



**Australian Government**  

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

**Risk Assessment and  
Risk Management Plan for  
DIR 100**

Limited and controlled release of wheat genetically  
modified for enhanced carbon assimilation in drought  
and heat prone environments

**Applicant: Commonwealth Scientific and Industrial  
Research Organisation**

**June 2010**

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# Executive Summary

## Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of licence application (DIR 100) from the Commonwealth Scientific and Industrial Research Organisation (CSIRO). The licence authorises dealings involving the limited and controlled release of up to 150 lines<sup>1</sup> of genetically modified (GM) wheat into the environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a genetically modified organism (GMO). The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public<sup>2</sup>.

## The application

CSIRO has applied for a licence for dealings involving the intentional release of up to 150 lines of GM wheat on a limited scale and under controlled conditions. The GM wheat lines have been genetically modified for enhanced carbon assimilation in drought and heat prone environments. The trial will take place at one site in the Queensland LGA of Redland, on a maximum area of 0.1 ha per growing season, between June 2010 and December 2013.

The applicant will release GM wheat modified to contain one or more of 26 genes derived from wheat and barley. Expression of the genes is expected to show improved grain weight and yield of the GM wheat in heat and drought prone environments through enhanced water use efficiency, photosynthesis and carbon assimilation. The applicant also intends to cross some of the lines using conventional breeding to produce GM wheat which contain a combination of these traits.

The GM wheat lines also contain a herbicide tolerance gene and an antibiotic resistance gene that were used as markers to select for successful genetic modifications during initial research and development work in the laboratory. The applicant does not intend to apply the herbicide in the field.

The purpose of the trial is to assess the agronomic performance of the GM wheat lines for biomass production, grain weight and yield under rain-fed and drought/heat prone conditions. The GM wheat would not be used for human food or animal feed.

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<sup>1</sup> The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event

<sup>2</sup> More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <[http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/raf-3/\\$FILE/raffinal3.pdf](http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/raf-3/$FILE/raffinal3.pdf)>

CSIRO proposed a number of controls to restrict the spread and persistence of the GM wheat lines and the introduced genetic materials in the environment. These controls were considered during the evaluation of the application.

### **Confidential Commercial Information**

Some details, including the identities of some of the genes and sequences, and associated references have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

### **Risk assessment**

The risk assessment took into account information in the application, relevant previous approvals, current scientific knowledge and advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology were postulated (risk scenarios), and these scenarios were evaluated to identify those that warrant detailed characterisation. This process is described as risk identification.

Eight risk scenarios were postulated, including consideration of whether or not expression of the introduced genes could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM wheat; or produce unintended changes in the biochemistry of the GMOs. The opportunity for gene flow to other organisms, and its effects if it were to occur, was also assessed.

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

The characterisation of the eight risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment.

Risks to the health and safety of people, or the environment, from the proposed release of the GM wheat into the environment are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

### **Risk management plan**

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, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through licence conditions.

As none of the eight risk scenarios characterised in the risk assessment give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be **negligible**. The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions are imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and to limit the proposed release to

the size and location requested by the applicant, as these were important considerations in establishing the context for assessing the risks.

The licence conditions require CSIRO to **limit** the release to a total area of 0.1 ha per growing season at one site between June 2010 and December 2013. The **control** measures include containment provisions at the trial site, prohibiting the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with Regulator's transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed.

### ***Conclusions of the RARMP***

The risk assessment concluded that this limited and controlled release of up to 150 GM wheat lines on a maximum total area of 0.1 ha per growing season over three years in the Queensland LGA of Redland, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

# Table of Contents

<b>EXECUTIVE SUMMARY</b> .....	<b>I</b>
INTRODUCTION .....	I
THE APPLICATION .....	I
CONFIDENTIAL COMMERCIAL INFORMATION.....	II
RISK ASSESSMENT.....	II
RISK MANAGEMENT PLAN .....	II
CONCLUSIONS OF THE RARMP .....	III
<b>TABLE OF CONTENTS</b> .....	<b>IV</b>
<b>ABBREVIATIONS</b> .....	<b>VI</b>
<b>TECHNICAL SUMMARY</b> .....	<b>1</b>
INTRODUCTION .....	1
THE APPLICATION .....	1
CONFIDENTIAL COMMERCIAL INFORMATION.....	2
RISK ASSESSMENT.....	2
RISK MANAGEMENT PLAN .....	3
LICENCE CONDITIONS.....	3
OTHER REGULATORY CONSIDERATIONS .....	4
IDENTIFICATION OF ISSUES TO BE ADDRESSED FOR FUTURE RELEASES .....	4
SUITABILITY OF THE APPLICANT .....	4
CONCLUSIONS OF THE RARMP .....	4
<b>CHAPTER 1 RISK CONTEXT</b> .....	<b>6</b>
SECTION 1 BACKGROUND .....	6
SECTION 2 THE LEGISLATIVE REQUIREMENTS .....	6
SECTION 3 THE PROPOSED DEALINGS.....	7
3.1 The proposed activities .....	8
3.2 The proposed limits of the dealings (size, locations and duration) .....	8
3.3 The proposed controls to restrict the spread and persistence of the GMOs and their genetic material in the environment.....	8
SECTION 4 THE PARENT ORGANISM.....	9
SECTION 5 THE GMOS, NATURE AND EFFECT OF THE GENETIC MODIFICATION .....	9
5.1 Introduction to the GMOs .....	9
5.2 The introduced genes, their encoded proteins and their associated effects .....	11
5.3 The regulatory sequences .....	19
5.4 Method of genetic modification .....	20
5.5 Characterisation of the GMO .....	20
SECTION 6 THE RECEIVING ENVIRONMENT.....	21
6.1 Relevant abiotic factors.....	22
6.2 Relevant biotic factors.....	22
6.3 Relevant agricultural practices .....	23
6.4 Presence of related plants in the receiving environment .....	24
6.5 Presence of the introduced genes or similar genes and encoded proteins in the environment ..	24
SECTION 7 AUSTRALIAN AND INTERNATIONAL APPROVALS .....	25
7.1 Australian approvals of GM wheat .....	25
7.2 International approvals of GM wheat.....	26
<b>CHAPTER 2 RISK ASSESSMENT</b> .....	<b>27</b>
SECTION 1 INTRODUCTION .....	27
SECTION 2 RISK IDENTIFICATION .....	28
2.1 Production of a substance toxic/allergenic to people or toxic to other organisms.....	30
2.2 Spread and persistence of the GM wheat plants in the environment.....	31
2.3 Vertical transfer of genes or genetic elements to sexually compatible plants .....	35
2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms .....	38
2.5 Unintended changes in biochemistry, physiology or ecology .....	39
2.6 Unauthorised activities .....	40
SECTION 3 RISK ESTIMATE PROCESS AND ASSESSMENT OF SIGNIFICANT RISK .....	41

SECTION 4	UNCERTAINTY .....	42
<b>CHAPTER 3</b>	<b>RISK MANAGEMENT PLAN.....</b>	<b>43</b>
SECTION 1	BACKGROUND .....	43
SECTION 2	RESPONSIBILITIES OF OTHER AUSTRALIAN REGULATORS .....	43
SECTION 3	RISK TREATMENT MEASURES FOR IDENTIFIED RISKS.....	44
SECTION 4	GENERAL RISK MANAGEMENT .....	44
4.1	Licence conditions .....	44
4.2	Other risk management considerations .....	50
SECTION 5	ISSUES TO BE ADDRESSED FOR FUTURE RELEASES .....	51
SECTION 6	CONCLUSIONS OF THE CONSULTATION RARMP.....	52
<b>REFERENCES</b>	<b>.....</b>	<b>53</b>
<b>APPENDIX A</b>	<b>SUMMARY OF ISSUES RAISED IN SUBMISSIONS RECEIVED FROM PRESCRIBED EXPERTS, AGENCIES AND AUTHORITIES ON THE CONSULTATION RARMP FOR DIR 100.....</b>	<b>66</b>

## Abbreviations

the Act	<i>Gene Technology Act 2000</i>
Act	Actin
APHIS	Animal and Plant Health Inspection Service
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
bar	phosphinothricin acetyl transferase (PAT)
CaMV	Cauliflower mosaic virus
CCI	Confidential Commercial Information as declared under section 185 of the <i>Gene Technology Act 2000</i>
DIR	Dealings Involving intentional Release
DNA	Deoxyribonucleic Acid
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
HGT	Horizontal gene transfer
m	metre
mm	millimetre
mRNA	Messenger Ribonucleic Acid
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase Chain Reaction
RARMP	Risk Assessment and Risk Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
nptII	Neomycin phosphotransferase II
PC2	Physical containment level 2
nos	Nopaline synthase
SSP	Seed Storage Proteins
TGA	Therapeutic Goods Administration
T-DNA	Transfer DNA
Ubi1	Ubiquitin1
US EPA	United States Environmental Protection Agency

# Technical Summary

## Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of licence application (DIR 100) from the Commonwealth Scientific and Industrial Research Organisation (CSIRO). The licence authorises dealings involving the limited and controlled release of up to 150 lines<sup>3</sup> of genetically modified (GM) wheat into the environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a genetically modified organism (GMO). The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public<sup>4</sup>.

## The application

CSIRO has applied for a licence for dealings involving the intentional release of up to 150 lines of GM wheat on a limited scale and under controlled conditions. The GM wheat lines have been genetically modified for enhanced carbon assimilation in drought and heat prone environments. The trial will take place at one site in the Queensland LGA of Redland, on a maximum area of 0.1 ha per growing season, between June 2010 and December 2013.

The applicant will release GM wheat modified to contain one or more of 26 genes derived from wheat and barley. Expression of the genes is expected to show improved grain weight and yield of the GM wheat in heat and drought prone environments through enhanced water use efficiency, photosynthesis and carbon assimilation. They will be under control of a constitutive promoter, a developmental specific promoter, or a drought inducible promoter derived from barley or maize. The applicant also intends to cross some of the lines using conventional breeding to produce GM wheat which contain a combination of these traits.

The GM wheat lines also contain two selectable marker genes, *nptII* and *bar*. The *nptII* gene encodes neomycin phosphotransferase type II which provides resistance to antibiotics such as kanamycin. The *bar* gene encodes phosphinothricin acetyl transferase which provides resistance to herbicides containing glufosinate ammonium. These were used as selectable markers during early stages of development of the GM plants in the laboratory.

Other short regulatory sequences that contribute to control of expression of the introduced genes are also present in the GM wheat. These are derived from maize, rice, Cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens* (a common soil bacterium). Although some of these sequences are derived from plant pathogens (*A. tumefaciens* and CaMV), the

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

<sup>4</sup> More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <[http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/raf-3/\\$FILE/raffinal3.pdf](http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/raf-3/$FILE/raffinal3.pdf)>

regulatory sequences comprise only a small part of the pathogen's total genome, and in themselves have no pathogenic properties.

The purpose of the trial is to assess the agronomic performance of the GM wheat lines for biomass production, grain weight and yield under rain-fed and drought/heat prone conditions. The GM wheat will not be used for human food or animal feed.

CSIRO proposed a number of controls to restrict the spread and persistence of the GM wheat lines and the introduced genetic materials in the environment. These controls were considered during the evaluation of the application.

### **Confidential Commercial Information**

Some details, including the identities of some of the genes and sequences, associated references and phenotypic data, have been declared, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

### **Risk assessment**

The risk assessment took into account information in the application (including proposed containment measures), relevant previous approvals, current scientific knowledge and issues relating to risks to human health and safety and the environment raised in submissions received from consultation with a wide range of prescribed experts, agencies and authorities (included in Appendix A of the RARMP). The public also had the opportunity to provide comments, however no submissions were received from members of the public.

A reference document, *The Biology of the Triticum aestivum L. em Thell (Bread Wheat)*, was produced to inform the risk assessment process for licence applications involving GM wheat plants. The document is available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology were postulated (risk scenarios), and these scenarios were evaluated to identify those that warrant detailed characterisation. This process is described as risk identification.

Eight risk scenarios were postulated, including consideration of whether or not expression of the introduced genes could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM wheat; or produce unintended changes in the biochemistry of the GMO. The opportunity for gene flow to other organisms, and its effects if it were to occur, was also assessed.

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

The characterisation of the eight risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principal reasons for this include:

- limits on the size, location and duration of the release proposed by CSIRO
- suitability of controls proposed by CSIRO to restrict the spread and persistence of the GM wheat plants and their genetic material

- limited ability and opportunity for the GM wheat to transfer the introduced genes to other wheat plants or other sexually compatible species
- none of the GM plant materials or products will be used for human food or animal feed
- widespread presence of the same or similar proteins encoded by the introduced genes in the environment and lack of known toxicity or evidence of harm from them.

Risks to the health and safety of people, or the environment, from the proposed release of the GMOs into the environment are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

### ***Risk management plan***

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, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through licence conditions.

As none of the eight risk scenarios characterised in the risk assessment give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be **negligible**. The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions have been imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and to limit the release to the size, location and duration requested by the applicant, as these were important considerations in establishing the context for assessing the risks.

### ***Licence conditions***

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

- limit the release to a total area of 0.1 ha at one site per growing season between June 2010 and December 2013
- locate the field trial site at least 50 m away from natural waterways
- establish a 10 m zone around the trial in which any related species are prevented from flowering and which is maintained in a manner that does not attract rodents
- surrounding the GM wheat and barley with an inspection zone of up to 200 m in which growth of sexually compatible species is controlled
- enclosing each trial site with a livestock-proof fence with lockable gate with mouse baiting inside the fence perimeter
- apply measures to promote germination of any wheat and barley seeds that may be present in the soil after harvest, including tillage and irrigation
- monitor the site for at least 24 months after harvest and until no volunteers are detected for a continuous 6 month period and destroy any wheat plants that may grow
- destroy all plant material from the trial not required for testing or future trials
- transport and storage of the GMOs in accordance with the Regulator's guidelines
- not allow the GM plant material or products to be used for human food or animal feed, or in the production of therapeutic goods.

## ***Other regulatory considerations***

Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)<sup>5</sup>.

APVMA has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia. The application of these herbicides is subject to regulation by the APVMA. While the GM wheat has been modified to be tolerant to glufosinate ammonium containing herbicides, the applicant does not intend to apply these herbicides during the trial.

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

## ***Identification of issues to be addressed for future releases***

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

- additional data on the potential toxicity and allergenicity of plant materials from the GM wheat lines
- phenotypic characterisation of the GM wheat lines, in particular of traits which may contribute to weediness, persistence, and ability to disperse in the environment
- molecular and biochemical characterisation of the GM wheat lines
- compositional analysis of the GM wheat lines.

## ***Suitability of the applicant***

The Regulator determined, at the commencement of the assessment process for this application, that CSIRO was suitable to hold a DIR licence under the requirements of section 58 of the Act. The Regulator is satisfied that CSIRO remains suitable as no relevant convictions have been recorded, and no licences or permits have been cancelled or suspended under laws relating to the health and safety of people or the environment.

## ***Conclusions of the RARMP***

The risk assessment concluded that this proposed limited and controlled release of up to 150 GM wheat lines on a maximum total area of 0.1 ha per growing season over three years in the Queensland LGA of Redland, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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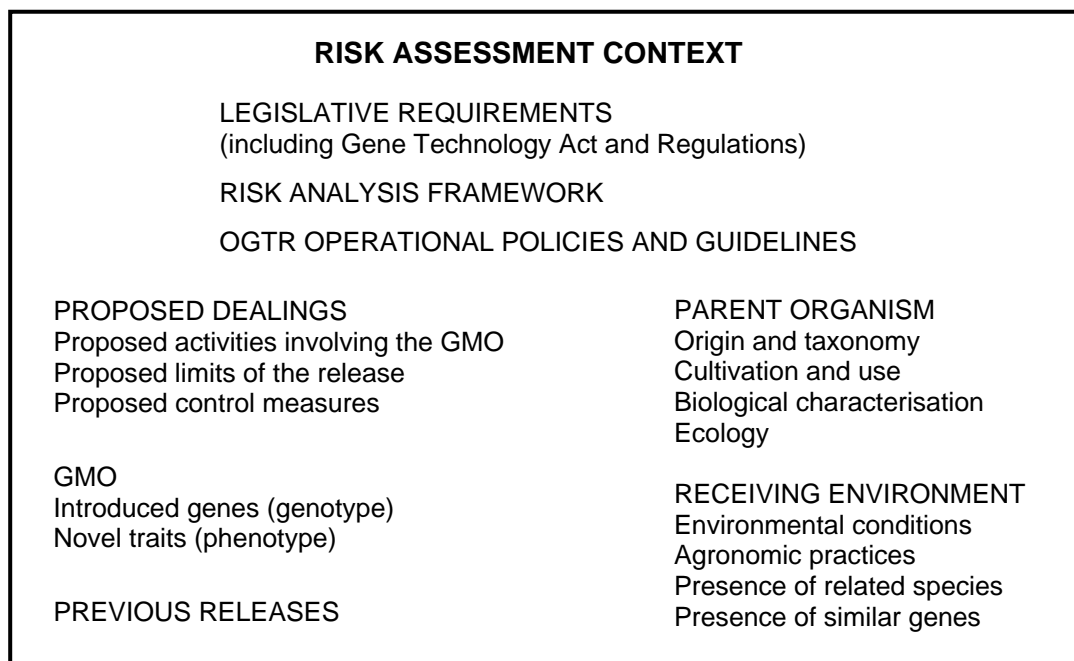
<sup>5</sup> More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

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

# Chapter 1 Risk context

## Section 1 Background

1. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed (Figure 1).



**Figure 1. Parameters used to establish the risk assessment context**

2. The risk assessment context is developed within the framework of the *Gene Technology Act 2000* and *Gene Technology Regulations 2001* (Section 2), the *Risk Analysis Framework*, and operational policies and guidelines (see <http://www.ogtr.gov.au>).

