the introduced genes under this limited and controlled trial.

24. The GM banana lines were produced using *Agrobacterium tumefaciens* mediated plant transformation. Information about this transformation method can be found in the document Methods of plant genetic modification available from the OGTR Risk Assessment References page.

5.2 Introduction to *Fusarium* wilt

25. Fusarium wilt (Panama disease) is caused by a soil-borne fungal pathogen *Fusarium oxysporum* forma specialis (f. sp.) *cubense* (Foc) of which four physiologically distinct races have been identified (referred to as races 1-4) (see Ploetz (2006) and Paul et al. (2011) and references therein). Races 2 and 3 do not infect commercially relevant banana cultivars and thus are not considered economically important. Foc race 1 infects commercially important cultivars and in the early 1950s, it decimated major exporting bananas cultivars such as Gros Michel (AAA) and Lady Finger (AAB) in South and Central America. This lead to wide-spread use of race 1 resistant cultivars from the Cavendish subgroup (AAA). Foc race 4 infects all race-1 susceptible banana cultivars as well as the Cavendish cultivars. Until recently race 4 only affected bananas in subtropical climates and was designated subtropical race 4. However, a new Foc variant called tropical race 4 (TR4) has been identified which affects Cavendish cultivars and locally important plantains growing in tropical regions. This variant is apparently spreading and is responsible for significant losses in South-east Asia, particularly Malaysia, China, Philippines and Indonesia as well as northern Australia.

26. Once a property becomes contaminated with Foc, the only option for continued banana production is replacement of susceptible cultivars with resistant ones. The Foc fungal spores can persist in the soil for decades and the use of fungicides and fumigants will not eradicate the pathogen. The best option for managing this disease is to minimise spread from infected areas by restricting movement of people, machinery, animal and water flow within the infected areas (Daly 2006).

Table 1. The genes introduced into the GM banana lines

Gene name	Gene – full name & description	Accession number/ genome identifier	Source	Intended Function	References
R1	*CCI*	*CCI*	banana (*CCI)*	Fusarium resistance	*CCI*
R2	*CCI*	*CCI*	banana (*CCI)*	Fusarium resistance	*CCI*
R3	*CCI*	*CCI*	banana (*CCI)*	Fusarium resistance	*CCI*
R4	*CCI*	*CCI*	banana (*CCI)*	Fusarium resistance	*CCI*
R5	*CCI*	*CCI*	banana (*CCI)*	Fusarium resistance	*CCI*
R6	*CCI*	*CCI*	banana (*CCI)*	Fusarium resistance	*CCI*
R7	*CCI*	*CCI*	banana (*CCI)*	Fusarium resistance	*CCI*
AA1	*CCI*	*CCI*	banana (*CCI)*	Enhanced stress tolerance & inhibition of apoptosis	*CCI*
RGA2[1]	*850-RGA2 - Banana nucleotide binding site-leucine rich repeat (NBS-LRR) type resistance gene*	*EU616673*	*Musa acuminate* ssp. *malaccensis* accession 850 (Mam 850)	Fusarium resistance	(Peraza-Echeverria et al. 2009; Peraza-Echeverria et al. 2008)
Ced-9	*Cell death abnormality gene-9*	*AAA20080*	*Caenorhabditis elegans*	Inhibition of apoptosis	(Hengartner et al. 1992)
nptII	*Neomycin phosphotransferase type II gene*	*M61162*	*Escherichia coli*	Selectable marker	(Beck et al. 1982)

[1]RGA2 was designated as RGC2 in licence application DIR 107.

Table 2. Regulatory genetic elements introduced into the GM banana lines

Genetic element	Function in GM plant	Source	Reference
pNos	Promoter from the nopaline synthase (*nos*) gene	*A. tumefaciens*	(Shaw et al. 1984)
tNos	Termination and polyadenylation signal from the *nos* gene	*A. tumefaciens*	As above
pR1	Promoter region from the banana R1 gene	banana (*CCI*)	*CCI*
tR1	Termination and polyadenylation signal from the banana R1 gene	banana (*CCI*)	*CCI*
pR2	Promoter region from the banana R2 gene	banana (*CCI*)	*CCI*
tR2	Termination and polyadenylation signal from the banana R2 gene	banana (*CCI*)	*CCI*
pR3	Promoter region from the banana R3 gene	banana (*CCI*)	*CCI*
tR3	Termination and polyadenylation signal from the banana R3 gene	banana (*CCI*)	*CCI*
pR4	Promoter region from the banana R4 gene	banana (*CCI*)	*CCI*
tR4	Termination and polyadenylation signal from the banana R4 gene	banana (*CCI*)	*CCI*
pR5	Promoter region from the banana R5 gene	banana (*CCI*)	*CCI*
tR5	Termination and polyadenylation signal from the banana R5 gene	banana (*CCI*)	*CCI*
pR6	Promoter region from the banana R6 gene	banana (*CCI*)	*CCI*
tR6	Termination and polyadenylation signal from the banana R6 gene	banana (*CCI*)	*CCI*
pR7	Promoter region from the banana R1 gene	banana (*CCI*)	*CCI*
tR7	Termination and polyadenylation signal from the banana R1 gene	banana (*CCI*)	*CCI*
pRGA2	Promoter from the RGA2 gene (*NBS-LRR type resistance gene)*	banana (*CCI*)	*CCI*
tRGA2	Termination and polyadenylation signal from the RGA2 gene *(NBS-LRR type resistance gene)*	banana (*CCI*)	*CCI*
Ma-pTIP2	Root specific promoter	*M. acuminate* ssp. *malaccensis*	*CCI*
pUbi	Promoter region from the *ubiquitin* gene	maize	(Christensen et al. 1992)
Ubi intron	Intron from *ubiquitin* gene used to enhance protein translation	maize	As above
TEV	Leader sequence to enhance protein translation	TEV	(Gallie et al. 1995) (Gallie & Browning 2001)
Ma-pAct	Promoter region from the *actin* gene	Musa spp. cv Bluggoe	(Hermann et al. 2001)
Ma-Act intron	Intron from *actin* gene used to enhance gene expression	Musa spp. cv Bluggoe	As above
Ma-tAct	Termination and polyadenylation signal from the *actin* gene	Musa spp. cv Bluggoe	As above
p35S	Promoter from 35S RNA	CaMV	(Guerineau et al. 1988)
t35S	Termination and polyadenylation signal from 35S RNA	CaMV	As above
pLacZ	Promoter from LacZα	*E. coli*	(Yanisch-Perron et al. 1985)

27. The tomato-Fusarium wilt pathosystem has become a well-established model system for the study of plant-Fusarium interactions at the genetic and molecular level (Gonzalez-Cendales et al. 2016 and references therein). The infection of tomato by Fusarium involves in-soil germination of spores in the vicinity of roots, attachment to the root surface, penetration of the root cortex and proliferation of hyphae within the root vascular system. Eventually the parenchyma of the dying plant is invaded and colonised by the fungus, followed by sporulation on the plant surface. Fungal hyphae within the xylem vessels, as well as tyloses, callose, gums and gels produced by the host plant, obstruct water and nutrient flow. The disease is characterised by yellowing, wilting and browning of the leaves, stunted growth and eventual death of the plant.

28. For banana, Fusarium enters through the roots and grows into the water-conducting tissues of the corm and pseudostem. This infection results in the initial symptoms of yellowing of margins of older leaves, followed by browning and drying out of the leaves. Collapse of the leaf occurs along the leaf stalk or at the leaf stalk junction with the pseudostem. Typically the dead outer leaves form a skirt of dead leaves around the plant with the inner (younger) leaves remaining upright giving a spikey appearance. The death of the parent pseudostem generally follows, but the suckers do not always die. Characteristically, the pseudostem of infected plants has a dark brown to black discoloration of the water-conducting tissues and infected corms also show this discoloration running through the tissues (Grice et al. 2009).

5.3 Introduction to plant-pathogen interactions

29. Plant-pathogen interactions are multi-facetted and complex. For example, plants encounter a multitude of potential pathogens including viruses, bacteria, fungi, nematodes and insects (Chen et al. 2006); however, only a few of these will be able to cause disease in any given plant. Plant pathogens can be grouped on the basis of feeding style: necrotrophs (such as Fusarium) feed on dead or dying plant material; biotrophs require live host cells for their establishment and survival; and hemi-biotrophs feed off live cells in the early stages of interaction and, as cells are dying, are able to switch to a necrotrophic life style.

30. Pathogens use diverse strategies for infection and proliferation in the host. Bacteria, for example, enter through stomata and hydathodes or through wounds and then proliferate in intercellular spaces. Fungi may directly enter epidermal cells or extend hyphae on top, between or through cells. Alternatively, pathogenic and symbiotic fungi can invaginate the host cell plasma membrane creating specialised feeding structures (haustoria). This interface between plant and pathogen allows for plant cells to detect and react to the pathogen (see review by Jones & Dangl 2006 and references therein).

31. Plants have evolved two strategies to detect pathogens (see review by Dodds & Rathjen 2010 and references therein). The first strategy involves the surface of the plant cell which contains receptor proteins called pattern recognition receptors (PRRs). These receptors recognise conserved microbial elicitors called pathogen associated molecular patterns (PAMPs). PAMPs tend to be essential components of the pathogen, such as bacterial flagellin or fungal chitin. PRRs may also recognise endogenous molecules such as cell wall fragments released by pathogen invasion. Stimulation of PRRs leads to PAMP-triggered immunity (PTI). However, pathogens deliver effector proteins into the host cell which often act to suppress PTI.

32. Thus, the second strategy of plant cells to detect pathogens involves intracellular recognition of the effector proteins (pathogen virulence molecules). This recognition induces effector-triggered immunity (ETI). This mode of recognition occurs inside the cell using protein receptors encoded by resistance (R) genes. Generally, PTI and ETI give rise to a similar response to pathogens, but the ETI is qualitatively stronger and faster and often involves a form of localised host cell death called a hypersensitive response (HR) (Dodds & Rathjen 2010).

33. Plant *R* genes confer resistance to pathogen strains carrying corresponding avirulence (*Avr*) genes, so called because their presence prevents growth on resistant plants (Dodds et al. 2006). This

system is known as a gene-for-gene resistance. Recognition of the products of the *R* and *Avr* genes triggers host defence response including a HR, limiting the spread of the pathogen from the infection site. Only in plant-pathogen interactions where both the specific *R* and the corresponding *Avr* gene products are present, is the plant resistant to the pathogen (Dodds et al. 2006).

34. Host cell death occurs during many interactions between plants and pathogens. In animal systems, the concept of programmed cell death (PCD) has long been established. Three different types of cell death are found: apoptosis, autophagic cell death and necrosis, with apoptosis being the most studied (see review by Reape et al. 2008 and references therein). Each type of animal cell death is characterised by certain distinguishing features. Apoptotic cells undergo a number of biochemical and morphological changes resulting in DNA laddering, cell shrinkage, membrane blebbing (protrusion of the cell membrane) and disassembly into apoptotic bodies (Pennell & Lamb 1997). Apoptosis is an active process requiring a coordinated cell death mechanism. In living cells, pro- and anti-apoptotic regulators are present. When pro-apoptotic stimuli override anti-apoptotic suppression of cell death in animals, a caspase cascade is activated and cell death occurs (Bossy-Wetzel & Green 1999; Salvesen 1999).

35. In plants, PCD is important for normal growth and development, as well as during pathogen and stress responses (see review by Reape et al. 2008 and references therein). Some cases of PCD in plants display characteristics found in animal cell apoptosis, such as DNA fragmentation and cleaving of caspase substrates, while at the same time there are differences, such as the lack of classical caspases. This type of PCD in plants is known as apoptosis-like PCD. In addition, evidence of inhibition of plant cell death by animal anti-apoptotic genes suggests that the genes involved in the control of PCD are conserved across wide evolutionary distances (Dickman 2004; Dickman et al. 2001; Khanna et al. 2007; Li & Dickman 2004; Shabala et al. 2007; Xu et al. 2004).

36. In plant-pathogen interactions, PCD occurs when the pathogen unsuccessfully parasitises the host, as well as during susceptible reactions in which the pathogen successfully causes disease (Greenberg & Yao 2004), suggesting common biochemical pathways during both interactions. The disease resistance response in plants is characterised by a HR at the infection site, which causes rapid, localised cell death which kills the cells near the site of infection, thereby limiting the spread of pathogens and providing the plant with disease resistance (Khurana et al. 2005). HR is associated with the expression of a variety of plant defence genes and the induction of PCD (Dickman 2004; Tadege et al. 1998).

37. HR cell death is effective at restricting biotrophic infections, but can provide a food source for invading necrotrophs (Dickman 2004; Glazebrook 2005). Thus, biotrophs actively suppress HR while necrotrophs promote HR-like cell death (Laluk & Mengiste 2010). The expression of anti-apoptotic genes in plants can therefore confer resistance to necrotrophic pathogens but can also increase susceptibility to biotrophic pathogens (Babaeizad et al. 2009).

5.4 The introduced genes, encoded proteins and their associated effects

5.4.1 The introduced genes for disease resistance and their encoded proteins

38. The introduced genes and their encoded proteins are described to illustrate their potential function in the GM banana lines. They have been grouped according to the trait associated with the introduced genes: specific disease resistance genes and genes conferring disease resistance through inhibition of apoptosis and/or stress tolerance.

Specific disease resistance genes – *R1- R7* and *RGA2*

39. Plant breeders have been using resistance genes to control disease in crop plants for close to 100 years. More recently, molecular studies have revealed that host resistance genes provide the capacity to recognise and respond to specific pathogens (Dodds & Rathjen 2010). The banana *R1-R7* genes encode proteins which recognise either specific molecules produced by a fungal pathogen or

other molecules produced as a result of infection by the pathogen. This recognition of infection results in the activation of a chain of signalling events leading to resistance against the pathogen.

