Australian Government
Department of Health and Aged Care
Office of the Gene Technology Regulator

September 2023

Risk Assessment and Risk Management Plan (Consultation version) for

DIR 199

Commercial release of banana genetically modified for resistance to Fusarium wilt tropical race 4 (TR4)

Applicant: Queensland University of Technology

This RARMP is open for consultation until 6 November 2023.

Written comments on the risks to human health and safety and the environment posed by this proposed release are invited. You may make your submission

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

or

via email to: ogtr@health.gov.au.

Please note that issues regarding food safety and labelling, the use of agricultural chemicals, and marketing and trade implications do **not** fall within the scope of these evaluations as they are the responsibilities of other agencies and authorities.

Summary of the Risk Assessment and Risk Management Plan (Consultation Version)

for

Licence Application No. DIR 199

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application for intentional, commercial-scale release of one line of genetically modified (GM) banana plants, QCAV-4, in Australia.

Parallel regulatory approval is being sought from Food Standards Australia New Zealand (FSANZ) for the use of the GM banana as food. The scope of FSANZ's assessment covers food safety, nutrition and food labelling, which is different to that of the Regulator's.

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed release poses negligible risks to human health and safety and the environment. Licence conditions have been drafted for the proposed release. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether or not to issue a licence.

The application

Application number	DIR 199
Applicant	Queensland University of Technology (QUT)
Project title	Commercial release of banana plants genetically modified for resistance to *Fusarium* wilt tropical race 4 (TR4)
Parent organism	Banana (*Musa acuminata* subgroup Cavendish cv Grand Nain)
Introduced genes and modified traits	Introduced gene conferring disease resistance: • *MamRGA2* – *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (TR4) resistance gene from *Musa acuminata ssp malaccensis* (wild banana) Introduced selectable marker gene: • *nptII* – antibiotic resistance gene from *Escherichia coli*
Proposed locations	Australia-wide
Primary purpose	Commercial cultivation of the GM banana plants

Risk assessment

The risk assessment process considers how the genetic modification and activities conducted with the GM banana plants 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 information in the application, relevant previous approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Both the short- and long-term risks are considered.

Credible pathways to potential harm that were considered included exposure of people or animals to the QCAV-4 GM banana plants, and commercial scale planting of the QCAV-4 GM banana plants. The potential harms considered were increased toxicity, allergenicity or weediness of the QCAV-4 GM banana plants compared to unmodified plants.

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings, either in the short or long term, are negligible. No specific risk treatment measures are required to manage these negligible risks.

The principal reasons for the conclusion of negligible risks are that the QCAV-4 GM banana plants have very limited ability to transfer the introduced genetic material to other banana plants; the QCAV-4 GM banana plants have limited ability to establish populations outside cultivation; the introduced proteins are not expected to be toxic or allergenic; and bananas are subject to strict biosecurity measures in the states and territories where bananas are commercially grown.

Risk management

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 evaluates and treats identified risks and considers general risk management measures. The risk management plan is given effect through licence conditions.

The risk management plan concludes that risks from the proposed dealings can be managed so as to protect people and the environment by imposing general conditions to ensure that there is ongoing oversight of the release.

As the level of risk is assessed as negligible, specific risk treatment is not required. However, licence conditions are proposed regarding post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP. The draft licence, detailed in Chapter 4 of the consultation RARMP, also contains several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

Table of contents

SUMMARY OF THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN I

INTRODUCTION I
THE APPLICATION I
RISK ASSESSMENT I
RISK MANAGEMENT II

TABLE OF CONTENTS III

ABBREVIATIONS V

CHAPTER 1 RISK ASSESSMENT CONTEXT 1

SECTION 1 BACKGROUND 1
1.1 Interface with other regulatory schemes 2
SECTION 2 THE PROPOSED RELEASE 2
SECTION 3 THE PARENT ORGANISM 3
SECTION 4 THE GMO, NATURE AND EFFECT OF THE GENETIC MODIFICATION 3
4.1 Introduction to the GMO 3
4.2 Introduction to Fusarium wilt tropical race 4 4
4.3 The introduced genes, encoded proteins and their associated effects 5
4.4 Characterisation of the GMO 6
4.5 Toxicity and allergenicity potential of the proteins encoded by the introduced genes 13
SECTION 5 THE RECEIVING ENVIRONMENT 14
5.1 Relevant agronomic practices 14
5.2 Relevant abiotic factors 15
5.3 Relevant biotic factors 15
5.4 Presence of the introduced genes and encoded proteins in the receiving environment 15
SECTION 6 PREVIOUS APPROVALS OF THE GM BANANAS 16
6.1 Australian approvals 16
6.2 International approvals 16

CHAPTER 2 RISK ASSESSMENT 17

SECTION 1 INTRODUCTION 17
SECTION 2 RISK IDENTIFICATION 18
2.1 Risk source 18
2.2 Causal pathway 19
2.3 Potential harm 19
2.4 Postulated risk scenarios 20
SECTION 3 UNCERTAINTY 26
SECTION 4 RISK EVALUATION 27

CHAPTER 3 RISK MANAGEMENT PLAN 29

SECTION 1 BACKGROUND 29
SECTION 2 RISK TREATMENT MEASURES FOR SUBSTANTIVE RISKS 29
SECTION 3 GENERAL RISK MANAGEMENT 29
3.1 Applicant suitability 29
3.2 Testing methodology 30
3.3 Identification of the persons or classes of persons covered by the licence 30
3.4 Reporting requirements 30
3.5 Monitoring for compliance 30
SECTION 4 POST RELEASE REVIEW 30
4.1 Adverse effects reporting system 31

4.2 Requirement to collect additional specific information 31
4.3 Review of the RARMP 31
SECTION 5 CONCLUSIONS OF THE CONSULTATION RARMP 32

CHAPTER 4 DRAFT LICENCE CONDITIONS 33

SECTION 1 INTERPRETATIONS AND DEFINITIONS 33
SECTION 2 LICENCE CONDITIONS AND OBLIGATIONS 33
2.1 General obligations of the licence holder 34
2.2 Provision of new information to the Regulator 34
2.3 Obligations of persons covered by the licence 35
SECTION 3 REPORTING AND DOCUMENTATION 35
3.1 Annual Report 35
3.2 Testing methodology 36
ATTACHMENT A 37

REFERENCES 38

APPENDIX A: SUMMARY OF SUBMISSIONS 42

Abbreviations

the Act	The *Gene Technology Act 2000*
BLAST	Basic Local Alignment Search Tool
bp	Base pair
CaMV	Cauliflower mosaic virus
CLR	Combined literature range
DAFF	Department of Agriculture, Fisheries and Forestry
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
FARRP	Food Allergy Research and Resource Program
f.sp.	forma specialis
FSANZ	Food Standards Australia New Zealand
g	Gram
GM	Genetically modified
GMO	Genetically modified organism
ha	hectare
HGT	Horizontal gene transfer
kg	Kilogram
MamRGA2	*Musa acuminata* ssp*. malaccensis* resistance analogue gene 2
mg	Milligram
mm	Millimetre
NBS-LRR	nucleotide binding site-leucine rich repeat
nos	Nopaline synthase gene
nptII	Neomycin phosphotransferase II gene
NSW	New South Wales
NT	Northern Territory
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
ORF	open reading frame
PCR	polymerase chain reaction
PRR	Post release review
pto	Resistance gene to *Pseudomonas syringae* pathovar *tomato*
QBAN	Queensland Banana Accredited Nursery
Qld	Queensland
QUT	Queensland University of Technology
R gene	Gene conferring resistance to a particular pathogen
RAF	Risk Analysis Framework
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator

RNA	Ribonucleic acid
RNA-Seq	RNA sequencing
RT	Reverse transcriptase
SD	Standard deviation
sp.	Species (singular)
spp.	Species (plural)
ssp.	Subspecies
t	Metric tonne
T-DNA	Transfer DNA
TR4	*Fusarium* wilt tropical race 4
TrEMBL	Translated European Molecular Biology Laboratory database
USDA	United States Department of Agriculture
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 and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, 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. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.

4. The *Risk Analysis Framework* (RAF) (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR) website.

5. Figure 1 shows the information that is considered, within the regulatory framework above, in establishing the risk assessment context. This information is specific for each application. Risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

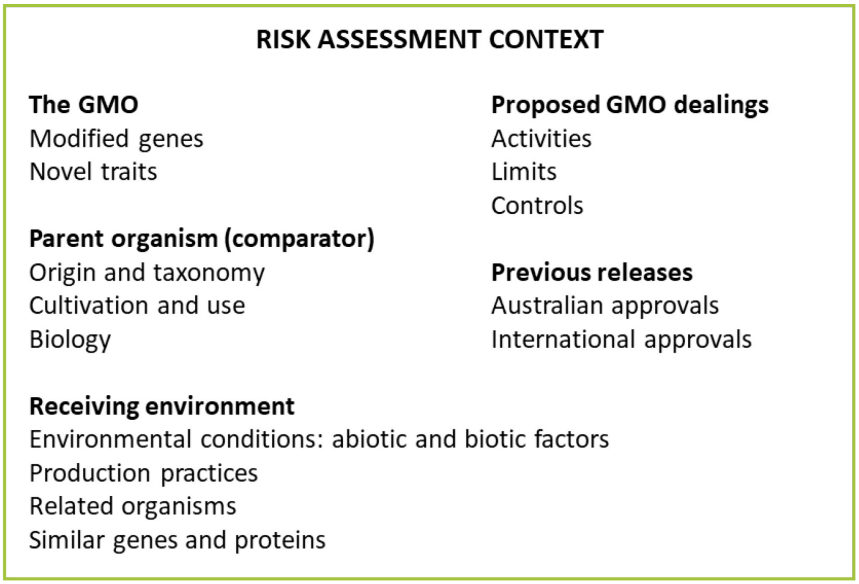

Figure 1 Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF

6. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. Therefore, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology

Technical Advisory Committee, State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, all Australian local councils, and the Minister for the Environment. A summary of issues contained in submissions received is provided in Appendix A.

7. Section 52 of the Act requires the Regulator, in a second round of consultation, to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public.

1.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings 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, the Therapeutic Goods Administration, the Australian Industrial Chemicals Introduction Scheme and the Department of Agriculture, Fisheries and Forestry (DAFF). These dealings may also be subject to the operation of State legislation recognising an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.

9. To avoid duplication of regulatory oversight, risks that have been considered by other regulatory agencies would not be re-assessed by the Regulator.

10. FSANZ assesses the safety of food produced using gene technology through administration of the *Australia New Zealand Food Standards Code*. FSANZ has also received an application, A1274, and is assessing the food safety of the GM banana line QCAV-4 and its products as food for human consumption.

Section 2 The proposed release

11. Queensland University of Technology (QUT) proposes commercial cultivation of a banana line (QCAV-4) that has been genetically modified (GM) for resistance to *Fusarium* wilt Tropical Race 4 (TR4) and contains an antibiotic marker gene that was used for the selection of plants during research. The GM banana line has been provisionally assigned the Organisation for Economic Co-operation and Development (OECD) identifier QUT-QCAV4-6. Throughout this document, the GMO will be referred to as QCAV-4 GM banana plants or the GMO.

12. The applicant indicated that the QCAV-4 GM banana plants are not intended to replace the current Cavendish banana cultivars growing in Australia, but rather to provide a safety net to the Australian banana industry should it be heavily impacted by TR4 in the future. If the banana industry wanted to grow it, then the QCAV-4 GM banana plants could be grown in all commercial banana growing areas or other areas suitable for banana production, subject to any moratoria imposed by States and Territories for marketing purposes. The products from the QCAV-4 GM banana plants would enter general commerce, including use in human food. For this RARMP, it is assumed that the QCAV-4 GM banana plants could be grown in all current and future areas in Australia that are suitable for banana cultivation.

13. The dealings involved in the proposed intentional release are to:

(a) conduct experiments with the GMO

(b) propagate the GMO

(c) use the GMO in the course of manufacture of a thing that is not a GMO

(d) grow the GMO

(e) transport of the GMO

(f) dispose of the GMO

and the possession, supply or use of the GMO for the purposes of, or in the course of, any of the above.

Section 3 The parent organism

14. The parent organism is banana (*Musa ssp*. L.). Most edible bananas are intraspecific or interspecific hybrids of *Musa acuminata* and *M. balbisiana*. Currently, bananas are grown commercially on the east coast of Australia from northern New South Wales (NSW) to far north Queensland (Qld). They are also grown in Western Australia (WA) around Carnarvon, Kununurra and Broome and in the Northern Territory (NT) near Darwin.

15. The parental variety for the GM banana is *Musa acuminata* subgroup Cavendish cultivar (cv) Grand Nain. Grand Nain belongs in the Cavendish subgroup of the triploid intraspecific hybrid of *M. acuminata* (AAA genome). Cultivars from the subgroup Cavendish account for approximately 97% of the bananas in the Australian market (HIA, 2022).

16. There are no known significant toxicities for bananas. Two possible allergic compounds have been noted. The first is a profilin, Mus xp 1, which may be related to oral allergy syndrome, and the second is latex, which can cause skin and gastrointestinal symptoms.