3. In addition, establishing the risk assessment context for this application includes consideration of:

- the proposed dealings (Section 3)
- the parent organism (Section 4)
- the GMOs, nature and effect of the genetic modification (Section 5)
- the receiving environment (Section 6)
- previous releases of these or other GMOs relevant to this application (Section 7).

## Section 2 The legislative requirements

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and with whom he must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of his decisions on licence applications. In addition, the Regulations outline matters the Regulator must consider when preparing a RARMP.

5. In accordance with section 50A of the Act, the Regulator considered information provided in the application and was satisfied that its principal purpose is to enable the

applicant to conduct experiments. In addition, limits have been proposed on the size, location and duration of the release and controls have been proposed by the applicant to restrict the spread and persistence of the GMOs and their genetic material in the environment. Those limits and controls are such that the Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application is considered to be a limited and controlled release and the Regulator has prepared a RARMP for this application.

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, local council(s) where the release is proposed to take place, and the public. The advice from the prescribed experts, agencies and authorities, and how it was taken into account, is summarised in Appendix A. No submissions were received from the public.

7. Section 52(2)(ab) of the Act requires the Regulator to decide whether or not one or more of the proposed dealings may pose a ‘significant risk’ to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d). This decision is provided in Section 3 of Chapter 2.

### ***Section 3 The proposed dealings***

8. The Commonwealth Scientific Industrial Research Organisation (CSIRO) proposes to release up to 150 lines<sup>6</sup> of GM wheat each containing one or in some cases more than one of 26 genes encoding proteins expected to enhance photosynthesis, biomass accumulation, carbon assimilation, grain yield, grain quality, water use efficiency, drought tolerance and/or heat tolerance. The applicant also intends to cross some of the lines using conventional breeding to produce GM wheat which contain a combination of these traits.

9. The dealings involved in the proposed intentional release would include:

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

10. These dealings are detailed further throughout the remainder of the current Chapter.

11. Some details, including the identities of some of the genes and sequences, associated references and phenotypic data, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was considered during the preparation of the RARMP and was made available to the prescribed expert groups and authorities that were consulted.

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<sup>6</sup> The term ‘line’ is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.

### 3.1 The proposed activities

The applicant has stated that the objective of the proposed trial is to evaluate agronomic properties of the GM wheat lines for biomass production, grain weight and yield under rain-fed and drought/heat prone conditions. The GM wheat and any products made from it would not be used for human food or animal feed.

12. The GM wheat lines proposed for release would be produced in PC2 facilities at CSIRO Plant Industry, St Lucia (Brisbane) before being transported to the trial site for planting in the field.

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

13. The release is proposed to take place at one site in the Redland LGA (Qld) on a maximum area of 0.1 ha per growing season between June 2010 and December 2013.

14. The majority of the wheat lines will be tested in miniplots (1.6 m<sup>2</sup>) in the first year. Those lines showing desirable phenotypes will be planted in full sized plots (9.6 m<sup>2</sup>) in subsequent years.

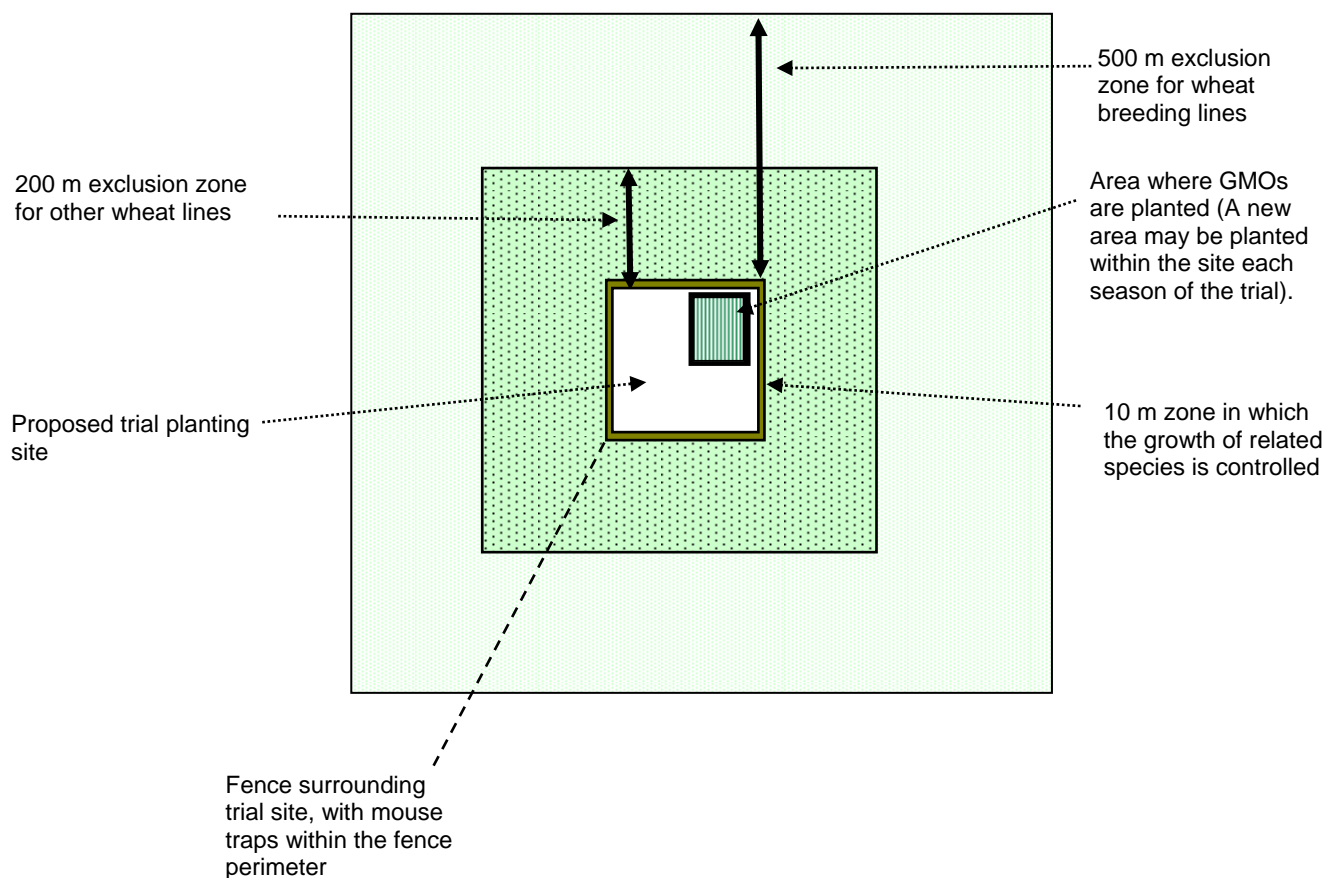
15. Only trained and authorised staff would be permitted access to the proposed location.

### 3.3 The proposed controls to restrict the spread and persistence of the GMOs and their genetic material in the environment

16. The applicant has proposed a number of controls to restrict the spread and persistence of the GM wheat lines and the introduced genetic material in the environment including:

- locating the trial site at least 100 m away from natural waterways
- enclosing each trial site with a fence with lockable gate with mouse baiting inside the fence perimeter
- locating the trial site at least 200 m from all other wheat plantings and at least 500 m from other wheat breeding lines
- surrounding the GM wheat with a 2 m wide buffer of non-GM wheat and a 10 m zone in which growth of related species is controlled
- promoting the germination of any residual seed following harvest through two six weekly irrigation cycles and destroying any volunteer wheat
- post harvest monitoring of the trial site for 12 months or until the site has been clear of volunteers for one growing season and destroying any volunteer wheat
- destroying all plant material from the trial not required for testing or future trials
- transporting and storing of the GMOs in accordance with the Regulator's guidelines
- not allowing the GM plant material or products to be used for human food or animal feed.

17. These controls (see also Figure 2), and the limits outlined above (Section 3.2), have been taken into account in establishing the risk assessment context (this chapter), and their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.2.1.



**Figure 2.** Diagram of some of the proposed containment measures (not drawn to scale).

## Section 4 The parent organism

18. The parent organism is the wheat (*Triticum aestivum* L.) cultivar Bob White, which is exotic to Australia. The wheat cultivar Bob White is not grown commercially in Australia but is commonly used in genetic modification work. Further detailed information about the parent organisms is contained in the reference document *The Biology of Triticum aestivum L. em Thell (Bread Wheat)*, that was produced to inform the risk assessment process for licence applications involving GM wheat plants (OGTR 2008). The document is available from the OGTR or from the website <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

19. Commercial wheat cultivation occurs in the wheat belt from south eastern Queensland through New South Wales, Victoria, southern South Australia and southern Western Australia (OGTR 2008).

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

### 5.1 Introduction to the GMOs

20. The applicant proposes to release up to 150 lines of GM wheat modified to contain one or more of 26 genes derived from wheat and barley (Table 1). Expression of the genes is expected to show improved grain weight and yield of the GM wheat in heat and drought

prone environments through enhanced water use efficiency, photosynthesis and carbon assimilation.

21. The GM wheat lines will also contain an antibiotic resistance selectable marker, the *neomycin phosphotransferase II (nptII)* gene and/or a herbicide tolerance selectable marker, the *phosphinothricin acetyl transferase (PAT) (bar)* gene (Table 1). The *nptII* and *bar* genes were used as selective markers during early stages of development of the GM plants in the laboratory. Three GM wheat lines will contain only the *nptII* or *bar* selectable marker gene and will serve as control lines.

**Table 1. The introduced genes, selectable markers and regulatory sequences of the GM wheat lines.**

Gene <sup>a</sup>	Selectable marker gene used in construct <sup>c</sup>	Promoter/source/expression <sup>d</sup>	3' region/source <sup>e</sup>
<i>TaNACdu1<sup>b</sup></i>	<i>Ubi1:bar:Nos3'</i> or <i>35S:nptII:Nos3'</i>	<i>Dhn8s</i> /barley/constitutive	<i>rbcS</i> /rice
<i>TaNf-YBps1<sup>b</sup></i>	<i>35S:nptII:Nos3'</i>	<i>Dhn4s</i> /barley/drought-inducible	<i>rbcS</i> /rice
<i>TaNf-YCps1<sup>b</sup></i>	<i>Act1:bar:Nos3'</i>	<i>Ubi1</i> /maize/constitutive	<i>rbcS</i> /rice
<i>TaNf-YAc1<sup>b</sup></i>	<i>Act1:bar:Nos3'</i>	<i>SFT1s</i> /barley/developmental	<i>rbcS</i> /rice
<i>TaDofsu1<sup>b</sup></i>	<i>Act1:bar:Nos3'</i>	<i>Ubi1</i> /maize/constitutive	<i>rbcS</i> /rice
<i>TaMybc1</i>	<i>Act1:bar:Nos3'</i>	<i>Ubi1</i> /maize/constitutive	<i>rbcS</i> /rice
<i>TaMyb807<sup>b</sup></i>	<i>Act1:bar:Nos3'</i>	<i>Dhn8s</i> /barley/constitutive	<i>rbcS</i> /rice
<i>TaMybc2</i>	<i>Act1:bar:Nos3'</i>	<i>SFT1s</i> /barley/developmental	<i>rbcS</i> /rice
<i>TaMyb83</i>	<i>Act1:bar:Nos3'</i>	<i>Ubi1</i> /maize/constitutive	<i>rbcS</i> /rice
<i>TaMyb96</i>	<i>Act1:bar:Nos3'</i>	<i>Dhn13s</i> /barley/constitutive	<i>rbcS</i> /rice
<i>TabZIP1</i>	<i>Act1:bar:Nos3'</i>	<i>Dhn13s</i> /barley/constitutive	<i>rbcS</i> /rice
<i>TabZIPc1</i>	<i>Act1:bar:Nos3'</i>	<i>Dhn8s</i> /barley/constitutive	<i>rbcS</i> /rice
<i>TaWRKY2</i>	<i>Act1:bar:Nos3'</i>	<i>SFT1s</i> /barley/developmental	<i>rbcS</i> /rice
<i>TaB3DBP1</i>	<i>Act1:bar:Nos3'</i>	<i>Dhn8s</i> /barley/constitutive	<i>rbcS</i> /rice
<i>TaRWD1</i>	<i>Act1:bar:Nos3'</i>	<i>SFT1s</i> /barley/constitutive	<i>rbcS</i> /rice
<i>TaZFPdu1<sup>b</sup></i>	<i>Act1:bar:Nos3'</i>	<i>HVA1s</i> /barley/drought-inducible	<i>rbcS</i> /rice
<i>TaHsf1</i>	<i>Act1:bar:Nos3'</i>	<i>HVA1s</i> /barley/drought-inducible	<i>rbcS</i> /rice
<i>TaHsf2</i>	<i>Act1:bar:Nos3'</i>	<i>HVA1s</i> /barley/drought-inducible	<i>rbcS</i> /rice
<i>HvCBF<sup>b</sup></i>	<i>Act1:bar:Nos3'</i>	<i>Dhn4s</i> /barley/drought-inducible	<i>rbcS</i> /rice
<i>TaCaT1</i>	<i>Act1:bar:Nos3'</i>	<i>Dhn13s</i> /barley/constitutive	<i>rbcS</i> /rice
<i>TaAuTP</i>	<i>Act1:bar:Nos3'</i>	<i>Ubi1</i> /maize/constitutive	<i>rbcS</i> /rice
<i>TaIP</i>	<i>Act1:bar:Nos3'</i>	<i>Ubi1</i> /maize/constitutive	<i>rbcS</i> /rice
<i>TaPSL1</i>	<i>Act1:bar:Nos3'</i>	<i>Ubi1</i> /maize/constitutive	<i>rbcS</i> /rice
<i>TaPGF1</i>	<i>Act1:bar:Nos3'</i>	<i>Ubi1</i> /maize/constitutive	<i>rbcS</i> /rice
<i>TaFBPase<sup>b</sup></i>	<i>Act1:bar:Nos3'</i>	<i>Dhn8s</i> /barley/constitutive	<i>rbcS</i> /rice
<i>HvEH1</i>	<i>Act1:bar:Nos3'</i>	<i>Dhn8s</i> /barley/constitutive	<i>rbcS</i> /rice

<sup>a</sup> The gene names, as supplied by the applicant, are explained and their general function described in Sections 5.2.1 and 5.2.2.

<sup>b</sup> Several genes names have been declared CCI. Therefore alternative names are used for these genes in this table and elsewhere in the RARMP.

<sup>c</sup> *Ubi*: ubiquitin, *35S*: Cauliflower Mosaic Virus 35S, *nptII*: neomycin phosphotransferase II, *bar*: barnase (*phosphinothricin acetyl transferase (PAT)*), *nos*: nopaline synthase terminator

<sup>d</sup> *Dhn*: dehydrin, *HVA*: later embryonic abundant protein, *SFT1*: sucrose:fructan 6-fructosyltransferase 1

<sup>e</sup> *rbcS*: rubisco.

22. In all the GM wheat lines, short regulatory elements would be used to control expression of the genes (Table 1). These sequences are derived from plants (including maize, rice and barley), a soil bacterium (*A. tumefaciens*) and a plant virus (Cauliflower mosaic virus; CaMV).

## 5.2 The introduced genes, their encoded proteins and their associated effects

23. The purpose of the genetic modifications is to enhance survival and grain quality production in heat and drought prone environments through enhancing water use efficiency, photosynthesis and carbon assimilation.
24. Stresses often impact negatively plant growth and as such can affect the productivity of crops. Therefore, plants need to be able to detect and adapt to changes in environmental conditions, including abiotic stresses such as heat and drought. For example, water stress can affect the ability to assimilate carbon efficiently which can result in reduced carbon sink and storage capacity (Silvius et al. 1977). Plants can adapt to stresses through employing a number of survival mechanisms; adaptation of plants to heat stress include morpho-anatomical, physiological and molecular responses, see for example Wahid et al (2007); adaptation to drought stress can include survival mechanisms such as altering of plant metabolism, growth and development, see for example Bartels and Sunkar (2006).
25. The initiation of the adaptive response of plants is one of the vital steps in surviving exposure to either abiotic or biotic stresses. Primary stresses such as heat, lack of water, cold and salt can lead to other physiological stresses such as osmotic and oxidative stresses. The often interactive response to these stresses involves the induction and expression of a large number of genes, which may encode functional proteins that regulate membrane transport or enzymes involved in biosynthetic pathways, or regulatory proteins such as the transcription factors, see for example Wang et al (2003). In the field, drought and heat stresses can often occur at the same time. Plants have been shown to modulate their response to a combination of stresses such as heat and drought; a number of genes may be expressed in response to either drought or heat, including genes which are specifically expressed when the plant is exposed to both these stresses (Rizhsky et al. 2004).
26. The genes introduced into the GM wheat proposed for release in this application include genes encoding enzymes, a growth factor, a calcium binding protein and several transcription factors.
27. Transcription factors (proteins that control expression of genes through binding to specific DNA sequences) are generally grouped into families according to their conserved structural domains which are involved in DNA binding. A number of these families have been shown to be important in developmental and general stress responses, including environmental stresses and exposure to pathogens. Transcription factors can be inducible by abscisic acid (ABA), see for example (Bartels & Sunkar 2006), which in turn seems to respond to the primary abiotic stress by which they are induced. For example, the transcription factors MYB, bZIP, WRKY, NAC and NF-Y are ABA dependent and induced by stresses such as drought and salt, while others such as the CBF1-3 transcription factors are ABA independent.
28. Transcription factors regulate expression of particular genes through binding to *cis* regulatory elements (ie DNA sequences in the same part of the genome as the gene); families of transcription factors bind to specific DNA sequences which can be specific for sets of genes with related functions. For example, in cereals the ACAA motif (Takaiwa et al. 1996; Diaz et al. 2002) and bipartite endosperm box (Albani et al. 1997), are found in the promoter region of the seed storage protein genes (SSP genes). The Dof, Myb and bZIP class of transcription factors are known to bind to these regulatory elements (Vicente-Carbajosa et al. 1997; Diaz et al. 2002; Yamamoto et al. 2006).
29. The effect of the introduced genes and their end-products have not been fully characterised in the GM wheat lines proposed for release. Their potential function of the

genes has been identified according to their up-regulation are a result of exposure to heat stress and drought conditions or they have been shown to be associated with drought/heat stress tolerance. In Table 2 and the following two sections, the genes and their encoded proteins are described in brief to illustrate their potential function within the GM wheat lines. These have been grouped according to the function of the encoded protein into the transcription factors and other proteins, which include membrane transport and enzymes involved in biosynthetic pathways.