40. The *R1- R7* genes are expected to confer resistance to the fungal pathogen Foc TR4. As all these genes were derived from banana, there is no expectation of unintended changes in the phenotype of the GM banana plants expressing these genes. Additionally, homologous genes are not known to affect other metabolic pathways in other plant species.

The RGA2 gene

41. Nucleotide binding site-leucine rich repeat (NBS-LRR[4]) genes are the largest class of resistance genes. Large numbers of this class of genes have been isolated from various plant species, from about 50 in papaya to 653 in rice (Marone et al. 2013).

42. Most NBS-LRR proteins lack a signal peptide or membrane spanning regions and thus are assumed to be located in the cytoplasm (McHale et al. 2006). The proteins act through a network of signalling pathways and induce a series of plant defence responses (McHale et al. 2006). The LRR domain is implicated in protein-protein interaction and more specifically in binding to pathogen-derived molecules. The LRR domain is thought to be the primary determinant of pathogen recognition specificity. The role of the NBS region is primarily as a signal transduction switch following pathogen recognition. NBS-LRR proteins can recognise a wide variety of pathogens including viruses, bacteria, fungi and insects. Activation of these genes results in a hypersensitive response, a localised form of host programmed cell death (Lozano et al. 2015).

43. Expression of *R* genes in several GM plants has been demonstrated to confer resistance to pathogens carrying the appropriate *Avr* gene (see review by Hulbert et al. 2001). For example, the *Pto* resistance gene from tomato (*Lycopersicum esculentum*) has been shown to function in tobacco (*Nicotiana tabacum*) and *N. benthamiana* (Rommens et al. 1995; Thilmony et al. 1995). Other than disease resistance, no other phenotypic changes were reported by the authors.

44. Resistance gene candidate RGA2 was isolated from a banana species, *Musa acuminata* ssp. *malaccensis*, which is resistant to Foc TR4. RGA2 shows sequence similarity to known *R* genes that encode NBS-LRR proteins, such as the tomato *I-2* gene for Fusarium wilt (Peraza-Echeverria et al. 2009; Peraza-Echeverria et al. 2008). In the previous field trial (DIR 107), several GM banana lines containing the candidate gene *RGA2* showed either immunity or increased resistance to Foc TR4; no unusual phenotypes were observed amongst these GM banana lines.

5.4.2 Genes conferring disease resistance through inhibition of apoptosis or enhanced stress tolerance

The ced-9 gene

45. The *ced-9* (cell death abnormality) gene, derived from the nematode *Caenorhabditis elegans*, is an anti-apoptotic gene that is active during the killing phase of PCD, where it protects cells from undergoing death (summarised in Conradt & Xue 2005). Thus, loss of function of the *ced-9* gene can lead to the death of cells that normally live (Hengartner et al. 1992). *Ced-9* is orthologous to the human *Bcl-2*, which has been found to promote cell survival by blocking PCD. *Bcl-2* belongs to a family of interacting proteins that regulate apoptosis, but also have other functions. These functions include roles in normal cell physiology related to neuronal activity, calcium handling, mitochondrial dynamics and energetics and other processes of normal healthy cells (Hardwick & Soane 2013).

46. Expression of the *ced-9* gene in plants has shown a range of effects, including improved plant survival under abiotic and biotic stresses, and developmental abnormalities. The *ced-9* gene has been previously expressed in banana embryonic cell suspensions where it was found to suppress cell death in *Agrobacterium tumefaciens* transformed plant cells (Khanna et al. 2007). In GM tobacco, the *ced-9* gene was shown to confer protective advantages against necrotrophic fungal pathogens (Dickman et

[4] The literature also refers to these as nucleotide binding-leucine rich repeat (NB-LRR) genes.

al. 2001) as well as a range of abiotic stresses including heat, cold, menadione[5] and hydrogen peroxide (Li & Dickman 2004), salt and oxidative stress (Shabala et al. 2007). GM tobacco plants expressing high levels of the *ced-9* gene were extremely resistant to the pathogens tested but also showed altered growth patterns, such as variegated leaf pigmentation. Moderately expressing GM tobacco plants did not show any developmental abnormalities but retained pathogen resistance (Dickman et al. 2001).

47. Expression of the *ced-9* gene in tomato plants enhanced plant survival by inhibiting PCD induced by virus infection and exposure to cold temperatures (Xu et al. 2004). Developmental abnormalities were also observed in the GM tomato plants expressing high levels of *ced-9* gene. These abnormalities included stunted growth and the formation of none or few viable seeds (Xu et al. 2004).

48. Homologues of *ced-9,* when expressed at high levels in GM tobacco and GM tomato plants showed various developmental abnormalities such as stunted growth, male sterility, reduced seed production, stem bleaching, flower deformation and altered leaf pigmentation (Dickman et al. 2001; Xu et al. 2004). In contrast, GM plants expressing these homologues at moderate levels retained pathogen resistance but did not show any of the developmental abnormalities (Dickman et al. 2001; Xu et al. 2004).

49. Based on the above, it is possible the GM bananas expressing *ced-9* may exhibit abnormal development. The applicant indicated that there were some phenotypic abnormalities or 'off-types' amongst the GM bananas, but that the off-types were unlikely to be due to the genetic modification (see discussion in Section 5.6.1). The applicant has indicated that abnormal development due to the genetic modification was not observed in previous field trials of GM bananas expressing *ced-9*. Lady Finger banana plants genetically modified with the *ced-9* gene showed increased tolerance to infection by Foc Race 1 in glasshouse studies (Paul et al. 2011). Similarly, *ced-9* GM Grand Naine banana plants showed increased tolerance to infection by Foc TR4 in the field (information provided by applicant).

50. The applicant has also indicated that in glasshouse studies, GM banana lines expressing the *ced-9* gene showed increased tolerance to water stress and the herbicide paraquat, and thus this gene may also provide tolerance to other stresses and/or confer resistance or susceptibility to other pathogens. These results are not unexpected as expression of *ced-9* in other GM plant species have shown increased tolerance to abiotic and biotic stress (see above).

The AA1 gene

51. The *AA1* gene was derived from a banana cultivar grown commercially in Australia and is expected to provide the GM banana plants with increased resistance to Foc TR4 under field conditions by increasing stress tolerance or through the regulation of apoptosis. These functions may also confer resistance or susceptibility to other pathogens and resistance to abiotic stresses. The applicant has indicated that, as *AA1* is involved in the regulation of apoptosis and plant development, it is possible the GM banana plants may exhibit abnormal development. Developmental abnormalities have been reported in other GM plants expressing homologues of the *AA1* gene. However, as this gene occurs naturally in bananas, there is no expectation of unintended physiological or phenotypical changes in the GM banana plants. Banana plants genetically modified with similar genes derived from Arabidopsis and rice showed no discernible negative effects during previous field trials under DIR 107 (information provided by applicant).

5.4.3 Reporter and selectable marker genes

Reporter gene - LacZ alpha (β-galactosidase)

52. The *lacZ* gene is present in pCambia2200 and pCambia2300 vectors used for transformation. The gene is derived from *E. coli* and encodes β-galactosidase that cleaves lactose to glucose and galactose which can then be used as an energy source (Juers et al. 2012). In the laboratory, the gene is

[5] Menadione is an analog of 1,4-naphthoquinone. In these experiments it was used to induce programmed cell death by causing oxidative stress through the production of a superoxide radical (Li & Dickman 2004).

used as a reporter to monitor gene expression in bacteria (Juers et al. 2012). The gene contains a number of cloning sites and the insertion of a candidate resistance gene into a cloning site inactivates the *lacZ* gene and allows for identification of successful cloning (Bevan 1984). As the bacterial *lacZ* promoter is not expressed in plants, the applicant would insert the candidate gene along with a promoter for plant expression into the cloning site. Thus, in the GM banana lines generated using the pCambia2200 and pCambia2300, there is no *lacZ* gene product.

The selectable marker gene – nptII

53. The *nptII* (also denoted *aph(3')-II*) gene was isolated from the common gut bacterium *E. coli* and encodes neomycin (or aminoglycoside) phosphotransferase type II, which inactivates aminoglycoside antibiotics such as kanamycin and neomycin. The *nptII* gene is used extensively as a selectable marker in the production of GM plants. Regulatory agencies in Australia and in other countries have assessed the use of the *nptII* gene in GM plants as not posing a risk to human health and safety or to the environment. Further information about this gene can be found in the document *Marker genes in GM plants* available from the Risk Assessment References page on the OGTR website.

54. Some of the transformation events will include the use of an excisable marker gene system to remove the *nptII* gene from the GM banana through the use of a recombinase. Recombinases have been used to remove the selectable marker gene from other GM crops (see review by Wang et al. 2011). The approach used by the applicant to develop the marker-free GM bananas involves the fusion of the cytosine deaminase gene (*codA*) from *E. coli* to the *nptII* gene adjacent to an inducible recombinase (R) gene (Figure 2). The *codA:nptII* fusion and R genes are sandwiched between two recombination sites (Rs). The R and Rs are derived from the yeast, *Zygosaccharomyces rouxi*. The above as well as the gene of interest (GOI), in this case any one of the ten resistance genes, are located between the Left and Right Border of the T-DNA.

55. Banana embryonic cell suspensions are transformed using *Agrobacterium* and then transferred to proliferation media containing the antibiotic kanamycin to select for genetically modified cells. Cells containing the *nptII* gene would survive the antibiotic treatment. The surviving cells are then transferred to media containing dexamethasone (DEX), and the selective agent 5-fluorocytosine (5FC). DEX activates the recombinase gene (Righetti et al. 2014), which recognises and excises everything between the two recombination sites. 5FC provides negative selection pressure in that the *codA* gene converts non-toxic 5FC to cytotoxic 5-fluorocytosine (5FU) (Schaart et al. 2004). Thus, cells with an excised *codA* gene would survive in the media containing 5FC. Surviving cells remain on media containing both DEX and 5FC until embryos are formed. Embryos are transferred to media without any selection pressure until plantlets are formed. The GM banana plants are screened using the polymerase chain reaction to confirm the absence of both *Agrobacterium* and the genes between the two recombination sites (i.e. the *codA:nptII* fusion and the recombinase gene). The applicant has indicated that following excision, only 84 bp of the untranslated region of the Rs site remained.

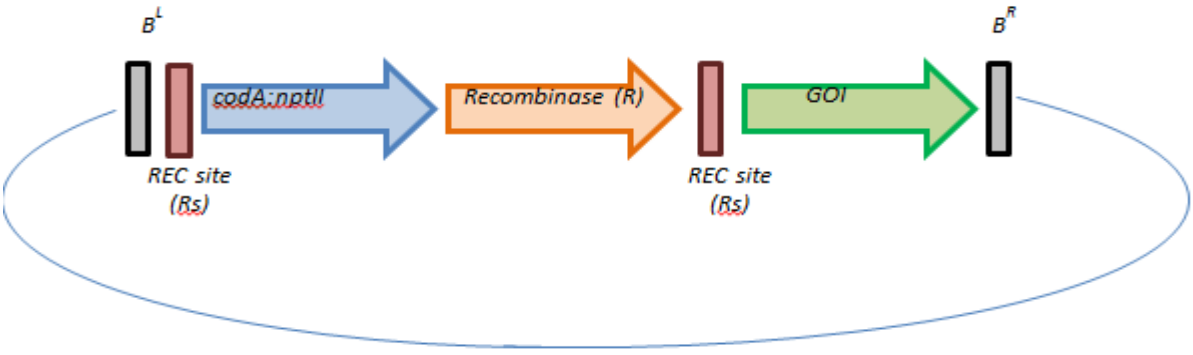

Figure 2. Generalised schematic of vector allowing excision of nptII marker gene.
Abbreviations: B^L - Left border; B^R - Right border; REC site (RS) - recombination site; codA:nptII - fusion of the codA and nptII genes; Recombinase (R) - recombinase gene; GOI - gene of interest (e.g. gene for resistance to Foc TR4).

5.5 Toxicity/allergenicity of the proteins associated with the introduced genes

56. Most of the candidate genes for Fusarium wilt resistance were derived from banana; *RGA2* is from *Musa acuminata* ssp. *malaccensis, AA1* is from a commercial cultivar grown in Australia, and the *R1-R7* genes are from banana species common to SE Asian and present in Australian germplasm collections. The only resistance gene not of banana origin is *ced-9* which was derived from a nematode, *C. elegans*.

57. Initially all of the GM banana lines would contain *nptII* from the common gut bacterium *E. coli* (Table 1). This gene confers antibiotic resistance on GM plant cells and was used during initial development in the laboratory to select plant cells containing the introduced genes.

58. However, in some GM banana lines, the *nptII* gene will be excised using an inducible recombinase system (see Section 5.4.3). The genetic elements of this system were derived from *E. coli* and *Z. rouxi*, which is a well-known yeast used in soy bean fermentation to make soy sauce. Once excision has occurred, all that remains of this system in the GM banana is an 84 bp untranslated region of the recombination site.

59. Similarly, although constructs created using pCambia2200 and pCambia2300 contain the *lacZ* gene derived from *E. coli*, cloning of the candidate genes into the multiple cloning site of the *lacZ* gene inactivates the *lacZ* gene and no gene product is produced.

60. Short regulatory sequences which control the expression of the introduced genes will also be introduced into the GM banana plants. These sequences are derived from maize and banana, the soil bacterium *A. tumefaciens* and the plant viruses CaMV and TEV (see Table 2). The promoter for the *lacZ* gene is derived from *E. coli,* but is not expressed in plant tissue.

