



**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 102**

Limited and controlled release of wheat and barley  
genetically modified for abiotic stress tolerance

Applicant: The University of Adelaide

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 102) from the University of Adelaide. The licence authorises dealings involving the limited and controlled release of up to 2340 lines<sup>1</sup> of genetically modified (GM) wheat and barley 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 or not 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**

The University of Adelaide has applied for a licence for dealings involving the intentional release of up to 1161 lines of GM wheat and 1179 lines of GM barley on a limited scale and under controlled conditions. The GM wheat and barley lines have been genetically modified to enhance nutrient utilisation and abiotic stress tolerance. The trial will take place at three sites, two in South Australia and one in Western Australia, on a maximum area of 0.75 ha per growing season, between June 2010 and December 2015.

The applicant will release GM wheat and barley modified to contain one of 35 genes encoding proteins expected to enhance nitrogen use efficiency, increase zinc uptake and enhance tolerance to a range of abiotic stresses including drought, cold, salt and low phosphorus. Most of the genes conferring the traits have been obtained from wheat, barley or maize. The remaining genes were derived from thale cress, moss or yeast, which are also widespread in the environment. All of the GM wheat and barley lines contain a selectable marker gene.

The purpose of the trial is to characterise growth and yield characteristics of the GM plants when grown under drought, rain fed or saline field conditions. The GM wheat and barley will not be used for human food or animal feed.

The University of Adelaide proposed a number of controls to restrict the spread and persistence of the GM wheat and barley lines and their genetic material into the environment. These controls have been considered during the evaluation of the application.

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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 resulting from a single 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/riskassessments-1>>.

## **Confidential Commercial Information**

Some details, including the name and sequence of some of the genes and promoters, 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 (including proposed containment measures), 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 are postulated (risk scenarios), and these scenarios are evaluated to identify those that warrant detailed characterisation. This process is described as risk identification.

Eight risk scenarios were postulated. This included consideration of whether or not expression of the introduced genes could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM wheat and barley; 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.

Risks to the health and safety of people, or the environment, from the proposed release of the GM wheat and barley 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 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 release to the size, locations and duration requested by the applicant, as these were important considerations in establishing the context for assessing the risks.

The licence conditions require the University of Adelaide to **limit** the release to a total area of 0.75 ha per growing season at three sites between June 2010 and December 2015. The **control** measures include containment provisions at the trial site, preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with the 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 1161 GM wheat lines and 1179 GM barley lines on a maximum total area of 0.75 ha per growing season over five years in the LGAs of Marion and Wakefield, South Australia, and Corrigin, Western Australia, 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, locations 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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## Abbreviations

the Act	<i>Gene Technology Act 2000</i>
APHIS	Animal and Plant Health Inspection Service
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
CCI	Confidential Commercial Information as declared under section 185 of the <i>Gene Technology Act 2000</i>
CaMV	Cauliflower mosaic virus
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
HGT	Horizontal gene transfer
HPT	Hygromycin phosphotransferase
km	kilometre
m	metre
mm	millimetre
mRNA	Messenger Ribonucleic Acid
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
nos	Nopaline synthase
NUE	Nitrogen Use Efficiency
OECD	Organisation for Economic Cooperation and Development
OGTR	Office of the Gene Technology Regulator
PC2	Physical containment level 2
PCR	Polymerase Chain Reaction
RARMP	Risk Assessment and Risk Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RNA	Ribonucleic Acid
TGA	Therapeutic Goods Administration
Ubi1	Ubiquitin1

# Technical Summary

## Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of licence application (DIR 102) from the University of Adelaide. The licence authorises dealings involving the limited and controlled release of up to 2340 lines<sup>3</sup> of genetically modified (GM) wheat and barley 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 or not 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

The University of Adelaide has applied for a licence for dealings involving the intentional release of up to 1161 lines of GM wheat and 1179 lines of GM barley on a limited scale and under controlled conditions. The GM wheat and barley lines have been genetically modified to enhance nutrient utilisation and abiotic stress tolerance. The trial will take place at three sites, two in South Australia and one in Western Australia, on a maximum area of 0.75 ha per growing season, between June 2010 and December 2015.