**Table 2. Identity, source and anticipated effect of the introduced genes for enhanced survival and grain quality production in heat and drought prone environments.**

Encoded protein type (Family or sub-family)	Gene Name	Source organism	Anticipated or potential phenotypic effect of the introduced gene (as specified by the applicant)
NAC Transcription factor	<i>TaNACdu1</i>	<i>T.aestivum</i>	Improved water use efficiency
NF-Y Transcription factors	<i>TaNf-YAc1</i>	<i>T.aestivum</i>	Improved photosynthesis, enhanced carbon assimilation and grain weight
	<i>TaNf-YCps1</i>	<i>T.aestivum</i>	
	<i>TaNf-YBps1</i>	<i>T.aestivum</i>	
MYB Transcription factors	<i>TaMYBc1</i>	<i>T.aestivum</i>	Enhanced carbon assimilation and grain weight
	<i>TaMYB807</i>	<i>T.aestivum</i>	
	<i>TaMYBc2</i>	<i>T.aestivum</i>	
	<i>TaMYB83</i>	<i>T.aestivum</i>	
	<i>TaMYB96</i>	<i>T.aestivum</i>	
Dof Transcription factor	<i>TaDofsu1</i>	<i>T.aestivum</i>	Improved biomass production
WRKY[Zn] Transcription factor	<i>TaWRKY2</i>	<i>T.aestivum</i>	Enhanced carbon assimilation and grain weight
bZIP Transcription factors	<i>TabZIP1</i>	<i>T.aestivum</i>	Enhanced carbon assimilation and grain weight
	<i>TabZIPc1</i>	<i>T.aestivum</i>	
CBF Transcription factor	<i>HvCBF</i>	<i>H. vulgare</i>	Improved drought tolerance and water use efficiency
C2H2 (ZFP) Transcription factor	<i>TaZFPdu1</i>	<i>T.aestivum</i>	Improved drought tolerance and water use efficiency
B3 Transcription factor	<i>TaB3DBP1</i>	<i>T.aestivum</i>	Enhanced carbon assimilation and grain weight
Heat Shock Transcription factors	<i>TaHsf1</i>	<i>T.aestivum</i>	Improved drought and heat tolerance and water use efficiency
	<i>TaHsf2</i>	<i>T.aestivum</i>	
RWD Transcription factor	<i>TaRWD1</i>	<i>T.aestivum</i>	Enhanced carbon assimilation and grain weight
Other proteins	<i>TaPGF1</i>	<i>T.aestivum</i>	Enhanced growth, carbon assimilation and grain weight
	<i>TaFBPase</i>	<i>T.aestivum</i>	Enhanced carbon assimilation and grain weight
	<i>TaPSL1</i>	<i>T.aestivum</i>	Enhanced grain weight and yield
	<i>HvEH1</i>	<i>H. vulgare</i>	Enhanced carbon assimilation and grain weight
	<i>TaIP</i>	<i>T.aestivum</i>	Enhanced grain weight and yield
	<i>TaCaT1</i>	<i>T.aestivum</i>	Enhanced carbon assimilation and grain weight
	<i>TaAuTP1</i>	<i>T.aestivum</i>	Enhanced carbon assimilation and grain weight

### 5.2.1 The transcription factor genes and encoded proteins

The introduced genes encoding transcription factors belong to a number of families and subfamilies (see table 2). The general characteristics of these families are described in this Section.

#### *NAC transcription factors*

30. The *TaNacdu1* gene encodes a NAC transcription factor. NAC transcription factors are a large group of plant specific transcription factors involved in developmental processes such as floral morphogenesis (Aida et al. 1997; Sablowski & Meyerowitz 1998), auxin mediated lateral root development (Xie et al. 2000), plant defence and in the response to a number of environmental stresses including drought and salinity (Hu et al. 2006). Most of the NAC

transcription factors contain a highly conserved N-terminal DNA binding domain (Olsen et al. 2005).

31. Involvement of NAC transcription factors in responses to drought was first reported in *Arabidopsis* (Tran et al. 2004) and has subsequently been confirmed in other plant species, including rice and soybean (Hu et al. 2006; Nakashima et al. 2007; Tran et al. 2009). Over-expression of the NAC transcription factor ATAF1 in *Arabidopsis* resulted in an increased susceptibility to fungus and bacterial pathogens, possibly through down regulation of defense genes (Wang et al. 2009). Interestingly, in *Arabidopsis* NAC transcription factors have also been shown to be involved in the plants response to low oxygen conditions such as water logging (Christianson et al. 2009).

32. In grasses such as rice, over-expression of the NAC transcription factor *OsNAC6* resulted in increased tolerance to water deficit and high salt conditions but these plants also showed growth retardation and lower grain yield (Nakashima et al. 2007). Hu et al (2006) report that over-expression of the stress responsive *NAC* genes, *SNAC1* and *SNAC2* in rice resulted in increased tolerance to drought and salt without growth retardation or yield penalties.

33. The *NAM-B1* gene of wheat encodes a NAC transcription factor that accelerates senescence and the remobilisation of nutrients to developing grains (Uauy et al. 2006). GM wheat expressing RNAi constructs that suppresses expression of this gene showed a delayed senescence and grains with reduced grain protein, zinc and iron content.

#### ***NF-Y transcription factors***

34. The *TaNF-YAc1*, *TaNF-YBps1* and *TaNF-YCps1* genes encode NF-Y transcription factors. The NF-Y transcription factors are functionally made up of three subunits; NF-YA, NF-YB and NF-YC. These subunits are encoded by separate genes and each subunit has its own conserved core region (Montovanni 1999). The NF-Y transcription factors bind to the CCAAT box, a highly conserved and ubiquitous eukaryotic promoter element.

35. The NF-Y proteins regulate many physiological processes with overlapping functions, and combinations of the encoded proteins for the three subunits allows for high combinatorial complexity. Due to this high degree of complexity it has been difficult to assign specific functions to specific *NF-Y* genes (Siefers et al. 2009). NF-Y genes can be expressed constitutively or be developmentally regulated. The *Arabidopsis* *NYA5* gene has been shown to be drought inducible in an ABA dependent manner (Li et al. 2008).

36. In rice, NF-YB proteins may have a role in chloroplast development as three subunits of NF-YB are expressed ubiquitously and repression of expression results in chloroplast degeneration and down regulation of photosynthesis genes (Miyoshi et al. 2003). In maize, over-expression of a single NF-YB subunit has been shown to increase chlorophyll content in the leaves and grain yield in field grown plants under water limiting conditions (Nelson et al. 2007).

#### ***MYB transcription factors***

37. The *TaMYBc1*, *TaMYBc2*, *TaMYB807*, *TaMYB83* and *TaMYB96* genes encode Myb transcription factors. The Myb family of transcription factors represents the largest family of transcription factors in plants. They can regulate secondary metabolism in response to cellular morphogenesis, hormone and stress signals.

38. The Myb transcription factors in plants contain a DNA binding domain with one, two or three imperfect repeats, each containing three helices. The Myb transcription factor family can be divided into three subfamilies, Myb1R, Myb3R and R2R3Myb, based on the number of adjacent repeats within the Myb domain (Romanel et al. 2009). These three subfamilies are

functionally diverse; Myb1R is involved in regulation of the circadian rhythm (Carre & Kim 2002); Myb3R is involved in the formation of B-type cyclin (Ito et al. 2001); and R2R3 Myb, the largest and most functionally diverse group, is involved in anthocyanin biosynthesis (Nesi et al. 2001; Piazza et al. 2002), response to giberillin acid (GA) (Gubler et al. 2002), dehydration, salicylic acid and pathogenesis (Vaillau et al. 2002; Nagaoka & Takano 2003; Denekamp & Smeekens 2003). In *Arabidopsis*, a R2R3Myb transcription factor has been shown to play a role in the regulation of expression of CBF transcription factors under cold stress (Agarwal et al. 2006a).

39. Himi and Noda (2005) reported that the *Red grain colour* gene (*R*) from wheat is a Myb type transcription factor and is associated with the production of grains with a red colour as a result of the production of flavonoid type compounds. The red colour of grain has been shown to be associated with grain dormancy.

40. Transcription factors such as Myb are known to bind to regulatory sequences of the *SSP* genes. In barley, a Myb transcription factor has been shown to interact with a Dof transcription factor and play a role in the regulation of endosperm specific genes during seed development (Diaz et al. 2002).

### ***Dof transcription factors***

41. The *TaDofsul* gene encodes a Dof transcription factor. Dof transcription factors belong to the zinc finger motif group of transcription factors; **D**N**A**-binding with **O**ne **F**inger. These proteins have a highly conserved DNA binding domain. The Dof proteins have been identified in a number of plants, including wheat, barley, pea, *Arabidopsis*, rice, tobacco and potato (Yanagisawa 2004). They have been shown to be associated with seed development and germination, phytohormone and defense responses (Yanagisawa 2002).

42. In maize, a possible role for Dof1 has been indicated in the regulation of light responsive genes such as C4 photosynthetic phosphoenolpyruvate carboxylase (Yanagisawa 2002). In *Arabidopsis*, the Dof transcription factor OBP1 has been shown to be involved in the regulation of gene expression in response to salicylic acid and oxidative stress (Chen et al. 1996; Yanagisawa 2002), while other Dof transcription factors such as DAG1 and DAG2 have been shown to control seed germination (Yanagisawa 2002; Gualberti et al. 2002) and may enhance nitrogen assimilation (Yanagisawa 2004).

43. Some Dof transcription factors are known to bind to regulatory sequences of the *SSP* genes including the wheat *wPbf* gene and an alpha-gliadin gene (Dong et al. 2007). Dof proteins regulating endosperm specific expression of storage proteins have also been isolated from barley and maize (Yanagisawa 2002).

44. In *Arabidopsis*, maize and wheat, Dofs with a bifunctional domain involved in both DNA binding and protein-protein interaction have been identified and specifically interact with the leucine zipper protein bZIP (Yanagisawa 2002).

### ***WRKY [Zn] transcription factors***

45. The *TaWRKY2* gene encodes a WRKY (Zn) transcription factor. These transcription factors contain a WRKY domain which is defined by the WRKYGQK amino acid sequence, or minimal variants thereof, at the N-terminus of the protein, as well as a zinc finger like motif (Eulgem et al. 2000). The WRKY(Zn) transcription factors can function as transcriptional activators or repressors and are involved in a range of biological functions in plants, including biotic and abiotic stress responses [(Eulgem et al. 2000; Ülker & Somssich 2004; Duan et al. 2007) and references therein]. Abiotic factors known to induce *WRKY* genes include structural damage, drought, cold and heat stress (Ross et al. 2007). Some WRKY transcription factors play a part in plant developmental programming (for example trichome

development), fruit ripening, flowering, hormone signalling, tannin synthesis, senescence and root development (Eulgem et al. 2000; Ülker & Somssich 2004).

46. In wheat, expression of WRKY has been shown to increase in response to exposure to low temperatures (Talanova et al. 2008). In wheat, barley and wild oat, the WRKY transcription factors, ABF1 and ABF2 are associated with the regulation of the  $\alpha$ -amylase genes (Rushton et al. 1995). The barley Hv-WRKY38 transcription factor has been shown to be involved in both cold and drought responses in an ABA independent manner (Mare et al. 2004). Also in barley, the SUSIBA2 transcription factor has been shown to be involved in regulation of starch synthesis (Sun et al. 2003).

47. In *Arabidopsis*, eight WRKY transcription factors were directly up-regulated by the transcription cofactor NPR1 as part of the systemic acquired resistance (SAR) which is an inducible plant defence against pathogens (Wang et al. 2006). Pandey and Somssich (2009) describe a role for WRKY transcription factors in general plant immunity activated in response to plant pathogens.

#### ***bZIP transcription factors***

48. The *TabZIP1* and *TabZIPc1* genes encode bZIP transcription factors. The bZIP transcription factors are ubiquitous in all eukaryotes and control numerous physiological and developmental processes. For example, in *Arabidopsis* bZIP has been shown to activate proline dehydrogenase (ProDH) an enzyme with roles in the regulation of L-proline levels, a compound of temporary carbon and nitrogen storage. The bZIP transcription factors are also regulators of light response (Ulm et al. 2004), seed storage (Lara et al. 2003) and pathogen defence (Kaminaka et al. 2006).

49. The bZIP transcription factors typically have a conserved domain of 40-80 amino acids which is made up of two distinct motifs; a leucine zipper which is required for dimerisation and a DNA binding region, (Hurst 1995). bZIP transcription factors are known to bind to regulatory sequences of the *SSP* genes (Albani et al. 1997).

50. In wheat, the TRAB1 bZIP transcription factor has been shown to regulate ABA signalling. TRAB1 has been shown to interact with the VP1 transcription factor to bind to the ABA-responsive elements (ABRE) to regulate seed dormancy and maturation (Hobo et al. 1999). Further studies in wheat by Kobayashi et al (2008) have shown that another bZIP transcription factor, LIP19, was induced by low temperatures, drought stress and ABA. Transcription of the wheat *TaOBF1* gene was also shown to be inducible by drought stress (2008).

51. In rice, expression profile analyses of *OsbZIP* have indicated a role in the regulation of floral and seed development. The *OsbZIP* genes were also regulated by light, and abiotic stresses such as cold and drought (Nijhawan et al. 2007).

#### ***CBF transcription factors***

52. The *HvCBF* gene encodes a CBF transcription factor. The CBF transcription factors bind a C-repeat/drought responsive element. Some of the CBFs are also known as DREB1 transcription factors (**D**ehydration **R**esponse **E**lement **B**inding factor) and are members of the *Apeta1/ethylene-responsive element binding protein* (AP2/EREBP) is made up of three gene families; *CBF1* (*DREB1b*), *CBF2* (*DREB1c*) and *CBF3* (*DREB1*) (Gilmour et al. 2004).

53. As described in (Agarwal et al. 2006) the *CBF1*, *CBF2* and *CBF3* genes are not regulated by ABA. CBFs bind to CRT/DRE elements which are found in the promoter region of cold-regulated (*COR*) genes. Low temperatures and water deficit conditions induce expression of these genes (Stockinger et al. 1997; Gilmour et al. 1998). Over-expression of *CBF/DREB* genes in rice also lead to increased drought tolerance.

### ***C<sub>2</sub>H<sub>2</sub> (ZFP) transcription factors***

54. The *TaZFPdu1* gene encodes a C<sub>2</sub>H<sub>2</sub> ZFP (zinc finger protein) transcription factor, which belongs to the ZFP family of transcription factors, one of the largest families of transcription factors in eukaryotes (Englbrecht et al. 2004). The C<sub>2</sub>H<sub>2</sub> ZFPs have two cysteines (C<sub>2</sub>) and two histidines (H<sub>2</sub>); known as a zinc finger domain. ZFPs are involved in a number of biological processes including the regulation of floral and vegetative development, see for example (Takasuji 2010).

55. A number of ZFP from species including *Arabidopsis*, rice and soybean respond to drought and cold stress (Seki et al. 2002; Vogel et al. 2005), and over-expression of ZFPs can enhance tolerance to abiotic stresses (Sakamoto et al. 2004; Huang et al. 2007). The majority of ZFP are thought to act as transcription suppressors during exposure to abiotic stresses, these proteins contain an ERF (ethylene response factor)-associated amphiphilic repressor (EAR) motif (see for example (Cifti-Yilmaz et al. 2007)). Similar observations of up-regulation of a ZFP by drought, salinity or ABA have been reported in rice (Agarwal et al. 2007; Huang et al. 2007).

### ***B3 transcription factors***

56. The *TaB3DBP1* gene encodes a transcription factor which contains a B3 domain. The B3 domain has been identified in a number of plant transcription factors. Some of the first identified B3 domain proteins include those encoded by the *Viviparous 1 (VP1)* gene from maize and *Abscisic acid-Insensitive 3 (ABI3)* gene from *Arabidopsis* (Giraudat et al. 1992). B3 domain-containing proteins have been found to be responsive to the phytohormones auxin (Auxin Response Factors- ARFs) and abscisic acid, and play important roles in developmental processes including seed maturation and plant growth, see for example (Stone et al. 2001). Further roles have since been identified in plant growth and development including involvement in flowering time regulation and hormone signalling pathways (Hu et al. 2004; Alvarez et al. 2006; Castillejo & Pelaz 2008; Romanel et al. 2009).

57. Several major classes of transcription factors have been identified to contain the B3 domain; the ABI3/ VP1 factors, the RAV like family and the ARF family (Riechmann et al. 2000). Some of these proteins have the B3 domains in addition to other known motifs such as the RAV1 protein (**R**elated to **A**BI3/**V**P1), which has a C-terminal B3 domain as well an N-terminal AP2/ERF domain (Kayaga et al. 1999). The AP2/ERF domains are found in the CBF type transcription factors and are known to induce tolerance to cold.

### ***Heat shock transcription factors***

58. The applicant states that the *Tahsf1* and *Tahsf2* genes encode heat shock transcription factors (hstf). Hstf regulate the expression of the heat shock proteins.

59. Heat shock proteins are a ubiquitous group of proteins that occur in both prokaryotes and eukaryotes. They have been shown to be involved in many cellular responses to heat stress and exposure to heavy metals, metabolic inhibitors and oxidative stress, see review (Miller & Mittler 2006). They are inducible by high temperatures but also many other stresses such as cold, drought and salinity. Many of the heat shock proteins can be induced by one stress or a combination of stresses, see for example (Swindell et al. 2007).