61. The encoded proteins have homologues that occur naturally in a range of organisms, including plants consumed by people and animals. On this basis, people and other organisms have a long history of exposure to the introduced proteins and their homologues.

62. No studies on toxicity/allergenicity have been performed on any of the GM banana plants or purified proteins as the proposed trial is at early research stage.

63. Although not required for this application, the applicant has provided a bioinformatic analysis of the introduced proteins encoded by the candidate genes. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the 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). There is little evidence to suggest that short stretches of shared identity lead to allergenic cross-reactivity (Aalberse 2000). The results that were presented suggest there may be some cross-reactivity with putative allergens, and may warrant further investigation if the GM bananas were to be used as human food. However, as these genes were derived from banana, it is unlikely they are novel allergens.

64. No adverse health effects were reported by the staff who handled the GMOs during the screening trials in the glasshouse.

5.6 Characterisation of the GMOs

5.6.1 Phenotypic characterisation

65. The purpose of the proposed trial is to conduct proof of concept experiments to assess the disease response and/or development of the GM banana lines. The applicant states that it is not possible to assess the response of banana to all pathogens in a glasshouse. Nor is it possible to grow numerous large banana plants for assessment of fruit characteristics in a glasshouse. Such phenotypic data will be collected during the proposed trial. The majority of genes being tested have not been assessed previously, thus most of the GM banana lines proposed for release have not been phenotypically characterised.

66. The GM banana lines containing the *ced-9* gene will be the same events assessed in the field trials for DIR 107, thus there has been some characterisation of these lines. GM Lady Finger lines containing the *ced-9* gene showed increased tolerance to infection by Foc Race 1 in glasshouse studies and *ced-9* GM Grand Naine banana plants showed increased tolerance to infection by Foc TR4 in the field (Paul et al. 2011; Paul 2009). Preliminary laboratory studies demonstrated that some GM banana lines, when treated with the herbicide paraquat, suffered slightly less damage than treated non-GM control bananas. Additional laboratory studies showed some GM lines had increased tolerance to water stress (Paul et al. 2011; Paul 2009). A field trial of the GM Grande Naine banana lines containing the *ced-9* gene construct has recently been completed by the applicant under licence DIR 079/2007. Results of this trial indicate that there are no adverse effects of the introduced *ced-9* gene on plant development or increased susceptibility to disease.

67. The applicant has indicated that most of the GM banana lines containing *ced-9* were phenotypically normal. However, one line had a slightly lower bunch weight and some of the other GM banana lines displayed a range of mild phenotypic abnormalities including altered leaf morphology, stunting and phyllotaxy (Paul 2011). The applicant considered that most of the abnormalities observed were conventional "off-types" most likely due to the tissue culture process because:

- off-types are commonly observed amongst non-GM banana plants arising from tissue-culture due to somaclonal variation (Israeli et al. 1995),
- the development of the GM banana plants includes regeneration via tissue-culture, and
- based on years of tissue culture experience, most of the off-types amongst the GM banana plants were phenotypically the same as the off-types observed in non-GM, tissue-cultured banana plants.

68. The applicant states that the GM banana plants will be monitored for aberrant phenotypes during the proposed trial.

Section 6 The receiving environment

69. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. Relevant information about the receiving environment includes abiotic and biotic interactions of the crop with the environment where the release would occur; agronomic practices for the crop; presence of plants that are sexually compatible with the GMO; and background presence of the gene(s) used in the genetic modification (OGTR 2013b).

70. The abiotic and biotic factors relevant to the growth and distribution of bananas in Australia are discussed in *The Biology of* Musa *L. (banana)*(OGTR 2016).

6.1 Relevant agronomic practices

71. It is anticipated that the cultivation practices used for planting and managing the proposed trial will not differ significantly from the standard practices used for commercial (non-GM) banana. These are outlined in *The Biology of* Musa L. (*banana*) (OGTR 2016).

72. Commercial production of bananas in Australia occurs on the east coast from northern NSW to northern QLD, around Carnarvon, Broome and Kununurra in WA, and around Darwin in the NT. *Musa* species have a limited range of temperature tolerances and sweet bananas are restricted to subtropical and tropical areas; none of the species are frost tolerant. Sweet bananas also require a mean rainfall of 100 mm per month with no more than a 3 month dry season. Generally for optimal production, bananas require 50 –100 mm rainfall or irrigation per week. Although bananas can grow well on a variety of soil types, they do require fertiliser, especially nitrogen and potassium, for optimal production. Bananas have a low tolerance for saline soils.

73. The applicant proposes to transport the planting material (tissue cultured plants) to DBFC farm from QUT and to acclimatise (harden off) the plants in a shadehouse before transferring them to the field location.

74. Cultivation and movement of all bananas (GM and non-GM) in Australia is subject to the relevant State and Territory authorities. Further information about cultivation and movement of plant material in QLD and the NT is available on the Queensland Government website and the NT Department of Primary Industry and Fisheries website. Of relevance to the proposed release is a recent National Banana Freckle Eradication Program commenced by the NT Government to eradicate the banana freckle fungal pathogen which was detected in the NT in 2013. All banana plants in six zones of NT infected with the fungal pathogen were destroyed, including the area of the proposed trial site. Special permits are now required for the movement, storage, cultivation and propagation of bananas in these zones. Further information can be found at the National Banana Freckle Eradication Program website.

75. The applicant has indicated they will consult with Queensland and NT authorities regarding the movement and planting of GM and non-GM banana material for this trial and adhere to all relevant regulations.

76. The applicant has indicated that prior to removal from the field site, any machinery used at the field site would be inspected and plant material removed. The plant material would be left to decompose at the trial site.

77. Standard commercial practice includes the removal of the male bell from inflorescences to increase bunch weight and remove feeding sites for pests (Broadley et al. 2004). The applicant proposes to remove the male bells from most inflorescences but wishes to observe the phenotype of some bells. In this case, the male inflorescence would be bagged rather than removed when it emerges. When removed, male inflorescences would be collected and placed in a container to prevent access by birds and bats. Once decomposed, they would be left on the ground at the field location.

78. During commercial cultivation of banana plants it is necessary to remove suckers and dead leaves for both disease management and to encourage plant vigour. Suckers would be cut off at ground level and a solvent (usually kerosene) poured down the centre of the pseudostem. The removed sucker and any detached leaf material is non-propagative and would be left on the ground at the field location to decompose. Waste plant material from the shadehouse and tissue culture facility would be collected and decomposed in a container and then left on the ground at the field location.

79. It is intended that the GM bananas be grown through to fruiting to allow assessment of fruit characteristics. Fruit would be obtained from the plant crop[6], which would then be ratooned and grown to fruiting before the proposed trial is concluded. Bunches would be bagged, as is done in commercial non-GM banana cultivation to protect developing fruit from being eaten or damaged by frugivores and to optimise ripening conditions (Broadley et al. 2004). Fruit would be harvested while still green (standard commercial practice). After assessment, fruit would be shredded and allowed to decompose on the ground at the field location. Similarly, at the end of the trial, any remaining bunches would also be shredded and placed on the ground to decompose at the field site.

80. At completion of the field trial, banana plants would be destroyed by injection with herbicide (e.g. glyphosate) or by mechanical means. Mechanical destruction involves multiple discing using a tractor towing a disc harrow which will uproot the plants and then shred the pseudostem and corm into small pieces. This process will be repeated 3-5 times with a 2-3 week interval between discing. If propagative material survives the discing, any emerging suckers would be injected with herbicide. The plant material will be left to decompose on the field trial site and the site would be subjected to monitoring for volunteer banana plants for a period of at least 12 months.

[6] 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'.

6.2 Relevant abiotic factors

81. The release is proposed to take place at one site of up to 6 ha, over a five year period, in the Litchfield Municipality (NT) on the property of the DBFC property. Tissue cultured GM banana lines, produced at QUT would be transported to DBFC and then hardened off in a separate, lockable area within the DBFC shadehouse. It is expected that the proposed field site will be located near the field site used for DIR 107 which is located a few hundred metres from the shadehouse. The field site is bordered on the west by a large shed and permanent dirt road, and on the east by a deep drainage channel.

82. The proposed release site is 500 m from a public road which runs alongside the DBFC property. The property adjoins a melon farm on one side and native bushland on the remaining sides. The proposed site is approximately 1 km from the boundary of Fogg Dam Conservation Reserve and 25km from Djukbinj National Park.

83. The closest population centre to the proposed release site is Humpty Doo (8 km), which has a population of approximately 5,500 people. The city of Palmerston is approximately 22 km from the release site and has a population of about 28,000 people. The area has a tropical savannah climate with distinct wet and dry seasons. The wet season is associated with tropical cyclones and monsoon rains. The major agricultural activities around the proposed release site are the cultivation of melons, mangoes, pineapples, Asian vegetables and cut flowers.

84. The applicant proposes to locate the GM banana trial site at least 1 km from the Adelaide River, the nearest natural waterway. The proposed release site is 15 m above sea level and the DBFC property owners state that the site has never been known to flood. The property was laser levelled five years ago and drainage channels were dug. The proposed release site is not adjacent to sloping ground or on a hill such that it would be prone to heavy run off or landslides. There is no recorded incident of storm or cyclone uprooting and dispersing propagative material at the proposed release site.

85. The DBFC property is surrounded by a fence with ring lock pig mesh, double barb wire on top and lockable gates. This fence will exclude pigs and water buffalo. During the duration of the previous trial (DIR 107) no pigs or water buffalo were ever observed inside the trial site and there was no sign of any breaches of the perimeter fencing. The applicant has also proposed that the trial site will be fenced to restrict access.

86. Due to the National Banana Freckle Eradication Program (see Section 6.1), growers in this area have only been able to apply for a permit to re-plant bananas from May 2016. Thus, it is possible that there may be banana plantations in the area to supply local markets. The applicant has indicated that previously DBFC was the only commercial grower in the area and that in the future it may apply for a permit to grow a small number of non-GM banana plants on the property. If this were to occur, the applicant has indicated the plants would be at least 10 m from the proposed field trial site.

6.3 Relevant biotic factors

6.3.1 Presence of related plants in the receiving environment

87. The proposed release site is part of a plantation where commercial bananas have previously been grown. Due to the detection of the banana freckle pathogen in this area, all the banana plants were destroyed (see Section 6.1). However, the applicant has indicated that the banana freckle pathogen has not been recorded at the proposed trial site or on the DBFC property. The applicant has indicated the property is currently planted to pineapple and mango.

88. There are two recognised *Musa* species native to Australia, *M. acuminata* subsp*. banksii* and *M. jackeyi* (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. *M. jackeyi* is rare and has only been reported at two locations in Queensland: Bellenden Ker and Cooktown. Neither of these species is known to be present in the NT, and neither species is classified as a weed in Australia (OGTR 2016).

6.3.2 Presence of other biotic factors

89. The management of pests and diseases is important in order to maximise yields, e.g. in northern QLD, about 20-25 pesticide applications may be needed to control leaf diseases. Banana plants can tolerate shade of up to 80%, but shading reduces plant growth, pseudostem thickness, suckering and yield. Weeds compete with bananas for water and nutrients, particularly nitrogen, and can harbor pests and pathogens. Outside of an agricultural situation, commercial bananas do not pose a weed problem in Australia. Commercial cultivars are effectively sterile and are propagated vegetatively, which limits their ability to spread. Without human intervention commercial banana cultivars would succumb to a number of pests and diseases, lack of nutrients, lack of moisture and/or extended drought, shading and poor competitive ability with other plants.

90. There are a number of diseases of banana present in Australia (Grice et al. 2009; OGTR 2016). The purpose of the proposed release is to assess the disease response of the GM banana lines to the fungal disease Foc TR4. The applicant states that the proposed release site was selected because the soil contains Foc TR4 at levels that would be expected to provide high, evenly distributed disease pressure.

6.4 Presence of similar genes and encoded proteins in the environment

91. The introduced genes for disease resistance have been sourced from banana and from the nematode *C. elegans*.

92. The source organism of the *RGA2* gene is the wild diploid banana *Musa acuminata* ssp. *malaccensis*. *M. acuminata* is widely distributed in Asia and considered one of the ancestors of modern eating banana (OGTR 2016). The sources of the *R1-R7* and *AA1* genes are from various banana species which are present in some Australian germplasm collections or commercially grown in Australia (the specific source of the genes is CCI). Homologues of these genes are widely distributed in plants, including many other species that are consumed by humans and animals.

93. *C. elegans* is widespread in Australia. The *ced-9* gene derived from *C. elegans* inhibits apoptosis, a process which is ubiquitous among multicellular organism. Therefore, it is expected that humans, animals and microorganisms routinely encounter the introduced genes for inhibition of apoptosis, homologues of these genes, or proteins with a similar function, through contact or ingestion of microorganisms, plants and animals, and food derived from plants and animals.

94. 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.

Section 7 Relevant Australian and international approvals

7.1 Australian approvals

7.1.1 Approvals by the Regulator

95. Some of the GM banana lines included in this application have previously been approved by the Regulator for release in Australia under licences DIR 079/2007 and DIR 107. The other lines have not been field trialled. The Regulator has also issued licences DIR 076/2007 and DIR 109 to QUT for limited and controlled release of banana genetically modified for enhanced nutrition.

96. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

7.1.2 Approvals by other government agencies

97. The movement and cultivation of GM and non-GM banana plants is subject to State and Territory legislation (see Section 6.1).