The applicant will release GM wheat and barley modified to contain one of 35 genes encoding proteins expected to enhance nitrogen use efficiency, increase zinc uptake and enhance tolerance to a range of abiotic stresses including drought, cold, salt and low phosphorus. Most of the genes conferring the traits have been obtained from wheat, barley or maize. The remaining genes were derived from thale cress, moss or yeast, which are also widespread in the environment. Expression of each of the genes will be under the control of a constitutive promoter or one of a number of drought, cold or salt inducible promoters.

All of the GM wheat and barley lines contain an antibiotic resistance gene, *hpt*. The gene provides resistance to the antibiotic hygromycin B and was used as a marker to select for successful genetic modifications during initial research and development work in the laboratory.

Short regulatory sequences that control expression of the genes will also be present in the GM wheat and barley lines. 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 regulatory

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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)>

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

The GM wheat lines were produced by transforming plants of the wheat cultivars Bobwhite, 'Drysdale' or 'Frame'. The GM barley lines were produced by transforming plants of the barley cultivars 'Golden Promise' or 'W14330'.

The purpose of the trial is to characterise growth and yield characteristics of the GM plants when grown under drought, rain fed or saline field conditions. The GM wheat and barley will not be used for human food or animal feed as part of this release.

The University of Adelaide proposed a number of controls to restrict the spread and persistence of the GM wheat and barley lines and their genetic material into the environment. These controls have been considered during the evaluation of the application.

### **Confidential Commercial Information**

Some details, including the names and sequences of some of the genes and promoters, 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 (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.

The reference documents, *The Biology of Triticum aestivum L. em Thell (bread wheat)* and *The Biology of Hordeum vulgare L. (barley)*, were produced to inform the risk assessment process for licence applications involving GM wheat and barley plants. The documents are available from the OGTR or from the website <<http://www.ogtr.gov.au>>.

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

Eight risk scenarios were postulated. This included consideration of whether or not expression of the introduced genes could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM wheat and barley; 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, locations and duration of the release proposed by the University of Adelaide

- suitability of controls proposed by the University of Adelaide to restrict the spread and persistence of the GM wheat and barley plants and their genetic material
- limited ability and opportunity for the GM wheat and barley lines to transfer the introduced genes to other wheat or barley crops or other sexually compatible species
- none of the GM plant materials or products will be used for human food or animal feed as part of this release
- 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 GM wheat and barley 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 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 proposed release to the size, locations 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 up to 0.75 ha per growing season at three sites, two in the LGAs of Marion and Wakefield (SA) and the other in the LGA of Corrigin (WA), between June 2010 and December 2015
- locate the trial sites at least 50 m away from natural waterways
- establish a 10 m zone around the trial sites in which any related species are prevented from flowering and which is maintained in a manner that does not attract or harbour rodents
- surround the GM wheat and barley with an inspection zone of up to 200 m in which growth of sexually compatible species is controlled
- ensure no other crops of wheat or barley are within 200 m of the trial sites
- enclose each trial site with a livestock-proof fence with lockable gate with rodent baiting inside the fence perimeter
- harvest the GM wheat and barley plant material separately from other crops
- clean the sites and equipment used on the sites following harvest
- apply measures to promote germination of any wheat and barley seeds that may be present in the soil after harvest, including irrigation

- monitor the site for at least 24 months after harvest and destroy any wheat and barley plants that may grow until no volunteers are detected for a continuous 6 month period
- destroy all GM plant material not required for further analysis or future trials
- transport and store material from the GMOs in accordance with Regulator's guidelines
- not permit any GM wheat or barley plant material to be used in human food, animal feed or in the production of therapeutic goods as part of this release.

### ***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>.

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 and barley lines proposed for release to be used for human food. Accordingly, the applicant has not applied to FSANZ to evaluate the GM wheat and barley 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 and barley lines, or to justify a reduction in containment conditions. This would include:

- additional data on the potential allergenicity or toxicity of plant materials from the GM wheat and barley lines
- additional phenotypic characterisation of the GM wheat and barley lines, in particular of characteristics indicative of weediness, including measurement of altered reproductive capacity and competitiveness, and information relating to cross tolerance to other abiotic stressors
- characterisation of the introduced genetic material in the plants, including copy number and genotypic stability.

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

The Regulator determined, at the commencement of the assessment process for this application, that the University of Adelaide was suitable to hold a DIR licence under the requirements of section 58 of the Act. The Regulator is satisfied that the University of Adelaide 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.

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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>>.