17. 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 (Simmonds, 1959; Ortiz and Vuylsteke, 1995) and triploid pollen viability has been reported as less than 10% in one study (Fortescue and Turner, 2004). Fruit develops largely by parthenocarpy (i.e. without prior fertilisation), thus preventing seed formation (Pillay and Tripathi, 2007). In addition, it has been noted that germination of seeds from widely grown cultivars of *Musa* in soil may be less than 1% (Pillay et al., 2002).

18. Detailed information about the parent organism is contained in a reference document, *The Biology of* Musa *L. (banana)* (OGTR, 2023) which was produced to inform the risk analysis process for licence applications involving GM banana plants. Baseline information from this document will be used and referred to throughout the RARMP.

Section 4 The GMO, nature and effect of the genetic modification

4.1 Introduction to the GMO

19. The applicant proposes to release plants of one GM banana line (QCAV-4) containing the *MamRGA2* gene that confers resistance to *Fusarium oxysporum* form a specialis (f.sp.) *cubense* TR4 (TR4), the pathogen causing Fusarium wilt Tropical Race 4 (Table 1).

20. The QCAV-4 GM banana plants also 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 QCAV-4 GM banana cells. The *nptII* gene was used during initial development of the GMO in the laboratory to select plant cells containing the introduced genetic modifications.

21. Short regulatory sequences which control the expression of the introduced genes have also been introduced into the QCAV-4 GM banana plants. These sequences are derived from a soil bacterium (*Agrobacterium tumefaciens*) and the plant virus Cauliflower mosaic virus (CaMV) (see Table 1). Both introduced promoters are constitutive promoters and the introduced genes are expected to be expressed in all tissues throughout the life cycle of the QCAV-4 GM banana plants.

4.1.1 Method of genetic modification

22. The QCAV-4 GM banana line was produced using *Agrobacterium*-mediated transformation. This method of transformation has been widely used in Australia and overseas for introducing genetic modifications into plants. More information can be found in the document *Methods of Plant Genetic Modification* which is available from the Risk assessment reference documents page on the OGTR website.

23. The QCAV-4 GM banana line was generated from the non-GM Grand Nain banana cell line GN212-12. Transformed cells were selected on media containing kanamycin. The bacteriostatic antibiotic Timentin® was used to eliminate *Agrobacterium* during *in vitro* selection of banana plants containing the introduced genetic modifications. GM banana plants were then multiplied *in vitro* by standard micropropagation techniques. GM banana plants that were shown to be negative for *Agrobacterium* using polymerase chain reaction (PCR) analysis were taken into the field following acclimatisation. One of these, the QCAV-4 GM banana line, was selected for commercialisation.

Table 1 Genetic elements introduced into the GM banana line

Name	Gene – full name and description	Accession number	Source	Intended Function	References
nos	Promoter from the nopaline synthase (*nos*) gene		*A. tumefaciens*	Promoter for the *MamRGA2* gene	(Bevan et al., 1983)
MamRGA2[1]	*MamRGA2* gene for *Fusarium oxysporum* forma specialis *cubense* tropical race 4 resistance	EU616673/ ACF21694	Banana (*Musa acuminata ssp. malaccensis* accession 850)	TR4 resistance	(Peraza-Echeverria et al., 2008; 2009)
nos 3' UTR	Termination and polyadenylation signal from the *nos* gene		*A. tumefaciens*	Promoter for *MamRGA2* gene	(Depicker et al., 1982; Bevan et al., 1983)
CaMV35S	Promoter from 35S RNA		CaMV	Promoter for *nptII* gene	(Odell et al., 1985)
nptII	Neomycin phosphotransferase type II gene	AAF65391/ AAA85506	*E. coli*	Antibiotic resistance, selectable marker	(Beck et al., 1982)
CaMV35S 3' UTR	Termination and polyadenylation signal from 35S RNA		CaMV	Terminator for *nptII* gene	(Guerineau et al., 1988)

[1]*MamRGA2* was designated as *RGC2* in licence application DIR 107 and *RGA2* in DIR 146.

4.2 Introduction to Fusarium wilt tropical race 4

24. Fusarium wilt (Panama disease) is caused by a soil-borne fungal pathogen *Fusarium oxysporum* f.sp. *cubense*. The pathogen enters through the roots and grows into the water-conducting tissues (the xylem) 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 (QDAF, 2016b; Business Queensland, 2020; PHA, 2021). The death of the parent pseudostem generally follows, but the suckers do not always die. Characteristically, the xylem in the pseudostem of infected plants has a dark brown to black discoloration and infected corms also show this discoloration running through the tissues (Grice et al., 2009). Fruit of infected plants appears symptomless (NSW DPI, 2017). Primary hosts of this disease include cultivated banana, *M. acuminata* (wild banana) and *M. textilis* (Manila hemp).

25. Four distinct races have been identified – Races 1, 2, 3 and 4 – based on their difference in pathogenicity (OGTR, 2023). Races 2 and 3 do not infect commercially relevant banana cultivars and thus are not considered economically important. Race 1 infects commercially important cultivars and in the early 1950s, it decimated major exported banana cultivars such as Gros Michel (AAA) and Lady Finger (AAB) in South and Central America. This led to wide-spread use of race 1 resistant cultivars from the Cavendish subgroup (AAA).

26. Race 4 consists of strains that infect Cavendish cultivars (Vézina and Rouard, 2021), but also affects cultivars susceptible to Races 1 and 2 (including Gros Michel, Silk, Pome and Bluggoe) and varieties not

affected by other races, such as 'Lakatan' and 'Pisang mas' (Vézina, 2022). This race has been subdivided further into another two strains, Subtropical race 4 and Tropical race 4 (TR4) (Vézina and Rouard, 2021; Vézina, 2022).

27. Worldwide, TR4 was found to be present in over 20 countries as of January 2020 (Vézina, 2022). It was detected near Darwin in 1997 and in Qld in 2015. It was detected in 160 plants at 5 properties in far north Qld (NSW DPI, 2017; QDAF, 2020; PHA, 2022).

28. TR4 is spread through infected planting material, via root contact, from parents to suckers and through movement of soil, water or contaminated equipment (QDAF, 2016b). Spores can persist in the soil for decades and the use of fungicides and fumigants will not eradicate the pathogen (QDAF, 2016b; Vézina, 2021). The disease has been found in a range of soil types (Biosecurity Queensland, 2021); however, some soil types may suppress the disease (Pegg et al., 2019; Biosecurity Queensland, 2021; Vézina, 2021).

29. 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 and Walduck, 2006). Strict quarantine practices have helped in restricting the spread of this disease. The Queensland Biosecurity Regulation 2016 (State of Queensland, 2016) and the Queensland Biosecurity Manual (Queensland Government, 2022) detail biosecurity requirements for Qld, including those related to banana production. A surveillance program for 2020/21 was implemented for commercial banana farms in far north Qld (QDAF, 2020).

30. Further details of Panama disease and its occurrence in Australia can be found in *The Biology of* Musa *L. (banana)* (OGTR, 2023).

4.3 The introduced genes, encoded proteins and their associated effects

4.3.1 Gene for disease resistance – MamRGA2

31. Expression of resistance (*R*) genes in several GM plants has been demonstrated to confer resistance to pathogens carrying the corresponding avirulence gene (see review by Hulbert et al., 2001). For example, the *R* gene *Pto* confers resistance to *Pseudomonas syringae* pathovar *tomato* in tomato (*Lycopersicum esculentum*) plants. It has been shown to also confer this resistance when introduced into tobacco (*Nicotiana tabacum*) and *N. benthamiana* both of which are naturally susceptible to infection by *Pseudomonas syringae* pathovar *tomato* (Rommens et al., 1995; Thilmony et al., 1995). Other than disease resistance, no other phenotypic changes were reported by the authors.

32. The *R* gene *MamRGA2* was isolated from a wild banana species, *Musa acuminata* ssp. *malaccensis* (Peraza-Echeverria et al., 2008; Peraza-Echeverria et al., 2009). This banana species is resistant to TR4 infection. *MamRGA2* shows sequence similarity to known *R* genes that encode Nucleotide Binding Site (NBS)-Leucine Rich Repeat (LRR) proteins for Fusarium wilt (Peraza-Echeverria et al., 2008; Peraza-Echeverria et al., 2009).

33. NBS-LRR[1] genes are the largest class of *R* genes. This class of genes is large, likely ancient, and present in a wide range of plant species, including commonly consumed plant species (Chang et al., 2020). Large numbers of this class of genes have been isolated from a range of edible plant species, for example, from about 50 in papaya to 653 in rice (Marone et al., 2013). The different NBS-LRR proteins can recognise a wide variety of pathogens including viruses, bacteria, fungi and insects. They act through a network of signalling pathways and induce a series of plant defence responses (McHale et al., 2006). Activation of downstream genes results in hypersensitive response, a localised form of host programmed

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

cell death used as a plant defence against certain pathogens (Lozano et al., 2015). Effects on abiotic stress tolerances upon introduction of *R* genes have not been reported.

34. 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 LRR domain is implicated in protein-protein interaction and more specifically in binding to pathogen-derived molecules. This domain is thought to be the primary determinant for specificity of pathogen recognition. The role of the NBS region is primarily as a signal transduction switch following pathogen recognition.

4.3.2 Selectable marker gene - nptII

35. The introduced *nptII* gene was used as a selectable marker in the laboratory to select transformed GM plants during early stages of development. This gene is derived from *E. coli* and encodes a neomycin phosphotransferase enzyme. It provides resistance to neomycin, kanamycin, paromonycin and related aminoglycoside antibiotics. More information on *nptII,* including information regarding its lack of toxicity or allergenicity, is available in the document *Risk Assessment Reference: Marker Genes in GM Plants* on the Risk assessment reference documents page on the OGTR website.

4.4 Characterisation of the GMO

4.4.1 Molecular characterisation

36. Molecular analyses of the QCAV-4 GM banana line were provided by the applicant to confirm the absence of integrated backbone sequences from the plasmid vector. PCR analysis of genomic DNA extracted from QCAV-4 GM banana leaf tissue confirmed the absence of unintended backbone sequences from the plasmid vector used for transformation. A comparison between whole genome sequencing of QCAV-4 GM banana against the plasmid vector sequence also confirmed the absence of integrated vector backbone sequences.

37. Whole genome sequencing combined with bioinformatics mapping was used to characterise the location and organisation of the introduced genetic modifications in QCAV-4 GM banana plants (see Figure 2 for more detail). This analysis revealed that, following transformation, a 26,849 bp insert was integrated in the antisense direction at a single site on chromosome 6 of the Grand Nain banana genome. This insertion corresponds with a 116 bp deletion of the original locus. Sequence analysis showed that the insertion was in an intergenic region between the gene sequences for two protein kinase domain-containing proteins, and no known open reading frames (ORFs) were interrupted.

38. Southern blot analysis showed that multiple copies of the introduced expression cassette were inserted in the QCAV-4 GM banana line. Further investigation using sequence analysis of the insert showed that the 26,849 bp insert contained:

- 3 identical copies of the 6,702 bp transfer DNA (T-DNA) (T-DNA1, T-DNA2, T-DNA3)
- two fragments of the introduced expression cassette recombined in opposite directions and inserted between T-DNA2 and T-DNA3 and
- additional sequence rearrangements:
  - between the 3’ genome flanking region and T-DNA1
  - between T-DNA1 and T-DNA2 and between T-DNA2 and T-DNA3 and
  - between T-DNA3 and the 5’ genome flanking region.

Sequence characterisation of the 26,849 bp insert identified 7 new ORFs larger than 30 amino acids.

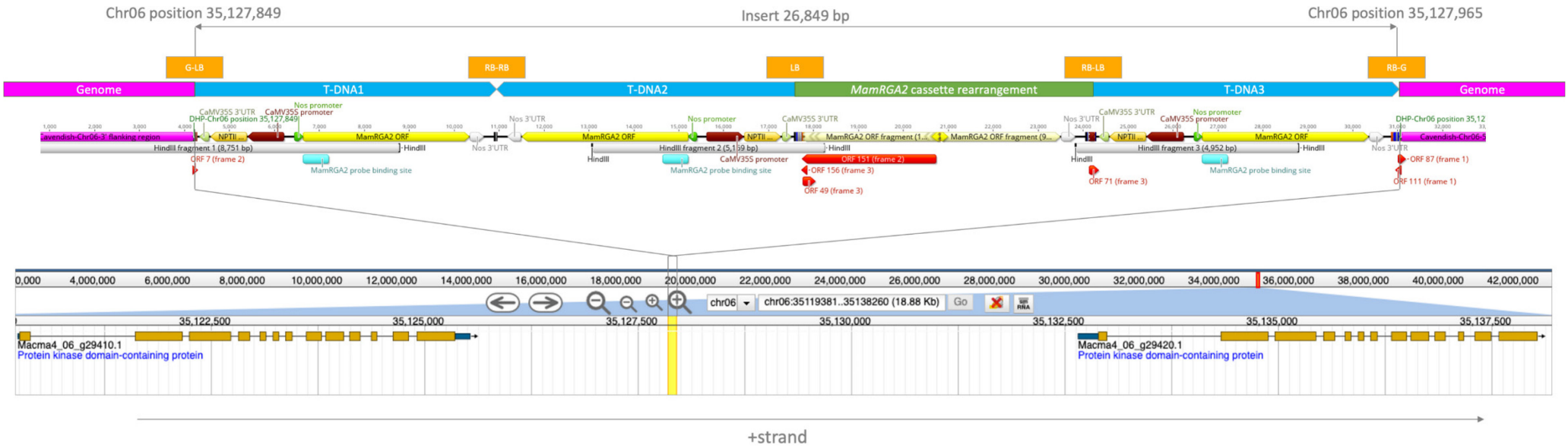

Figure 2 Diagrammatic representation of the organisation of the insert in event QCAV-4. A T-DNA insertion of 26,849 bp is located on banana chromosome 6.
The insert contains 3 full, intact and functional copies of the 6,702 bp T-DNA (T-DNA 1 to 3, blue arrows) as well as two fragmented portions of the expression cassette recombined in opposite direction and inserted between T-DNA2 and T-DNA3 (green box). The two genome-T-DNA and 3 inter T-DNA junctions contain various levels of rearrangement and are indicated with orange boxes. Seven new ORFs larger than 30 amino acids were identified (red arrows) in the inter T-DNA regions only. No evidence of any vector backbone sequence was detected.