60. The hstf family in *Arabidopsis* appears to be larger than described for any animal species. This family responded to heat and other stresses, such as salt and oxidative stress (Swindell et al. 2007). Mishra et al (2002) has suggested a master regulatory role for HsfA1 in the acquisition of heat tolerance in tomato through regulating the expression of a number of other hstf; the posttranscriptional silencing of HsfA1 resulted in an effective absence of heat shock protein synthesis and adaptation to heat stress.

61. In wheat, hstf were shown to be upregulated in response to heat stress (Qin et al. 2008). The response to heat shock and the induction of heat shock proteins can differ between wheat cultivars (Yildiz & Terzi 2008).

### ***The RWD transcription factor***

62. The *TaRWD1* gene encodes a RWD domain protein which functions as a transcription factor. The RWD domain was identified in a screen of protein sequences containing known nuclear domains. Doerks et al (2002) describe RWD as a defined region related to the **R**ING finger and **W**D-domain-containing proteins and **D**EAD (DEXD)-like helicases. The domain is found in several species including *Arabidopsis*, yeast, humans and *C. elegans* (Doerks et al. 2002).

63. In plants, RWD domains have been found in RING E3 ligases from, for example *Arabidopsis* and rice (Stone et al. 2005). RING domain proteins have been shown to act as E3 ubiquitin ligases, which are involved in ubiquitylation of target proteins (Haglund & Dikic 2005; Mazzucotelli et al. 2006). In tobacco, over-expression of RING-H2 encoded by the *PtaRHE1* gene was shown to upregulate defense genes and expression of WRKY transcription factors. Expression of the *PtaRHE1* gene could also be induced by ABA and salicylic acid (Bopopi et al. 2010).

### **5.2.2 Other genes and their encoded proteins**

#### ***TaPGF1***

64. The *TaPGF1* gene encodes a peptide growth factor. In plants, peptide growth factors function in peptide signalling during plant growth.

#### ***TaFBPase***

65. The *TaFBPase* gene encodes a fructose 1,6-bisphosphatase enzyme. There are two forms of fructose 1,6-bisphosphatase, a chloroplast and cytosolic form. The chloroplast form of FBPase converts fructose 1,6-bisphosphate to fructose 6-phosphatase. Its activity is modulated by a number of factors including magnesium concentration which changes with exposure to light (Anderson et al. 1979). The cytosolic enzyme catalyses the conversion of triose phosphates to sucrose (Daie 1993).

#### ***TaPSL***

The *TaPSL* gene encodes a protein with unknown function highly associated with stem watersoluble carbohydrate, enhanced grain weight and yield.

#### ***HvEHI***

66. The *HvEHI* gene encodes an EH-domain protein, a class of proteins found in plants, mammals and other organisms (Naslavsky & Caplan 2005). In plants EH-domain containing proteins usually consist of an EH-domain, at the N-terminus of the protein, a central coiled-coil region and a C-terminal nucleotide binding domain (Bar et al. 2008). These proteins can perform a wide range of functions including transcriptional regulation of the endocytosis pathway. In *Arabidopsis*, two EH-domain containing proteins, AtEHD1 and AtEHD2, have been shown to be involved in endocytosis in this plant species (Bar et al. 2008).

67. Another study with *Arabidopsis* has shown that EDH2 may regulate auxin signalling possibly through clathrin dependent regulation of the PIN receptor endocytosis (Dhonukshe et al. 2007).

#### ***TaIP***

68. The *TaIP* gene encodes an enzyme, which is involved in the isopentenyl synthesis pathway, a component of the isoprene synthetic pathway. Compounds derived from this

pathway include photosynthetic pigments, electron carriers, secondary metabolites and hormones (McGarvey & Croteau 1995).

69. Studies by Sharkey and Singsaas (1995) have shown that isoprene in leaves could improve resistance to heat in relation to photosynthesis. More recent studies indicate that isoprene protects leaves against high temperatures (Loreto & Velikova 2001). It has been suggested that increased isoprene can stabilise lipid membranes thereby providing protection against heat damage (Siwko et al. 2007). As such, high levels of isoprene have been shown to provide thermotolerance, and emission of isoprene by plants appears to be regulated by temperature at different levels, see reviews by (Sharkey 2005; Sharkey et al. 2008). In *Arabidopsis*, high levels of isoprene applied ectopically appeared to accelerate the flowering process (Terry 1995).

### ***TaCaT1***

70. The *TaCaT1* gene encodes a Calcium binding protein. Calcium levels can be transiently increased by several biological signals including responses to abiotic and biotic stresses.

71. In plants, the Calcium binding proteins have several broad structural classes; the calcium binding proteins with an EF-hand motif; calcium sensors such as the calcium binding proteins without an EF-hand motif; calcium regulated protein kinases; and the calmodulin class of proteins as described in Tuteja and Mahajan (Tuteja & Mahajan 2007).

### ***TaAuTPI***

The *TaAuTPI* gene encodes an auxin carrier. Auxin regulates plant developmental and growth processes. It has been implicated in adaptive responses to abiotic and biotic stresses; the adaptation to stresses often includes growth retardation and reduced metabolism, see for example (Park et al. 2007). Regulation of the auxin pool is one mechanism that facilitates the maintenance of the auxin mediated processes. Conjugation of auxin to other compounds such as amino acids and sugars or the distribution through transportation of auxin to other plant organs have been identified as mechanisms that achieve auxin homeostasis (Park et al. 2007).

## **5.2.3 Toxicity/allergenicity of the proteins encoded by the introduced genes**

72. The genes conferring enhanced survival in heat and drought prone environments were obtained from wheat or barley, which are plants widely consumed by people and animals. On this basis humans and other organisms have a long history of exposure to these genes and their expressed proteins.

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

74. No studies on the toxicity or allergenicity of the GM wheat lines and their products have been undertaken to date as the proposed trial is at an early stage. Such studies would have to be conducted if approval was sought for the GMOs or their products to be considered for human consumption in Australia.

## **5.2.4 The plant antibiotic resistance marker gene (*nptII*) and the encoded protein**

75. The *nptII* gene is used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004). As discussed in previous DIR RARMPs, and in more detail in the RARMPs for DIR 070/2006 and DIR 074/2007 (available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir070-2006>> and <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir074-2007>> or by contacting the OGTR), regulatory agencies in Australia and in other countries have assessed the use of the *nptII* gene in GM plants as not posing a risk to human or animal health or to the environment. The most recent detailed international evaluation of *nptII* in terms of human

safety was by the European Food Safety Authority, which concluded that the use of the *nptII* gene as a selectable marker in GM plants (and derived food or feed) does not pose a risk to human or animal health or to the environment (EFSA 2009).

### **5.2.5 The herbicide resistance marker gene (*bar*) and the encoded protein**

76. Most of the GM wheat lines proposed for release also contain the *bar* herbicide tolerance marker gene. The *bar* gene was isolated from *Streptomyces hygroscopicus*, a common saprophytic, soil-borne microorganism (Thompson et al. 1987). The *bar* gene encodes the PAT protein, which confers tolerance to glufosinate ammonium, the active component in a number of herbicides. The *bar* gene has been discussed most recently in DIR 091 and in more detail in previous RARMPs for DIR 062/2005 and DIR 071/2006.

77. PAT proteins are widespread in the environment; they are present in naturally occurring bacteria as well as in other GM crops approved for commercial release. Extensive toxicity studies using the purified form of the PAT protein have shown that the PAT protein is not likely to be toxic or allergenic to humans. Detailed descriptions of the results of these studies are available in the RARMPs for DIR 021/2002 and DIR 062/2005.

78. Other regulatory agencies, both in Australia and in other countries, have previously assessed the *bar* gene, or related *pat* gene encoding the same PAT enzyme, as safe for use in human food. Food Standards Australia New Zealand (FSANZ) has approved the use of food derived from other GM plants containing either the *bar* or *pat* gene, including GM cotton, corn, canola, rice and soybean, concluding that the PAT protein is not toxic (for example ANZFA 2001a; ANZFA 2001b; ANZFA 2001c; FSANZ 2003; FSANZ 2005; FSANZ 2008). The studies submitted in support of the food uses for this protein indicate that it has none of the properties associated with protein toxins or allergens.

79. A number of GM crops, including food crops, containing the *bar* or *pat* gene encoding the PAT protein, have been approved for commercial release both in Australia (DIR 021/2003, DIR 062/2005 and DIR 091) and overseas. No adverse effects on humans, animals or the environment have been reported from any releases.

## **5.3 The regulatory sequences**

### **5.3.1 Regulatory sequences for expression of the introduced genes**

80. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. The promoters used in the GM wheat lines are listed in Table 1, Section 5.1. The introduced genes for enhanced survival and grain quality production in heat and drought prone environments are under the control of a range of different promoters. Constitutive promoters have been derived from the following genes; dehydrin 8 (*Dhn8*) and dehydrin 13 (*Dhn13*) from barley and *Ubiquitin1* (*Ubi1*) from maize. The developmental promoter derived from the sucrose:fructan 6-fructosyltransferase 1 (*6-SFT1*) gene was obtained from barley. The drought-inducible promoters from the dehydrin 4 (*HvDhn4*) or later embryonic abundant protein promoters (*HVA1*) genes were obtained from barley. The *Dhn4*, *Dhn8* and *Dhn13*, *Dehydrin* genes from barley, have been characterised in some detail and are expressed constitutively or induced in response to stresses such as water deficit and low temperatures (Choi et al. 1999; Rodriguez et al. 2005). The *Hv6-SFT1* gene is inducible by abiotic stresses (Livingston III et al. 2009). The *HVA1* gene encodes a group 3 LEA (Late Embryogenesis Abundant) protein, which is inducible by ABA and conditions of water stress (Lal et al. 2008). The *Ubi1* promoter has been widely used in plant genetic modification (Christensen et al. 1992).

81. Also required for gene expression in plants are mRNA terminators, including a poly-adenylation signal. The mRNA terminators used in the GM wheat lines were derived from the *nopaline synthase* (*nos*) gene from *A. tumefaciens* and the *rubisco* (*rbcS*) gene from rice. The

*nos* terminator has been used in a wide variety of constructs for plant genetic modification (Reiting et al. 2007). The *nptII* selectable marker gene is under the control of the 35S promoter from CaMV. The introduced *nptII* gene is under the control of the constitutive CaMV 35S promoter. The *bar* gene is under the control of the maize *Ubi1* or the rice Actin promoter. Both the *nptII* gene and *bar* gene have the *A. tumefaciens nos* as the mRNA terminator.

82. Humans, animals and other organisms are commonly exposed maize and barley and rice as they have been consumed safely by humans and animals for centuries. Although CaMV and *A. tumefaciens* are plant pathogens, the regulatory sequences comprise only a small part of its total genome, and are not in themselves capable of causing disease.

#### **5.4 Method of genetic modification**

83. The GM wheat lines were generated using biolistics (Pellegrineschi et al. 2002), which involved coating very small gold particles with two transformation constructs. For the majority of transformations, a candidate gene and selectable markers genes, with vector sequences removed, were coated onto the gold particles. In the case of the GM wheat lines over expressing the *TaNACdu1* gene, the whole plasmid vector that also contained the *nptII* selectable marker gene was used. Coated particles were then “shot” into immature embryos from *T. aestivum* cultivars Bob White. Transformed plant tissue was selected by exposure to Bialophos or geneticin. Once the plantlet had developed roots, they were transferred to soil in a PC2 certified glasshouse. The putative GM plants were screened by PCR to confirm presence of the introduced gene(s) for enhanced survival and grain quality production in heat and drought prone environments. and expression was verified by quantitative RT-PCR.

84. Some of the GM wheat lines will contain more than one gene; these will be generated through co-transformation using biolistic transformation of a mixture of candidate genes, or generated through conventional crossing of the GM lines containing single inserted genes for enhanced survival and grain quality production in heat and drought prone environments. These GM lines will also be tested in field trial if they pass phenotypic screening at the T1 stage in PC2 certified controlled environmental facilities (CEF).

#### **5.5 Characterisation of the GMOs**

##### **5.5.1 Stability and molecular characterisation**

85. The applicant states that all genes to be introduced into wheat have been sequenced, and the constructs used to generate the GM wheat lines to date were sequenced prior to transformation. As the project is at an early stage, molecular characterisation of the GM wheat lines has not been carried out and the stability of the genetic modifications is only known for some lines. For example, the GM wheat lines containing the *TaNACdu1* gene were found to be stable for the 3 generations examined. The genomic locations of the introduced DNA has not been characterised, and the number of copies of the transgenes present in each line is unknown.

##### **5.5.2 Characterisation of the phenotype of the GM wheat**

86. The purpose of the proposed trial is to characterise the phenotypes of the GM wheat lines in the field. Drought adaptation traits under field conditions are complex, for example due to soil-root interactions, and these conditions can be difficult to replicate in controlled environment facilities. As such, trialling in the field is necessary to demonstrate that the GM wheat lines have improved productivity (grain weight, grain yield and biomass) in environments with limited water availability.

87. The GM wheat lines have not undergone any detailed phenotypic characterisation. Preliminary data show that the GM lines produce strong tillers and long spikes, factors which

can potentially lead to a higher harvest index. Candidate genes were selected because they have been shown to be associated with drought tolerance/heat stress, or correlate positively with grain weight/yield of wheat under drought/heat prone environments such as the *TaCaT1*, *TaPSL1*, *TaRWD1* and *TaFBPase4* genes. No abnormal morphological characteristics were observed so far for those GM wheat lines selected for field trial and any plants that show abnormal phenotypes under field conditions will be eliminated from the trial.

88. In experiments conducted in PC2 facilities, limited data was obtained on some of the GM wheat lines in relation to water use efficiency using a “500 ml bottle test” (500g soil+ 120 ml 0.3 g/L Aquasol); the seeds were soaked for 5 days before planting and no further water was added during growth. The above ground shoots were harvested after 31 days when almost all plants were completely wilted. The results are summarised in Table 3.

**Table 3. Water use efficiency test for GM wheat lines over-expressingTaNACdu1**

GM lines (T2)	WUE (g biomass/100ml water) ( $\pm$ SD)	Ratio GM to control	p-value
Dhn8NACdu1 L36_bar	0.577 ( $\pm$ 0.032)	1.12	<0.01
Dhn8NACdu1 L10_nptII	0.607 ( $\pm$ 0.039)	1.18	<0.01
Dhn8NACdu1 L6_nptII	0.618 ( $\pm$ 0.026)	1.20	<0.01
GM mean	0.601 ( $\pm$ 0.021)	1.17	<0.01
<b>Control lines</b>			
'Bob White' (control)	0.526 ( $\pm$ 0.039)	NA	NA
Dhn8NACdu1_bar -ve	0.513 ( $\pm$ 0.026)	NA	NA
Dhn8NACdu1_nptII -ve	0.505 ( $\pm$ 0.026)	NA	NA
Non-GM mean	0.515 ( $\pm$ 0.009)	NA	NA

89. The applicant provided some data to illustrate that expression of the introduced genes produced increased biomass under simulated stress conditions. For example, *TaNACdu1* under the control of the drought inducible promoter Dhn4s, showed higher biomass production under mild salt and drought conditions compared to the 'Bob White' control.

90. More detailed analysis of the GM wheat lines will include the analysis of biomass samples and grains through destructive and non-destructive testing in order to determine morphological (height, stem and leaf size and weight), agronomic (biomass at anthesis and maturity, tiller number and weight, stem carbohydrate content, head weight, grains per spike), grain characteristics (dimensions, grain weight, grain number) and grain yield. Samples will also be assessed and processed for stem carbohydrate content measurements.

## **Section 6 The receiving environment**

91. The receiving environment forms part of the context in which the risks associated with dealings involving the GMO are assessed. This includes the geographic regions where the release would occur and any relevant biotic/abiotic properties of these locations; the intended agronomic practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2009).

92. The proposed dealings involve planting of GM wheat at one site on the outskirts of Cleveland, Qld, within the Queensland Department of Primary Industries Redlands Research Station (RRS).

93. The RRS is bordered by roads on two sides, with residential/light commercial activities on the other sides of both roads. The remaining two sides are bordered by Council land and by residential land. Although the RRS borders onto some residential land, the proposed trial site is located in a central position within the RRS and is approximately 250 m from the nearest residential block in the suburb of Cleveland.

94. The trial will be conducted in an enclosure (50 m x 100 m) which will be surrounded by a fence with lockable gates and a station manager is resides at the experimental station.

## 6.1 Relevant abiotic factors

95. The abiotic factors relevant to the growth and distribution of commercial wheat in Australia are discussed in *The Biology of Triticum aestivum L. em Thell (Bread Wheat)* (OGTR 2008).

96. The release is proposed to take place in the Redland LGA in Qld with a typical sub-tropical climate, with high summer rainfall (as defined by the Koeppen Classification system used by the Australian Bureau of Meteorology). The rainfall and temperature statistics for the area around the proposed release site are given in Table 5.

**Table 4. Climatic data for Cleveland (Redland), Qld**

Climatic statistic	Average figure for Cleveland (Redland)
Average daily max/min temperature (winter)	21 °C /9°C
Average daily max/min temperature (spring)	25.2 °C /13.9 °C
Average daily max/min temperature (summer)	28.6 °C /19.5 °C
Average daily max/min temperature (autumn)	25.6 °C /15.6 °C
Average monthly rainfall (winter)	67.4 mm
Average monthly rainfall (spring)	75.2 mm
Average monthly rainfall (summer)	151.4 mm
Average monthly rainfall (autumn)	125.9 mm

\*data taken from the Australian Bureau of Meteorology website (<http://www.bom.gov.au/climate/averages/>). Temperature and rainfall data are an average of 56 to 110 years of records.

Summer entries are averages of monthly data from December to February, spring entries are averages of monthly data from September to November, and winter entries are averages of monthly data from June to August, autumn entries are averages of monthly data from March to May.