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

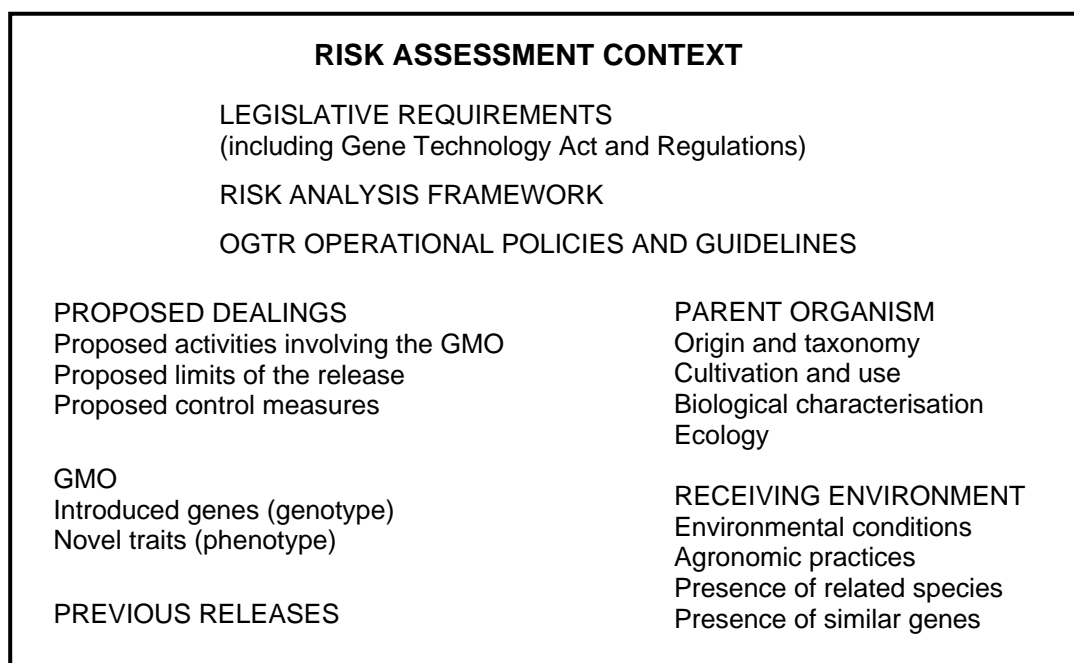
The risk assessment concluded that this limited and controlled release of up to 1161 GM wheat lines and 1179 GM barley lines on a maximum total area of 0.75 ha per growing season over five years in the LGAs of Marion and Wakefield, South Australia, and Corrigin, Western Australia, poses **negligible** 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, locations 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 assessment 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 and the 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 *Gene Technology Act 2000* (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 *Gene Technology Regulations 2001* (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, locations

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 any 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). The decision is provided in Section 3 of Chapter 2.

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

8. The University of Adelaide proposes to release up to 1161 lines<sup>6</sup> of genetically modified (GM) wheat and 1179 lines of GM barley into the environment under limited and controlled conditions. The GM wheat and barley plants will contain one of 35 genes encoding proteins expected to enhance nitrogen use efficiency, increase zinc uptake, or confer tolerance to a range of abiotic stresses including drought, cold, salt and low phosphorus.

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

- propagating, growing, raising or culturing the GMOs
- breeding the GMOs
- conducting experiments with 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 of the application including the names and sequences of some of the genes and promoters, and associated references 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.

#### **3.1 The proposed activities**

12. The applicant has stated that the purpose of the trial is to conduct experiments to assess whether or not expression of the introduced genes for abiotic stress tolerance, nitrogen use efficiency and zinc biofortification results in increased yield in the GM wheat and barley plants when grown under field conditions. Seed would be collected and retained for analysis

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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.

and possible future trials, subject to further approval(s). Plant materials from the GM wheat and barley will not be used for human food or animal feed.

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

13. The release is proposed to take place at three sites, two in South Australia and one in Western Australia on a total maximum area of 0.75 ha per growing season between June 2010 and December 2015.