Note: the insert sequence and diagrams provided are oriented in the antisense of their real orientation on Chromosome 6 (source: applicant supplied).

39. Small or large deletions, duplications or rearrangements may occur following *Agrobacterium*-mediated transformation (see *Methods of Plant Genetic Modification* which is available from the Risk assessment reference documents page on the OGTR website).

40. The transcriptional potential of the 7 newly identified ORFs was assessed by the applicant using two approaches – *in silico* analyses and an RNA sequencing (RNA-Seq) approach. *In silico* analyses examined the upstream and downstream sequences of the 7 ORFs for regulatory elements which may result in the transcription of these ORFs, including searches for plant promoter-like sequences, transcription factor binding sites and 3' UTR-like sequences. This analysis was done using software applications for genomic research available on the PlantCARE database (Lescot et al., 2002) and the Softberry website (Salamov and Solovyev, 1997; Solovyev et al., 2010). These searches were inconclusive because of the high number of small motif sequences identified, which may not be evidence of functional protein binding sites (Hernandez-Garcia and Finer, 2014). The applicant also used RNA-Seq to assess any expression of the 7 ORFs. RNA was extracted from QCAV-4 GM banana leaf, root and ripe fruit tissue and used to generate RNA-Seq data. This data was then mapped to the 26,849 bp insert sequence, which also included 3' and 5' flanking sequences of chromosome 6. This analysis did not show any evidence that the ORFs were transcribed in these QCAV-4 GM banana tissues.

4.4.2 Molecular stability

41. The applicant used Southern blot analysis to determine the stability of the insert in the QCAV-4 GM banana over 4 generations (Table 2). As discussed in *The Biology of* Musa *L. (banana)* (OGTR, 2023), banana is a perennial crop that is propagated vegetatively, so each generation is genetically highly similar. 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', whereby the pseudostem that has just borne fruit is cut down and replaced by a sucker from the corm (i.e. the underground true stem). Generations 1 - 3 listed in Table 2 are successive crops.

Table 2 Sample information for Southern blot analysis

Generation		DNA source
	Non-GM control (GN212-12)	Tissue culture cells
	QCAV-4 original mother plant (121-12)	
	QCAV-4 (DIR-107 plants)	
1	QCAV-4 - plant crop	Leaf tissue from DIR-146 plants
2	QCAV-4 - 1st ratoon crop	
3	QCAV-4 - 2nd ratoon crop	

42. Consistent hybridisation banding patterns between the original QCAV-4 GM banana plants maintained in tissue culture and the 3 successive generations of QCAV-4 GM banana plants in the field indicate stable integration of the introduced genes. Endogenous homologs of the introduced *MamRGA2* gene in Grand Nain banana were also detected in each of the samples. These homologs were distinguishable from the introduced *MamRGA2* gene due to their predicted hybridisation patterns which differ from that of the introduced *R* gene.

4.4.3 Expression of the introduced MamRGA2 *gene*

43. As discussed in Sections 4.1 and 4.3, QCAV-4 GM banana plants contain the *MamRGA2* gene from wild banana *M. acuminata* ssp. *malaccensis*, which provides resistance to TR4, the fungal pathogen that causes Panama disease. The presence of this gene should provide QCAV-4 GM banana plants with TR4

resistance, and the two previous field trials conducted under licences DIR 107 and DIR 146 confirmed this. Both trials were conducted on a commercial banana farm in the Litchfield Municipality (NT) which has high TR4 pressure. Non-GM banana plants and QCAV-4 GM banana plants were visually inspected for the presence of Panama disease symptoms, including wilting, leaf yellowing and discolouration of vascular tissue in the pseudostem. PCR-based assays were also conducted to confirm presence of TR4 in the banana plants. Vascular tissue discolouration was found to be an accurate diagnostic indicator of TR4 infection (Dale et al., 2017). The field trials included replicates for both the QCAV-4 GM banana plants and the non-GM Grand Nain control. For the DIR 107 trial, in samples from the 4th generation (i.e. the third ratoon), 87.5% of the non-GM controls were infected with TR4, compared to 20% of the QCAV-4 GM plants (Dale et al., 2017). Field trials under DIR 146 are ongoing, but in samples from the 5th generation (i.e. the 4th ratoon), the disease incidence in the QCAV-4 GM banana plants was 2%, while 66% of the non-GM controls were infected.

44. Quantitative reverse transcriptase-PCR was used to assess gene expression levels in QCAV-4 plants from the DIR 107 field trial. Results showed a strong correlation between *MamRGA2* RNA expression and TR4 protection (Dale et al., 2017). Additional results also indicated that the non-GM Grand Nain cultivar has analogous endogenous *RGA2* homologs that are expressed at too low levels to provide protection against TR4 infection (Dale et al., 2017).

4.4.4 Compositional analysis of QCAV-4 GM banana fruit and peel

45. The applicant provided nutritional compositional data for QCAV-4 GM banana fruit and peel tissue compared to the non-GM control (Grand Nain GN212-12). Fruit was harvested from 10 QCAV-4 GM and 10 non-GM field grown ratoon 4 and ratoon 5 (5th and 6th generations, respectively) banana plants. Peel samples of ethylene-ripened fruit were taken from 6 QCAV-4 GM and two non-GM field grown ratoon 6 (7th generation) banana plants.

46. The fruit and peel samples were tested for:

- proximates (moisture, total fat, total protein, ash, carbohydrates and energy)
- minerals (magnesium, manganese and potassium) and
- vitamins C (ascorbic acid) and B6 (pyridoxine).

47. The mean (± standard deviation (SD)) and range of the data for each analyte for fruit and peel are shown in Table 3 and Table 4, respectively, for QCAV-4 GM and non-GM control plants. The tables also show the combined literature range (CLR) of values for each analyte, based on data from the Australian Food Composition Database (FSANZ, 2022) and the FoodData Central database of the USDA (USDA, 2019). The CLR value for peel samples is from Grand Nain data published by Emaga et al. (2007) as published databases were not available for banana peel composition.

Compositional analysis of QCAV-4 GM banana fruit

48. For ratoon 4 fruit samples, significant differences were detected between QCAV-4 GM and non-GM banana fruit mean values for all analytes except fat, manganese and pyridoxine, although there was not a clear pattern of differences (Table 3). The mean values of QCAV-4 GM and non-GM banana fruit analytes were within the CLR range of values, except for manganese, ascorbic acid and pyridoxine, for which both QCAV-4 GM and non-GM banana fruit were all lower than the CLR value range. For ratoon 5 fruit samples, a significant difference (p<0.05) between QCAV-4 GM and non-GM banana fruit mean values was only detected for manganese (Table 3), and both QCAV-4 GM and non-GM banana fruit mean values were lower in manganese and ascorbic acid and higher for ash than the CLR value range.

Table 3 Nutritional compositional data for QCAV-4 GM and non-GM banana fruit

Analyte	Genotype	Ratoon 4		Ratoon 5		CLR (min - max)
		Mean ± SD	Mean range (min - max)	Mean ± SD	Mean range (min - max)	
Moisture (g/100 g)	QCAV-4	**79.1 ± 1.0**	77.6 - 80.4	79.2 ± 1.2	78.1 - 81.3	71.3 - 80.6
	non-GM	**76.9 ± 1.1**	75.5 - 78.6	78.1 ± 1.7	75.5 - 80.8	
Fat (g/100 g)	QCAV-4	0.19 ± 0.00	0.19 - 0.19	0.19 ± 0.00	0.19 - 0.19	0.00 - 0.72
	non-GM	0.23 ± 0.05	0.19 - 0.30	0.19 ± 0.00	0.19 - 0.19	
Protein (g/100 g)	QCAV-4	**0.97 ± 0.09**	0.80 - 1.10	1.07 ± 0.29	0.30 - 1.40	0.62 - 1.40
	non-GM	**1.08 ± 0.06**	1.00 - 1.20	1.24 ± 0.13	1.10 - 1.40	
Ash (g/100 g)	QCAV-4	**0.79 ± 0.11**	0.70 - 1.00	1.05 ± 0.41	0.60 - 1.90	0.43 - 1.00
	non-GM	**0.89 ± 0.10**	0.70 - 1.00	1.10 ± 0.43	0.70 - 1.80	
Carbohydrates (g/100 g)	QCAV-4	**19.1 ± 1.0**	18.0 - 21.0	18.8 ± 1.5	14.0 - 21.0	17.3 - 27.5
	non-GM	**20.9 ± 1.2**	19.0 - 22.0	19.5 ± 1.9	17.0 - 22.0	
Energy (kJ/100 g)	QCAV-4	**340 ± 17**	320 - 370	336 ± 25	250 - 360	287 - 426
	non-GM	**377 ± 19**	350 - 400	352 ± 32	310 - 400	
Magnesium (mg/kg)	QCAV-4	**261 ± 14**	230 - 280	295 ± 20	260 - 330	180 - 380
	non-GM	**294 ± 20**	270 - 340	308 ± 28	270 - 370	
Manganese (mg/kg)	QCAV-4	**0.52 ± 0.08**	0.39 - 0.64	**0.63 ± 0.14**	0.42 - 0.92	0.93 - 8.29
	non-GM	**0.65 ± 0.23**	0.39 - 0.98	**0.88 ± 0.34**	0.55 - 1.70	
Potassium (mg/kg)	QCAV-4	3,852 ± 178	3,550 - 4,120	3,788 ± 204	3,450 - 4,110	3,000 - 4,260
	non-GM	3,652 ± 231	3,360 - 4,030	3,760 ± 214	3,510 - 4,120	
Ascorbic Acid (mg/100 g)	QCAV-4	**1.96 ± 0.18**	1.70 - 2.20	2.28 ± 0.43	1.40 - 3.00	4.0 - 15.1
	non-GM	**1.72 ± 0.31**	1.30 - 2.30	1.97 ± 0.37	1.30 - 2.60	
Pyridoxine (mg/100 g)	QCAV-4	0.12 ± 0.04	0.10 - 0.20	0.33 ± 0.03	0.27 - 0.39	0.19 - 0.42
	non-GM	0.13 ± 0.05	0.10 - 0.20	0.34 ± 0.03	0.30 - 0.40	

Mean values (±SD) highlighted in **bold** show a significant difference ($p < 0.05$) between the QCAV-4 GM and non-GM banana fruit for a particular analyte.

Compositional analysis of QCAV-4 GM banana peel

49. Differences between QCAV-4 GM and non-GM banana peel samples were observed for several measures, however only the differences for protein and magnesium were statistically significant ($p<0.05$) (Table 4). Mean protein concentration was significantly higher in QCAV-4 GM banana peel than in non-GM banana peel. The range for protein was higher for QCAV-4 GM banana peel than for non-GM banana peel. The mean value for manganese from QCAV-4 GM banana peel was higher than the mean of the non-GM banana peel values (and also above the range). The small sample size from field trial data and the lack of reliable data from published literature make it difficult to interpret the biological significance of these differences.