97. Hilliards Creek, a major source of irrigation water for the site, bisects the RSS and is buffered by a vegetated riparian zone. The proposed field location is located within the RSS on flat arable land not subject to flooding (information supplied by the applicant) at least 100 m from Hilliards Creek.

## 6.2 Relevant biotic factors

98. Wheat has been grown in several research blocks within the RRS, as part of barley trials (which includes the wheat marker rows), over the last several years. According to the cultivation patterns undertaken at RRS encourage germination, and the warm and wet environment at RRS provides ideal conditions for germination and rapidly exhausts the soil seed bank. The proposed trial will be planted in a location that has not grown wheat or barley for minimum of two years. However, sections of the 200 m zone surrounding the proposed trial site will be within 200 m of where wheat marker rows have been grown in the past two

years, but not since harvest in November 2009. In general, this is a horticultural not a cereal cultivation area.

99. The biotic factors relating to the growth and distribution of commercial wheat and barley in Australia are discussed in the reference document, *The Biology of Triticum aestivum L.em Thell* (Bread Wheat) (OGTR 2008).

100. Of relevance to this proposed release are the following points:

- The nearest commercial wheat crop will not be within 50 km of the trial.
- Invertebrates, vertebrates and microorganisms could be exposed to the introduced genes and their associated effects. In particular, native birds and rodents (either introduced or native).

### 6.3 Relevant agricultural practices

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

102. As stated in Section 3.2, the GM wheat lines will be evaluated in replicated plots (2-4 replicates), in mini (1.6 m<sup>2</sup>) and full (9.6 m<sup>2</sup>) sized plots during the release.

The GM wheat lines will be grown from seed and sown either by hand or using a mechanical hand planter. Single seed descent will have been followed for 2-4 generations in a PC2 facility. The seeds from selected lines will be bulked up in a PC2 facility for planting, seed from T2<sup>7</sup> GM wheat lines will only be planted in the mini plots for the first year, which will serve as an initial field screening. Promising lines will then be planted in full sized plots which consist of 8 rows which are 6 m in length and separated by 0.2 m. The applicant aims to avoid overplanting the GM wheat on areas grown to the GM wheat in previous seasons, but indicates that some areas may be need to be used in two consecutive years.

103. In Australia, spring wheat varieties are commonly grown as a winter crop and are usually planted in May and June. Harvest of the mature wheat generally occurs from mid-November to late December. Wheat is exotic to Australia and the parental wheat cultivar Bob White is not grown commercially in Australia.

104. The applicant proposes to harvest the wheat using a small mechanical single row harvester or plot harvester. Conventional cultivation practices for wheat is outlined in more detail in *The Biology of Triticum aestivum L. em Thell* (Bread Wheat) (OGTR 2008).

105. Non-propagative plant material remaining at the field location after harvest (for example, residual stem stubble) would be ploughed into the ground after the trial. The harvested areas would then be watered twice at 6 weekly intervals to encourage germination of any fallen seed. Any volunteers will be destroyed with herbicide before they flower. The applicant proposes to plant a break crop such as lucerne or forage brassica on areas within the site not planted with the GM wheat. Any wheat plants that grow in the area sown with a break crop will be physically removed or sprayed with a non-selective herbicide to ensure that they do not set seed. Excess seed not required for experimental analysis or future trials would be removed from the site and destroyed.

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<sup>7</sup> The generation of a GM plant is identified by the letter T (transgenic) followed by a numeral indicating the number of generations for which it has been maintained, beginning at T0 for the original transformed plant.

106. There are a number of pests and diseases of wheat (OGTR 2008), which may require management (for example application of pesticides such as herbicides or insecticides) during the growing season. Weed control using specific classes of herbicides may involve a pre- or post-emergence application.

#### 6.4 Presence of related plants in the receiving environment

107. The applicant proposes to maintain a 500 m zone around the site of the trial in which there is no cultivation of wheat breeding lines for the duration of the trial. A 200 m isolation zone for all other wheat cultivation will also be maintained. Barley (*Hordeum vulgare*) trials may take place at the research station, which will include wheat as markers (about 20 wheat plants in marker plots). These barley trials will be separated by more than 200 m from the proposed GM wheat trial.

108. Wheat is sexually compatible with many species within the genus *Triticum*. Apart from commercially cultivated bread and durum wheat, other *Triticum* species are not known to be present in Australia. Other species belonging to the genera *Elytrigia*, *Elymus*, *Hordeum* and *Secale*, members of which have some capacity to form hybrids with wheat, are known to occur in Australia. The applicant has indicated that they are not aware of the presence of sexually compatible species at the site of the proposed release. The related species *H. vulgare* occurs at the RRS. *Elymus scaber* has not been observed at the RRS, although its presence can not be discounted with certainty.

109. Hybridisation of wheat with *Hordeum vulgare* has not been reported. Wheat can hybridise with *Hordeum marinum* but only with substantial human intervention (Perschina et al. 1998; Islam & Colmer 2008) and the resultant hybrids are usually infertile (Islam et al. 2007). Of the species that may perhaps hybridise with bread wheat under natural conditions, few are known to be present in Australia. *Aegilops* spp are recognised as quarantine weeds in Australia and are not known to be present naturally. The interspecific crossing potential of wheat is discussed in more detail in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008).

#### 6.5 Presence of the introduced genes or similar genes and encoded proteins in the environment

110. The introduced genes for enhanced survival and grain quality production in heat and drought prone environments were originally isolated from wheat and barley, which are already widespread and prevalent in the environment in Australia and elsewhere. Therefore, it is expected humans and animals routinely encounter the introduced genes and their products.

111. Several of the introduced genes are derived from common bacteria. The marker gene *nptII* was derived from *E. coli*, which is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997), and as such it is expected to be routinely encountered by humans. The PAT protein is widespread in the environment, through the presence of the bacteria from which it is derived. PAT proteins are produced naturally by the common soil bacteria *Streptomyces viridochromogenes* and *S. hygroscopicus*, encoded by the *pat* and *bar* genes, respectively (Wohlleben et al. 1988; Strauch et al. 1988). These species of *Streptomyces* are saprophytic, soil-borne bacteria and are not considered pathogens of plants, humans or other animals (OECD 1999a). Genes encoding PAT or similar enzymes are present in a wide variety of bacteria. Acetyltransferases, the class of enzymes to which PAT belongs, are common enzymes in all microorganisms, plants and animals. Different versions of PAT protein have also been expressed in other GM crop plants trialled in Australia (DIRs 010/2001, 015/2002, 016/2002, 036/2003, 038/2003, 040/2003 and 044/2003) or commercially approved (canola DIR 021/2003, cotton DIR 062/2005 and cotton DIR 091).

112. Short regulatory sequences are derived from plants (including maize, barley and rice), a soil bacterium (*A. tumefaciens*) and a plant virus (Cauliflower mosaic virus; CaMV). Although some of these sequences are derived from plant pathogens (*A. tumefaciens* and CaMV), the regulatory sequences comprise a small part of the pathogen's total genome, and are not in themselves capable of causing disease.

## **Section 7 Australian and international approvals**

### **7.1 Australian approvals of GM wheat**

#### **7.1.1 Previous releases approved by Genetic Manipulation Advisory Committee or the Regulator**

113. There has been no release of these GM wheat lines in Australia. Dealings with the lines proposed for release have been undertaken in PC2 facilities under Notifiable Low Risk Dealings; NLRD 907/2003.

114. The Regulator has issued seven licences for the limited and controlled release of other GM wheat lines:

- DIR 053/2004 was issued to Grain Biotech for GM salt tolerant wheat on an area of 0.45 ha in Western Australia
- DIR 054/2004 was issued to CSIRO for GM wheat with altered starch content on 0.412 ha in the Australian Capital Territory
- DIR 071/2006 was issued to the Victorian Department of Primary Industries, for GM drought tolerant wheat on 0.315 ha in Victoria
- DIR 077/2007 was issued to the University of Adelaide for GM wheat and barley with enhanced tolerance to abiotic stresses or increased beta glucan on 0.04 ha in South Australia
- DIR 080/2007 was issued to the Victorian Department of Primary Industries, for GM drought tolerant wheat on 0.225 ha in Victoria;
- DIR 092 was issued to CSIRO for GM wheat with altered grain composition on 1 ha in May 2009 in the ACT.
- DIR 093 was issued to CSIRO for GM wheat and barley with altered grain starch composition on 1 ha in June 2009 in the ACT.
- DIR 094 was issued to CSIRO for GM wheat and barley with enhanced nutrient utilisation efficiency on 1 ha in July 2009 in the ACT.
- DIR 099 was issued to CSIRO for GM wheat and barley with enhanced nutrient utilisation efficiency and altered grain composition on 2 ha in June 2010 in New South Wales and Western Australia.

115. Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), there have been five field trials of different types of GM wheat ranging in size from 325–1500 plants: PR65 (1996), PR66 (1996), PR102 (1998), PR102X (2000), and PR107 (1999).

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

#### **7.1.2 Approvals by other Australian government agencies**

117. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. Other government regulatory requirements may also have to be met in respect of release of GMOs, including those of the

Australian Quarantine and Inspection Service (AQIS), Food Standards Australia New Zealand (FSANZ), and the Australian Pesticides and Veterinary Medicines Authority (APVMA). This is discussed further in Chapter 3.

118. APVMA has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia. While the GM wheat has been modified to be tolerant to glufosinate ammonium containing herbicides, the applicant does not intend to apply these herbicides during the trial.

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

## **7.2 International approvals of GM wheat**

120. There have been no releases of these GM wheat lines internationally. However, there have been releases of other GM wheat plants in field trials. The traits which have been modified include novel protein production, disease resistance, altered grain properties and herbicide tolerance<sup>8</sup>.

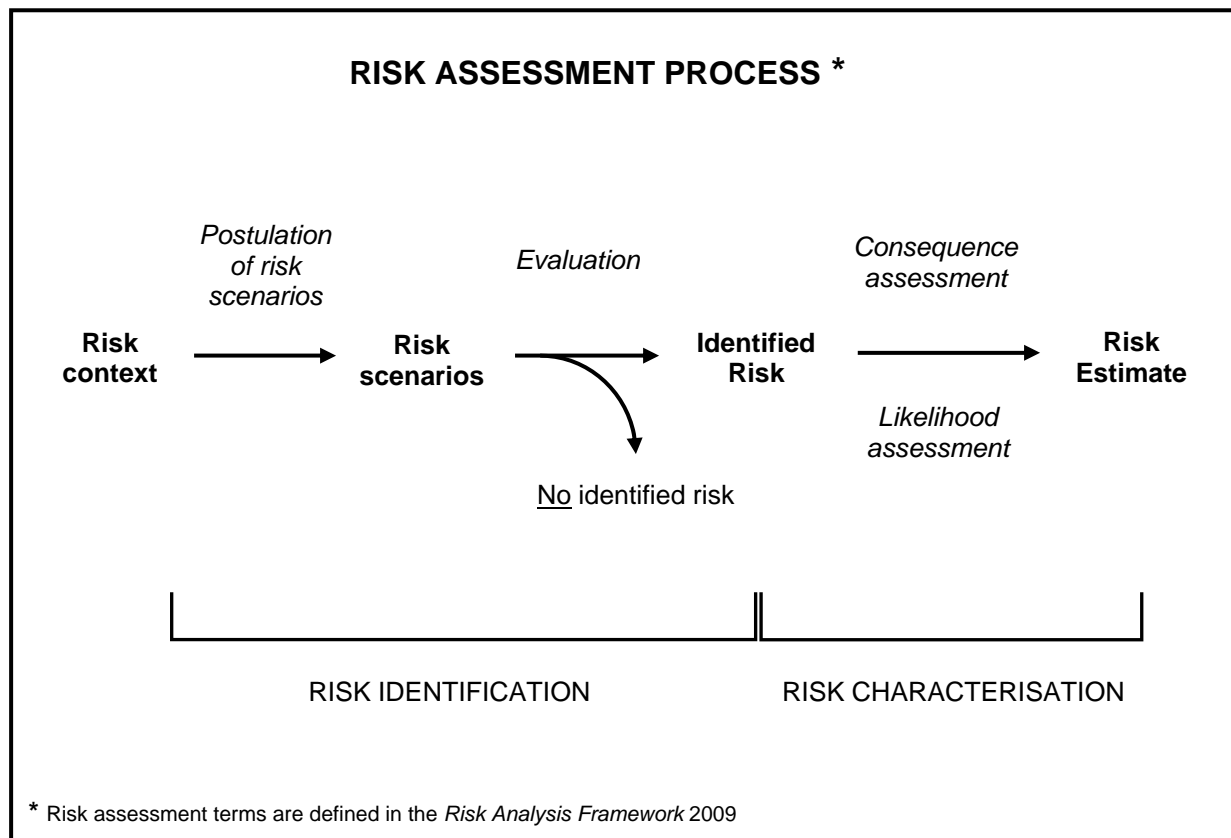
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<sup>8</sup> <<http://www.aphis.usda.gov/brs/status/relday.html>>, <<http://gmoinfo.jrc.ec.europa.eu/>> accessed 10 February 2010.

## Chapter 2 Risk assessment

### Section 1 Introduction

121. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 4). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.



**Figure 3. The risk assessment process**

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

123. Each risk scenario is evaluated to identify those risks that warrant detailed characterisation. A risk is only identified when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

124. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2009). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

125. Identified risks are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments.

## **Section 2 Risk identification**

126. The following factors are taken into account when postulating relevant risk scenarios:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
- the proposed limits
- the proposed controls
- characteristics of the parent organism(s)
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- agronomic management practices for the GMOs.

127. Eight risk scenarios were identified and evaluated. These are summarised in Table 5, where circumstances that share a number of common features are grouped together in broader risk categories. None of the risk scenarios were considered to lead to an identified risk that required further assessment. More detail of the evaluation of these scenarios is provided later in this Section.

128. As discussed in Chapter 1, Section 5, some of the GM wheat plants would contain the antibiotic resistance selectable marker genes *nptII*. The *nptII* genes and their products have already been considered in detail in previous RARMPs and by other regulators (for example (EFSA 2007)). Since neither of these genes has been found to pose risks to either people or the environment, their potential effects will not be further assessed for this application.

**Table 5. Summary of risk scenarios from dealings with the GM wheat modified for enhanced survival and grain quality production in heat and drought prone environments.**

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Exposure to GM plant material containing the introduced genes, or their end products	Increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>The introduced genes and their encoded proteins occur naturally in the environment and are unlikely to be toxic or allergenic to people or toxic to other organisms</li> <li>None of the GM wheat material would be used for human food or animal feed</li> <li>The limited scale, short duration and other proposed limits and controls, minimise exposure of people and other organisms to products of the introduced genes</li> </ul>
Section 2.2 Spread and persistence of the GM wheat plants in the environment	2. Expression of the introduced genes improving the survival of the GM wheat plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>Cultivated wheat is not considered to be weedy and the genetic modifications are not expected to change the weediness characteristic of the GMOs.</li> <li>Many factors other than water availability and heat restrict the spread and persistence of wheat in the areas proposed for release, including low intrinsic competitive ability, nutrient availability, pests and diseases</li> <li>Glufosinate ammonium is not used as the main method to control wheat in other crops</li> <li>The limits and controls proposed for the release would restrict spread and persistence</li> </ul>
	3. Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including animals and extreme weather conditions	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>The proposed limits and controls would minimise dispersal, such as locating the field trial sites at least 50 m from natural waterways, measures to exclude livestock and control rodent numbers, and transporting material according to the Regulator's guidelines.</li> </ul>
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible	4. Expression of the introduced genes in other wheat plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>Wheat is predominately self-pollinating and outcrossing is limited.</li> <li>Risk scenarios 1 – 3 associated with expression of the introduced genes did not constitute identified risks for people or the environment</li> <li>The limits and controls proposed for the release would restrict gene flow</li> </ul>

Risk category plants	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
	5. Expression of the introduced genes in other sexually compatible plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>• There is limited sexual compatibility with relatives of wheat</li> <li>• Risk scenario 1- 4, associated with expression of the introduced genes did not constitute identified risks for people or the environment</li> <li>• The limits and controls proposed for the release, including isolation from other wheat crops, would restrict gene flow</li> </ul>
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	6. Expression of the introduced genes in other organisms as a result of gene transfer	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>• The introduced genes and regulatory sequences or components thereof are already present in the environment and are available for transfer via demonstrated natural mechanisms</li> <li>• Risk scenarios 1 – 5 associated with expression of the introduced genes did not constitute identified risks for people or the environment</li> </ul>
Section 2.5 Unintended changes in biochemistry, physiology or ecology	7. Changes to biochemistry, physiology or ecology of the GM wheat plants resulting from expression, or random insertion, of the introduced genes or regulatory sequences	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>• Gross morphological changes are likely to have been detected and eliminated during the production and laboratory screening of the GM wheat lines.</li> <li>• Unintended adverse effects, if any, would be minimised by the proposed limits and controls.</li> </ul>
Section 2.6 Unauthorised activities	8. Use of the GMOs outside the proposed licence conditions	Potential adverse outcomes mentioned in Sections 2.1 to 2.6	No	<ul style="list-style-type: none"> <li>• The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator</li> </ul>

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

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

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

131. A range of organisms may be exposed directly or indirectly to proteins encoded by the introduced genes and their end products. Workers cultivating the GM wheat would be exposed to all plant parts. Organisms may be exposed directly to the end products of the introduced genes through biotic interactions with GM wheat plants (vertebrates, invertebrates,

symbiotic microorganisms and/or pathogenic fungi) or through contact with root exudates or dead plant material (soil biota). Indirect exposure would include organisms that feed on organisms that feed on GM wheat plant parts or degrade them (vertebrates, insects, fungi and/or bacteria).