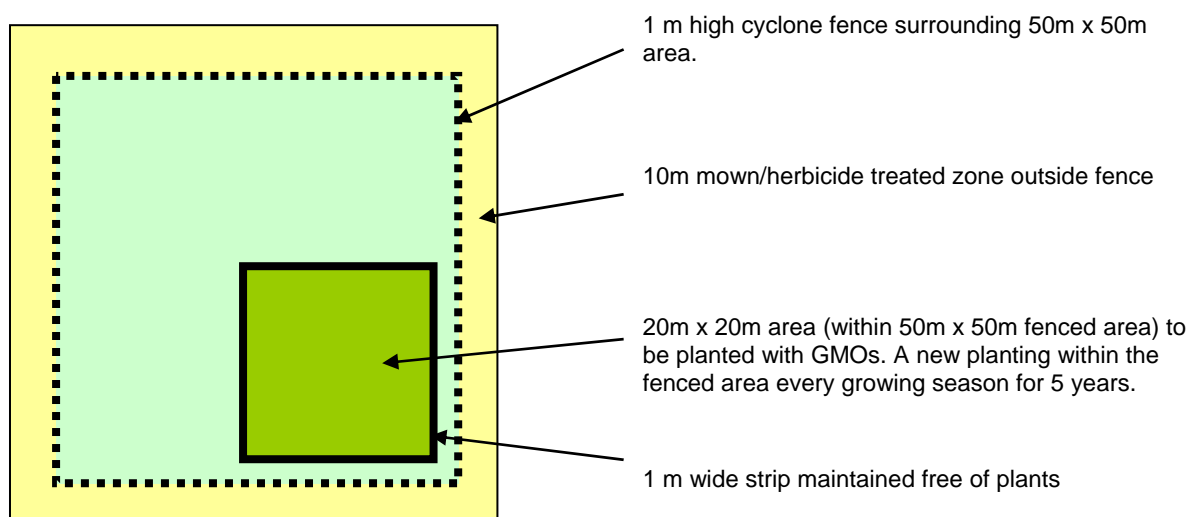
14. Only trained and authorised staff will be permitted access to the proposed locations.

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

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

- locating the trial site at least 50 m away from natural waterways
- enclosing each trial site with a livestock-proof fence with lockable gate with rodent baiting inside the fence perimeter
- locating the trial site at least 200 m away from all other commercial wheat and barley plantings, and at least 500 m away from plantings of wheat and barley breeding lines
- surrounding the trial with a 10 m wide monitoring zone free of vegetation (Figure 2)
- maintaining a 50 m zone (in addition to the 10 m monitoring zone) around the trial sites free of any sexually compatible species
- promoting the germination of any residual seed following harvest through irrigating the site and destroying any volunteer wheat and barley with herbicide
- post harvest monitoring of the site for 24 months or until the site has been clear of volunteers for one growing season and destroying any volunteer wheat and barley identified during this period
- destroying all plant materials from the trial site not required for analysis or future trials
- transporting and storing of the GMOs in accordance with the Regulator's guidelines
- not allowing the GM plant materials or products to be used for human food or animal feed.

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



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

## Section 4 The parent organism

17. The parent organisms are bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), both of which are exotic to Australia. Commercial wheat and barley cultivation occurs in the wheat belt from south eastern Queensland through New South Wales, Victoria, southern South Australia and southern Western Australia (OGTR 2008b). A small amount of barley is also grown in Tasmania (OGTR 2008a).

18. The wheat cultivars used to generate the GM wheat lines are ‘Bobwhite’, ‘Drysdale’ and ‘Frame’. The ‘Bobwhite’ cultivar is not favoured as a commercial bread wheat as it is considered to be of lower quality than most commercial cultivars (Bhalla et al. 2006), but is commonly used in genetic modification work because it is relatively easy to genetically modify and has previously been used in conventional (non-GM) wheat breeding programs. The cultivars ‘Frame’ and ‘Drysdale’ are used in commercial growing operations throughout Australia and have some degree of drought tolerance as they have been bred for Australian conditions.

19. The GM barley lines in the proposed release were derived from the barley cultivars ‘Golden Promise’ and ‘WI4330’. The barley cultivar ‘Golden Promise’ was derived from the ‘Maythorpe’ cultivar following modification by the use of gamma-ray irradiation. It is a semi-dwarf, malting cultivar that has been found to have greater tolerance to soil salinity than ‘Maythorpe’ (Forster 2001). While the precise genetic changes are not known, salt tolerance in ‘Golden Promise’ is a consequence of the plants’ ability to limit the uptake of salt from the soil and results in this cultivar having a higher grain yield than its parental cultivar. ‘Golden Promise’ is also reported to have some tolerance to drought (Forster 2001) but is not used in commercial plantings. ‘WI4330’ is a former breeding line, developed by the SA Barley Improvement Program at the University of Adelaide but found not to perform well under drought conditions.