Table 4 Composition data for QCAV-4 GM and non-GM banana peel

Analyte	Genotype	Mean ± SD	Mean range (min - max)	CLR
Moisture (g/100 g)	QCAV-4	90.6 ± 1.3	89.5 - 92.8	89.8
	non-GM	90.3 ± 1.8	89.0 - 91.5	
Fat (g/100 g)	QCAV-4	0.47 ± 0.12	0.30 - 0.60	0.58
	non-GM	0.45 ± 0.07	0.40 - 0.50	
Protein (g/100 g)	QCAV-4	**0.73 ± 0.10**	0.60 - 0.90	0.83
	non-GM	**0.45 ± 0.07**	0.40 - 0.50	
Ash (g/100 g)	QCAV-4	2.25 ± 0.79	1.70 - 3.80	1.31
	non-GM	2.75 ± 1.48	1.70 - 3.80	
Carbohydrates (g/100 g)	QCAV-4	5.8 ± 1.5	5.0 - 8.0	8.7
	non-GM	6.0 ± 2.8	4.0 - 8.0	
Energy (kJ/100 g)	QCAV-4	128 ± 26	90 - 160	NA
	non-GM	125 ± 49	90 - 160	
Magnesium (mg/kg)	QCAV-4	125 ± 12	110 - 140	140
	non-GM	145 ± 7	140 - 150	
Manganese (mg/kg)	QCAV-4	**2.55 ± 1.22**	1.10 - 4.00	2.25
	non-GM	**2.10 ± 0.57**	1.70 - 2.50	
Potassium (mg/kg)	QCAV-4	8,147 ± 241	7,840 - 8,520	6,479
	non-GM	7,645 ± 431	7,340 - 7,950	
Ascorbic Acid (mg/100 g)	QCAV-4	0.90 ± 0.00	0.90 - 0.90	N/A
	non-GM	0.90 ± 0.00	0.90 - 0.90	
Pyridoxine	QCAV-4	0.10 ± 0.02	0.08 - 0.12	N/A

Analyte	Genotype	Mean ± SD	Mean range (min - max)	CLR
(mg/100 g)	non-GM	0.08 ± 0.02	0.06 - 0.09	

Mean values (±SD) highlighted in **bold** show a significant difference ($p < 0.05$) between the QCAV-4 and non-GM value for a particular analyte.

50. In summary, the data from Table 3 and Table 4 do not show a consistent pattern of significant differences between QCAV-4 GM and non-GM banana analytes. Furthermore, most of the analyte mean values for QCAV-4 GM and non-GM banana samples are within the range of values reported in the literature.

4.4.5 Phenotypic and agronomic characterisation

51. Phenotypic and agronomic performance of QCAV-4 GM banana plants was assessed in the trial conducted under the DIR-146 licence that was conducted in the Litchfield Municipality (NT).

52. Data was collected from QCAV-4 GM banana plants and the non-GM Grand Nain (GN212-12) control for the following phenotypic and agronomic characteristics:

- bunch weight
- yield
- cycle time
- plant girth and
- plant height.

Bunch weight, yield and cycle time data was collected from 5 generations (plant crop and 4 ratoons), while plant height and plant girth data were collected from fruit-bearing banana plants in ratoons 6 and 7 (7^{th} and 8^{th} generation).

53. Over 5 generations, the average bunch weight for QCAV-4 GM banana plants fitted within the range of the non-GM banana control plants (Table 5). The value for the plant crop was significantly lower ($p<0.001$) for the first two generations (plant crop and ratoon 1) but were comparable from generation 3 onwards. The applicant states that a reduction in productivity early in the trial may be a result of the QCAV-4 GM banana plants adjusting to field conditions following *in vitro* generation and cultivation in the laboratory.

54. Cycle time for the QCAV-4 GM and non-GM banana plants are also shown in Table 5. Cycle time is the period from planting to harvest for the plant crop, and the time between harvests for subsequent generations (i.e. ratoon crops). QCAV-4 GM banana plants had a longer cycle time at plant crop and ratoon 2, but these differences were not significant. Significantly shorter cycle times were shown in ratoon 1 and ratoon 3. Overall, the results do not show an obvious trend in cycle time difference between QCAV-4 GM and non-GM banana plants.

55. Yield measurements for the QCAV-4 GM and non-GM banana plants are also shown in Table 5. For the field trialled plants, these measurements were calculated using an industry standard of 1670 plants per hectare (ha). QCAV-4 GM banana plants produced a higher yield than non-GM control banana plants in all generations, except for the plant crop (generation 1). Reported average yield of banana plantations in Australia ranges from 11.2 to 37.5 tonnes (t)/ha across different states and territories, with a national average of 29 t/ha (OGTR, 2023). The calculated values for both QCAV-4 GM and non-GM controls are above the national average until ratoon crop 3, where the yield values for non-GM banana controls drop below the Australian average while the QCAV-4 banana plants continue to generate higher yields. It is uncertain whether the differences from the national average are meaningful considering the values were calculated based on a small sample size rather than measured in a planting at least 1 ha in size. However, a higher yield of the QCAV-4 GM banana plants compared to the controls is expected as QCAV-4 would

not have suffered from Panama disease whereas the non-GM banana controls were adversely affected by TR4.

Table 5 Bunch weight and cycle time

		Bunch weight (kg)			Cycle time (days)			Yield (t/ha)
	Genotype	n	Average ±SD	Range	n	Average ±SD	Range	
Plant crop	QCAV-4	50	**28.1 ± 4.3**	15.5 – 39.4	50	331.1 ± 13.6	317 - 372	47
	non-GM	46	33.1 ± 4.7	18.5 – 42.0	46	327.3 ± 11.0	317 - 362	51
Ratoon 1	QCAV-4	49	**24.3 ± 5.1**	13.5 – 36.2	49	**199.2 ± 27.0**	134 - 277	40
	non-GM	37	29.9 ± 5.7	9.6 – 40.3	36	214.4 ± 22.5	169 – 270	37
Ratoon 2	QCAV-4	42	31.7 ± 6.0	0.60 - 0.90	42	207.3 ± 15.2	179 – 249	44
	non-GM	32	31.5 ± 8.8	10.0 – 49.8	28	206.1 ± 13.1	183 – 224	34
Ratoon 3	QCAV-4	45	28.6 ± 4.6	15.0 – 42.3	40	**174.9 ± 27.3**	141 – 275	43
	non-GM	25	29.8 ± 8.0	17.5 – 45.5	18	206.2 ± 38.2	156 – 273	25
Ratoon 4	QCAV-4	34	34.8 ± 3.8	25.5 – 42.0	31	193.6 ± 39.5	104 – 306	40
	non-GM	15	35.7 ± 5.4	27.5 – 45.4	13	213.5 ± 33.9	147 - 257	18

Yield calculated based on 1670 plant/ha. Values in bold represent significant differences (p<0.05).

56. Plant height and plant girth measurements for QCAV-4 GM banana plants and non-GM banana plants from ratoon 6 or ratoon 7 are shown in Table 6. The applicant standardised measurements to the same developmental stage by assessing only plants with bunches. Data collection was limited for the non-GM plants because of the high adverse impact of TR4 on these plants. No significant differences were observed between QCAV-4 GM and the assessed non-GM banana plants. The applicant also assessed a range of botanical characteristics, including immature and mature plant characteristics and fruit characteristics, from ratoon crop 5 (6th generation) of QCAV-4 and from the non-GM parent Grand Nain (United States Plant Patent application (Patent number: PP34398)). Based on comparisons with the non-GM parent for these botanical descriptors, they concluded that in the absence of disease pressure, the QCAV-4 GM banana plants were phenotypically identical to the non-GM banana plants.

Table 6 Plant girth and height measurements

		Plant girth (mm)		Plant height (mm)	
Genotype	n	Average ± SD	Range	Average ± SD	Range
QCAV-4	30	757 ± 53	660 - 870	2,615 ± 127	2,280 - 2,840
non-GM	10	774 ± 94	650 - 880	2,546 ± 173	2,350 - 2,810

4.5 Toxicity and allergenicity potential of the proteins encoded by the introduced genes

57. The applicant provided bioinformatic analyses that assessed the toxicity and allergenicity potential of the protein encoded by *MamRGA2*. Bioinformatic analyses may assist in the assessment process by predicting, on a theoretical basis, the toxic or allergenic potential of a protein. The results of such

analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing.

58. The sequence similarity of *MamRA2* against proteins with known or putative toxicity or allergenicity was assessed, as were the 7 ORFs in QCAV-4 GM banana plants (Section 4.4.1).

59. Sequence similarity searches were conducted to assess the potential for increased toxicity, using the Basic Local Alignment Search Tool (BLAST) available within the Geneious Prime® program containing a 92,851-sequence subset from two databases, Swiss-Prot and the Translated European Molecular Biology Laboratory database (TrEMBL). Swiss-Prot contains manually annotated and reviewed sequences, whereas TrEMBL contains automatically annotated, unreviewed sequences. The sequence subset was generated by searching the two databases for the keyword 'toxin' (29 August 2022). Neither the MamRGA2 query sequence nor the translated sequences of the 7 predicted ORFs showed significant sequence similarity to any proteins known, or suspected, to be of mammalian toxicological concern. However, as expected, some homology (< 23%) was found for MamRGA2 to two wheat and one barley plant resistance-like proteins that provide protection from pathogenic microorganisms.

60. Sequence similarity searches on the MamRGA2 sequence and the translated sequences of the 7 predicted ORFs were conducted to assess the potential for increased allergenicity. The Food Allergy Research and Resource Program (FARRP) contains 2,233 protein or amino acid sequence entries of unique proven or putative allergens (food, airway, venom/salivary and contact) from 430 species. These sequences were interrogated using the BLAST available within the Geneious Prime® program. Three analyses, i.e. a full-length sequence search, 80-mer sliding window search and 8-mer exact match search, were undertaken for MamRGA2 and each of the 7 new predicted peptides/proteins. None of the searches identified immunologically relevant similarities with any of the known or putative allergens in the database.

61. There is no evidence that the *nptII* gene or the protein it encodes is toxic or allergenic (OGTR Risk Assessment documents and references therein). GM foods containing the *nptII* gene has been assessed and approved for sale in Australia (FSANZ website, accessed 3 May 2023).

Section 5 The receiving environment

62. The receiving environment forms part of the context in which the risks associated with dealings involving the GMO are assessed. Relevant information about the receiving environment includes abiotic and biotic interactions of the plant 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, 2013).

63. 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, 2023).

64. The applicant has proposed to commercially grow the QCAV-4 GM banana plants, if wanted by the industry. Therefore, for this licence application, it is considered that the receiving environment is all the banana-growing areas of Australia.

5.1 Relevant agronomic practices

65. It is anticipated that the cultivation practices for the proposed release will not differ from the standard practices used for current commercial non-GM banana. These are outlined in *The Biology of* Musa L. (*banana*) (OGTR, 2023).

66. 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.

67. A range of measures including restrictions on movement and cultivation of bananas (GM and non-GM) and movement of related material are in place in the states and territories. Further information

about State regulations can be found in the Queensland Biosecurity Manual (Queensland Government, 2022), Banana Industry Biosecurity Guideline (QDAF, 2016a), Plant Quarantine Manual for New South Wales (NSW DPI, 2016), NT Government website and WA DPIRD website. These are important in the control of banana pests and diseases, which is vital to maintain the industry in Australia.

68. Most sweet banana cultivars are effectively sterile and therefore propagated vegetatively (see Section 3 for more detail). Tissue cultured material is considered the best method to propagate banana planting material that is free of pests and diseases (QDAF, 2016a; State of Queensland, 2017; WA DPIRD, 2020). This is done using virus indexed material obtained from accredited nurseries and tissue culture facilities (QDAF, 2016a). Both Qld and NSW have adopted the Queensland Banana Accredited Nursery (QBAN) system that provides both vegetative and tissue cultured planting material, and WA also has requirements for use of QBAN material. The QBAN scheme includes monitoring and recording of all aspects of the propagation process to ensure 'traceable clean planting material that is free of targeted pests' (PHA and Queensland DEEDI, 2009). The applicant has indicated that QCAV-4 GM banana plants will be propagated using industry standard micropropagation techniques and sucker propagation.

5.2 Relevant abiotic factors

69. *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. 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.

70. More detailed information regarding abiotic factors impacting the growth and distribution of bananas in Australia is discussed in the reference document, *The Biology of* Musa L. (*banana*) (OGTR, 2023).

5.3 Relevant biotic factors

5.3.1 Presence related plants in the receiving environment

71. There are two recognised *Musa* species native to Australia, *M. acuminata* subspecies (ssp.) *banksii* and *M. jackeyi* (Ross 1987). *M. acuminata* ssp. *banksii,* a fertile diploid, is the most common and can be found along the tip of Cape York and other parts of northern Qld. *M. jackeyi* is rare and has only been reported at two locations in Qld: Bellenden Ker and Cooktown. Neither of these species are classified as a weed in Australia (OGTR, 2023).

5.3.2 Presence of other biotic factors

72. The control of pests and diseases in the banana industry is vital to maintain the industry in Australia (OGTR, 2023). Banana plants can tolerate shade of up to 80%, but shading reduces plant growth, pseudostem thickness, suckering and yield (Simmonds, 1962; Nelson et al., 2006). 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 pests and diseases, lack of nutrients, lack of moisture and/or extended drought, shading and poor competitive ability with other plants.

5.4 Presence of the introduced genes and encoded proteins in the receiving environment

73. The source organism of the *MamRGA2* gene is the wild diploid banana *M. acuminata* ssp. *malaccensis*. *M. acuminata* is widely distributed in Asia and considered one of the ancestors of modern eating banana (OGTR, 2023). It is not grown commercially in Australia but is present in some germplasm collections as either tissue cultured plants (QUT, Brisbane and Maroochy Research Facility, Department

of Agriculture and Fisheries (DAF), Nambour) or growing in the field (South Johnstone Research Facility, DAF).

74. The *nptII* gene was isolated from *E. coli*, a common bacterium that is widespread in human and animal digestive systems and in the environment in Australia (Gordon and Cowling, 2003). As such, it is expected that humans, animals and microorganisms routinely encounter the encoded protein.