7.2 International approvals

98. None of the GM banana lines covered in this application has been approved for release in any other countries.

Chapter 2 Risk assessment

Section 1 Introduction

99. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of, gene technology (Figure 3). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

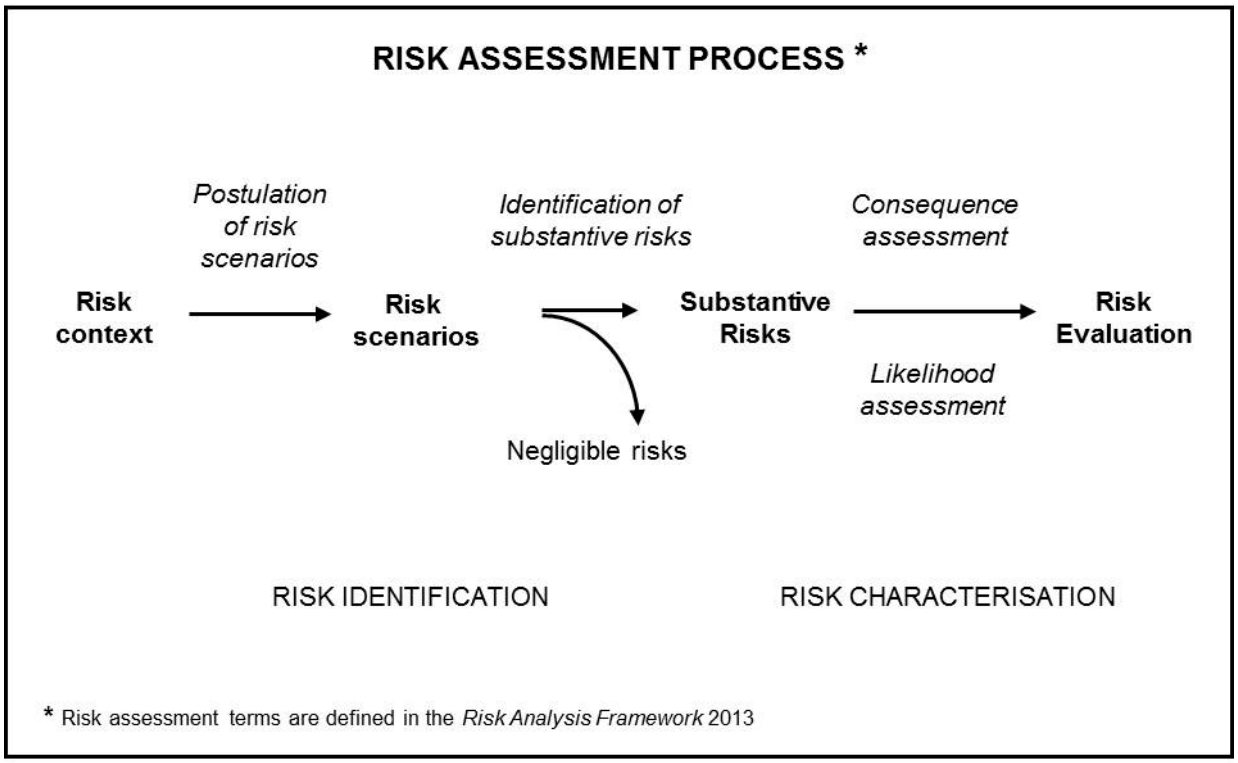

Figure 2. The risk assessment process

100. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios) in the short and long term.

101. Postulated risk scenarios are screened to identify substantive risks that warrant detailed characterisation. A substantive risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

102. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, reported international experience and consultation (OGTR 2013a). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are considered in postulating risk scenarios (Keese et al. 2014). In addition, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

103. Substantive risks (i.e. those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments. The level of risk, together with analysis of interactions between potential risks, is used to evaluate these risks to determine if risk treatment measures are required.

Section 2 Risk Identification

104. Postulated risk scenarios are comprised of three components (Figure 4):

i. The source of potential harm (risk source).

ii. A plausible causal linkage to potential harm (causal pathway).

iii. Potential harm to an object of value (people or the environment).

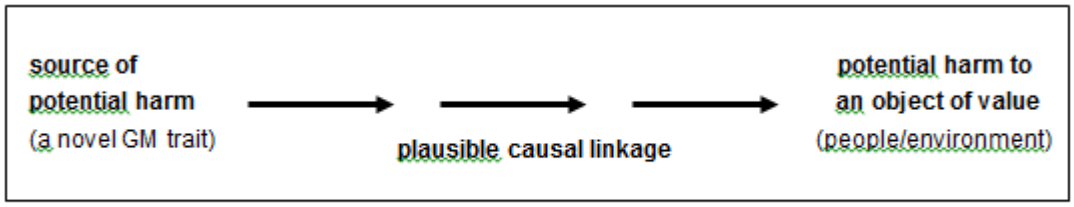

Figure 4. Risk scenario

105. In addition, the following factors are taken into account when postulating relevant risk scenarios:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
- the proposed limits including the extent and scale of the proposed dealings
- the proposed controls to limit the spread and persistence of the GMOs
- characteristics of the parent organism(s).

2.1 Risk source

2.1.1 The introduced genes for Fusarium wilt resistance

106. The source of potential harms can be intended novel GM traits associated with one or more introduced genetic elements, or unintended effects/traits arising from the use of gene technology.

107. As discussed in Chapter 1, the GM banana lines have been modified by the introduction of one of ten candidate genes conferring resistance to Foc TR4. The genes were sourced from banana (*Musa* spp) and the nematode, *C. elegans*. These introduced genes are considered further as potential sources of risk.

2.1.2 The reporter and selectable marker genes

108. The *lacZ* gene is present in the two pCambia vectors (see 5.4.3) and is used in the laboratory as a reporter to monitor gene expression in bacteria. The gene contains a number of cloning sites and the insertion of a candidate resistance gene into a cloning site inactivates the *lacZ* gene and allows for identification of successful cloning. The bacterial *lacZ* promoter is not expressed in plants. Thus, in the GM banana lines generated using the pCambia vectors, there is no *lacZ* gene product produced and potential effects will **not** be further considered for this application.

109. Some of the GM banana lines contain the *nptII* gene which confers antibiotic resistance and was used as a selectable marker gene. This gene and its product have already been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator as well as by other regulatory agencies in Australia and overseas. Further information about this gene can be found in the document *Marker genes in GM plants* available from the Risk Assessment References page on the OGTR website. As the gene has not been found to pose a substantive risk to either people or the environment, its potential effects will **not** be further considered for this application.

110. The remaining GM banana lines proposed for release will not contain the *nptII* gene through the use of an excisable marker gene system (Chapter 1, Section 5.4.3). The use of this system results in an 84 bp untranslated region of the recombination site remaining in the GM banana lines. As this DNA is not

expressed as a protein and dietary DNA has no toxicity (Society of Toxicology 2003), potential effects will **not** be further considered for this application.

2.1.3 The regulatory sequences

111. The introduced genes are controlled by introduced regulatory sequences. These were derived from plants, bacteria and plant viruses (see Chapter 1, Table 2). Regulatory sequences are naturally present in plants and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein and dietary DNA has no toxicity (Society of Toxicology 2003). Hence, risks from these regulatory sequences will **not** be further assessed for this application.

2.1.4 Unintended effects resulting from the process of genetic modification

112. The genetic modifications have the potential to cause unintended effects in several ways, including altered expression of endogenous genes by random insertion of introduced DNA in the genome, increased metabolic burden due to expression of the proteins encoded by the introduced genes, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, the range of unintended effects produced by genetic modification is not likely to be greater than that from accepted traditional breeding techniques. These types of effects also occur spontaneously and in plants generated by conventional breeding (Bradford et al. 2005; Ladics et al. 2015; Schnell et al. 2015). In general, the crossing of plants, each of which will possess a range of innate traits, does not lead to the generation of progeny that have health or environmental effects significantly different from the parents (Steiner et al. 2013; Weber et al. 2012). Therefore, although unintended effects resulting from the introduced genes will be considered further, unintended effects resulting from the process of genetic modification will **not** be considered further in this application.

2.2 Causal pathway

113. The following factors are taken into account when postulating plausible causal pathways to potential harm:

- 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) on the properties of the organism
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- agronomic management practices for the GMOs
- spread and persistence of the GM plants (e.g. reproductive characteristics, dispersal pathways and establishment potential)
- tolerance to abiotic conditions (e.g. climate, soil and rainfall patterns)
- tolerance to biotic stressors (e.g. pest, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organism
- gene transfer by horizontal gene transfer
- unauthorised activities.

114. Although all of these factors are taken into account, some may have been considered in previous RARMPs or are **not** expected to give rise to substantive risks (see Sections 2.2.1 to 2.2.4 below).

2.2.1 Tolerance to abiotic and biotic factors

115. The intent of the introduced genes is to confer resistance to Foc TR4 and/or enhance stress tolerance in the GM banana plants (Chapter 1, Section 5.4). In previous trials of GM banana lines, some of the candidate genes have also conferred enhanced tolerance to water stress and other GM plants expressing homologous genes have shown tolerance to various biotic and abiotic stresses. Thus, it is possible the GM

banana plants may be more tolerant to abiotic and biotic stresses that are naturally encountered in the environment. Therefore, tolerance to abiotic and biotic stresses will be assessed further.

2.2.2 Gene transfer to sexually compatible relatives

116. As discussed in Chapter 1, Section 4, the banana cultivars that will be genetically modified are essentially male and female sterile. Banana pollen has low viability and male flowers will be removed or bagged which would restrict pollen flow. There are only two recognised *Musa* species native to Australia and neither is known to be present in the NT (OGTR 2016) where the field trial is proposed to take place. Thus, gene transfer is not expected from the GM banana plants to sexually compatible species and will **not** be assessed further.

2.2.3 Horizontal gene transfer

117. The potential for horizontal gene transfer and any possible adverse outcomes has been reviewed in the literature (Keese 2008) and has been assessed in many previous RARMPs. Horizontal gene transfer was most recently considered in detail in the RARMP for DIR 108. No risk greater than negligible was identified due to the rarity of these events and because the gene sequences (or sequences which are homologous to those in the current application) are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, horizontal gene transfer will **not** be assessed further.

2.2.4 Unauthorised activities

118. Previous RARMPs have considered the potential for unauthorised activities to lead to an adverse outcome. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires the Regulator to have regard to 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, and no risk greater than negligible was identified in previous RARMPs. Therefore unauthorised activities will **not** be considered further.

2.3 Potential harm

119. Potential harms from GM plants include:

- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced biodiversity through harm to other organisms or ecosystems
- reduced establishment of desirable plants, including having an advantage in comparison to related plants
- reduced yield of desirable vegetation
- reduced products or services from the land use
- restricted movement of people, animals, vehicles, machinery and/or water
- reduced quality of the biotic environment (e.g. providing food or shelter for pests or pathogens) or abiotic environment (e.g. negative effects on fire regimes, nutrient levels, soil salinity, soil stability or soil water table).

120. These harms are based on those used to assess risk from weeds (Standards Australia Ltd et al. 2006). Judgements of what is considered harm depend on the management objectives of the land into which the GM plant is expected to spread and persist. A plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.3.1 Production of a substance toxic or allergenic to people or toxic to other organisms

121. 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).

122. Allergenicity is the potential of a substance 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).

123. Expression of the introduced candidate genes could result in production of novel toxic or allergenic compounds, or alter the production of endogenous compounds of banana that are toxic or allergenic. The potential for the production of novel toxins or allergens and for altered production of endogenous banana toxins and allergens will be considered further.

2.4 Postulated risk scenarios

124. Two risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 3, and discussed individually below. Postulation of risk scenarios considers impacts of the GM banana or its products on people undertaking the dealings, as well as impacts on people and the environment if the GM plants or genetic material were to spread and/or persist.

125. In the context of the activities proposed by the applicant and considering both the short and long term, neither of the two risk scenarios gave rise to any substantive risks.

Table 3. Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced genes for resistance to Foc TR4	Growing GM banana plants at the field trial site ⬇ Expression of the genes in GM plants ⬇ Exposure of humans and other desirable organisms by ingestion of, or contact with, GM banana plant material or products, or inhalation of GM banana pollen	Increased toxicity or allergenicity in humans or increased toxicity to other desirable organisms	No	• GM plant material would not be used in human food or animal feed. • The limited scale, short duration and other proposed limits and controls minimise exposure of people and other organisms to the GM plant material. • The evidence indicates that the introduced proteins are unlikely to be toxic or allergenic.
2	Introduced genes for resistance to Foc TR4	Growing GM banana plants at the field trial site ⬇ Expression of the genes in GM plants ⬇ Dispersal of propagules to nature reserves, roadsides, drains or intensive use areas ⬇ Establishment of volunteer GM bananas plants in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity in humans or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants OR Reduced utility or quality of the environment	No	• The proposed limits and controls would minimise the likelihood of persistence at the trial site and spread from the site. • There is no expectation the introduced Foc TR4 resistance genes confer other characteristics to enhance the spread and persistence of the GM bananas. • The introduced genes for regulation of apoptosis or enhanced stress tolerance may enhance tolerance to other abiotic and biotic stresses. • Bananas have limited ability to survive outside agricultural settings.

Risk scenario 1

Risk source	Introduced genes for resistance to Foc TR4
Causal pathway	⬇ Growing GM banana plants at the field trial site ⬇ Expression of the genes in GM plants ⬇ Exposure of humans and other desirable organisms by ingestion of, or contact with, GM banana plant material or products, or inhalation of GM banana pollen
Potential harm	Increased toxicity or allergenicity in humans or increased toxicity to other desirable organisms

Risk source

126. The source of potential harm for this postulated risk scenario is the introduced candidate genes for resistance to Foc TR4.