**Risk scenario 1. Exposure to GM plant material containing the introduced genes, encoded proteins or their end products**

132. Expression of the introduced genes for enhanced survival in heat and drought prone environments, and the herbicide tolerance marker gene, could potentially result in the production of novel toxic or allergenic compounds in the GM wheat lines, or alter the expression of endogenous wheat proteins. If humans or other organisms were exposed to the resulting compounds through ingestion, contact or inhalation of the GM plant materials, this may give rise to detrimental biochemical or physiological effects on the health of these humans or other organisms.

133. Non-GM wheat flour can produce allergic responses in susceptible individuals on inhalation or ingestion. Individuals suffering from coeliac disease can have a sensitivity response upon ingestion of wheat flour, caused by the prolamin fraction of the storage protein complex, gluten (OGTR 2008). There are no known major toxic properties of non-GM wheat. These properties are not expected to be altered in the GM wheat lines proposed for release (OGTR 2008).

134. People and animals are exposed to most of the proteins and their end products, produced by these genes through their diet and the environment. No information was found to suggest that any of these proteins or products are toxic or allergenic to people or other organisms (Chapter 1, Section 5.2.3). Assessment of the potential toxicity or allergenicity of the *bar* gene and its encoded protein is discussed in detail in the RARMPs for DIR 021/2003, DIR 062/2005 and DIR 091.

135. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of exposure of people and other organisms to GM plant materials. Human contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff. The trial site is located on a research station and therefore access to the general public would be minimised. There is little potential for exposure of the public to GM plant material via ingestion, skin contact or inhalation as the trial is of limited scale and of short duration and no GM plant material would be used for human food. Livestock would not be intentionally exposed as the GM plant material would not be used as animal feed.

136. After harvest, the applicant proposes to destroy GM wheat materials produced, apart from retaining some plant materials for research purposes and future releases. These measures would minimise exposure to the GM plant material.

137. **Conclusion:** The potential for allergic reactions in people, or toxicity in people and other organisms as a result of exposure to GM plant materials containing proteins encoded by the introduced genes or their endproducts is **not an identified risk** and will not be assessed further.

## **2.2 Spread and persistence of the GM wheat plants in the environment**

138. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM wheat plants in particular, is given in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008). In summary, wheat shares some characteristics with known weeds, such as wind-pollination (although it is predominantly self-pollinating) and the ability to germinate or to produce some seed in a range of environmental

conditions. However, wheat lacks most characteristics that are common to many weeds, 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 volume seed dispersal and long-distance seed dispersal (Keeler 1989). In addition, wheat has been bred to avoid seed shattering and white wheats have little seed dormancy (OGTR 2008).

139. Scenarios that could lead to increased spread and persistence of the GM wheat plants include expression of the introduced genes conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials. These risk scenarios could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the encoded proteins and their endproducts.

***Risk scenario 2. Expression of the introduced genes improving the survival of the GM wheat plants***

140. If the GM wheat lines were to establish or persist in the environment they could increase the exposure of humans and other organisms to the GM plant material. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with GM plant materials has been considered in Risk scenario 1 and was not considered an identified risk.

141. Survival of wheat is limited by a number of factors including temperature, competitive ability, nutrient availability, pests and diseases (Slee 2003; Condon 2004).

142. If the expression of the introduced genes were to provide the GM wheat with a significant selective advantage over non-GM wheat plants and they were able to establish and persist in favourable non-agricultural environments this may give rise to undesirable changes in species composition in these environments.

143. Several commercial cultivars with tolerance to drought are already available in Australia. For example, the variety Gladius, released by Australian Grain Technologies in February 2007, produces yields 20-30% higher under drought conditions than the benchmark variety Yitpi (Wheeler 2007). The GM wheat lines in the proposed release were derived from the wheat cultivar 'Bob White 26' which is poorly adapted to the Australian cropping environment and consequently is not used in commercial plantings. The GM wheat lines are therefore unlikely to be more competitive than existing elite varieties, even if an increase in drought and/or heat tolerance is achieved.

144. The GM wheat lines express genes that encode proteins that are expected to enhance drought tolerance and/or heat tolerance. Plants respond to different abiotic stresses often through an interconnecting series of signalling and transcription controls. Therefore, the regulatory nature of the introduced genes may mean that the encoded proteins could also confer tolerances to other environmental stresses. During the trial the applicant proposes to cross some of the GM plants to produce offspring containing more than one of the introduced genes. This may create a plant with more than one gene for enhanced drought tolerance, or result in a plant that has enhanced stress tolerance. This could lead to the spread and persistence of the GM wheat lines if these environmental stresses were the main limiting factors.

145. There are examples of genes isolated from other organisms, belonging to the same gene families as the introduced genes, which confer tolerances to abiotic stresses other than drought and/or heat stress to GM plants. Over-expression of several of the genes in other plant species has shown that this can result in improved tolerances to other abiotic stresses such as salt and cold (see Chapter 1 Section 5.2.1. and 5.2.2). Over-expressed forms of some of the

introduced genes have been shown to either increase or decrease the resistance or sensitivity of the GM plants to pathogen infection (Chapter 1 Section 5.2.1. and 5.2.2).

146. Additionally, plants expressing the genes for heat and/or drought tolerance could have increased seed dormancy, viability, and/or improved seedling germination rates under stresses (other than drought and heat). For example, some of the GM wheat lines contain the Myb transcription factor. The *Red grain colour* gene (*R*) from wheat has been shown to be a Myb type transcription factor and is associated with the production of grains with a red colour as a result of the production of flavonoid compounds. The red colour of grain has been shown to be associated with grain dormancy. As such these lines could display some enhanced seed dormancy.

147. The applicant states that preliminary data on some of the GM wheat lines indicate that, if rainfall is low and sporadic during the growing season, some of the transgenic plants may survive better than the controls and may thus have a competitive advantage over non-GM wheat.

148. Some of the categories of GM wheat plants contain the *bar* gene for herbicide tolerance (Chapter 1, Section 5.2). These GM wheat lines could have a selective advantage in an environment where the application of these herbicides was standard practice. Such a scenario could occur within the cultivation setting at the Redlands Research Station, where the GM wheat could spread and persist if the herbicide to which the GM wheat is tolerant were used exclusively as part of the agricultural practices to manage the cultivation of wheat, such as when removing unwanted volunteers. In the highly unlikely event that the GM wheat dispersed into an area where the herbicides to which the GM wheat is tolerant were applied, it may have an advantage over non-GM wheat. However, alternative herbicides are available and the applicant has indicated that they intend to use glyphosate based herbicides to control any GM wheat and prevent spread and persistence. Additionally, non-chemical methods of destruction are also proposed as a component of the control measures (Chapter 1, Sections 3.3 and 6.3).

149. Modern wheat cultivars, some of which are bred for high vigour, are not recognised as significant weeds in Australia, and there have been no reports of bread wheat becoming an invasive pest in Australia or overseas. Additionally, the spread and persistence of the GM wheat plants would still be limited by lack of seed shattering, low intrinsic competitive ability, nutrient availability, pests and diseases and environmental factors that normally limit the spread and persistence of wheat plants in Australia (Slee 2003; Condon 2004).

150. However, even if there were any significant advantages conferred to the GM wheat lines as a result of the genetic modification, the proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of the spread and persistence of the GM wheat lines proposed for release. The release would be of limited size and short duration and the applicant proposes a number of control measures, including destruction of all plant materials not required for further analysis, repeated post harvest irrigation of the site to encourage germination of remaining seed followed by herbicide treatments to destroy volunteers and post harvest monitoring of the release site.

151. **Conclusion:** The potential for increased weediness, allergenicity or toxicity due to expression of the introduced genes for improving the survival of the GM wheat plants is **not an identified risk** and will not be assessed further.

***Risk scenario 3. Dispersal of reproductive GM plant materials through various means, including animals and extreme weather conditions***

152. If the GM wheat lines were to be dispersed from the release site they could increase the exposure of humans and other organisms to the GM plant material and/or establish and persist in the environment. The effects of contact, inhalation or ingestion of the GM wheat lines have been assessed in Risk scenario 1 and were not an identified risk in the context of the proposed limits and controls. The potential for the introduced genes to result in improved survival of the GM wheat lines in the environment was assessed in Risk scenario 2 and was not an identified risk. Therefore the dispersal of reproductive GM plant material is not expected to adversely affect the health of humans or other animals; or to increase the weediness of the GM wheat lines compared to non-GM wheat.

153. Dispersal of reproductive GM plant materials, for example viable grain, could occur through a variety of ways including endozoochory (dispersal through ingestion by animals), the activity of animals such as rodents and herbivores or through extremes of weather such as flooding or high winds.

154. Seed dispersal for wheat through endozoochory has not been reported, however, it is possible that wheat seeds could germinate after passage through the digestive system of some mammals. For example, viable wheat seeds have been detected in cattle dung (Kaiser 1999). Seeds which survive chewing and digestion by animals are typically small and dormant (Malo & Suárez 1995). The GM wheat lines proposed for release are in white wheat parental backgrounds, which have large seeds with low dormancy and thin seed coats (Hansen 1994; DPI Vic 2005), and are therefore likely to be easily broken down in the digestive system of mammals.

155. Rabbits are known pests of wheat. Rabbits favour soft, green, lush grass (Myers & Poole 1963) and select the most succulent and nutritious plants first (Croft et al. 2002). Although viable seeds from a variety of plant species have been found in rabbit dung, viable wheat seeds were not among them (Malo & Suárez 1995).

156. Habitat modifications such as reduced plant cover have been reported to be a deterrent to the movement of mice (White et al. 1998; Central Science Laboratory 2001; AGRI-FACTS 2002; Brown et al. 2004) and therefore the proposed 10 m wide zone of reduced plant cover around the trial site is expected to discourage dispersal by mice. Rodent baits and traps will be placed around the fence surrounding the site, which will further limit any potential for seed dispersal by rodents.

157. Preliminary data provided by the applicant show that some of the GM wheat lines produce strong tillers and long spikes, factors which can potentially lead to a higher harvest index. An increase in grain number per spike may result in extra seed remaining at the site after harvest. The characteristics of the release environment such as warm temperatures and potentially good rainfall promote the germination of seeds. This together with the limits and controls proposed by the applicant, would minimise the opportunity for seed dispersal from volunteer plants. Proposed controls include, surrounding the site by a fence to prevent livestock access and repeated post harvest irrigation of the site to encourage germination of remaining seed, followed by herbicide treatments to destroy volunteers and post harvest monitoring of the site (Chapter 1, Section 3.3).

158. Wheat lacks seed dispersal characteristics such as stickiness, burrs, and hooks, which contribute to seed dispersal via animal fur (Howe & Smallwood 1982). The release site will be surrounded by a fence with a locked gate, which would reduce the possibility of livestock or unauthorised people accessing the site, and minimise the potential for seed dispersal via

this route. All GM plant material will be transported in accordance with the Regulator's transport guidelines which will minimise the opportunity for dispersal of the GM material.

159. Wheat seed could also be transferred from the GM trial site via water run-off. However, irrigation of the site or rainfall will produce minimal water run-off as the site is reasonably flat (information supplied by the applicant).

160. Extremes of weather may cause dispersal of plant parts. The proposed field location is located within the RSS on flat arable land not subject to flooding (information supplied by the applicant) at least 100 m from the nearest creek. Furthermore, in addition to locating the proposed release away from natural waterways to prevent dispersal in the event of flooding, control measures have been proposed by the applicant to minimise dispersal and persistence. These include having a buffer zone and monitoring zone, and surrounding the location by a 190 m isolation zone free of wheat or sexually compatible species.

161. **Conclusion:** The potential for allergenicity, toxicity or increased weediness due to the dispersal of reproductive (sexual or asexual) GM plant materials through various means including animals and extreme weather conditions is **not an identified risk** and will not be assessed further.

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

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

163. Baseline information on vertical gene transfer associated with non-GM wheat plants can be found in *The Biology of Triticum aestivum L. em Thell. (Wheat)* (OGTR 2005). In summary, wheat plants are primarily self-pollinating and while natural hybrids with other species can occur at low frequencies, they are usually sterile.

#### **Risk scenario 4. Expression of the introduced genes in other wheat plants**

164. Transfer and expression of the introduced genes to other wheat plants could increase the weediness potential, or alter the potential allergenicity and/or toxicity of the resulting plants.

165. As discussed in Risk scenario 1, allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM wheat plants by the introduced genes. This will be the same if the introduced genes are expressed in other wheat plants.

166. As discussed in Risk scenario 2, the survival of the GM wheat plants proposed for release would be limited by a diverse range of environmental factors that normally limit the spread and persistence of wheat plants in Australia. The combination of traits as a result of cross pollination between GM wheat plants is likely to contribute only incrementally to the potential weediness of the GM wheat plants. The conditions that limit the spread and persistence of non-GM wheat would be expected to limit the spread and persistence of any GM wheat plants with a combination of the introduced genes.

167. Wheat is predominantly self-pollinating (Hucl 1996), and outcrossing occurs primarily through wind mediated pollination. The extent to which plants shed pollen varies between cultivars and is influenced by environmental conditions (Waines & Hegde 2003; OGTR 2008), discussed in detail more recently in the RARMP for DIR 094. Under field conditions wheat pollen has a viable lifespan of less than 30 minutes (OECD 1999b). Environmental

conditions including temperature, relative humidity and wind intensity have a great influence on pollen viability and pollen movement.

168. Gene flow rates in wheat have been studied from both experimental (small scale)- and commercial-scale fields, with gene flow over long distances only detectable from commercial-scale fields (reviewed in OGTR 2008). The majority of gene flow from small scale fields occurs up to ten metres from the pollen source, and only low levels of gene flow have been detected as far as 300 m away (Matus-Cadiz et al. 2004). Studies under Australian field conditions (south Australia and the ACT), indicate that gene flow occurs at extremely low frequencies and over very short distances, less than 12 m; 0.012% and 0.0037% in the ACT and South Australia respectively (Gatford et al. 2006). From commercial scale fields, low levels of gene flow have been reported up to 300 m for wheat, with rare incidences occurring at greater distances (2.75 km); gene flow levels are highly variable and can depend greatly on prevailing winds (Matus-Cadiz et al. 2004; Matus-Cadiz et al. 2007). The likelihood of gene transfer declines rapidly as the distance from the pollen source increases.

169. Wheat has been grown at RRS in several research blocks as part of barley trials (20 wheat plants per plot), over the last several years. The applicant states that there is no residual seed bank as cultivation patterns to encourage germination and the warm and wet environment provide ideal conditions for germination and rapidly exhaust soil seed bank. The proposed trial will be planted in a location that has not grown wheat or barley for minimum of two years. However, the area surrounding the trial may be within 200 m of where wheat and barley have been grown in the past two years.

170. Cross-pollination between the different GM wheat lines proposed for release must also be considered in relation to unintentional stacking of the GM traits, possibly contributing to weediness of the resultant GM wheat lines. As discussed in Risk scenario 2, the GM wheat lines proposed for release may show greater survival or recovery under abiotic stress conditions such as cold, drought or saline soils. The combination of these traits with those in the other GM wheat lines is likely to contribute only incrementally to the potential weediness of the GM plants, the spread and persistence of which would still be restricted by factors such as lack of seed shattering, low intrinsic competitive ability, a range of pests and diseases and other environmental factors that normally restrict the spread and persistence of wheat plants in Australia. The persistence of such plants would also be controlled by measures proposed by the applicant to restrict the persistence of the GM lines at the release site (see Risk scenario Risk scenario 2 and Chapter 1 Sections 3.2 and 3.3).

171. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the potential for pollen flow and gene transfer to non-GM wheat plants. In particular, the applicant has proposed to isolate the trial site from other wheat plantings and the majority of the pollen is expected to fall within the trial site or the 10 m herbicide-treated area directly surrounding the trial site. The applicant also proposes to perform post harvest monitoring of the site for twelve months or until the site has been clear of volunteers for one growing season and to destroy any volunteer plants found at the site. These latter measures would ensure any remaining GM wheat seeds, or plants that were potentially the product of gene flow, in these areas would be destroyed.

172. **Conclusion:** The potential for allergenicity in people, or toxicity in people and other organisms or increased weediness due to the expression of the introduced genes and regulatory sequences in other wheat plants as a result of gene transfer is **not an identified risk** and will not be assessed further.

**Risk scenario 5. Expression of the introduced genes in other sexually compatible plants**

173. Transfer and expression of the introduced genes for enhanced heat and/or drought tolerance, and the herbicide tolerance gene, in other sexually compatible plants could confer a selective advantage to these plants under conditions of environmental stress or where glufosinate ammonium is applied.

174. As discussed in Risk scenario 2, the survival of the GM wheat plants proposed for release would be limited by a diverse range of environmental factors that normally limit the spread and persistence of wheat plants in Australia. Furthermore, the combination of traits is likely to contribute only incrementally to the potential weediness of the GM wheat plants. The conditions that restrict the spread and persistence of any hybrids between non-GM wheat and other sexually compatible plants would be expected to restrict the spread and persistence of any hybrids between the GM wheat and other sexually compatible species. Therefore, the expression of the introduced genes in other sexually compatible species is also unlikely to give these plants a significant selective advantage.

175. As discussed in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008), there are few species outside the *Triticum* genus that are sexually compatible with wheat and known to form hybrids under natural conditions. Examples include: *Aegilops cylindrica*, *Ae. ovata*, *Ae. biuncialis* and possibly *Secale cereale*. The hybrids obtained are generally male sterile and often have reduced female fertility. Hybridisation between wheat and other species in the *Elymus* and *Hordeum* genera have been recorded, and typically result in sterile hybrids. Artificial hybrids between wheat and *Secale cereale* have been reported, but no natural hybrids between these species have been observed in Europe or the USA (Eastham & Sweet 2002). However, some non-peer reviewed reports exist of naturally formed hybrids from Canada (Hegde & Waines 2004). Hybrids obtained between wheat and *S. cereale* are completely male sterile but female fertile (Hegde & Waines 2004). Similarly artificial crosses between rye and wheat give rise to triticale, there are no reports of hybridisation between bread wheat and triticale occurring in nature (Eastham & Sweet 2002). Furthermore, any hybridisation would require synchronicity of flowering between the GM wheat lines and compatible species to enable cross-pollination and gene flow to occur.