Section 6 Previous approvals of the GM bananas

6.1 Australian approvals

6.1.1 Approvals by the Regulator

75. QCAV-4 GM banana planting has previously been approved by the Regulator for two field trials under licences DIR 107 and DIR 146. These trials were conducted under limited and controlled conditions on commercial banana farms in the Litchfield Municipality (NT), where there is high Panama disease pressure.

76. The DIR-107 licence was issued in 2011, and the trial completed in June 2016. The DIR-146 licence was issued in 2016 and is ongoing. The GM banana lines grown in these trials, including QCAV-4, were evaluated for a range of agronomic traits, including disease incidence.

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

6.1.2 Approvals by other government agencies

78. The movement and cultivation of banana plants is subject to State and Territory legislation (see Section 5.1).

6.2 International approvals

79. QCAV-4 GM banana plants have not been approved for release overseas.

Chapter 2 Risk assessment

Section 1 Introduction

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

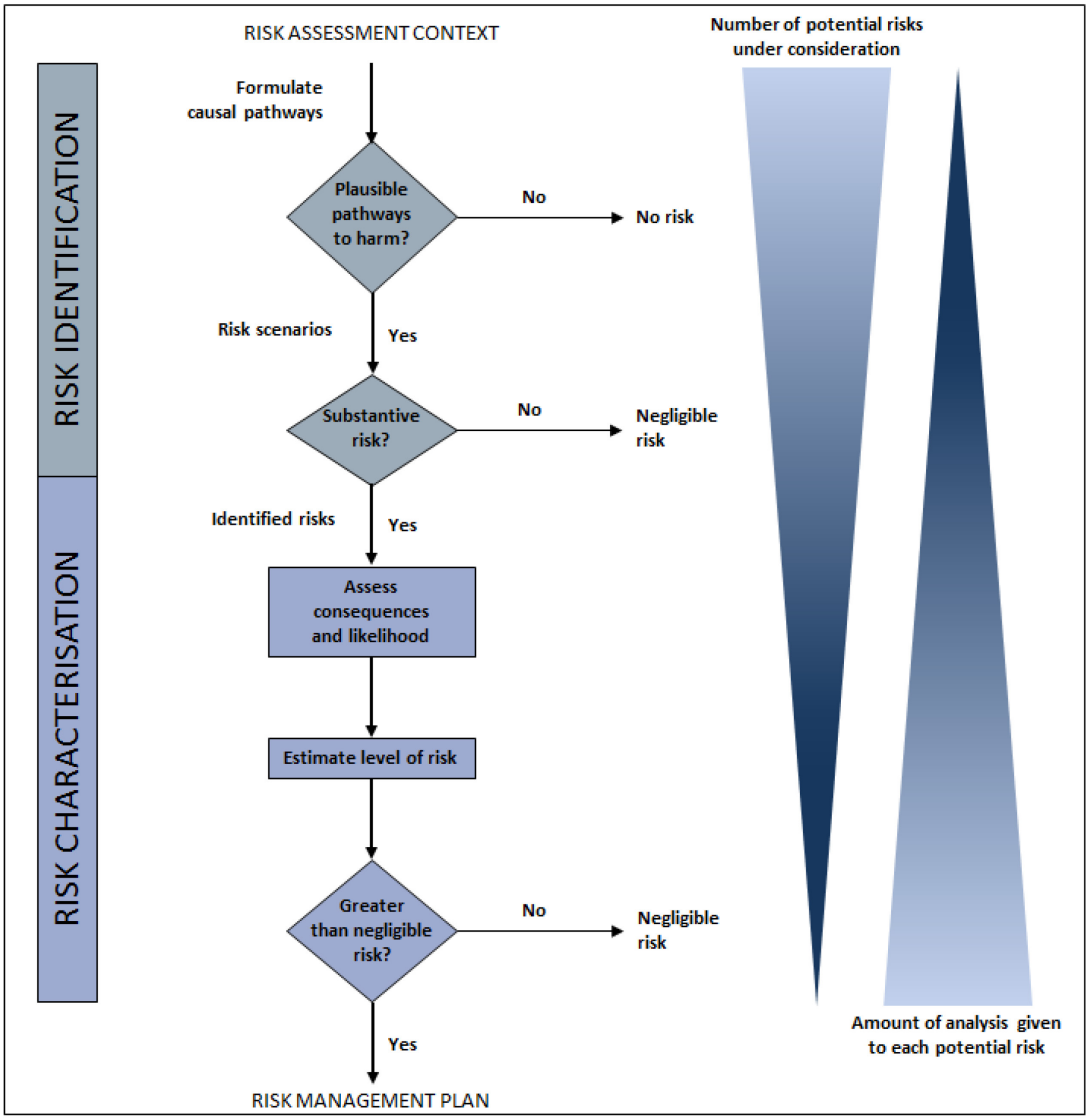

Figure 3 The risk assessment process

81. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, previous agency experience, reported international experience and consultation (OGTR, 2013).

82. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are called risk scenarios.

83. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly occur, or do not

lead to harm in the short and long term, do not advance in the risk assessment process (Figure 3), i.e. the risk is considered no greater than negligible.

84. Risk scenarios identified as substantive risks are further characterised in terms of the potential seriousness of harm (consequence assessment) and the likelihood of harm (likelihood assessment). The consequence and likelihood assessments are combined to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

85. A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants, as this approach addresses the full range of potential adverse outcomes associated with 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). Risk scenarios postulated in previous RARMPs prepared for licence applications for the same or similar GMO are also considered.

Section 2 Risk identification

86. 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), and

iii. Potential harm to people or the environment.

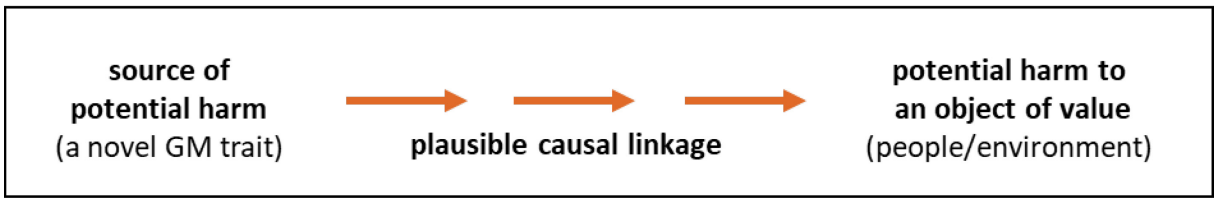

Figure 4 Components of a risk scenario

87. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors detailed in Chapter 1:

- the proposed dealings,
- any proposed limits including the extent and scale of the proposed dealings,
- any proposed controls to limit the spread and persistence of the GMO, and
- the characteristics of the parent organism(s).

2.1 Risk source

88. The sources 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.

89. As discussed in Chapter 1, Section 4.1, QCAV-4 GM banana plants contain a small deletion on chromosome 6 where the insertion of 3 complete copies of the introduced expression cassette and two fragments of the cassette occurred as well as 7 new ORFs and a number of small genome rearrangements where the copies of the inserted expression cassettes meet. The intended effect of insertion of the *MamRGA2* gene is to provide resistance to infection by TR4, and the introduced *nptII* gene was important for selecting GM banana cells in the early development of the QCAV-4 GM banana line. The GMO containing the introduced genetic modifications is further considered as a potential source of risk.

90. The introduced genes are controlled by introduced regulatory sequences. These are derived from a soil bacterium and a plant virus (see 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, so exposure is to the DNA only and dietary DNA has no toxicity (Delaney et al., 2018). As described in Chapter 1, these sequences have been widely used in

other GMO, without reports of adverse effects. Hence, potential for harm from the regulatory elements will not be considered further.

2.2 Causal pathway

91. The following factors are considered when postulating plausible causal pathways to potential harm:

- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- 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
- 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. pests, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organisms
- gene transfer by horizontal gene transfer
- unauthorised activities.

92. Although all of these factors are taken into account, some are not included in risk scenarios because they have been considered in previous RARMPs and are not expected to give rise to substantive risks.

93. As discussed in Chapter 1, Section 3, the non-GM Grand Nain banana cultivar is essentially male and female sterile, and the genetic modification is not expected to alter this. Banana pollen has low viability and the Grand Nain cultivar is a triploid, for which vegetative propagation is regarded as the only form of reproduction (OGTR, 2023). Triploid pollen viability has been reported as less than 10% in one study (Fortescue and Turner, 2004). Fruit develops largely by parthenocarpy (i.e. without prior fertilisation), thus preventing seed formation (Pillay and Tripathi, 2007). Thus, gene transfer is not expected from the GM banana plants to sexually compatible species and will **not** be assessed further.

94. If the QCAV-4 GM banana plants lost their resistance to TR4 infection, either due loss of the insert; natural mutation in the GM banana plants causing low levels or lack of expression of the introduced *MamRGA2* gene; or TR4 overcoming the resistance imparted by the expression of the introduced *MamRGA2* gene, the resulting interaction between banana and TR4 would either be similar or identical to *status quo* and the risks to the health and safety of people and risks to the environment would be identical to the current situation. Therefore, loss of resistance to TR4 infection will **not** be assessed further.

95. The potential for horizontal gene transfer (HGT) and any possible adverse outcomes has been reviewed in the literature (Keese, 2008; Philips et al., 2022) and assessed in previous RARMPs. No risk greater than negligible was identified, due to the rarity of HGT 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, HGT will **not** be assessed further.

96. 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

97. Potential harms from GM plants are based on those used to assess the risk from weeds (Standards Australia et al., 2006; Keese et al., 2014), including:

- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced biodiversity for nature conservation
- reduced establishment or yield of desirable plants
- 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).

98. Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. For example, a plant species may have a different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.4 Postulated risk scenarios

99. Two risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 7 and discussed in depth in Sections 2.4.1 and 2.4.2. Postulation of risk scenarios considers impacts of the GM banana plants on people undertaking the dealings, as well as impacts on people and the environment exposed to the GM banana plants as the result of commercial use or spread and persistence of plant material.

100. 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 7 Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	GM banana plants	Growing GM banana plants ⬇ Expression of the introduced genetic changes in the GM banana plants ⬇ Exposure of people and other organisms to GM banana plants and plant material via ingestion, contact or inhalation	• Increased toxicity or allergenicity in people • Increased toxicity in other organisms	No	• The introduced *MamRGA2* gene is from an edible wild banana. • The *nptII* gene and its product are present in other GM food and feed plants without causing adverse effects in people and other organisms. • There is no significant sequence similarity of the introduced genetic modifications to known and putative allergens and toxins. • The insertion occurred in an intergenic region and is flanked by genes encoding proteins without similarity to known banana allergens. • The new open reading frames are not expressed in leaf, root and fruit tissue. • Food safety assessment is being conducted by FSANZ.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
2	GM banana plants	Growing GM banana plants ⬇ Expression of the introduced genetic changes in GM banana plants ⬇ Dispersal of propagules to nature reserves or intensive use areas ⬇ Establishment of volunteer GM banana plants in conservation and natural environments or intensive use areas	• Increased toxicity or allergenicity in people or increased toxicity in other organisms • Reduced establishment or yield of plants • Reduced utility or quality of the environment	No	• Bananas have limited ability to spread, establish and persist outside cultivation. • There is no expectation the introduced genetic modifications would give QCAV-4 GM banana plants greater ability to withstand abiotic stressors than non-GM bananas. • Bananas are subject to strict biosecurity measures both within and outside agricultural settings.

2.4.1 Risk scenario 1

Risk source	GM banana plants
Causal pathway	⬇ Growing GM banana plants ⬇ Expression of the introduced genetic changes in the GM banana plants ⬇ Exposure of people and other organisms to the GM banana plants and plant material via ingestion, contact or inhalation
Potential harm	Increased toxicity or allergenicity for people OR Increased toxicity for other organisms

Risk source

101. The sources of potential harm for this postulated risk scenario are the GM banana plants.

Causal pathway

102. The applicant proposes that the QCAV-4 GM banana plants could be cultivated on a commercial scale in all suitable Australian agricultural cropping areas. Both the *nos* (Bevan et al., 1983; Ebert et al., 1987) and the CaMV35S (Odell et al., 1985) promoters used to drive expression of the *MamRGA2* and *nptII* genes, respectively, in the QCAV-4 GM banana plants are regarded as constitutive promoters. Thus, the QCAV-4 GM banana plants are expected to express the introduced MamRGA2 and NPTII proteins in all tissues throughout the life cycle of the plants. The other, unintended genetic changes may lead to expression of peptides or proteins, such as possible expression of one or more of the 7 introduced ORFs. Therefore, people and other organisms in the environment could be exposed to the introduced *MamRGA2* and *nptII* genes and their products and may be exposed to other inadvertently produced peptides or proteins.

103. People involved in the banana industry would be exposed to the QCAV-4 GM banana plants and plant material through contact and inhalation. It is noted that contact with pollen from the QCAV-4 GM banana plants would occur at low levels, considering that pollen production in the non-GM parent cultivar is considered low. The introduced genes and their products are not known to be involved in the biochemical pathway for pollen production, only in the signalling pathway in TR4 recognition and resistance to antibiotics. Therefore, no change in pollen production in the GM banana plants compared to the parent cultivar is anticipated.