Causal pathway

127. People may be exposed to the GM banana or its products through contact, consumption, or inhalation of pollen. The proposed limits and controls of the trial (Chapter 1, Sections 3.1 and 3.2) would minimise the likelihood of exposure of people and other organisms to GM plant material. The GM banana fruit will not be used for human consumption, and the male inflorescences will either be bagged or removed. The trial site will be located on a rural, commercial property approximately 500 m from the nearest roadway which would limit access by people. Therefore, the people that will be exposed to the introduced genes and their products will be limited to trained staff involved in cultivating, harvesting, and transporting the GM banana. Access would be further limited by an existing fence with a lockable gate (see Chapter 1, Section 6.2) which surrounds the entire property and an additional fence proposed by the applicant to surround the trial site.

128. The proposal to bag fruit and to either bag or remove male inflorescences would also minimise exposure of frugivores and flower feeding animals such as insects, birds and bats. Fruit will be harvested while still green, which is a standard practice of commercial banana production. After harvest and weighing the green bananas, the applicant has indicated the fruit will be shredded and left to decompose on the ground. In tropical environments, shredded fruit is expected to rapidly decompose and become unpalatable to vertebrates. This method of destroying fruit was used at other banana field trials in northern QLD and the NT (DIRs 076/2007, 079/2007 and 107) and the applicant states that no vertebrates have been observed consuming shredded fruit from these trials. Thus, the shredding and decomposition of the banana fruit may further limit exposure to vertebrates such as birds and bats.

129. It is possible that larger animals such as feral pigs and water buffalo or domestic livestock would attempt to access the site and thus be exposed to the GM banana or its products. However, exposure of larger animals to the GM banana plants would be minimal as the proposed trial is limited to one site with a maximum size of 6 ha.

130. Other organisms would be exposed to the GM banana plants as they grow and to any GM plant material that may fall to the ground or is left to decompose on the ground of the trial site.

Potential harm

131. Potentially, people exposed to the proteins expressed by the introduced genes may show increased toxic reactions or increased allergenicity. From consideration of the causal pathway, these are limited to staff involved in handling and harvesting the GM banana plants during the course of the field trial. Similarly, exposure to the proteins expressed by the introduced genes may lead to increased toxicity to other desirable organisms.

132. The introduction of the candidate genes does not lead to expression of a novel protein which is from any class of proteins having known toxic or allergenic members (Radauer & Breiteneder 2007). Although proteins are not generally associated with toxicity, all known food allergens are proteins. Plant derived allergens come chiefly from peanut, tree nuts, wheat and soybean (Delaney et al. 2008; Herman & Ladics 2011). As the candidate genes are not derived from these plant species, they are unlikely to be allergenic. Exposure of staff to the GM plant material either in the glasshouse or, in the case of GM banana lines containing ced-9 or RGA2, through previous field trials, did not result in adverse reactions.

133. Non-GM banana is not known to be toxic to humans or other organisms. Although no toxicity or allergenicity studies have been performed on the GM banana plant material, the introduced genes were isolated from naturally occurring organisms that are already widespread and prevalent in the environment, including common food plants (banana) and a soil organism (nematode) (Chapter 1, Section 5.5). Thus, people and other organisms are exposed to the same or similar proteins through their diet and the

environment. Therefore, the allergenic and toxic properties are not expected to be altered in the GM banana lines proposed for release.

Conclusion

134. Risk scenario 1 is not identified as a substantive risk, due to limited exposure and the lack of toxicity or allergenicity of the introduced proteins to humans or other desirable organisms. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Risk scenario 2

Risk source	Introduced genes for resistance to Foc TR4
Causal pathway	⬇ Growing GM banana plants at the field trial site ⬇ Expression of the genes in GM plants ⬇ Dispersal of propagules to nature reserves, roadsides, drains or intensive use areas ⬇ Establishment and persistence of volunteer GM bananas plants in nature reserves, roadside areas or intensive use areas ⬇
Potential harm	Increased exposure to and thus increased toxicity or allergenicity for humans and increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants OR Reduced utility or quality of the environment

Risk source

135. The source of potential harm for this postulated risk scenario is the introduced candidate genes for resistance to Foc TR4.

Causal pathway

136. If the expression of the introduced genes were to provide the GM banana plants with a significant selective advantage over non-GM bananas or the follow on crop, then this may lead to persistence of the GM bananas at the release site. Additionally, if the GM banana plants were dispersed and were able to establish and persist in non-agricultural environments, this may give rise to adverse outcomes.

137. Extremes of weather, such as flooding or strong winds may disperse plant parts. However, according to the applicant, the property has been laser levelled and is not prone to heavy run-off. Although strong winds may remove leaves or fruit, these are not capable of vegetative propagation. Banana plants can propagate vegetatively from sections of the corm containing buds from which suckers are produced. There have been no reports of cyclones or storms uprooting and dispersing propagative material at the release site. Additionally, dispersal of vegetative, propagative material has not been observed for other trials of GM banana due to extreme weather conditions. Therefore, dispersal of propagative plant material by flooding or strong winds is unlikely.

138. It is possible that large animals such as feral pigs and water buffalo or domestic livestock may be able to disperse vegetative plant material. Bananas are a large plant with a fibrous root system and suckers are firmly attached to the corm. Although large animals may damage plants at ground level, there have been no records of animals uprooting banana plants or detaching suckers. Thus these animals are unlikely to disperse propagative material from the site.

139. People could also disperse propagative material from the release site. Control measures proposed by the applicant as well as the proposed limits of the release will minimise dispersal outside the trial site (Chapter 1, Section 3.2). GM plant materials will be transported in accordance with the Regulator's transportation guidelines as well as any State and Territory regulations. Only plant materials needed for

experimentation will be transported outside the site and will be contained to prevent any loss of material. Plant material will be cleaned from any equipment, including clothing, prior to its removal from the trial site. Banana bunches would be harvested green, assessed and then destroyed. Therefore, exposure to the GMOs and GM plant material would be restricted to trained staff and this would minimise dispersal outside the trial site.

140. There has been limited characterisation of the GM banana lines; most of the introduced genes have not previously been characterised in banana. As discussed in Chapter 1, Section 5.4.1, the *R1-R7* genes are expected to confer resistance to Foc TR4. All of the R genes were derived from banana, thus there is no expectation of other changes to phenotype. Genes homologous to *R1-R7* in other plants are not known to affect other metabolic pathways in other plant species. In previous studies, GM bananas expressing *RGA2* (also an *R* gene) showed increased resistance or immunity to Foc TR4; no other unusual phenotypes were observed.

141. As discussed in Chapter 1, Section 5.4.2, c*ed-9* is involved in regulation of apoptosis, but may also be involved in normal cell physiology and development. Work with homologous genes in other plant species indicates possible increased tolerance to other stressors such as heat, cold, salt, oxidative stress and resistance to other pathogens. In previous trials, GM banana expressing *ced-9* showed increased resistance to Foc race 1, Foc TR4, and in glasshouse trials, increased tolerance to water stress and the herbicide paraquat. As this gene has a role in the regulation of apoptosis, it may confer resistance or susceptibility to other pathogens. Based on the above, the *ced-9* gene may confer traits which could provide the GM banana lines with a selective advantage compared to non-GM bananas.

142. As discussed in Chapter 1, Section 5.4.2, the *AA1* gene is expected to provide the GM banana plants with increased resistance to Foc TR4 by increasing stress tolerance or through the regulation of apoptosis. In previous trials, GM banana plants expressing similar genes derived from Arabidopsis and rice showed no discernible negative effects.

143. Baseline information on the weediness of banana, including factors limiting the spread and persistence of non-GM plants of these species, is given in *The Biology of* Musa L. (banana) (OGTR 2016). In summary, commercial cultivars of bananas are not considered weedy, they lack the ability to compete with other plants and are unlikely to persist outside areas of intensive cultivation aimed at banana production. Because commercial cultivars of bananas are effectively sterile and rarely produce seed, they lack many characteristics of invasive plants, such as the ability to produce a persisting seed bank, rapid growth to flowering, continuous seed production as long as growing conditions permit, high seed output, high seed dispersal and long-distance seed dispersal (Keeler 1989).

144. The geographic range of non-GM banana in Australia is limited by a number of abiotic factors, particularly water and temperature stress (OGTR 2016). Bananas are not frost tolerant and are restricted to subtropical and tropical areas with a mean air temperature of 26.7°C. Generally they require 50-100 mm of water per week. Although candidate genes such as *ced-9* and *AA1* may provide enhanced tolerance to some of these abiotic factors, they are unlikely to enhance tolerance to all the abiotic factors which limited spread and persistence of non-GM banana.

145. The most conspicuous biotic factor affecting bananas is competition with other plants. They are quickly killed by deep shade and intolerant of root competition, with particular sensitivity to the presence of grasses (OGTR 2016). Other biotic factors, such as pests and diseases can affect their ability to establish, spread and persist. All the candidate genes are expected to confer resistance to Foc TR4 and possibly to other diseases as well. Thus, the introduced candidate genes are unlikely to overcome the multitude of biotic factors that limit the spread and persistence of banana in the environment.

146. The GM bananas are unlikely to persist at the release site once the trial has been completed. The applicant has proposed to disc the trial site multiple times and treat any volunteers with herbicide. The site would be monitored for at least one year post-harvest and any volunteers would be destroyed. These measures are considered to minimise the likelihood of persistence of GMOs after completion of the trial and have been effective in previous trials of GM bananas.

Potential harm

147. If the GM banana plants were to persist at the release site or be dispersed, establish and persist in non-agricultural environments, this may give rise to adverse outcomes such as:

- increased exposure to and thus increased toxicity or allergenicity for humans and increased toxicity to other desirable organisms
- reduced establishment or yield of desirable plants, or
- reduced utility or quality of the environment.

148. If GM banana plants were to establish beyond the trial limits, they could potentially cause toxicity or allergenicity in people, or toxicity to desirable organisms, or reduced establishment or yield of desirable plants. However, as discussed in risk scenario 1, the introduced gene products are not expected to be toxic or allergenic to people or to other organisms. This would apply even if the GM banana plants established beyond the trial limits.

149. If GM banana plants were to establish and persist beyond the trial limits, this could potentially impact the environment, e.g. it could reduce establishment or yield of desirable agricultural crops; reduce establishment of desirable native vegetation; reduce utility of roadsides, drains, channels and other intensive use areas; or reduce the quality of the biotic environment by providing a reservoir for pathogens or pests. As discussed above, the causal pathways which may lead to increased spread and persistence of the GM bananas are unlikely to occur. Therefore, the presence in banana of any of the introduced genes is unlikely to lead to any of the potential harms listed above.

Conclusion

150. Risk scenario 2 is not identified as a substantive risk due to the extremely limited ability of the GM banana to spread and persist outside cultivation, the proposed limits and controls designed to restrict dispersal, and the susceptibility of the GM banana to post-harvest controls which will minimise persistence at the release site. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

151. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis[7].

152. There are several types of uncertainty in risk analysis (Bammer & Smithson 2008; Clark & Brinkley 2001; Hayes 2004). These include:

- uncertainty about facts:
  - knowledge – data gaps, errors, small sample size, use of surrogate data
  - variability – inherent fluctuations or differences over time, space or group, associated with diversity and heterogeneity
- uncertainty about ideas:
  - description – expression of ideas with symbols, language or models can be subject to vagueness, ambiguity, context dependence, indeterminacy or under-specificity
  - perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

153. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

[7] A more detailed discussion of uncertainty is contained in the Regulator's *Risk Analysis Framework* available from the OGTR website or via Free call 1800 181 030.

154. As field trials of GMOs are designed to gather data, there are generally data gaps when assessing the risks of a field trial application. However, field trial applications are required to be limited and controlled. Even if there is uncertainty about the characteristics of a GMO, limits and controls restrict exposure to the GMO, and thus decrease the likelihood of harm.

155. For DIR 146, uncertainty is noted particularly in relation to:

- potential increases in toxicity or allergenicity as a result of the genetic modification
- potential for increased spread and persistence of the GMOs, including in land uses outside of agriculture.

156. Additional data, including information to address these uncertainties, may be required to assess possible future applications with reduced limits and controls, such as a larger scale trial or the commercial release of these GMOs.

157. Chapter 3, Section 4, discusses information that may be required for future release.

Section 4 Risk Evaluation

158. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

159. Factors used to determine which risks need treatment may include:

- risk criteria
- level of risk
- uncertainty associated with risk characterisation
- interactions between substantive risks.

160. Two risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the control measures proposed by the applicant, and considering both the short and long term, neither of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 3 and include:

- none of the GM plant material or products will used for human food or animal feed
- the evidence indicates that the introduced proteins are unlikely to be toxic or allergenic
- limited ability of the GM banana plants to establish populations outside cultivation
- limited ability of the GM banana plants to transfer the introduced genetic material to other plants
- limits on the size, location and duration of the release proposed by QUT
- suitability of controls proposed by QUT to restrict the spread and persistence of the GM banana plants and their genetic material.

161. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM banana plants into the environment are considered to be negligible. The Risk Analysis Framework, which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Chapter 3 Risk management plan

Section 1 Background

162. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan addresses risks evaluated as requiring treatment and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through licence conditions.

163. 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.

164. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: section 64 requires the licence holder to provide access to premises to OGTR inspectors 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.

165. The licence is also 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. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Risk treatment measures for substantive risks

166. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed field trial of GM banana. These risk scenarios were considered in the context of the scale of the proposed release, the proposed containment measures, and the receiving environment, and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

167. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, licence conditions have been imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this chapter and listed in detail in the licence.