176. Information in addition to that discussed in the *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008) has been identified in relation to the possibility of hybrids forming between *Triticum* and *Aegilops*. In Europe, hybrids between *Triticum* and *Aegilops* species have been reported. However these were obtained from seed resulting from cross hybridising plants at close proximity from established mixed populations, hand crosses and crosses conducted under controlled conditions. The fertility of these hybrids varied greatly depending on the *Aegilops* species and wheat cultivars used in the experiments. Although some fertile hybrids were obtained, most showed compromised fertility and were generally male sterile (Schoenenberger et al. 2005; Schoenenberger et al. 2006; Loureiro et al. 2006; Loureiro et al. 2009).

177. Of the species that might hybridise with bread wheat under natural conditions, few are known to be present in Australia. Apart from commercially cultivated bread and durum wheat, other *Triticum* species are not known to be present in Australia. Durum wheat (*Triticum turgidum* subsp. Durum) can cross with wheat, although there are no reports of gene flow beyond 40 m (Matus-Cadiz et al. 2004). Other species belonging to the genera *Elytrigia*, *Elymus*, *Hordeum* and *Secale* are known to occur in Australia. *Aegilops* spp are recognised as a quarantine weed species but are not known to be present naturally (see Chapter 1, Section 6.4).

178. The applicant has indicated that they are not aware of the presence of sexually compatible species at the site of the proposed release. The general area in the Redland LGA is a horticultural, not a cereal cultivation area. Research on barley is carried out at the research station; however wheat and barley are not sexually compatible. Wheat will be grown as marker plots within barley trials conducted at RRS. These will be isolated from the GM wheat trial by at least 200 m. *Elymus scaber* has not been observed, although its presence can not be discounted with certainty.

179. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the potential for pollen flow and gene transfer to sexually compatible plants. In particular, the applicant proposes to isolate the trial site from other sexually compatible crop plants, and the majority of the pollen is expected to fall within the trial site or the 10 m herbicide-treated area directly surrounding the trial site. The applicant proposes to manually remove any sexually compatible plants that are found within 100 m from the GM wheat.

180. **Conclusion:** The potential for allergenicity in people, or toxicity in people and other organisms or increased weediness due to the expression of the introduced genes in other sexually compatible plant species as a result of gene transfer is **not an identified risk** and will not be assessed further.

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

181. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). Data is accumulating to show that HGT occurs more frequently than first assumed and can occur between plants, as well as between plants and less complex organisms (Bock 2010). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but could be part of a scenario potentially leading to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or by altering the expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

182. Risks that might arise from horizontal gene transfer have been considered in previous RARMPs (for example DIR 057/2004 and DIR 085/2008), which are available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office. From the current scientific evidence, HGT from GM plants to other organisms presents negligible risks to human health and safety or the environment due to the rarity of such risk scenarios, relative to those HGT events that occur in nature, and the limited chance of providing a selective advantage to the recipient organism.

183. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 6.5. All of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

### ***Risk scenario 6. Expression of the introduced genetic material in other organisms as a result of horizontal gene transfer***

184. Possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of potential recipient organisms and the nature of the introduced genetic material. Risks that might arise through HGT from a GMO to another organism have been recently reviewed (Keese 2008) and considered in detail in a previous RARMP (DIR 085/2008) which is available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office.

185. HGT could result in the presence of the introduced genes for herbicide tolerance in bacteria, plants, animals or other eucaryotes. However, the introduced sequences were isolated from plants and bacteria, which are already widespread in the environment (See Chapter 1, Section 6.5), and are thus already available for transfer from those sources via demonstrated natural mechanisms.

186. A key consideration in the risk assessment process should be the safety of the protein product resulting from the expression of the introduced genes rather than horizontal gene transfer *per se* (Thomson 2000). If the introduced genes or their end products are not associated with any risk then even in the unlikely event of HGT occurring, they should not pose any risk to humans, animals or the environment. Conclusions reached for Risk scenarios 1 - 4 associated with the expression of the introduced sequences did not represent an identified risk. Therefore, any rare occurrence of HGT of introduced genetic material to other organisms is expected to be unlikely to persist and/or result in an adverse effect.

187. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 6.5. The introduced genetic elements are derived from organisms that are already present in the wider Australian environment.

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

## 2.5 Unintended changes in biochemistry, physiology or ecology

189. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy<sup>9</sup> (Haslberger 2003). Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such unintended effects may include:

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

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

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<sup>9</sup> Pleiotropy is the effect of one particular gene on other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

***Risk scenario 7. Changes to biochemistry, physiology or ecology of the GM wheat plants resulting from expression or random insertion of the introduced genes***

191. For the majority of the GM wheat lines, no phenotypic differences between the GM wheat plants and the non-GM parental lines have been observed under glasshouse conditions, although the applicant indicates that the GM lines have undergone very limited phenotypic characterisation beyond examination of grain properties. The applicant indicates that any plants that show abnormal phenotypes under field conditions will be eliminated from the trial.

192. The GM wheat lines contain one or more introduced genes for improved water use efficiency and tolerance to heat stress. Increased heat and/or reduced water availability would induce stress on the plants and plants respond to stress often through interconnected signals and transcriptional controls (see Chapter 1, Section 5.2 for further details). Therefore, there is potential for a number of interrelated biochemical pathways to be affected by the introduced genes. The possible effects of the introduced genes were considered in the light of information obtained from published literature of the gene (if available) or closely related genes and/or gene families as a whole. Therefore, a much broader range of anticipated effects were considered in the previous risk scenarios than would likely result from any single introduced gene. Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced genes are already discussed for Risk Scenarios 1 - 3, which were not considered to be identified risks.

193. Various biochemical pathways of the GM wheat plants could be changed by the expression of the introduced genes, resulting in the production of novel or higher levels of endogenous toxins, allergens or anti-nutritional compounds.

194. The outcome of random insertion of an introduced gene is impossible to predict. Such outcomes may include, for example, alteration to reproductive capacity, altered capacity to deal with environmental stress, production of novel substances, and changes to levels of endogenous substances. Unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003). During this limited and controlled release the applicant proposes to measure the agronomic performance of the GM wheat lines, and any substantial unexpected alterations will be detected and eliminated during the trial.

195. The likelihood of any pleiotropic effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2, and 3.3. In particular, the scale of the trial would minimise the potential for adverse effects. The trial is located within a research station to which only authorised people have access, which limits exposure of the public to the GM plant material. Humans and livestock would not be intentionally exposed as the GM plant material will not be used as food or animal feed.

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

## **2.6 Unauthorised activities**

***Risk scenario 8. Use of GMOs outside the proposed licence conditions (non-compliance)***

197. Non-compliance with the conditions of the licence could lead to spread and persistence of the GM wheat plants outside of the proposed release areas and/or increased exposure of people and other organisms to GM material. The adverse outcomes that this Risk scenario could cause are the same as those discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator 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.

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

### **Section 3 Risk estimate process and assessment of significant risk**

199. The risk assessment begins with postulation of potential pathways that might lead to harm to the health and safety of people or the environment during the proposed release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and within the context of the receiving environment.

200. Eight risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

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

202. The characterisation of the eight risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principal reasons for this include:

- limits on the size, location and duration of the release proposed by CSIRO
- suitability of controls proposed by CSIRO to restrict the spread and persistence of the GM wheat plants and their genetic material
- limited ability and opportunity for the GM wheat plants to transfer the introduced genes to commercial wheat crops or other sexually compatible species
- none of the GM plant materials or products will be used human food or animal feed
- widespread presence of the same genes or sequences in the environment and lack of known toxicity or evidence of harm from them.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM wheat plants into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment<sup>10</sup>.

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<sup>10</sup> 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 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

## Section 4 Uncertainty

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

204. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability<sup>11</sup>. For field trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, rather than necessarily to treat an identified risk.

205. For DIR 100, which involves early stage research, uncertainty is noted in particularly in relation to the characterisation of:

- Risk scenario 1, regarding potential increases in toxicity or allergenicity as a result of the introduced genes
- Risk scenario 2, associated with a potential for increased survival of the GMOs
- Risk scenario 7, associated with the potential for any unintended effects as a result of changes in biochemistry, physiology or ecology of the GM wheat plants

206. Additional data, including information to address these uncertainties, would be required to assess possible future applications for a larger scale trial, reduced containment conditions, or the commercial release of these GM wheat lines if they are selected for further development.

207. Chapter 3, Section 5 discusses information that may be required for future release.

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<sup>11</sup> A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2009) available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>> or via Free call 1800 181 030.

## Chapter 3 Risk management plan

208. 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, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through proposed licence conditions. The risk management plan informs the Regulator's decision-making process. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

### **Section 1 Background**

209. 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.

210. All licences are required to be subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions contemplate the Regulator maintaining 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.

211. It is further provided that the licence be subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

### **Section 2 Responsibilities of other Australian regulators**

212. Australia's gene technology regulatory system operates as part of an integrated legislative framework. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies<sup>12</sup>.

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

214. FSANZ is responsible for human food safety assessment, including GM food. As the trial involves early stage research, the applicant does not intend any material from the GM wheat plants to be used for human food. Accordingly, the applicant has not applied to FSANZ

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<sup>12</sup> More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <[http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/raf-3/\\$FILE/raffinal3.pdf](http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/raf-3/$FILE/raffinal3.pdf)>.

to evaluate the GM wheat plants. However, in the event of a commercial release, FSANZ approval would need to be obtained before materials from the GM wheat plants could be sold for human consumption.

APVMA has regulatory responsibility for the use of agricultural chemicals, including herbicides and insecticidal products, in Australia. The application of these herbicides is subject to regulation by the APVMA. While the GM wheat has been modified to be tolerant to glufosinate ammonium containing herbicides, the applicant does not intend to apply these herbicides during the trial.

215. No other approvals are required.

### **Section 3 Risk treatment measures for identified risks**

216. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are **negligible** risks to people and the environment from the proposed trial of GM wheat. The *Risk Analysis Framework* (OGTR 2009) which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

217. These risk scenarios were considered in the context of the scale of the proposed release (a maximum area of 0.1 ha per growing season on one site in Qld between May 2010 and December 2013), the proposed containment measures (Chapter 1, Section 3), and the receiving environment (Chapter 1, Section 6).

### **Section 4 General risk management**

218. Licence conditions are imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and limit the release to the size, location and duration requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are detailed in Chapter 4 and summarised in Section 4.1.2.

#### **4.1 Licence conditions**

##### **4.1.1 Consideration of limits and controls proposed by CSIRO**

219. Sections 3.2 and 3.3 of Chapter 1 provide details of the limits and controls proposed by CSIRO in their application. These are discussed in the eight risk scenarios characterised for the proposed release in Chapter 2. The appropriateness of these controls is considered further below.

220. The permitted dealings are confined to a maximum of 0.1 ha per growing season within the Redlands Research Station (RRS), in the Redland LGA in Qld and the duration of the proposed release has been limited to three years. The applicant does not intend to use any of the GM plant material as human food or animal feed. Only staff with appropriate training would be allowed access to the trial sites. These measures will minimise the potential exposure of humans, vertebrates and other organisms to the GMOs (Risk scenario 1) and the potential for the GM wheat lines to disperse and establish outside the proposed release site (Risk scenario 3). To further minimise the exposure of humans to the products of the GM wheat, a licence condition has been imposed which prohibits the use of the GM products in the production of therapeutic goods as defined in the *Therapeutic Goods Act 1989*.

221. The applicant has stated that the trial site will be at least 100 metres from the nearest waterway. Although a small natural waterway flows through the RRS, the site has no history of flooding, which would reduce the likelihood of plant material being washed away from the

site (Risk scenario 3). It is a standard DIR licence condition that a trial site be located at least 50 metres from a natural waterway to limit the dispersal of viable GM plant material in the event of flooding.

222. The applicant has proposed to limit gene flow from the GM wheat (Risk scenario 4) by surrounding the release site with 2 m buffer zone planted with non-GM wheat, a 10 m wide herbicide treated area, a 200 m zone in which no other wheat plants are to be grown and a 500 m zone in which no wheat breeding lines would be cultivated. The applicant proposed to inspect for and destroy any related species if detected close to and during flowering of the GM wheat.

223. Differences in pollen flow have been observed in different pollen flow studies in wheat. A number of variables, particularly pollen source size, climatic conditions and the difficulty of detecting rare events, could influence the accuracy and reproducibility of these measurements. Low rates of outcrossing occur up to 100 m from the pollen source (reviewed by OGTR 2008), and very low rates of outcrossing have been recorded up to 2.75 km from a commercial wheat field (Matus-Cadiz et al. 2007).

224. Isolation distances for GM wheat field trials and other wheat crops vary greatly among different countries. For example, Canada requires a 30 m isolation distance whereas the United States requires 20 feet (approximately 6.1 m) (USDA-APHIS 1994; Canadian Food Inspection Agency 2006). In the European Union, a number of GM wheat field trials have been approved, and isolation distances from other wheat planting range from 2 to 50 m (European Commission Directorate General for the Environment 2009).

225. In Australia, requirements for basic and certified seed production for both wheat and barley are aligned with Organisation for Economic Cooperation and Development (OECD) rules (Australian Seeds Authority Ltd. 2006). OECD rules (OECD 2008) stipulate a maximum acceptable level of off-types or other cultivars of the same species of 0.1% for basic wheat seed and 0.3% for certified wheat seed (1<sup>st</sup> generation). Seed crops of self-fertilising cereals such as wheat are required to be separated from other cereal crops by a barrier or space sufficient to prevent seed mixture during harvest. Similarly, the United States *Federal Seed Act Regulations* does not specify an isolation distance for wheat used for seed production. However, for hybrid seed production (where the phenotype may be variable and determination of contamination levels is difficult) a distance of 300 feet (approximately 100 m) is required for the US and 25–100 m for the OECD (Code of Federal Regulations 2006; OECD 2008).

226. The applicant proposed to maintain a 500 m isolation zone to separate the GM wheat lines from the cultivation of wheat breeding lines and a 200 m separation between the GM lines and other wheat cultivation. On the basis of the scientific literature on gene flow, international containment measures for GM wheat trials, and the rules for producing basic and certified seed, preventing the planting of *Triticum* species within 200 m of the GM trial is considered adequate to minimise gene flow from the GM wheat plants (including wheat breeding lines) (Risk scenario 4 and Risk scenario 5) and is therefore imposed as a licence condition. This consideration is reflected in a licence condition in recent DIR wheat licences (eg DIRs 092, 093 and 094), where an isolation zone of 200 m is imposed. This 200 m zone must also be inspected for sexually compatible species, which if found must be destroyed before flowering.

227. While the applicant proposes to maintain the 200 m separation from any other non-GM wheat plantings, they propose that inspections are carried out in this 200 m zone for the first growing season but then reduce this to a 50 m zone in the following growing seasons<sup>13</sup>. This reduction in inspection requirements is based on data indicating that gene flow for wheat is low over distances of 60 m for wheat (see discussion above). In effect the applicant's proposal is for the GMOs to be surrounded by a 10 m monitoring zone which, coupled with a 50 m of the inspection zone would give 60 m in total requiring inspection and control/removal of sexually compatible species by herbicide treatment, ploughing back into the soil, or uprooting before they flower.

228. The likelihood of non-GM wheat or any sexually compatible species occurring within the 200 m zone will depend on the occurrence of sexually compatible species as well as the environmental/climatic context, previous cropping history and management practices for the site. The cropping history is likely to have a great influence on the number of volunteer wheat within the proposed 200 m isolation zone. The general area surrounding the RRS in the Redland LGA is a horticultural, not a cereal, cultivation area. However, wheat has been grown in several research blocks within the RRS, as part of barley trials, over the last several years. According to the applicant, cultivation patterns undertaken at RRS encourage germination, and the warm and wet environment provides ideal conditions for germination and rapidly exhausts the soil seed bank. Specifically, the cultivation practices used, such as incorporating crop residue into the soil by offset disk, provides germination opportunities for the seed. Following germination, the area is again cultivated, before any volunteer plants reach tillering stage, destroying these plants and providing further germination opportunities for any remaining seed. In previous seasons at RRS, in areas where these cultivation practices have been used, no or very few volunteers have emerged in the first three months. The applicant states that after six months, the crop residue is almost completely broken down and no emergence of seedlings is observed. These cultivation practices have been used on previously cultivated areas within the 200 m isolation zone.

229. The areas where the GMOs are proposed to be planted will be on an area that has not had barley trials grown for at least two years. Given the post-harvest practices, any seed that would have remained in the soil following the harvest of the last barley trial (with wheat marker rows) is expected to have germinated in the subsequent two years. However, the proposed trial site will be within 200 m of where wheat marker rows were grown in the past two years, but not since harvest in November 2009. As a result, there is the possibility that non-GM wheat volunteers may germinate in some parts of the 200 m isolation zone in the first year of the proposed trial.

230. As discussed above, a 200 m isolation zone clear of sexually compatible species is considered adequate to minimise gene flow from the GM wheat plants to any other wheat (including breeding lines). Considering the cropping history, cultivation practices and environmental/climatic context at the site, it is possible that a small number of non-GM wheat plants could occur within the 200 m isolation zone in the first year of the trial. Therefore, a licence condition has been imposed that initially requires inspection for, and destruction of, sexually compatible species in the total area of the 200 m isolation zone. However, if no wheat has been grown in the isolation zone for two years and no wheat or sexually compatible species have been found during monitoring of the isolation zone in the first year, a condition allows CSIRO to request a reduction in the inspection requirements to the first 50 m around

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<sup>13</sup> CSIRO requested a reduction in inspection requires after the release of the consultation RARMP

the trial site. A requirement to maintain a minimum separation distance of 200 m from other wheat crops will not be affected by any reduction in inspection area.