104. The QCAV-4 GM banana plants and their products would enter general commerce and be used in the same way as non-GM banana. The general public could be exposed to the fruit and other products from the

QCAV-4 GM banana plants containing the introduced genetic changes and resulting proteins through contact and consumption.

105. Native animals, such as birds, bats and insects, could enter banana cropping areas and feed on the fruit or other parts of the GM banana plants. Native vertebrate and invertebrate ground- or soil-dwelling organisms, such as earthworms, snakes or rodents, could come into contact with or consume GM plant material that falls to the ground or is left to decompose on the ground. Therefore, desirable organisms would be exposed to the GM banana plants and material derived from them.

Potential harms

106. Toxicity is an adverse effect of exposure to a substance (Klaassen and Watkins, 2010). The effect of a toxic agent depends on the dose, duration of exposure and exposure route, e.g. inhalation, ingestion or via the skin. Responses may be either immediate or delayed. Non-GM banana plants and their products are not known to cause substantial toxicity in people and animals (OGTR, 2023). The evaluation below seeks to determine whether people or other desirable organisms exposed to the products from the introduced genetic changes in the GM banana plants may show increased toxicity. FSANZ is currently assessing the safety of fruit and other products from this GMO for people in the context of commercial human food, and these aspects will not be covered in this document.

107. Allergic reactions are an adverse effect resulting from sensitisation to a chemical, followed by an allergic response upon subsequent exposure (Klaassen and Watkins, 2010). Allergenicity is the potential for a chemical to be recognised by a person's body as a foreign substance and to elicit a (disproportionate) immunological reaction. Non-GM banana plants are known to contain several allergens which can cause allergic reactions in people, with effects ranging from oral allergy syndrome or urticaria to anaphylaxis. Examples of allergens in non-GM banana are particular profilins, class I chitinases and thaumatin-like proteins (OGTR, 2023). The following evaluation examines whether people exposed to the products resulting from the introduced genetic changes may show increased allergic reactions.

<u>The introduced MamRGA2 gene and its products</u>

108. As discussed in Chapter 1, 4.3.1, the introduced *MamRGA2* gene is an NBS-LRR class gene. These genes are involved in pathogen recognition and downstream signalling of the presence of a pathogen. NBS-LRR class genes are commonly present and expressed in food and feed plants, including bananas (Chang et al., 2020). Thus, people and other organisms are exposed to similar proteins through their diet and the environment.

109. The *MamRGA2* gene was isolated from an edible wild banana, *Musa acuminata* ssp. *malaccensis* (see Chapter 1, Section 4.5). This species occurs in Asia and is a progenitor of Cavendish bananas (OECD, 2009). In its native range, it is consumed by people and animals and is not known to be toxic.

110. *In silico* analysis of the MamRGA2 sequence did not show significant sequence similarity to any proteins known, or suspected, to be of mammalian toxicological concern.

111. Although proteins are not generally associated with toxicity, all known food allergens are proteins. Plant derived allergens come chiefly from peanut, tree nuts, wheat, soybean and sesame (<u>FDA website</u>, accessed June 2023). As the *MamRGA2* gene is not derived from these plant species, it is unlikely to be allergenic. In addition, the *MamRGA2* gene is not the member of a class of known allergens identified in its parent, the Cavendish banana.

112. *In silico* analyses of the MamRGA2 sequence did not reveal immunologically relevant similarities with any of the known or putative allergens in the FARRP database.

113. The above information indicates that allergenic and toxic potential is not altered in the GM banana plants due to the introduction of the *MamRGA2* gene.

<u>The introduced nptII gene and its products</u>

114. The GMO contains 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 various GMOs containing this gene have been assessed as posing negligible risk to human or animal health and to the

environment by the Regulator as well as by other regulatory agencies both in Australia and overseas. Introduction of this gene to the GM banana plants is not considered to result in toxic or allergenic effects in people or other desirable organisms.

Other introduced genetic changes

115. Other genetic changes were introduced unintentionally (see Chapter 1, 4.4.1). The observed genetic changes include:

a) a small deletion at the site of insertion

b) insertion of multiple copies of transgenes (in this case, 3 copies of the transgenes were integrated)

c) insertion of fragments of transgenes (two fragments of the *MamRGA2* gene, one of which was inverted)

d) generation of new ORFs at the site of integration and

e) other small genetic changes, such as indels or point mutations, between the flanking regions and insert as well as between the intact copies of the introduced gene cassette.

116. The types of genetic changes characterised in the GM banana plants are common in genetic modification as well as conventional plant breeding and natural mutations, and their presence does not mean that these changes would be harmful to the resulting plant or to organisms exposed to the plant or derived materials.

117. Investigations into these additional genetic changes have been conducted:

a) The deletion of 116 bp at the site of integration occurred in an intergenic region. The location of the deletion makes it implausible that it would lead to the generation of a novel toxin or allergen. However, if this deletion were in a regulatory sequence, then a change in the expression level/s of one or more neighbouring genes could occur, and increased expression of a native allergen could occur as a consequence. The genes on either side of the insert are predicted to encode protein kinase domain-containing proteins. The currently identified banana allergens are not protein kinases and it is highly unlikely that the deletion would lead to increased allergenicity in people. It is noted that non-GM banana does not cause substantial toxicity in people or animals. It is considered that a change in the expression levels of a limited number of proteins would not alter the toxicity of the GM banana plants and materials.

b) Insertion of multiple copies of the introduced *MamRGA2* and *nptII* genes is highly unlikely to lead to increased toxicity to people and other organisms, or to an increase in allergenicity in people, as the products of each gene are not implicated in these adverse effects (see above) and there is no reasonable expectation that this would be different due to the presence of multiple copies in the GMO.

c) Two fragments of the *MamRGA2* gene and some of the introduced regulatory sequences are inserted in opposite directions between the second and third copy of the introduced expression cassette in the insert. Any resulting new ORFs are discussed in (d) below. The fragment sequences themselves are truncated and therefore non-functional. The presence of these additional DNA sequences is highly unlikely to affect the potential of the GM banana plants to cause an increased level of toxicity or a change in allergenicity in people, or an increased level of toxicity in other organisms.

d) After inconclusive *in silico* database searches for similarity of the 7 new ORFs with plant promoter-like sequences and transcription factor binding sites, an RNA-Seq approach was used by the applicant to assess if one or more of the new ORFs were expressed. The results from the RNA-Seq approach indicate that none of the ORFs were transcribed in the tissues that were analysed, i.e. leaf, root and ripe fruit. As no RNA from these ORFs is produced in these tissues, there will be no production of peptides or protein from them. Thus, it is implausible that the presence of the new ORFs would lead to increased toxicity in people and other organisms, or to increased allergenicity in people. In addition, *in silico* analyses of the sequences of the 7 new ORFs did not reveal significant similarities to known or putative toxins and allergens.

e) Other small genetic changes occurred between the flanking regions and insert as well as between the intact copies of the introduced gene cassette. These regions contained the 7 new ORFs discussed in d). It is considered implausible that these small changes, which are not expressed in the GM banana, could give rise to novel toxins or allergens, or lead to expression of a greater amount of the known allergens in the GM banana plants.

118. It is noted that no adverse reports were received by the Regulator after contact of staff with the GM banana plants during the field trials authorised under licences DIR-107 and DIR-146.

119. The applicant has also supplied a compositional analysis for the fruit and peel tissue from the QCAV-4 GM banana plants and of fruit and peel tissue derived from its non-GM Grand Nain counterpart (GN212-12) as a control. Samples were analysed for the content of proximates (moisture, total fat, total protein, ash, carbohydrates and energy); minerals (magnesium, manganese and potassium); and vitamins (ascorbic acid and pyridoxine). While there were statistical differences in the levels of some of the analytes between QCAV-4 GM and non-GM banana fruit and peel datasets, most mean values for proximates, vitamins and minerals from fruit and peel were within the compositional range reported in the literature. Further, the fact that no consistent pattern was observed indicates that the expression of the inserted expression cassettes did not impact the nutritional composition of fruit and peel in the QCAV-4 GM banana. The samples in this study were taken from the 5th and 6th ratoon crop of the GM and non-GM banana plants at a time when TR4 would have already had adverse effects on the non-GM banana plants, meaning that differences between QCAV-4 GM and non-GM samples could have resulted from the diseased state of the non-GM banana plants. Taken together, it is highly unlikely that any differences observed would have an adverse nutritional impact on people and other organisms consuming the QCAV-4 GM banana fruit or peel. As noted earlier, matters relevant to human food safety of the fruit and other products from the QCAV-4 GM banana plants are being assessed by FSANZ. Furthermore, the GM bananas are unlikely to be a primary source of food for animals and therefore they are likely to consume it only as a small proportion of their diet. As such, slight differences in composition from non-GM bananas are unlikely to have an adverse impact.

Conclusion

120. Risk scenario 1 is not identified as a substantive risk for an increase in toxicity or allergenicity in people, or in toxicity in other organisms because: the insertion occurred in an intergenic region, flanked by genes encoding proteins without similarity to known banana allergens; the *MamRGA2* gene was sourced from an edible wild banana; the *nptII* gene and its product are present in other GM food and feed plants without causing adverse effects; the new ORFs are not expressed; and there is no significant sequence similarity between the introduced genetic modifications to known and putative allergens and toxins. This risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	GM banana plants
Causal pathway	⬇ Growing GM banana plants ⬇ Expression of the introduced genetic changes in GM banana plants ⬇ Dispersal of propagules to nature reserves or intensive use areas ⬇ Establishment of volunteer GM banana plants in conservation and natural environments or intensive use areas ⬇
Potential harm	Increased toxicity or allergenicity in people or increased toxicity to other organisms OR Reduced establishment or yield of plants OR Reduced utility or quality of the environment

Risk source

121. The sources of potential harm for this postulated risk scenario are the GM banana plants.

Causal pathway

122. If presence of the introduced genetic changes was to provide the QCAV-4 GM banana plants with a significant selective advantage over non-GM bananas, this may lead to persistence of the QCAV-4 GM bananas in areas where they are cultivated. It is noted that persistence of healthy banana plants is a desirable outcome in commercial banana cultivation and persistence during banana cultivation is not considered an adverse effect. However, if the QCAV-4 GM banana plants were dispersed outside the area they are cultivated in, and were able to establish and persist in environments, such as conservation and natural environments or intensive use areas, this may give rise to adverse outcomes. This assessment assumes that QCAV-4 GM banana plants could be present in all current and potential banana growing areas in Australia due to deliberate planting, if a licence is issued.

123. 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, 2023). 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 managed to optimise 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).

124. The geographic range of non-GM banana in Australia is limited by a number of abiotic factors, particularly water and temperature, as well as soil nutrient levels (OGTR, 2023). Sweet bananas are restricted to subtropical or tropical areas between 30^{o}N and 30^{o}S that have a mean air temperature of 26.7^{o}C. Optimal root growth occurs between 22-25^{o}C, and lower temperatures will slow root growth. Bananas prefer a mean rainfall of 100 mm per month with no more than a 3-month dry season. Generally, bananas require 20-60 mm of water per week as rainfall or supplied through irrigation. A north-easterly or north-westerly aspect, frost free and protected from cold, strong winds is preferred, with a slope of less than 15%. Bananas are adversely affected by competition with other plants. Other biotic factors, such as pests and diseases, can further limit their ability to establish, spread and persist.

125. Movement of banana material is tightly regulated due to biosecurity concerns, and penalties apply for unauthorised movement of banana plant material. State and territory biosecurity legislation regulates movement of banana material, and such legislation would apply to any GM banana material,. This would limit long distance spread of QCAV-4 GM banana material by people. The biosecurity concerns around banana plants in general also results in removal of any banana plants found outside areas where they are deliberately cultivated.

126. The *MamRGA2* gene confers resistance to TR4 and while it is theoretically possible that it may contribute to resistance to other biotic stressors (diseases) as well, there is no evidence from the literature or from the previous trials to support or refute this. However, this is an area of uncertainty for this risk assessment. Since the introduced *MamRGA2* gene is an *R* gene, it is not expected that it would give the QCAV-4 GM banana plants any ability to overcome abiotic stressors, such as temperature and water requirements.

127. Other than TR4 resistance, the following agronomic characteristics were taken for field grown QCAV-4 GM banana plants (see Chapter 1, 4.4.5): bunch weight, yield, cycle time, plant girth and plant height. Bunch weight, yield and cycle time data was collected from 5 generations (plant crop and 4 ratoons), while plant height and plant girth data were collected from fruit-bearing banana plants in ratoons 6 and 7 (7th and 8th generation). Overall, the results show that bunch weight, yields, plant girth and plant height are similar to non-GM banana plants. Yields of non-GM banana in TR4 affected areas may be lower than yields of QCAV-4 GM banana plants. Although some the QCAV-4 plants had shorter cycle times (time from planting to harvest for the plant crop, or time between harvests for ratoon crops), these differences were not always statistically significant, and as such these results do not imply meaningful differences in

cycle time between QCAV-4 GM and non-GM banana plants. Other plant characteristics, such as immature and mature plant characteristics, and fruit characteristics, were identical to the non-GM parent.