20. Further detailed information about the parent organism is contained in the reference documents, *The Biology of Triticum aestivum L. em Thell (bread wheat)* and *The Biology of Hordeum vulgare L. (barley)*, that were produced to inform the risk assessment process for licence applications involving GM wheat and/or barley plants (OGTR 2008a; OGTR 2008b). These documents are available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

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

### 5.1 Introduction to the GMOs

21. The applicant proposes to release up to 2340 GM wheat and barley lines, each with a selectable marker gene plus one introduced gene from among thirty five genes of interest. Details of the genes and constructs used to generate these lines, including the regulatory sequences, are listed in Table 1 and Table 2; the genes are broadly grouped according to encoded protein type and expected phenotypic effect. In each GM line the gene of interest is under the control of one of twelve different promoters (Table 2). GM wheat plants were generated using the biolistic transformation method and GM barley plants were generated using the *Agrobacterium* mediated transformation method. Genes belonging to groups 1 to 4 (Table 1) will be used to generate GM lines in three wheat and two barley cultivars; genes in groups 5 and 6 will be used to generate lines in barley cultivars only.

22. The introduced genes encode proteins that are intended to confer tolerance to a range of abiotic stresses, including drought, salt and low soil phosphorus, to improve nitrogen use efficiency, or to enhance zinc uptake and translocation with the aim of increasing zinc content of grain. All GM wheat and barley lines would also contain the antibiotic resistance selectable marker gene *hpt*.

23. The GM wheat and barley plants contain genes derived from wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), maize (*Zea mays*), thale cress (*Arabidopsis thaliana*), moss (*Physcomitrella patens*) or yeast (*Saccharomyces cerevisiae*). The different classes of GM lines can be summarised as follows:

GM wheat and barley lines containing

- A gene (*AtAVPI*) from thale cress that encodes a protein associated with salinity, drought and low phosphorus tolerance (Group 1)
- An aminotransferase gene from barley that encodes a protein involved in nitrogen utilisation efficiency (Group 2)
- One of twenty five genes from wheat, maize and barley that encode transcription factors<sup>7</sup> involved in regulation of drought and cold/frost tolerance (Group 3)
- One of four genes from wheat and maize encoding proteins involved in drought tolerance. Three of the genes encode protein kinases expected to confer increased drought tolerance through activation of transcription factors, and the fourth gene encodes a potential kinase substrate (Group 4)

GM Barley lines containing

- A gene (*HvZIP7*) from barley that encodes a protein associated with zinc uptake and translocation, and increased zinc content in grain (Group 5)
- One of three genes from moss, thale cress and yeast that encode proteins involved in salinity tolerance (*PpENAI*, *AtCIPK16* and *ScNHA1*) (Group 6)

24. Each of the transcription factor genes would be expressed in both wheat and barley via ten different constitutive, tissue specific or inducible promoters derived from wheat, barley,

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<sup>7</sup> A transcription factor is any protein required for recognition, by RNA polymerases, of specific regulatory sequences in genes (eg a promoter) (Lewin 1994).

maize and cauliflower mosaic virus (CaMV). Nine of these promoters would also be used in combination with each of the four protein kinase or protein kinase substrate genes (Table 2). The remaining genes are expressed via promoter sequences derived from CaMV, rice or wheat. The transcription termination region for the introduced genes is derived from *Agrobacterium*. Regulatory elements are discussed further in Section 5.3 of this Chapter.

25. The genes proposed for use in this application and their end-products have not been fully characterised in the GM wheat and barley lines. Their potential functions have been identified according to their up-regulation upon exposure to drought, drought/heat or cold/frost conditions and their homology to other genes shown to be associated with stress responses. In Tables 1 and 2 and the following sections, the genes and their encoded products are described in brief to illustrate their potential function within the GM wheat and barley lines.

26. The majority of the introduced genes encode transcription factors or protein kinases, some of which, or their homologues<sup>8</sup>, are also known to confer other agronomic effects in plants, such as tolerance to other abiotic and biotic stresses. At this early stage of research, the nature and extent of such stress tolerance is not known and this uncertainty is taken into account in the risk analysis process.