3.1 Licence conditions to limit and control the release

3.1.1 Consideration of limits and controls proposed by QUT

168. Sections 3.1 and 3.2 of Chapter 1 provide details of the limits and controls proposed by QUT in their application. Many of these are discussed in the two risk scenarios characterised for the proposed release in Chapter 2. The appropriateness of these controls is considered further below.

169. The release will be limited to a maximum of 6 ha on one site in the Litchfield Municipality LGA (NT) and the duration of the release will be limited to five years. Only staff with appropriate training will be allowed access to the trial site, shadehouse and tissue culture facility. The applicant does not intend to use any of the GM plant material for human food or animal feed. These measures will limit the potential

exposure of humans, vertebrates and other organisms to the GMOs (Risk Scenario 1) and the potential for the GM banana lines to disperse and establish outside the proposed release site (Risk Scenario 2).

170. The proposed trial site is located within a property that is surrounded by a fence with lockable gates. In addition, the applicant has proposed surrounding the trial site with fencing. Although the fencing may further reduce access to the trial site by people and large animals, due to the lack of toxicity of the GM bananas, lack of likelihood of dispersal of propagative plant material and the small size and short duration of the trial, (as discussed in Risk Scenarios 1 and 2)fencing has not been imposed as a licence condition.

171. The field location is bordered on one side by a deep (approximately 1 m) drainage channel which is not connected to any waterway. The applicant has stated that the proposed trial site is at least 1 km from the nearest waterways and that the site is level and not prone to flooding, heavy runoff or landslips, which reduces the likelihood of plant material being washed away from the site (Risk Scenario 2). It is a standard DIR licence condition that trial sites must be located at least 50 metres from a waterway to limit the dispersal of viable GM plant material in the event of flooding. In addition, a licence condition has been imposed requiring immediate notification of any extreme weather conditions affecting the site during the release.

172. During the previous trial at this site (DIR 107) the licence holder was required to maintain a 10 m isolation zone between the GM banana trial and the commercial banana production on the property. The purpose of the isolation zone was to provide a clear boundary for the trial. The DBFC property no longer grows bananas commercially and thus QUT did not propose this control measure for the current application. However, it is possible that commercial banana production may commence again during the proposed 5 year trial, thus a licence condition has been imposed for a 10 m isolation zone to ensure the trial clearly delineated from other bananas on the DBFC property.

173. In addition, the applicant has proposed a number of other measures to ensure segregation. Specifically, only staff with appropriate training will be permitted to enter the trial site. All plants grown within the trial site will be treated as GMOs. Except for experimental purposes, fruit and other plant material will not be removed from the trial site.

174. The covering of bunches is a standard practice used in commercial, non-GM banana cultivation. This practice as applied to GM fruit would limit the potential exposure of frugivores to the GMOs (Risk Scenario 1) as well as limit the potential for the GM banana fruit to disperse, in the unlikely event that any seed is produced (Risk Scenario 2), and has been imposed as a licence condition.

175. The applicant proposes to harvest fruit while it is still hard and green (unripe) and firmly attached to the plant, as is standard commercial practice. Unripe fruit is less appealing to frugivores such as bats and birds than fully ripe fruit. Fruit that is not required for experimental analysis would be destroyed by shredding and placing it on the ground at the field location to decompose. In tropical environments, shredded fruit is expected to rapidly decompose and become unpalatable to vertebrates. This method of destroying fruit was used at other banana field trials in northern QLD and the NT (DIRs 076/2007, 079/2007 and 107) and the applicant states that no vertebrates have been observed consuming shredded fruit from these trials. These practices have been imposed as licence conditions which would further limit the potential for exposure of animals to, or dispersal of, the GMOs (Risk Scenarios 1 and 2).

176. The applicant proposes to remove and dispose of male flowers, or bag any male flowers that are left on the plant for analysis. Removing or bagging of male flowers would occur prior to the opening of the floral bracts that enclose the flowers. Removed flowers would be placed in a secure container and allowed to decompose. Decomposed material would be removed from the container and left on the ground at the field location. Although sexual reproduction is unlikely, these measures would further reduce the chances of any pollen that may be produced remaining viable or being dispersed into the environment (Risk Scenario 2) and have been imposed as licence conditions. They would also limit the potential exposure of nectar feeding animals including birds, marsupials and bats (Risk Scenario 1). Since bananas sometimes produce hermaphrodite flowers that can contain small amounts of pollen, it is considered appropriate to also remove or bag hermaphrodite flowers.

177. The applicant proposes to desucker plants at the field location by cutting off suckers at ground level, gouging out the centre of the pseudostem and pouring in kerosene or distillate. This method is commonly used in commercial banana cultivation (Broadley et al. 2004) and kills the growing point of the sucker while it is still firmly attached to the corm. Removed pseudostems and leaf material would be left on the ground at the field location to decompose. This method is considered to be appropriate for preventing dispersal of the GMO (Scenario 2) and has been imposed as a licence condition.

178. The applicant proposes to collect plant waste, including unwanted GMOs, from the shadehouse and tissue culture facility in a decomposition bin located within each structure. Both the shadehouse and tissue culture facility are permanent, lockable structures with access restricted to trained staff only. Once the plant material had decomposed it would be transported and emptied onto the ground at the field trial site. As the decomposed waste material left on the ground would therefore be non-propagative, this destruction method is considered to be appropriate for preventing dispersal (Risk Scenario 2) and has been imposed as a licence condition.

179. The applicant proposes to destroy plants at the end of the trial using either of two methods. The first method of destruction is by injection with herbicide (e.g. glyphosate). For the first method, the systemic nature of these herbicides means that the whole plant, including the corm, starts to die and decay rapidly and virtually no regrowth occurs. 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 (Lindsay et al. 2003). The second method is by repeated discing of the trial site to break-up the banana corm, followed by injection with herbicide of any volunteer plants which may emerge. The discing cuts up the plant material and corm and thus leads to faster decomposition compared to the first method. Both methods have been used successfully by the applicant in previous field trials. Following either method of destruction, the applicant has proposed to monitor the field site for 12 months for volunteer banana plants, and to destroy by herbicide treatment any volunteers found. In addition to this, the applicant has proposed that monitoring of the release site would continue until no volunteers are detected for at least six continuous months. These measures would minimise the persistence of the GMOs in the environment (Risk Scenario 2) and have been imposed as licence conditions.

180. The applicant has stated that any plant material taken to or from the site will be transported according to the Regulator's Guidelines for the Transport of GMOs. The guidelines are standard protocols for the handling of GMOs to minimise exposure of the GMOs to human and other organisms (Risk Scenario 1) and dispersal into the environment (Risk Scenario 2).

181. In addition to the above points, QLD and NT Government legislation targeted to the cultivation and transport of bananas and control of plant diseases (see Chapter 1, Section 6.1) would also apply to the release of GM bananas and would act as an effective adjunct to the imposed control measures.

3.1.2 Summary of draft licence conditions to be implemented to limit and control the release

182. A number of licence conditions have been drafted to limit and control the release, based on the above considerations. These include requirements to:

- limit the release to a maximum total area of 6 ha at one site on the DBFC property in the Litchfield Municipality LGA for a period of 5 years, from January 2017 to January 2022
- locate the trial site at least 50 m away from waterways
- maintain a 10 m zone around the GM bananas in which no bananas may be grown
- 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
- harvest the GM banana separately from other crops
- clean all equipment used in connection with the GMOs

- monitor the field site for at least 12 months after harvest and destroy any volunteer banana plants that may grow, until no volunteers are detected for a continuous 6 month period
- destroy all GM plant material, including fruit, not required for further analysis
- transport and store all GMOs in accordance with the Regulator's guidelines
- not permit any GM banana plant material to be used in human food or animal feed.

3.2 Other risk management considerations

183. All DIR licences issued by the Regulator contain a number of conditions that relate to general risk management. These include conditions relating to:

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting requirements
- access for the purpose of monitoring for compliance.

3.2.1 Applicant suitability

184. 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, for either an individual applicant or a body corporate, include:

- any relevant convictions of the applicant
- 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 capacity of the applicant to meet the conditions of the licence.

185. If a licence were issued, the conditions would include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

186. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

3.2.2 Contingency plan

187. If a licence were issued, QUT would be required to submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any unintended presence of the GM banana outside permitted areas.

188. QUT would also be required to provide the Regulator with a method to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism. This methodology would be required before planting the GMOs.

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

189. If a licence were issued, the persons covered by the licence would be 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. Prior to growing the GMOs, QUT would be required to provide a list of people and organisations that will be covered by the licence, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

190. If issued, the licence would require 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.

191. A number of written notices would also be required under the licence to assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices would include:

- expected and actual dates of planting
- details of areas planted to the GMOs
- expected and actual dates of harvest and cleaning after harvest
- details of inspection activities.

3.2.5 Monitoring for compliance

192. 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 release site.

193. 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.

194. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides 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 4 Issues to be addressed for future releases

195. Additional information has been identified that may be required to assess an application for a commercial release of these GM banana lines, or to justify a reduction in limits and controls. This includes:

- additional molecular and biochemical characterisation of the GM banana lines, particularly with respect to potential for increased toxicity and allergenicity, and
- additional phenotypic characterisation of the GM banana lines, particularly with respect to traits which may contribute to weediness.

Section 5 Conclusions of the consultation RARMP

196. The RARMP concludes that the proposed limited and controlled release of GM banana poses negligible risks to the health and safety of people or the environment as a result of gene technology, and that these negligible risks do not require specific risk treatment measures.

197. However, conditions have been imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.

References

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

Babaeizad, V., Imani, J., Kogel, K.H., Eichmann, R., Huckelhoven, R. (2009) Over-expression of the cell death regulator BAX inhibitor-1 in barley confers reduced or enhanced susceptibility to distinct fungal pathogens. *Theoretical and Applied Genetics* **118**: 455-463.

Bammer, G., Smithson, M. (2008) *Uncertainty and risk: Multidisciplinary perspectives.* Bammer, G., Smithson, M., eds. Earthscan, London.

Beck, E., Ludwig, G., Auerswald, E.A., Reiss, B., Schaller, H. (1982) Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. *Gene* **19**: 327-336.

Bevan, M. (1984) Binary *Agrobacterium* vectors for plant transformation. *Nucleic Acids Research* **12**: 8711-8721.

Blattner, F.R., Plunkett, G.I., Bloch, C.A., Perna, N.T., Burland, V., Riley, M. et al. (1997) The complete genome sequence of *Escherichia coli* K-12. *Science* **277**: 1453-1462.

Bossy-Wetzel, E., Green, D.R. (1999) Caspases induce cytochrome c release from mitochondria by activating cytosolic factors. *Journal of Biological Chemistry* **274**: 17484-17490.

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

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

Chen, X., Shang, J., Chen, D., Lei, C., Zou, Y., Zhai, W. et al. (2006) A β-lectin receptor kinase gene conferring rice blast resistance. *The Plant Journal* **46**: 794-804.

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.

Clark, A.J. and Brinkley, T. (2001) Risk management: for climate, agriculture and policy. Commonwealth of Australia, Canberra.

Conradt, B., Xue, D. (2005) Programmed cell death. In: *Wormbook*, The C.elegans Research Community, ed . Wormbook, doi/10.1895/wormbook.1.32.1.

Daly, A. (2006) *Fusarium* wilt of bananas (Panama disease) (*Fusarium oxysporum* f. sp. *cubense*). Northern Territory Government.

Delaney, B., Astwood, J.D., Cunny, H., Conn, R.E., Herouet-Guicheney, C., MacIntosh, S. et al. (2008) Evaluation of protein safety in the context of agricultural biotechnology. *Food and Chemical Toxicology* **46**: S71-S97.

Dickman, M.B. (2004) Can model plants help banana improvement through biotechnology? *Infomusa* **13**: 6-11.

Dickman, M.B., Park, Y.K., Oltersdorf, T., Li, W., Clemente, T., French, R. (2001) Abrogation of disease development in plants expressing animal antiapoptotic genes. *Proceedings of the National Academy of Sciences* **98**: 6957-6962.

Dodds, P.N., Lawrence, G.J., Catanzariti, A.M., Teh, T., Wang, C.I.A., Ayliffe, M.A. et al. (2006) Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proc Natl Acad Sci U S A* **103**: 8888-8893.

Dodds, P.N., Rathjen, J.P. (2010) Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat Rev Genet* **11**: 539-548.

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

Gallie, D.R., Browning, K.S. (2001) eIF4G functionally differs from eIFiso4G in promoting internal initiation, cap-independent translation, and translation of structured mRNAs. *Journal of Biological Chemistry* **276**: 36951-36960.

Gallie, D.R., Tanguay, R.L., Leathers, V. (1995) The tobacco etch viral 5' leader and poly(A) tail are functionally synergistic regulators of translation. *Gene* **165**: 233-238.

Glazebrook, J. (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* **43**: 205-227.

Gonzalez-Cendales, Y., Catanzariti, A.M., Baker, B., Mcgrath, D.J., Jones, D.A. (2016) Identification of I-7 expands the repertoire of genes for resistance to *Fusarium* wilt in tomato to three resistance gene classes. *Mol Plant Pathol* **17**: 448-463.

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

Greenberg, J.T., Yao, N. (2004) The role and regulation of programmed cell death in plant-pathogen interactions. *Cellular Microbiology* **6**: 201-211.

Grice, K., Henderson, J., Pattison, T., Thomas, J., Vawdrey, L., Young, A. (2009) Chapter 5: Banana. In: *Diseases of Fruit Crops in Australia*, Cooke, T., Persley D., House S., eds . CSIRO Publishing Collingwood, VIC. 65-89.

Guerineau, F., Woolston, S., Brooks, L., Mullineaux, P. (1988) An expression cassette for targeting foreign proteins into chloroplasts. *Nucleic Acids Research* **16**: 11380.