128. Taken together, the introduced genetic changes are expected to provide the QCAV-4 GM banana plants with an advantage over the parental non-GM banana plants in areas where TR4 is present. However, the introduced genetic changes will not result in the QCAV-4 GM banana plants overcoming the abiotic and other biotic factors that limit the spread and persistence of banana in the environment.

Potential harm

129. If the QCAV-4 GM banana plants were to persist in banana cultivation areas, or be dispersed, establish and persist in non-agricultural environments, such as conservation and natural environments or intensive use areas, this may potentially give rise to adverse outcomes.

130. If QCAV-4 GM banana plants were to establish beyond growing areas, they could 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 genetic changes are not expected to cause increased toxicity or altered allergic responses in people, or to cause increased toxicity in other desirable organisms.

131. If QCAV-4 GM banana plants were to spread, establish and persist beyond growing areas, this could 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.

132. As discussed above, the causal pathways which may lead to increased spread and persistence of the QCAV-4 GM bananas are highly unlikely to occur. Therefore, the presence of the introduced genetic changes in QCAV-4 GM banana plants is highly unlikely to lead to any of the potential harms listed above.

Conclusion

133. Risk scenario 2 is not identified as a substantive risk due to bananas having limited ability to spread, establish and persist outside cultivation; the introduced genetic modifications not giving QCAV-4 GM banana plants greater ability to withstand abiotic stressors than non-GM bananas; and bananas being subject to strict biosecurity measures both within and outside agricultural settings. Therefore, this risk could not be considered greater than negligible and does **not** warrant further detailed assessment.

Section 3 Uncertainty

134. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis[2]. There are several types of uncertainty in risk analysis (Clark and Brinkley, 2001; Hayes, 2004; Bammer and Smithson, 2008). 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:

[2] 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.

- 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.

135. Uncertainty is addressed by approaches including balance of evidence, conservative assumptions, and applying measures that reduce the potential for 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.

136. The QCAV-4 GM banana plants have been approved by the Regulator for limited and controlled release (field trial) under licences DIR 107 and DIR 146. The RARMPs for these field trials identified additional information that may be required for large-scale trials with reduced limits and controls or for commercial release of QCAV-4 GM banana plants. For DIR 107, phenotypic characterisation of the GM banana lines with respect to weediness including tolerance to environmental stress or disease susceptibility and additional molecular and biochemical characterisation of the GM banana plants were identified. For DIR 146, uncertainty was raised associated with the potential for any increase in toxicity or allergenicity, and the potential for increased spread and persistence of the GMOs, including in land uses outside of agriculture. Information provided by the applicant addressing these areas of uncertainty is presented in Chapter 1, Sections 4.4 and 4.5 and discussed in relevant sections in Chapter 1 and in the risk scenarios. In addition, matters relevant to human food safety of the fruit and other products from the QCAV-4 GM banana plants are being assessed by FSANZ.

137. Uncertainty can arise from a lack of experience with the GMO. For this GMO, QCAV-4 GM banana plants have been grown in field trials authorised under licences for DIR 107 and DIR 146 over several years. Relevant data from those releases have been considered in the DIR 146 RARMP (for DIR 107 data), and in relevant sections of Chapter 1 of this document and in the risk scenarios. No unintended effects or adverse events have been reported as part of those trials.

138. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.

139. Post release review (PRR) will be also used to address uncertainty regarding future changes to knowledge about the GMO or the receiving environment (Chapter 3, Section 4). PRR is typically required for commercial releases of GMOs, which generally do not have limited duration.

Section 4 Risk evaluation

140. 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.

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

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

142. Two risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. The level of risk for each scenario was considered negligible, considering both the short and long term. The principal reasons for these conclusions are summarised in Table 7.

143. The *Risk Analysis Framework* (OGTR, 2013), 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. The Regulator considers that

the dealings involved in this proposed release do not pose a significant risk to either people or the environment[3].

[3] As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed up to six weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Chapter 3 Risk management plan

Section 1 Background

144. 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 proposed licence conditions.

145. 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.

146. All licences are subject to 3 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.

147. 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 and to manage risk to people or the environment. 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

148. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed release of QCAV-4 GM banana plants. These risk scenarios were considered in the context of the scale of the proposed release and the receiving environment. The risk evaluation concluded that no control measures are required to treat these negligible risks.

Section 3 General risk management

149. 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
- testing methodology
- identification of the persons or classes of persons covered by the licence
- reporting structures
- access for the purpose of monitoring for compliance.

3.1 Applicant suitability

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

- any relevant convictions of the applicant

- 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.

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

152. 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 Testing methodology

153. If a licence were issued, QUT would be required to provide a method to the Regulator for the reliable detection of the GMO. This instrument would be required prior to conducting any dealings with the GMO.

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

154. If a licence were issued, any person, including the licence holder, could conduct any permitted dealing with the GMO.

3.4 Reporting requirements

155. If issued, the licence would oblige the licence holder to report without delay any of the following to the Regulator:

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

156. The licence holder would also be obliged to submit an Annual Report containing any information required by the licence.

157. There are also provisions that would enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 5, below).

3.5 Monitoring for compliance

158. 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 the Regulator, or a person authorised by the Regulator, to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

159. 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 the health and safety of people or the environment could result.

Section 4 Post release review

160. Paragraph 10 of the Regulations requires the Regulator to consider the short and the long term when assessing risks. The Regulator takes account of the likelihood and impact of an adverse outcome over the foreseeable future and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

161. The Regulator engages in ongoing oversight of licences to take account of future findings or changes in circumstances. If a licence was issued, this ongoing oversight would be achieved through post release review (PRR) activities. The three components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to collect additional specific information (Section 4.2)
- review of the RARMP (Section 4.3).

162. The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting system

163. Any member of the public can report adverse experiences/effects resulting from an intentional release of a GMO to the OGTR through the Free-call number (1800 181 030), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see Section 4.3 below) as well as the RARMPs of future applications involving similar GMOs.

4.2 Requirement to collect additional specific information

164. Collection of additional specific information on an intentional release provides a mechanism for 'closing the loop' in the risk analysis process and for verifying findings of the RARMP.

165. This may involve monitoring specific indicators of harm that have been identified in the risk assessment. The term 'specific indicators of harm' does not mean that it is expected that harm would necessarily occur if a licence was issued. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. Should a licence be issued, the licence holder would be required to monitor these specific indicators of harm as mandated by the licence.

166. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

167. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks greater than negligible. Therefore, they were not considered substantive risks that warranted further detailed assessment. No specific indicators of harm have been identified in this RARMP for application DIR 199. However, specific indicators of harm may also be identified during later stages, e.g. following the consideration of comments received on the consultation version of the RARMP, or if a licence were issued, through either of the other components of PRR.

168. Conditions have also been included in the draft licence to allow the Regulator to request further information from the licence holder about any matter to do with the release, including research to verify predictions of the risk assessment.

4.3 Review of the RARMP

169. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR, or by relevant new scientific information or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s) or identified new risks to people or to the environment that require management, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the consultation RARMP

170. The risk assessment concludes that the proposed commercial release of QCAV-4 GM banana plants poses negligible risks to the health and safety of people or the environment as a result of gene technology.

171. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, if a licence were to be issued, general conditions are proposed to ensure that there is ongoing oversight of the release.

Chapter 4 Draft licence conditions

Section 1 Interpretations and Definitions

1. In this licence:

   (a) unless defined otherwise in this licence, words and phrases used in this licence have the same meaning as they do in the Act and the Gene Technology Regulations 2001;

   (b) words importing a gender include every other gender;

   (c) words in the singular number include the plural and words in the plural number include the singular;

   (d) expressions used to denote persons generally (such as “person”, “party”, “someone”, “anyone”, “no one”, “one”, “another” and “whoever”), include a body politic or corporate as well as an individual;

   (e) references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;

   (f) where a word or phrase is given a particular meaning, other grammatical forms of that word or phrase have corresponding meanings;

   (g) specific conditions prevail over general conditions to the extent of any inconsistency.

2. In this licence:

‘**Act**’ means the *Gene Technology Act 2000* (Cth) or the corresponding State legislation under which this licence is issued.

‘**GM**’ means genetically modified.

‘**GMO**’ means the genetically modified organism that is the subject of the dealings authorised by this licence.

‘**OGTR**’ means the Office of the Gene Technology Regulator.

‘**Regulator**’ means the Gene Technology Regulator.

Section 2 Licence conditions and obligations

3. This licence does not authorise dealings with the GMO that are otherwise prohibited as a result of the operation of State legislation recognising an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.

4. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with the GMO are authorised during any period of suspension.

5. The licence holder is Queensland University of Technology.

6. Any person, including the licence holder, may conduct any authorised dealing(s) with the GMO.

7. Except as restricted by condition 3, all dealings with the GMO are permitted.

8. Dealings with the GMO may be conducted in all areas of Australia.

9. This licence authorises dealings with the GMO described in **Attachment A**.

2.1 General obligations of the licence holder

10. The licence holder must notify the Regulator as soon as practicable if any of its contact details change.

Note: please address correspondence to OGTR.M&C@health.gov.au.

Prior to issuing a licence, the Regulator considers suitability of the applicant to hold a licence. The following two conditions address ongoing suitability of the licence holder.

11. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and must comply with its instrument of accreditation.

12. The licence holder must:

    (a) inform the Regulator as soon as practicable after any of these events occur:

        i. any relevant conviction of the licence holder; or

        ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; or

        iii. any event or circumstances that would affect the capacity of the licence holder to meet the conditions of the licence; and

    (b) provide any information related to the licence holder's ongoing suitability to hold a licence, if requested by the Regulator, within the timeframe stipulated by the Regulator.

13. The licence holder must inform any person covered by this licence, to whom a particular condition of the licence applies, of the following:

    (a) the particular condition (including any variations of it); and

    (b) the cancellation or suspension of the licence; and

    (c) the surrender of the licence.

2.2 Provision of new information to the Regulator

Licence conditions are based on the risk assessment and risk management plan developed in relation to the application using information available at the time of assessment. The following two conditions require that any new information that may affect the risk assessment is communicated to the Regulator.

14. The licence holder must inform the Regulator if the licence holder becomes aware of:

    (a) additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or

    (b) any contraventions of the licence by a person covered by the licence; or

    (c) any unintended effects of the dealings authorised by the licence.

Note: The Act requires, for the purposes of the above condition, that:

(a) the licence holder will be taken to have become aware of additional information of a kind mentioned in condition 14 if he or she was reckless as to whether such information existed; and

(b) the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in condition 14, if he or she was reckless as to whether such contraventions had occurred, or such unintended effects existed.

Note: Contraventions of the licence may occur through the action or inaction of a person.

15. If the licence holder is required to inform the Regulator under condition 14, the Regulator must be informed without delay.

Note: An example of informing without delay is contact made within a day of becoming aware of new information via the OGTR free call phone number 1800 181 030, which provides emergency numbers for incidents that occur out of business hours.

16. If at any time the Regulator requests the licence holder to collect and provide information about any matter to do with the progress of the dealings authorised by this licence, including but not confined to:

    (a) additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence, whether or not the licence holder has provided information to the Regulator under condition 14(a);

    (b) any contraventions of the licence by a person covered by the licence, whether or not the licence holder has provided information to the Regulator under condition 14(b);

    (c) any unintended effects of the dealings authorised by the licence, whether or not the licence holder has provided information to the Regulator under condition 14(c);

    (d) research, including by way of survey, to verify predictions of the risk assessment, or for any purpose related to risks to the health and safety of people, or to the environment;

    (e) scientific literature and reports in respect of the GMO authorised by this licence, for a nominated period;

    (f) details of any refusals of applications for licences or permits (however described) to deal with the GMO made pursuant to the regulatory laws of a foreign country;

and the request is reasonable, having regard to consistency with the Act and relevance to its purpose, then the licence holder must collect the information and provide it to the Regulator at a time and in the manner requested by the Regulator.

Note: The Regulator may invite the licence holder to make a submission on the reasonability of a request by the Regulator to collect and provide information relevant to the progress of the dealings with the GMO.

2.3 Obligations of persons covered by the licence

17. Persons covered by this licence must not deal with the GMO except as expressly permitted by this licence.

18. If a person is authorised by this licence to deal with the GMO and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Section 3 Reporting and documentation

3.1 Annual Report

19. The licence holder must provide an annual report to the Regulator by the end of September each year covering the previous financial year. An annual report must include:

    (a) information about any adverse impacts, unintended effects, or new information relating to risks, to human health and safety or the environment caused by the GMO or material from the GMO;

    (b) information about the numbers of QCAV-4-6 GM banana plants sold for commercial purposes, in each State and Territory for each growing season in the period; and

(c) information about the numbers of QCAV-4-6 GM banana plants grown for non-commercial (e.g. research) purposes in each State and Territory for each growing season in the period.

Note: nil plantings should also be reported under sub-conditions (b) and (c).

3.2 Testing methodology

20. At least 14 days prior to conducting any dealings with the GMO, the licence holder must provide to the Regulator a written methodology to reliably detect the GMO, or the presence of the genetic modifications described in this licence in a recipient organism. The detection method(s) must be capable of identifying, to the satisfaction of the Regulator, the genetic modifications described in this licence.