**Table 1. Name, source and anticipated effect of the introduced genes.**

Gene Number	Gene designation <sup>†</sup>	Gene name* and Source organism	Anticipated phenotypic effect of introduced gene or their homologs	GMO
<b>H<sup>+</sup> translocating pyrophosphatase</b>				<b>Group 1</b>
1	<i>AtAVP1</i>	<i>AtAVP1</i> <i>A. thaliana</i>	Improved salinity and drought tolerance, and low phosphorus tolerance.	
<b>Aminotransferase</b>				<b>Group 2</b>
2	<i>Aminotransferase</i>	<i>H. vulgare</i>	Improved nitrogen use efficiency.	
<b>TRANSCRIPTION FACTORS</b>				<b>Group 3</b>
<b>3A</b>				<b>3A</b>
3	<i>CELLWALL1</i>	CCI	Possible secondary cell wall biosynthesis regulator in vascular tissues, quality of light-dependent regulation of stem length.	
4	<i>CELLWALL2</i>	CCI	Unknown. Possibly cell wall and/or chloroplast gene regulator.	
5	<i>DROUGHT1</i>	CCI	Drought-inducible, may be involved in drought induced male sterility, may be responsible for chloroplast stability.	
6	<i>DROUGHT2</i>	CCI	Drought-inducible, may be involved in drought induced male sterility, may be responsible for chloroplast stability.	
7	<i>DROUGHT3</i>	CCI	Drought inducible, may provide a connection between cell expansion and plant turgor, chloroplast stability, protection from pathogens under drought.	
<b>DREB</b>				<b>3B</b>
8	<i>TaDREB2</i>	<i>TaDREB2</i> <i>T. aestivum</i>	Recovery after drought and no undesirable phenotype in wheat under inducible Rab17 promoter, regulator of drought and cold inducible genes.	
9	<i>TaDREB3</i>	<i>TaDREB3</i> <i>T. aestivum</i>	Recovery after drought in wheat under Rab17 promoter, frost tolerance, regulator of drought and cold inducible genes..	
10	<i>ZmDREB2</i>	<i>ZmDREB2</i> <i>Z. mays</i>	Drought inducible. Expected increase in drought and cold tolerance.	
<b>3C</b>				<b>3C</b>
11	<i>DROUGHT4</i>	CCI	Isolated from drought/high temperature library. Expected to be regulator of drought/frost tolerance.	
12	<i>COLD1</i>	CCI	Isolated from 'cold'/frost' gene library. No induction by cold, but can activate cold/drought/salt inducible promoter of <i>PROMOTER2</i> (see Table 2).	
13	<i>COLD2</i>	CCI	Isolated from 'cold/frost' gene library. Strongly and specifically	

<sup>8</sup> Homologous genes refer to genes within a single species that diverged by gene duplication or to genes in different species with similar structures and evolutionary origin.