Hardwick, J.M., Soane, L. (2013) Multiple Functions of BCL-2 Family Proteins. *Cold Spring Harbor Perspectives in Biology* **5**: a008722.

Hayes, K. R. (Accessed:11-8-2004) Ecological implications of GMOs: robust methodologies for ecological risk assessment. Best practice and current practice in ecological risk assessment for genetically modified organisms. CSIRO Division of Marine Research.

Hengartner, M.O., Ellis, R., Horvitz, R. (1992) *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. *Nature* **356**: 494-499.

Herman, R.A., Ladics, G.S. (2011) Endogenous allergen upregulation: transgenic vs. tradtionally bred crops. *Food and Chemical Toxicology* **49**: 2667-2669.

Hermann, S.R., Harding, R.M., Dale, J.L. (2001) The banana actin 1 promoter drives near-constitutive transgene expression in vegetative tissues of banana (*Musa* spp.). *Plant Cell Reports* **20**: 525-530.

Israeli, Y., Lahav, E., Reuveni, O. (1995) Chapter 6: *In vitro* culture of bananas. In: *Bananas and Plantains*, Gowen, S., ed . Chapman & Hall London, UK. 147-178.

Jones, J.D.G., Dangl, J.L. (2006) The plant immune system. *Nature* **444**: 323-329.

Juers, D.H., Matthews, B.W., Huber, R.E. (2012) LacZ beta-galactosidase: structure and function of an enzyme of historical and molecular biological importance. *Protein Sci* **21**: 1792-1807.

Keeler, K.H. (1989) Can genetically engineered crops become weeds? *Bio/Technology* **7**: 1134-1139.

Keese, P. (2008) Risks from GMOs due to horizontal gene transfer. *Environmental Biosafety Research* **7**: 123-149.

Keese, P.K., Robold, A.V., Myers, R.C., Weisman, S., Smith, J. (2014) Applying a weed risk assessment approach to GM crops. *Transgenic Research* **23**: 957-969.

Khanna, H.K., Paul, J.Y., Harding, R.M., Dickman, M.B., Dale, J.L. (2007) Inhibition of *Agrobacterium*-Induced Cell Death by Antiapoptotic Gene Expression Leads to Very High Transformation Efficiency of Banana. *Molecular Plant-Microbe Interactions* **20**: 1048-1054.

Khurana, S.M.P., Pandey, S.K., Sarkar, D., Chanemougasoundharam, A. (2005) Apoptosis in plant pathogen disease response: A close encounter of the pathogen kind. *Current Science* **88**: 740-752.

Ladics, G.S., Bartholomaeus, A., Bregitzer, P., Doerrer, N.G., Gray, A., Holzhauser, T. et al. (2015) Genetic basis and detection of unintended effects in genetically modified crop plants. *Transgenic Research* **24**: 587-603.

Laluk, K., Mengiste, T. (2010) Necrotroph Attacks on Plants: Wanton Destruction or Covert Extortion? In: *The Arabidopsis Book*, null Edition The American Society of Plant Biologists. 1-34.

Li, W., Dickman, M.B. (2004) Abiotic stress induces apoptotic-like features in tobacco that is inhibited by expression of human Bcl-2. *Biotechnology Letters* **26**: 87-95.

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.

Lozano, R., Hamblin, M.T., Prochnik, S., Jannink, J.L. (2015) Identification and distribution of the NBS-LRR gene family in the Cassava genome. *BMC Genomics* **16**: 360.

Marone, D., Russo, M.A., Laido, G., De Leonardis, A.M., Mastrangelo, A.M. (2013) Plant nucleotide binding site-leucine-rich repeat (NBS-LRR) genes: active guardians in host defense responses. *Int J Mol Sci* **14**: 7302-7326.

McHale, L., Tan, X., Koehl, P., Michelmore, R.W. (2006) Plant NBS-LRR proteins: adaptable guards. *Genome Biol* **7**: 212.

OGTR (2013a) *Risk Analysis Framework.* Office of the Gene Technology Regulator, Canberra, Australia.

OGTR (2013b) Risk Analysis Framework 2013. Australian Government Office of the Gene Technology Regulator, Canberra.

OGTR (2016) The biology of *Musa* L. (banana). Office of the Gene Technology Regulator.

Paul, J.-Y., Becker, D.K., Dickman, M.B., Harding, R.M., Khanna, H.K., Dale, J.L. (2011) Apoptosis–related genes confer resistance to Fusarium wilt in transgenic 'Lady Finger' bananas. *Plant Biotechnology Journal* **9**: 1141-1148.

Paul, J.Y. (2009) [Thesis] The manipulation of apoptosis-related genes to generate resistance to fusarium wilt and water stress in banana. Queensland University of Technology.

Pennell, R.I., Lamb, C. (1997) Programmed Cell Death in Plants. *The Plant Cell* **9**: 1157-1168.

Peraza-Echeverria, S., Dale, J.L., Harding, R.M., Collet, C. (2009) Molecular cloning and in silico analysis of potential Fusarium resistance genes in banana. *Molecular Breeding* **23**: 431-443.

Peraza-Echeverria, S., Dale, J.L., Harding, R.M., Smith, M.K., Collet, C. (2008) Characterization of disease resistance gene candidates of the nucleotide binding site (NBS) type from banana and correlation of a transcriptional polymorphism with resistance to Fusarium oxysporum f.sp. cubense race 4. *Molecular Breeding* **22**: 565-579.

Ploetz, R.C. (2006) Fusarium Wilt of Banana Is Caused by Several Pathogens Referred to as *Fusarium oxysporum* f. sp. *cubense*. *Phytopathology* **96**: 653-656.

Radauer, C., Breiteneder, H. (2007) Evolutionary biology of plant food allergens. *Journal of Allergy and Clinical Immunology* **120**: 518-525.

Reape, T.J., Molony, E.M., McCabe, P.F. (2008) Programmed cell death in plants: distinguishing between different modes. *Jornal of Experimetal Botany* **59**: 435-444.

Righetti, L., Djennane, S., Berthelot, P., Cournol, R., Wilmot, N., Loridon, K. et al. (2014) Elimination of the nptII marker gene in transgenic apple and pear with a chemically inducible R/Rs recombinase. *Plant Cell Tissue and Organ Culture* **117**: 335-348.

Ross, E.M. (1987) Musaceae. In: *Flora of Australia Volume 45, Hydatellaceae to Liliaceae*, George, A.S., ed . Australian Government Publishing Service Canberra. 16-19.

Salvesen, G.S. (1999) Programmed cell death and the caspases. *APMIS* **107**: 73-79.

Schaart, J.G., Krens, F.A., Pelgrom, K.T., Mendes, O., Rouwendal, G.J. (2004) Effective production of marker-free transgenic strawberry plants using inducible site-specific recombination and a bifunctional selectable marker gene. *Plant Biotechnol J* **2**: 233-240.

Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N. et al. (2015) A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. *Transgenic Research* **24**: 1-17.

Shabala, S., Cuin, T., Prismall, L., Nemchinov, L. (2007) Expression of animal CED-9 anti-apoptotic gene in tobacco modifies plasma membrane ion fluxes in response to salinity and oxidative stress. *Planta* **227**: 189-197.

Shaw, C.H., Carter, G.H., Watson, M.D., Shaw, C.H. (1984) A functional map of the nopaline synthase promoter. *Nucleic Acids Research* **12**: 7831-7846.

Society of Toxicology (2003) Society of Toxicology position paper: The safety of genetically modified foods produced through biotechnology. *Toxicological Sciences* **71**: 2-8.

Standards Australia Ltd, Standards New Zealand, CRC for Australian Weed Management (2006) *HB294:2006 National Post-Border Weed Risk Management Protocol.* Available online.

Steiner, H.Y., Halpin, C., Jez, J.M., Kough, J., Parrott, W., Underhill, L. et al. (2013) Evaluating the potential for adverse interactions within genetically engineered breeding stacks. *Plant Physiology* **161**: 1587-1594.

Tadege, M., Bucher, M., Stahli, W., Suter, M., Dupuis, I., Kuhlemeier, C. (1998) Activation of plant defense responses and sugar efflux by expression of pyruvate decarboxylase in potato leaves. *The Plant Journal* **16**: 661-671.

Wang, Y., Yau, Y.Y., Perkins-Balding, D., Thomson, J.G. (2011) Recombinase technology: applications and possibilities. *Plant Cell Rep* **30**: 267-285.

Weber, N., Halpin, C., Hannah, L.C., Jez, J.M., Kough, J., Parrott, W. (2012) Crop genome plasticity and its relevance to food and feed safety of genetically engineered breeding stacks. *Plant Physiology* **160**: 1842-1853.

Xu, P., Rogers, S.J., Roossinck, M.J. (2004) Expression of antiapoptotic genes *bcl-xL* and *ced-9* in tomato enhances tolerance to viral-induced necrosis and abiotic stress. *Proceedings of the National Academy of Sciences* **101**: 15805-15810.

Yanisch-Perron, C., Vieira, J., Messing, J. (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mpl8 and pUC19 vectors. *Gene* **33**: 103-119.

Appendix A Summary of submissions from prescribed experts, agencies and authorities[8]

Advice received by the Regulator from prescribed experts, agencies and authorities on the consultation RARMP is summarised below. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Abbreviations: GM: Genetically Modified; **RARMP**: Risk Assessment and Risk Management Plan; **Sub. No:** submission number.

Sub. No:	Summary of issues raised	Comment
1	No objections to the issue of a licence for DIR 146.	Noted.
2	Supports the conclusion that DIR 146 poses negligible risk of harm to human health and safety and the environment.	Noted.
3	The risk of unintended release into the environment of GM plant material, or gene transfer to non-GM banana or related species is appropriately minimised by the proposed limits and controls.	Noted.
	The consultation RARMP notes all the proteins encoded by the introduced genes for resistance to *Fusarium* have homologues that occur naturally in a range of organisms that are used as food sources by both people and animals, and hence people and animals have a history of exposure (§55-63, 91). Believes it should be stated more directly that the proteins that are derived from banana (not just their homologues) have been part of the food source of both people and animals without any known adverse effects.	Noted. The RARMP does make this point in Chapter 2, Risk Scenario 1, indicating that the introduced genes are derived from naturally occurring organisms that are widespread and prevalent in the environment, i.e. common food plants such as banana. Furthermore it states that people and other organisms are exposed to the same or similar proteins through their diet and the environment. Additional text has been added to RARMP.
	Discussion of toxicity and allergenicity could be expanded by recording that none of the banana proteins expressed from the introduced genes in the GM plants belong to classes of plant proteins that have been associated with the adverse effects in people, and likely therefore higher organisms (such as native animals).	Noted. Risk Scenario 1 has been amended using relevant references.
	The consultation RARMP notes that as the resistance genes are derived from banana, there is not expected to be any other change to phenotype other than disease resistance (§138). In the case of *ced-9,* the consultation RARMP notes that work with homologous genes indicates that it could possibly increase tolerance to some abiotic and biotic stress tolerances, which may provide a selective advantage (§139). Believes that this information is important and should be supported by references.	The paragraph refers to Chapter 1, Section 5.4.2, where the potential for increased tolerance to some abiotic and biotic stressors is discussed more fully. The discussion is supported by published references and data provided by the applicant generated from previous trials of GM banana containing the *ced-9* gene.

[8] Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Sub. No:	Summary of issues raised	Comment
	The consultation RARMP notes that there are two native *Musa* species in Australia, but neither occurs in the region where the field trial will occur (§115). If gene flow occurred to a sexually compatible species, any trait conferred (most obviously disease resistance) would not increase the toxicity, allergenicity or weediness of the recipient or hybrid plants. The basis for this conclusion is the experience gained from conventional breeding. Believes that greater emphasis could be placed in the risk assessment on the accumulated experience gained from conventional breeding suggesting that the risks to the environment of the GM plants will be negligible. In particular, the trait of disease resistance (in many species) has been associated with a history of safe use.	Agreed, the experience gained through conventional breeding is relevant.
	While the risks of increased toxicity or increased weediness due to the genetic modification are low, there is always uncertainty regarding potential unintended effects of genetic modifications. Generally agrees that there is uncertainty with respect to potential increases in toxicity, allergenicity, and weediness (§153 of the consultation RARMP), but the experience of conventional breeding with resistance genes is that these factors are unlikely to be of significance. As such, in view of any commercial release, consideration should be given to whether tests for toxicity and allergenicity with the plant derived resistance proteins are necessary. Regardless, it is suggested that the text in § 193 be modified to include reference to exposure, for example "---respect to potential for increased toxicity and allergenicity, exposure due to a large scale release, and ".	Noted. A commercial release involving the sale of fruit from the GM bananas for human food would require approval from FSANZ. Chapter 3, Section 4 refers to data that may be required for an application involving commercial release or a release with reduced limits and controls. The assessment of such an application would consider exposure to humans and other organisms. As such, the exposure is likely to be greater than that of this field trial.
4	The procedures for cultivation and handling the bananas are pretty standard for the industry.	Noted.
	The Consultation Summary states: *The GM banana plants may also contain a marker gene, from a common soil bacterium that allowed the GM plants to be selected during their initial development in the laboratory*. • It would be more helpful in this evaluation/comment stage if a more definitive statement was provided – what percentage of the plants being evaluated will still have the marker gene present? • Overall, agree that the risk is low but the provided information on the selection marker is not as good as I would like to see when making a comment on risk analysis type situations.	Based on previous risk and safety assessments (see below), the percentage of GM banana plants containing *nptII* is highly unlikely to impact the risk assessment for the proposed field trial. *NptII* has been used extensively as a marker gene in the production of GM plants. Chapter 1, Section 5.4.3 provides a link to a separate OGTR document about the use and risk assessment of *nptII* and other frequently used marker genes. The *nptII* gene has been previously thoroughly assessed in GM plants already approved for commercial release.
5	Agrees with the overall conclusions of the RARMP.	Noted.