Note: please address correspondence to OGTR.M&C@health.gov.au.

ATTACHMENT A

DIR No: 199

Full Title: Commercial release of banana genetically modified for resistance to *Fusarium* wilt tropical race 4 (TR4)

Organisation Details

Postal address: Queensland University of Technology (QUT)

Level 4 Building X, 88 Musk Avenue

Kelvin Grove QLD 4059

Accreditation No: 066

GMO Description

GMO covered by this licence

One banana line genetically modified by the introduction of only the genes listed below, known by the (provisional) OECD unique identifier QUT-QCAV4-6.

Parent Organism

Common Name: Banana

Scientific Name: *Musa acuminata*

Modified traits

Category: Disease resistance

Selectable marker - antibiotic

Description: The GMO contains multiple copies of one introduced gene conferring *Fusarium* wilt TR4 disease resistance and one introduced selectable antibiotic marker gene (Table 1, below)

Purpose of the dealings with the GMO

The purpose of the dealings is commercial production of the GM banana plants in all areas of Australia, and for products of the GMO to enter general commerce.

Table 1 Introduced genes in the QCAV-4 GM banana plants

Gene (source)	Promoter (source)	Terminator (source)	Protein produced	Protein function
Musa acuminata ssp. *malaccensis* resistance analogue gene 2, *MamRGA2* (*Musa acuminata* ssp. *malaccensis*)	Promoter from the nopaline synthase *(nos)* gene (*Agrobacterium tumefaciens*)	Termination and polyadenylation signal from the *nos* gene (*Agrobacterium tumefaciens*)	*Musa acuminata* ssp. *malaccensis* resistance analogue gene 2 protein (MamRGA2)	Protein for resistance to *Fusarium oxysporum* f.sp. *cubense* tropical race 4
Neomycin phosphotransferase type II gene *nptII* (*Escherichia coli*)	Promoter from 35S RNA *CaMV35S* (Cauliflower Mosaic Virus)	Termination and polyadenylation signal from 35S RNA *CaMV35S 3' UTR* (Cauliflower Mosaic Virus)	Neomycin phosphotransferase type II (NPTII)	Antibiotic resistance, selectable marker

References

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

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

Bevan, M., Barnes, W.M., and Chilton, M.-D. (1983). Strcuture and transcription of the nopaline synthase gene region of T-DNA. Nucleic Acids Research *11*, 369-385.

Biosecurity Queensland (2021). 2021 Panama TR4 Epidemiological review. (Biosecurity Queensland).

Business Queensland (2020). Panama disease tropical race 4 (TR4). (Queensland Government. Business Queensland) Accessed: 10 February 2021.

Chang, W., Li, H., Chen, H., Qiao, F., and Zeng, H. (2020). NBS-LRR gene family in banana (Musa acuminata): genome-wide identification and responses to Fusarium oxysporum f. sp. cubense race 1 and tropical race 4. European Journal of Plant Pathology *157*, 549-563.

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

Dale, J., James, A., Paul, J.Y., Khanna, H., Smith, M., Peraza-Echeverria, S., Garcia-Bastidas, F.*, et al.* (2017). Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race 4. Nature Communications *8*, 1496.

Daly, A., and Walduck, G. (2006). *Fusarium* Wilt of Bananas (Panama Disease), F.a.M. Department of Primary Industry, NT Government, ed. (Department of Primary Industry, Fisheries and Mines, NT Government).

Delaney, B., Goodman, R.E., and Ladics, G.S. (2018). Food and Feed Safety of Genetically Engineered Food Crops. Toxicol Sci *162*, 361-371.

Depicker, A., Stachel, S., Dhaese, P., Zambryski, P., and Goodman, H.M. (1982). Nopaline synthase: transcript mapping and DNA sequence. Journal of Molecular and Applied Genetics *1*, 561-573.

Ebert, P.R., Ha, S.B., and An, G. (1987). Identification of an essential upstream element in the nopaline synthase promoter by stable and transient assays. Proc Natl Acad Sci U S A *84*, 5745-5749.

Emaga, T.H., Andrianaivo, R.H., Wathelet, B., Tchango, J.T., and Paquot, M. (2007). Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels. Food Chemistry *103*, 590-600.

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

FSANZ (2022). Australian Food Composition Database - Release 2.0 (Canberra: Food Standards Australia New Zealand).

Gordon, D.M., and Cowling, A. (2003). The distribution and genetic structure of Escherichia coli in Australian vertebrates: Host and geographic effects. Microbiology (Reading) *149*, 3575-3586.

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

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

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

Hernandez-Garcia, C.M., and Finer, J.J. (2014). Identification and validation of promoters and cis-acting regulatory elements. Plant Science *217-218*, 109-119.

HIA (2022). Australian Horticulture Statistics Handbook 2020/21 - Fruit. (Sydney: Horticulture Innovation Australia Limited).

Hulbert, S.H., Webb, C.A., Smith, S.M., and Sun, Q. (2001). Resistance gene complexes: evolution and utilization. Annu Rev Phytopathol *39*, 285-312.

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., and Smith, J. (2014). Applying a weed risk assessment approach to GM crops. Transgenic Research *23*, 957-969.

Klaassen, C.D., and Watkins, J.B., eds. (2010). Casarett & Doull's Essentials of Toxicology, 2nd edn (New York, USA: McGraw-Hill).

Lescot, M., Déhais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., Rouzé, P.*, et al.* (2002). PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Research *30*, 325-327.

Lozano, R., Hamblin, M.T., Prochnik, S., and 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., and Mastrangelo, A.M. (2013). Plant nucleotide binding site-leucine-rich repeat (NBS-LRR) genes: active guardians in host defense responses. International Journal of Molecular Sciences *14*, 7302-7326.

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

Nelson, S.C., Ploetz, R.C., and Kepler, A.K. (2006). *Musa* species (bananas and plantains). In Species Profiles for Pacific Island Agroforestry, C.R. Elevitch, ed. (Holualoa, Hawai'i: Permanent Agricultural Resources).

NSW DPI (2016). Plant Quarantine manual for New South Wales Version 1.0 January 2016. Plant biosecurity plant product integrity & standards. (New South Wales Department of Primary Industries).

NSW DPI (2017). Panama disease - tropical race 4. (New South Wales Department of Primary Industries) Accessed: 10 February 2021.

Odell, J.T., Nagy, F., and Chua, N.H. (1985). Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature *313*, 810-812.

OECD (2009). Consensus document on the biology of bananas and plantains (*Musa* spp.) (France: Organisation for Economic Co-operation and Development), pp. 87.

OGTR (2013). Risk Analysis Framework 2013, 4th edn (Canberra, Australia: Office of the Gene Technology Regulator).

OGTR (2023). The biology of *Musa* L. (banana) Version 3. (Canberra: Office of the Gene Technology Regulator).

Ortiz, R., and Vuylsteke, D. (1995). Factors influencing seed set in triploid *Musa* spp. and production of euploid hybrids. Annals of Botany *75*, 151-155.

Pegg, K.G., Coates, L.M., O'Neill, W.T., and Turner, D.W. (2019). The epidemiology of Fusarium wilt of banana. Frontiers in Plant Science *10*, doi: 10.3389/fpls.2019.01395.

Peraza-Echeverria, S., Dale, J.L., Harding, R.M., and 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., and 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.

PHA (2021). Plant Health Australia Fact Sheet - Panama disease. (Plant Health Australia).

PHA (2022). Panama TR4 Protect. (Plant Health Australia) Accessed: 24 March 2022.

PHA, and Queensland DEEDI (2009). Farm biosecurity manual for the banana industry. Reducing the risk of exotic and damaging pests becoming established in crops. Version 1.0. (Plant Health Australia, Queensland Government Department of Employment, Economic Development and Innovation).

Philips, J.G., Martin-Avila, E., and Robold, A.V. (2022). Horizontal gene transfer from genetically modified plants - Regulatory considerations. Frontiers in Bioengineering and Biotechnology *10*.

Pillay, M., Tenkouano, A., and Hartman, J. (2002). Bananas and Plantains: Future Challenges in *Musa* Breeding. In Crop Improvement, Challenges in the Twenty-First Century (New York: Food Products Press), pp. 223-252.

Pillay, M., and Tripathi, L. (2007). Banana. In Genome Mapping and Molecular Breeding in Plants, Volume 4 Fruits and Nuts, C. Kole, ed. (Berlin: Springer-Verlag), pp. 281-301.

QDAF (2016a). Banana Industry Biosecurity Guideline. (Queensland Department of Agriculture and Fisheries).

QDAF (2016b). Panama disease overview. (Queensland Department of Agriculture and Fisheries) Accessed: 24 September 2015.

QDAF (2020). Latest Panama TR4 news. (Queensland Government - Department of Agriculture and Fisheries) Accessed: 16 September 2021.

Queensland Government (2022). Queensland biosecurity manual. Edition 2021, version 17. (Biosecurity Queensland, Queensland Department of Agriculture and Fisheries).

Rommens, C.M.T., Salmeron, J.M., Oldroyd, G.E.D., and Staskawicz, B.J. (1995). Intergeneric Transfer and Functional Expression of the Tomato Disease Resistance Gene Pto. The Plant Cell *7*, 1537-1544.

Salamov, A.A., and Solovyev, V.V. (1997). Recognition of 3' -processing sites of human mRNA precursors. Bioinformatics *13*, 23-28.

Simmonds, N.W. (1959). Bananas (London, UK: Longmans, Green & Co.).

Simmonds, N.W. (1962). The Evolution of Bananas (London: Longmans, Green and Co. Ltd).

Solovyev, V.V., Shahmuradov, I.A., and Salamov, A.A. (2010). Identification of Promoter Regions and Regulatory Sites. In Computational Biology of Transcription Factor Binding, I. Ladunga, ed. (Totowa, NJ: Humana Press), pp. 57-83.

Standards Australia, Standards New Zealand, and CRC for Australian Weed Management (2006). HB 294:2006 National Post-Border Weed Risk Management Protocol (Standards Australia and Standards New Zealand).

State of Queensland (2016). Queensland Biosecurity regulation 2016. (Current as at 14 August 2020) (State of Queensland).

State of Queensland (2017). Banana best management practices. On-farm biosecurity. Report No. BA14013. (Queensland Government Department of Agriculture and Fisheries).

Thilmony, R.L., Chen, Z., Bressan, R.A., and Martin, G.B. (1995). Expression of the Tomato Pto Gene in Tobacco Enhances Resistance to Pseudomonas syringae pv tabaci Expressing avrPto. The Plant Cell *7*, 1529-1536.

USDA (2019). FoodData Central (United States Department of Agriculture).

Vézina, A. (2021). Fusarium wilt of banana. (Musapedia, the banana knowledge compedium) Accessed: 28 January 2021.

Vézina, A. (2022). Tropical race 4. (Musapedia, the banana knowledge compedium) Accessed: 24 January 2023.

Vézina, A., and Rouard, M. (2021). *Fusarium oxysporum* f. sp. *cubense*. (Musapedia, the banana knowledge compedium) Accessed: 28 January 2021.

WA DPIRD (2020). Propagation of bananas. (Government of Western Australia, Department of Primary Industries and Regional Development) Accessed: 10 February 2021.

Appendix A: Summary of submissions

The Regulator received several submissions from prescribed experts, agencies and authorities[4] on matters relevant to preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. These issues, and where they are addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	Agrees that the following should be included in the RARMP: • the potential for the GM banana to be harmful to people through toxicity or allergenicity • the potential for the GM banana to be harmful to other organisms through toxicity • the potential for the introduced traits to increase the weediness of the GM banana, leading to harm to the environment • the potential for harm to result from gene flow to related species • the potential for commercial release to result in changes to agricultural practices that may have an adverse environmental impact.	These were addressed in Chapters 1 and 2 of the RARMP.
	Noted that Food Standards Australia New Zealand will assess the use of the GM banana and its products as food for human consumption.	Noted.
	Agreed that the scope of the RARMP should be for commercial scale production of the GMO and not assume any limitations on the release.	The evaluation did not assume any limitations on the proposed release (see RARMP).
2	The application is not applicable as there are no bananas grown in the area.	Noted.
3	Has no comment at this point.	Noted.
4	Has no comment on the application.	Noted.

[4] Prescribed experts, agencies and authorities include the Gene Technology Technical Advisory Committee, State and Territory Governments, relevant local governments, Australian government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
5	Agrees that the following matters should be considered: • the potential for the GM banana plants to be harmful to people through toxicity or allergenicity • the potential for the GM banana plants to be harmful to other organisms through toxicity • the potential for the introduced trait to increase the weediness of the GM banana plants, leading to harm to the environment • the potential for harm to result from gene flow to other banana plants or related species • the potential for commercial release to result in changes to agricultural practices that may have an environmental impact.	These were addressed in Chapters 1 and 2 of the RARMP.
	In addition, recommends including data that were identified in previous authorisations as 'may be required' with regard to toxicity, spread and persistence.	Licence application DIR-199 was evaluated on the merits of the information it contained, including data obtained from field trials authorised under previous limited and controlled releases.