231. The applicant has proposed to surround the trial by a 10 m monitoring zone which would be treated with herbicide to limit growth of sexually compatible species. The applicant has also indicated that in order to restrict gene flow, a 2 m wide buffer of non-GM wheat would be planted around the area planted to the GMOs. However, the use of physical barrier plants is not expected to effectively reduce pollen dispersal by wind. Therefore, the requirement of a 2 m wide buffer of non-GM wheat is not imposed as a licence condition.

232. In addition to maintaining the 200 m separation to any other non-GM wheat plantings, the applicant proposes to remove any related species, such as *E. scaber* and *Hordeum spp*, in the 100 m area surrounding the GM wheat lines.

233. As discussed in Risk scenario 5, natural hybrids between *T. aestivum* and *Secale* or *T. aestivum* and *Elytrigia (Elymus)* have not been recorded. There is no evidence to indicate that viable hybrids could be generated between wheat and *H. vulgare*, and wheat and *E. scaber*. Other species, with which *T. aestivum* could potentially form hybrids, albeit with highly compromised fertility, include a few *Aegilops* species. *Aegilops spp* are recognised as quarantine weeds in Australia and are not known to be present naturally. The applicant has indicated that no sexually compatible species have been observed at the release site. The related plant species *H. vulgare* has been identified at the RRS. The species *E. scaber* has not been observed at RRS to date, but its presence can not be discounted with certainty. In the context of the release site and on the basis of the scientific literature on interspecific and intergeneric gene flow, there will no requirement imposed to keep the isolation zone free of these species.

234. In determining post-harvest monitoring requirements, it is important to consider the potential dispersal of grain during sowing and harvesting (mechanical dispersal). This is most likely to result in dispersal of grain into the area immediately around the trial, including the buffer zone.

235. The applicant proposed to conduct the trial within an enclosure surrounded by a deer-mesh fence, the bottom 0.85 m of which also consists of rabbit mesh, around the perimeter of which they will conduct rodent baiting. These measures will prevent access by livestock and rabbits to the GM wheat and aid in reducing the size of rodent populations. As viable seed may remain on the soil surface after harvest, a licence condition is imposed requiring rodent reduction measures to continue after harvest and until all remaining seeds have been incorporated into the soil through post harvest tillage. Additionally, the 10 m herbicide-treated zone will serve as a measure to control rodent activity at the proposed release site (Risk scenario 3). Whilst there are differing reports regarding the average territory size of mice, the use of reduced vegetation has been shown to help reduce rodent numbers in agricultural settings. This will minimise the potential exposure of vertebrates to the GMOs (Risk scenario 1) and the potential dispersal of the GMOs (Risk scenario 3).

236. Although the applicant has indicated that rabbit mesh in the lower half of the fence can be included, this has not been imposed as a licence condition because seed dispersal by rabbits is not expected. Rabbits prefer soft, green, lush grass (Myers & Poole 1963), and evidence suggests that viable wheat seeds are unlikely to pass through the digestive system of rabbits (Risk scenario 3). Therefore, a rabbit-proof fence has not been imposed as a licence condition.

237. The applicant has proposed a number of measures to reduce the persistence of any GM wheat plants and seeds in the seed bank at the proposed release site after harvest of the proposed trial (Risk scenario 2). These measures include treating harvested areas to encourage

germination of seed dropped at harvest with three cycles of irrigation followed by destroying volunteer wheat plants. Plant material remaining at the site after harvest will be ploughed in after harvest. The applicant has also proposed to monitor the proposed release site for 12 months after harvest. All volunteers will be destroyed before flowering by herbicide application.

238. The loss of wheat seed at harvest is estimated to range between 0.8 and 6%, depending upon factors including the harvest machinery used, the genetic tendency of a variety to shed seed and weather conditions (reviewed by Anderson & Soper 2003). The applicant has proposed to harvest the GM wheat plants with a hand-operated plot harvester or plot harvester. Wicks et al. (2000) reported that small plot headers are less efficient than commercial harvesters, so self-sown wheat may be a greater problem under experimental conditions.

239. Viable wheat seeds persist in the soil for longer periods in dry than in moist conditions, and wheat seeds present as un-threshed ears have longer dormancy than loose seeds (Komatsuzaki & Endo 1996). The applicant has indicated that it is highly unlikely that seed banks would persist at the RRS due to the prevailing environmental condition of warm temperatures and high rainfall. Shallow tillage after harvest, combined with irrigation, will ensure germination of the grain dropped at harvest (Ogg & Parker 2000); deep tillage encourages burial-induced dormancy (reviewed by Anderson & Soper 2003). Shallow tillage concurrent with irrigation would also serve to enable degradation of the plant material remaining at the site after harvest.

240. There is high variability in volunteer wheat emergence, and various field studies report volunteer emergence up to two years following harvest (reviewed by Anderson & Soper 2003). A Canadian field study of spring wheat persistence reported low levels of volunteer germination three years after wheat seeds were dropped in test plots (Harker et al. 2005). However, the relevance of these results to Australian field conditions is questionable, because of the harsh winter conditions in the western Canadian field sites studied. It is thought that dormancy of cereals is reduced in warmer temperatures (reviewed by Pickett 1989), and so dormancy is expected to be reduced in Australian field conditions compared to western Canada. Furthermore, there is evidence that the proposed irrigation and cultivation treatments are effective in promoting germination of wheat seed, which will remove seed from the soil seed bank.

241. The applicant has proposed to water the harvested area twice at 6 weekly intervals to encourage germination of any fallen seed and a post harvest monitoring regime of 12 months. However, viable wheat seeds can persist in soil for periods of up to 24 months under Australian conditions. Therefore, the licence imposes post harvest monitoring of the release site for at least 24 months after harvest with no volunteers observed in the most recent six months, before an application that inspection conditions no longer apply can be made to the Regulator. Additionally, it is considered that three irrigations, combined with an appropriate tillage regime, and monitoring for and destruction of volunteers for at least 24 months, would effectively reduce survival and persistence of viable wheat seeds in the soil. These treatments will further promote germination by ensuring any remaining seeds are exposed to sufficient moisture and placed at an appropriate depth for germination and will also encourage the microbial decomposition of any residual seed. These measures will minimise the persistence of the GMOs in the environment (Risk scenario 2).

242. The applicant has proposed to plant break crops in the areas of the trial site not being used for growing the GM wheat. The crops proposed are lucerne (*Medicago sativa*) and forage brassica (*Brassica campestris*). The break crop would be grown for approximately three months before being ploughed back into the soil. During growth of the break crops, any

volunteer wheat plants present within the breakcrop would be treated with selective herbicides or physically removed.

243. Although, as indicated above, volunteer wheat plants should be destroyed if present, their presence may go undetected in the break crop. This possibility would be taken into account by the Regulator in considering any application for site off, if the break crop was grown in the six month period prior to the application for sign off.

244. Non-GM wheat will be planted amongst the GM plants at the site. As there is a possibility of viable GM material being present in the non-GM plants, a licence condition has been imposed requiring any non-GM plants to be treated as the GMOs and therefore subject to all the same licence conditions. This will restrict the dispersal of viable GM plant material (Risk scenario 3).

245. The proposed release site will be surrounded by a fence with a locked gate, preventing the possibility of seed dispersal by any large animals or by unauthorised people accessing the site. Dispersal by authorised people entering the proposed trial site would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing.

246. The applicant has stated that any plant material taken off-site for experimental analysis will be transported according to the Regulator's *Guidelines for the transport of GMOs*, <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/transport-guide-1>>. These are standard protocols for the handling of GMOs to minimize exposure of the GMOs to human and other organisms (Risk scenario 1), and dispersal of GMOs or GM material into the environment (Risk scenario 3, 4 and 5). Adherence to those Guidelines is a condition of this licence.

#### **4.1.2 Summary of measures imposed by the Regulator to be implemented to limit and control the proposed release**

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

- limit the release to a total area of 0.1 ha at one site per growing season between June 2010 and December 2013
- locate the field trial site at least 50 m away from natural waterways
- establish a 10 m zone around the trial in which any related species are prevented from flowering and which is maintained in a manner that does not attract rodents
- ensure no other crops of wheat are within 200 m of the trial sites
- surround the GM wheat and barley with an inspection zone of up to 200 m in which growth of sexually compatible species is controlled
- enclosing each trial site with a livestock-proof fence with lockable gate with mouse baiting inside the fence perimeter
- apply measures to promote germination of any wheat and barley seeds that may be present in the soil after harvest, including tillage and irrigation
- monitor the site for at least 24 months after harvest and until no volunteers are detected for a continuous 6 month period and destroy any wheat plants that may grow
- destroying all plant material from the trial not required for testing or future trials
- transporting and storing of the GMOs in accordance with the Regulator's guidelines
- not allow the GM plant material or products to be used for human food or animal feed, or in the production of therapeutic goods.

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

## **4.2 Other risk management considerations**

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

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment
- a requirement that the applicant allows access to the trial sites by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

### **4.2.1 Applicant suitability**

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

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

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

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

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

### **4.2.2 Contingency plan**

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

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

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

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

#### **4.2.4 Reporting structures**

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

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

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

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

- expected and actual dates of planting
- expected and actual dates of commencement of flowering
- expected and actual dates of harvest and cleaning after harvest.

#### **4.2.5 Monitoring for Compliance**

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

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

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

### **Section 5 Issues to be addressed for future releases**

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

- additional data on the potential toxicity and allergenicity of plant materials from the GM wheat lines
- phenotypic characterisation of the GM wheat lines, in particular of traits which may contribute to weediness, persistence, and ability to disperse in the environment
- molecular and biochemical characterisation of the GM wheat lines
- compositional analysis of the GM wheat lines.

## **Section 6 Conclusions of the RARMP**

264. The risk assessment concluded that this proposed limited and controlled release of up to 150 GM wheat lines on a maximum total area of 0.1 ha over three years in the Queensland local government area of Redland, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

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## Appendix A Summary of issues raised in submissions received from prescribed experts, agencies and authorities<sup>14</sup> on the consultation RARMP for DIR 100

The Regulator received several submissions from prescribed experts, agencies and authorities on the consultation RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence that was used in finalising the RARMP that formed the basis of the Regulator’s decision to issue the licence. A number of submissions received raised issues relating to risks to the health and safety of people and the environment as summarised below.

Summary of issues raised	Comments
<p>Inactive ingredients produced from grain, eg gluten and sugars, are often used in medicine capsules, tablet coating or fillings.</p> <p>Consequently, where ever the risk assessment refers to exclusion of grains from ‘human food or animal feed’ reference should also be made to the exclusion of grains from ‘therapeutic goods’.</p>	<p>A statement that the GMO should be excluded from use as ingredients in ‘therapeutic goods’ has been included into Chapter 3, Section 4 of the RARMP. The Licence has been amended to ensure the GMO or products thereof will not be used in the production of therapeutic goods.</p>
<p>Transfer of seeds from harvesters and seeders pose the greatest risk of contamination of the environment. Notes that proposed licence conditions require equipment to be cleaned but no standard is specified. They consider it is important for the OGTR to provide guidance on the required cleaning standards.</p>	<p>The proposed licence conditions did not specify a method of cleaning equipment, but rather the outcome which must be achieved – that the GMOs be removed from equipment and destroyed.</p>
<p>The authors have categorically stated that there is no identified risk to unintended changes in biochemistry, physiology and ecology (eg. Table 5 section 2.5 and throughout document). Since transcription factors declared in the application have a range of effects and there may be a risk of unintended changes in biochemistry and physiology.</p> <p>Suggests the conclusions (Section 152, 162 and others throughout document) need to be changed to reflect a level of risk of unintended changes in biochemistry, physiology or ecology which requires further investigation prior to release of the GM wheat for human or animal consumption.</p>	<p>As discussed in Section 2.5 of chapter 2, there is potential for a number of interrelated biochemical pathways to be affected by the introduced genes. The possible effects of the introduced genes were considered in the light of information obtained from published literature on the genes (if available) or closely related genes and/or gene families as a whole.</p> <p>The risk has been assessed as negligible within the context of the proposed release. This includes the limits and controls proposed as part of this release. In particular, the small scale of the trial would minimise the potential for adverse effects on the environment, and exposure of the public and livestock to the GM plant material is unlikely due to restricted access to the site and because GM plant material will not be used as food or animal feed.</p> <p>The RARMP outlines future research requirements should the applicant seek a licence for a larger scale or commercial release (Chapter 3). These include further information on the potential allergenicity or toxicity of the GM plants, additional phenotypic characterisation</p>

<sup>14</sup> GTTAC, State and Territory Governments, Australian Government agencies and the Minister for the Environment.

Summary of issues raised	Comments
	(including characteristics indicative of weediness), and characterisation of the genetic material in the plants. Additionally, approval by FSANZ would be required before material from the GM wheat could be sold as food.
Basic agronomic performance is not sufficient to identify unintended changes (Para 193).	This is a limited and controlled release. The purpose is to evaluate the GM wheat lines under heat and drought prone conditions. It is likely that substantial unintended effects resulting in morphological and/or physiological abnormalities and/or detrimental phenotypes will be detected during these early stages of development. Paragraph 193 has been modified to reflect this. For any future larger scale releases, such as a commercial release, additional data would need to be provided to identify and characterise any unintended effects, for example compositional analyses and detailed phenotypic characterisation.
There are contradictory statements in Section 191 that recognises the potential of unintended effects, but is not reflected throughout the document.	This is a limited and controlled release. The paragraph indicates the likelihood of unintended effects occurring but notes that the assessment had considered a wide range of potential phenotypes based on the range of functions described for the introduced genes and other similar genes. Unintended effects are discussed in a number of areas in the RARMP including Chapter 1 and Risk scenario 2. Any areas of uncertainty requiring more information and data for future larger scale releases have been identified in Chapter 2, Section 4 of the RARMP. Also see above.
Is supportive of the application, however suggests that a rodent-proof fence be imposed as an added licence condition, to further control rodent activity around the fenced area of the proposed release site.	Chapter 2, Risk Scenario 3 discusses dispersal of wheat by rodents. Mice are known pests of grain crops. Habitat modification (for example, cultivation and maintenance of grassland vegetation below 10 cm) has been shown to reduce rodent numbers in irrigated farming systems in Australia, and in and around farm buildings. Reduced plant cover has also been reported to be a deterrent to the movement of mice. This has been discussed in detail in the RARMP for DIR 077/2007. The applicant has proposed several measures to control rodent numbers at the proposed release site, including a 10 m wide zone in which plant cover would be kept at or below 10 cm and placing rodent baits inside the fence line. These measures are considered adequate to minimise dispersal of the GM wheat by rodents and have been imposed in the licence.
Draft RARMP does not include discussion of the potential gene stacking on the weediness of the GM wheat lines. The applicant proposes to deliberately cross some of the GM wheat lines proposed for release. The effects of combining multiple traits on the ability to establish and persist in non-agricultural environments are not known. A more detailed examination of potential effects of gene stacking could assist in the identification of more directed data collection requirements.	In the RARMP, the potential for the genetic modifications to increase the weediness of the GM wheat through intended and unintended effects is discussed in risk scenario 2. Although any potential stacking events are not characterised, the combination of traits as a result of crossing of the GM wheat lines is likely to contribute only incrementally to the potential weediness of the GM wheat plants. The conditions that normally limit the spread and persistence of non-GM wheat such as temperature, competitive ability, nutrient availability and susceptibility to pests and diseases would be expected to limit the spread and persistence of any GM wheat plants. Furthermore, the spread and persistence of the GM wheat would be managed by measures proposed by the applicant and imposed in the licence. Further discussion has been added to the RARMP to provide additional general consideration of the potential stacking of the traits (see Chapter 2, Risk scenario 4).  The RARMP outlines future research requirements should the applicant seek a licence for a larger scale release or commercial release.

Summary of issues raised	Comments
<p>Draft RARMP does not include discussion of the potential interaction or stacking of the GM traits in this trial with those traits present in other current wheat trials. Early consideration of possible interactions of the expanding number of traits may lead to enhanced risk assessment outcomes. Recommends inclusion of a discussion of this nature and indicates prior to large scale trials, it would be prudent to conduct a foresighting exercise on all of the wheat trials in Australia to determine data gaps and whether the interaction of wheat with the Australian environment may change.</p>	<p>Stacking of the GM traits in this release with other limited &amp; controlled releases of GM wheat was not discussed because it is highly unlikely that stacking between GM plants in different trials will occur. The different trials are geographically remote and licence conditions imposed for each release effectively restrict gene flow. Furthermore, this is early stage research and GM lines to be grown as part of this application may not undergo further development or reach the commercial release stage. Therefore, a discussion on this issue is not warranted at this stage. A consideration of stacking and any potential risks that might arise as a consequence would be undertaken for a release where controls on gene flow were minimal or absent, such as for an application to commercially release GM wheat.</p>
<p>Paragraph 236 of the draft RARMP concludes that “preventing the planting of <i>Triticace</i> species within 200m ... adequate to minimise gene flow ...”. Recommends that the definition of “Related Species” be changed to mean plants of the Tribe <i>Triticace</i>. This would enhance the inspection and monitoring procedures to minimise gene flow to sexually compatible species.</p>	<p>In paragraph 236, '<i>Triticace</i> species' refers to species in the genus <i>Triticum</i> not to the tribe <i>Triticeae</i>. Of the species that might hybridise with bread wheat under natural conditions, few are known to be present in Australia. Apart from commercially cultivated bread and durum wheat, other species of <i>Triticum</i> are not known to be present in Australia. <i>Aegilops</i> spp are recognised as quarantine weeds in Australia and are not known to be present naturally. Natural hybrids between <i>T. aestivum</i> and <i>Secale</i> or <i>T. aestivum</i> and <i>Elytrigia</i> (<i>Elymus</i>) have not been recorded. Other species with which <i>T. aestivum</i> could potentially form hybrids, albeit with highly compromised fertility, do not occur naturally in Australia. In the context of the proposed release site and on the basis of the scientific literature on interspecific and intergeneric gene flow monitoring for the presence of species in the tribe <i>Triticeae</i> was not warranted.</p>