Sub. No:	Summary of issues raised	Comment
	The Regulator should consider clarifying the need for fencing of the trial site/property or implementation of feral animal control measures.	Fencing of the trial site and property was determined to be unnecessary for mitigation of identified risks and this has been clarified in the RARMP. Chapter 2, Risk Scenarios 1 and 2 considered risks associated with exposure of feral animals to the GM banana plants and dispersal of viable GM plant material from the trial site by feral animals. The Risk Scenarios concluded that the risks were negligible, with no need to invoke actions for mitigation.
	The Regulator should consider clarifying the description of the likelihood and mitigation measures for extreme weather events.	The proposed site has been identified in the RARMP as being in an area where extreme weather events may occur. Chapter 2, Risk Scenario 2 considered that the risk of dispersal of propagative GM plant material by extreme weather conditions was negligible. The likelihood of an extreme weather event for a particular 6ha site in the NT would be difficult to predict. Standard licence conditions require both the submission of a contingency plan prior to conducting any dealings with the GMOs and reporting of extreme weather events to the Regulator.

Appendix B Summary of submissions from the public

The Regulator received 5 submissions from the public on the consultation RARMP. The issues raised in these submissions are summarised in the table below. All issues raised in the submissions that related to risks to the health and safety of people and the environment were 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.

Issues raised **A:** Agricultural production systems; **AR:** Antibiotic resistance; **B:** Biosecurity issues; **CCI:** commercial confidential information; **GS:** gene source; **HHS:** human health and safety; **HGT:** Horizontal Gene Transfer; **HU**: Herbicide use; **LC:** limits and controls; **NBFEP**: The National Banana Freckle Eradication Program; **OSA:** outside the scope of the Act; **R:** Reporting requirements; **RARMP:** risk assessment and risk management plan; **SS:** site selection.

Other abbreviations the **Act:** *Gene Technology Act 2000*; **APVMA**: Australian Pesticides and Veterinary Medicines Authority; **Ch:** Chapter; **FSANZ:** Food Standards Australia New Zealand; **GM**: genetically modified; **L:** Licence; **RARMP**: Risk Assessment and Risk Management Plan; the **Regulator**: Gene Technology Regulator; **Sub. No.:** submission number.

Sub. No:	Issue	Summary of issues raised	Comment
1, 2, 4, 5	**SS**	Questions why the applicant is seeking approval to conduct this trial in NT rather than in northern QLD where the vast majority of Cavendish commercial banana production takes place.	The Act requires the Regulator to prepare a RARMP for licence applications, which takes into account risks to the health and safety of people and risks to the environment. The risk assessment considers the context of the trial as proposed by the applicant, including specific parameters such as the location of the trial. The applicant's motivation for choosing this particular site is outside the scope of the Act.
1, 4	**CCI, RARMP**	Finds it inappropriate that certain gene names were declared as CCI and/or questions that the genes are indeed for resistance against Fusarium wilt.	Applicants may apply for a declaration that specified information is CCI under the Act. Under section 185 of the Act, the Regulator must declare information as CCI if it meets certain criteria. The CCI was used by OGTR staff in preparing the RARMP and was made available to prescribed experts and agencies during consultation on the RARMP.
1	**NBFEP**	Resents destruction of all the mostly healthy heritage local bananas as a result of the NBFEP in and around the Litchfield Municipality. Adds that the NBFEP has destroyed healthy heritage food crops, lives, local incomes and local environment to protect the economic interests ONLY of the Queensland-based members of the Australian Banana Growers Council.	Implementation of the NBFEP is outside the scope of the Act.
1, 2, 4, 5	**NBFEP**	Requests start of the trial to be delayed until after the Red Zone requirements for authorised sentinel plants has finished in June 2017 to ensure fairness to all local banana growers.	In addition to requirements by the Regulator, this field trial will remain subject to State and Territory laws that cover the cultivation and transport of bananas and control of plant diseases, including Banana Freckle.
1	**GS**	Is concerned about the gene from a soil nematode and the marker from a soil bacterium. Questions the geographic origin of the bacterium.	The gene from the soil nematode and the marker gene are not CCI and are discussed in the RARMP. The marker gene was derived from the bacterium *Escherichia coli* which is widespread in human and animal digestive systems as well as

Sub. No:	Issue	Summary of issues raised	Comment
			in the environment.
1	**RARMP LC**	Concerned that there is no reliable way to prevent the GM bananas being used for human food or animal feed after a severe weather event destroys property fences and other bio-security measures. Also concerned about dispersal of GM bananas to the surrounding area by feral pigs.	The risks associated with the potential for extreme weather events and large animals to disperse viable GM plant material was considered negligible. Licence conditions require the submission of a contingency plan and methodology to detect GMOs prior to conducting any dealings with the GM banana plants. Licence conditions also require that extreme weather events and the presence of the GMO outside the field trial site must be reported to the Regulator without delay.
1	**LC**	The Darwin Banana Farm operators grow patented pineapples from QLD and go back and forth for this. No amount of control measures will isolate the trial site from other banana and pineapple crops.	There is no need to isolate the GM trial from pineapple crops as no risk could be identified if GM bananas were grown in close proximity to pineapples. Licence conditions require the GM banana trial to be surrounded by a 10 m isolation zone where no other banana is permitted to be grown.
1	**LC**	Transport is not secure in the Wet season and storage and resultant destruction of the GMOs cannot be assured.	Storage of plant material would be on the DBFC property within the tissue culture facility, if required. Destruction of the GMOs would be on the trial site or in bins within the facilities and thus transport, if needed, would be minimal. There is no evidence to suggest that the shredded bunches or other GM plant material would not decompose on the ground during the wet season.
1	**LC**	A sign on the gate asking people to heed bio-security measures has not been heeded before, so what makes you believe such signs would be heeded in the future?	Licence conditions require signage only on the shadehouse and tissue culture facility. People are required to notify the Regulator immediately of any suspected unauthorised activity with a GMO.
1	**LC**	Concerned about the possible expansion of the trial and use of the GM bananas as human food	Licence conditions only allow for planting of the GM bananas under the conditions of the licence. There is no provision in the licence to cultivate the GM banana outside of the trial site or for human trials involving consumption of the fruit. Licence conditions do not permit the use of the GM bananas for human food or animal feed.
1	**A**	Voiced concerns regarding commercial banana growing practices and corporate control of banana germplasm. Indicated that due regard was not given to alternative production methods and the rights of all banana growers to grow and consume heritage varieties of banana.	The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. Consideration of alternative farming methods and compliance with State or Territory legislation is outside the scope of the Act.
1	**A**	We challenge you to convince us – all 50,000 of us who once grew healthy banana plants – that a QUT GM trial in the Red Zone of the Litchfield Municipality of the NT – in the capital interests of QLD monoculture banana growers – will in any way benefit local banana production for local consumption.	The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. Consideration of any benefits that may be derived from gene technology are outside the scope of assessments conducted by the Regulator.
3	**L**	Wants the Regulator to reject the application, because: It remains unresolved who is responsible for the control, cleanup,	The banana cultivars are effectively male sterile and seed is rarely produced. Strict licence conditions have been imposed to minimise

Sub. No:	Issue	Summary of issues raised	Comment
		compensation and restoration of damage caused by GM contamination. In this regard, there is little or no education and training for non-GM growers, government instrumentalities and the general public. How do neighbours test for gene escapes and appropriately manage and mitigate risk?	spread and persistence of the GM banana and the introduced genetic material in the environment. Based on current information and experience, the control measures imposed are considered to be effective for restricting spread of the GM banana. There has been no documented escape of GM bananas from any field trial authorised by the Regulator. If suspected that GM plant material has dispersed from the trial site then this must be reported to the Regulator immediately.
3	**LC**	Calls for a freeze on all new approvals of GM crops, including open-air trials of GM varieties. Litchfield's extraordinary natural landscape and geography, akin to Kakadu, should not be put at risk by this GM banana trial.	The RARMP concluded that the proposed limited and controlled release of GM banana poses negligible risks to the health and safety of people and the environment as a result of gene technology, and that these negligible risks do not require specific risk treatment measures. However, conditions have been imposed to limit the release to the proposed size, location and duration as these were important considerations in establishing the context for assessing the risks. The adequacy of proposed control measures is discussed in Chapter 3 of the RARMP and is given effect through the licence.
3	**HU**	Science suggests that the excessive use of agrichemicals, particularly glyphosate-based Roundup, associated with widespread Roundup Ready GM crops is a contributing cause of Fusarium wilt. It is unwise to combat a problem using the same thinking as created it.	The GM bananas will contain genes for resistance to disease. They do not contain genes for herbicide tolerance. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia.
4	**HGT**	Challenges the assumption that horizontal gene transfer has negligible risk. The related claim that the gene sequences are '*already present in the environment and available for transfer via demonstrated natural mechanisms*' seems to overlook the significance of using this invasive scientific technology to introduce genes from other kingdoms into a plant species.	Risks resulting from horizontal gene transfer were considered negligible due to the rarity of these events and because the gene sequences (or sequences which are homologous to those in the current application) are already present in the environment and available for transfer via demonstrated natural mechanisms.
4	**B**	No detail is provided regarding the intended activities to evaluate resistance to Fusarium wilt, or how risks posed by handling the disease will be managed. While some actions from the draft license conditions may be relevant, there is no detail specifically estimating these risks, or describing the adequacy of management plans.	In addition to requirements by the Regulator, this field trial will remain subject to State and Territory laws that cover the cultivation and transport of bananas and control of plant diseases, including Fusarium wilt.
5	**OSA**	Notes that food safety and labelling, the use of agricultural chemicals and marketing and trade implications do not fall within the scope of the evaluations that the Regulator is required to conduct and strongly recommends that the Regulator broaden her scope to ensure the health and safety of the community.	During development of the gene technology legislation, it was determined that the Regulator's activities should form part of an integrated legislative framework that also includes a number of other regulatory authorities with complementary responsibilities and expertise. This arrangement both enhances coordinated decision-making and avoids duplication. While the Regulator must consider risks to human health and safety and the environment relating to dealings with GMOs,

Sub. No:	Issue	Summary of issues raised	Comment
			other agencies have responsibility for regulating GMOs or genetically modified (GM) products.
5	**LC**	If GM bananas were dispersed outside the trial, this would cause additional restrictions on local banana growers, and this potential harm should be included in Risk Scenario 2. Additionally, a Contingency Plan, and written methodology to reliably detect GMO's be submitted by the applicant before any license is granted. Any instances of GMO's outside an approved area should be notified to the Regulator in writing.	Licence requirements require the submission of a contingency plan and methodology to detect GMOs prior to conducting any dealings with the GM banana plants. It is also a condition of the licence that the presence of the GMO outside the field trial site must be reported to the Regulator without delay.
5	**LC**	Recommends that a condition of any license being granted be the construction of a secure pig proof fence around the planting area as the applicant has already suggested. The assurance by the DBFC property manager that pigs or buffalo "have not been seen on the property for numerous years" is vague, and does not indicate whether the boundary fence has been breached, or how often.	Risk scenarios 1 and 2 considered risks associated with exposure of feral animals to the GM banana plants and dispersal of viable GM plant material from the trial site by feral animals. The Risk scenarios concluded that the risks were negligible, with no present need to invoke actions for mitigation. This has been clarified in the RARMP.
5	**HU**	Notes that one of the GM banana lines included in the application (with the ced-9 gene) suffered less damage, and has shown increased tolerance to the herbicide Paraquat. Wishes to register concern that even higher levels of dangerous herbicides will be used in the planting area than what (we suspect) is used in current practice, with possible detrimental health impacts to waterways and the native bushland and wildlife which surrounds the property on three sides.	The APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. The use of paraquat on the GM bananas would require approval from the APVMA.
5	**LC**	The separation of GMO's from conventional plant material in the shade house and tissue culture facilities by a mere 50cm is not acceptable if the intention is to ensure they are always kept separate during the five year project. Recommends separate facilities for GMO's or at the very least, separate enclosures within the facility with a lockable door.	Licence conditions require separation by at least 50 cm as well as labelling to clearly distinguish between GM and non-GM banana plants. No incidents regarding distinguishing GMOs from other banana plants have been reported from previous banana field trials.
5	**-**	The Regulator makes reference in Ch 2 to short and long-term impacts without defining or elaborating on what long-term actually means. Wishes for the OGTR to clarify what a long term timeframe would be when considering GMO's and the risks and safety implications into the future. There are many examples of both the intentional and unintentional introduction of species and organisms to this country where the detrimental effects have not been apparent to authorities until many years after their release.	This licence is for a field trial of GM banana. The licence conditions imposed on the trial are sufficient to ensure that the presence of the GM bananas and their genetic material in the environment will be limited to the term of the licence. Therefore, no adverse effects from this trial are foreseen in the near and far future.
5	**L**	Recommends that the definition of Waterways in the licence be changed to “all permanent and **seasonal natural** waterways and man-made waterways that flow into natural waterways.” Obviously, in major rainfall events, a seasonal waterway is just as capable of transporting plant material as a permanent waterway.	Noted. The definition of waterways is designed to capture the everyday or normal context of the release site. If extreme weather events were to occur then other licence conditions would require reporting of the extreme weather conditions, reporting of GMOs occurring outside the proposed trial site and implementation of a

Sub. No:	Issue	Summary of issues raised	Comment
			contingency plan.