Gene Number	Gene designation <sup>†</sup>	Gene name* and Source organism	Anticipated phenotypic effect of introduced gene or their homologs	GMO	
			induced by cold.		
<b>3D</b>				<b>3D</b>	
14	<i>DROUGHT5</i>	CCI	Drought tolerance (if overexpressed). Stomatal closure, wax.		
15	<i>DROUGHT6</i>	CCI			
<b>CBF</b>				<b>3E</b>	
16	<i>ZmCBF1</i>	<i>ZmCBF1</i> <i>Z. mays</i>	Involved in plant cold acclimation; drought, salt, cold and freezing tolerance; pathogen defence; chloroplast development and tolerance to oxidative stress; stunted phenotype.		
17	<i>ZmCBF4</i>	<i>ZmCBF4</i> <i>Z. mays</i>			
<b>3F</b>				<b>3F</b>	
18	<i>DROUGHT7</i>	CCI	Drought inducible, strongly activates <i>PROMOTER2</i> , increase in biotic stress tolerance expected.		
19	<i>DROUGHT8</i>	CCI	Binds and strongly activates <i>PROMOTER2</i> , stress inducible, resistance to pathogens also expected.		
20	<i>DROUGHT9</i>	CCI	Binds and strongly activates <i>PROMOTER2</i> , stress inducible, resistance to pathogens also expected.		
21	<i>DROUGHT10</i>	CCI	Binds and strongly activates <i>PROMOTER2</i> , stress inducible, resistance to pathogens also expected.		
22	<i>DROUGHT11</i>	CCI	Drought inducible, homologue of <i>DROUGHT7</i> .		
<b>3G</b>					<b>3G</b>
23	<i>DROUGHT12</i>	CCI	Some drought tolerance. Binds and strongly activates <i>PROMOTER2</i> , potential substrate for drought/salt inducible kinases.		
24	<i>DROUGHT13</i>	CCI	Improvement of drought tolerance in GM plants, homologue of <i>DROUGHT12</i> , potential substrate of stress inducible kinases		
25	<i>DROUGHT14</i>	CCI	Drought/cold inducible		
26	<i>DROUGHT15</i>	CCI	Drought inducible		
27	<i>COLD3</i>	CCI	From 'cold/frost' gene library, induced during recovery after stress.		
<b>PROTEIN KINASES</b>					<b>Group 4</b>
28	<i>KINASE1</i>	CCI	Abscisic acid (ABA), drought, salt and cold inducible kinase. May regulate binding of bZip factors to ABA-inducible promoters. GM plants show better recovery after drought stress than control plants		
29	<i>KINASE2</i>	CCI	ABA, drought, salt and cold inducible kinase. May regulate binding of bZip factors to ABA-inducible promoters.		
30	<i>KINASE3</i>	CCI	Drought inducible kinase.		
31	<i>KINASE SUBSTRATE4</i>	CCI	Induced by salt stress. Kinase substrate, isolated from cDNA drought stress library.		
<b>OTHER PROTEINS</b>					
<b>Zn-regulated transporters</b>				<b>Group 5</b>	
32	<i>HvZIP7</i>	<i>H. vulgare</i>	Involved in zinc translocation, expected to increase zinc uptake and translocation and increase zinc content of grain.		
<b>Na<sup>+</sup> pumping ATPase</b>					<b>Group 6</b>
33	<i>PpENAI</i>	<i>P. patens</i>	Na <sup>+</sup> transporter, expected to confer increased salt tolerance.		
<b>Calcineurin B-like interacting protein kinase</b>					
34	<i>AtCIPK16</i>	<i>A. thaliana</i>	Protein interacts with calcineurin B-like proteins, upregulated under salt stress.		
<b>Na<sup>+</sup>/H<sup>+</sup> antiporter</b>					
35	<i>ScNHA1</i>	<i>S. cerevisiae</i>	Na <sup>+</sup> , K <sup>+</sup> /H <sup>+</sup> , important for salinity tolerance in yeast, may improve salinity tolerance in plants.		

\*Two-letter prefixes to the gene names are used to denote the species of origin (source organism) for the genes: *Hv*=*Hordeum vulgare*; *Ta*=*Triticum aestivum*; *Zm*=*Zea mays*, *Pp*=*Physcomitrella patens*; *A*=*Arabidopsis thaliana*; *Sc*=*Saccharomyces cerevisiae*.

<sup>†</sup>Identities of some of the genes have been declared CCI. The applicant has assigned a designation to each of the genes and these are listed in Column 2. Where the true gene name can be disclosed, they are listed in Column 3 together with the source organism.

**Table 2. Gene constructs used to generate the GM wheat and barley lines proposed for release**

Gene of interest	Promoters <sup>†</sup>	Origin, expression pattern and/or inducibility*	Terminator	Max no lines	
				Barley	Wheat
<i>Gene 1</i>	2x35S pUbi pRab17	CaMV (Constitutive) Maize (Constitutive) Maize (DI, SI, CI)	nos	12	12
<i>Gene 2</i>	OsAnt1	Rice (Root specific)	nos	5	5
Transcription Factors					
<i>Genes 3-27</i>	2x35S (transcription factors only)  pUbi pRab17 <i>PROMOTER1</i> <i>PROMOTER2</i>	CaMV (Constitutive)  Maize (Constitutive) Maize (DI, SI) Barley (DI, SI, CI) Wheat (DI, SI, CI)	nos	1000	1000
Kinase/kinase substrate					
<i>Genes 28-31</i>	<i>PROMOTER3</i> <i>PROMOTER4</i> <i>PROMOTER5</i> <i>PROMOTER6</i> <i>PROMOTER7</i>	Wheat (DI, SI, CI) Wheat (DI, SI, CI) Wheat (DI, CI) Wheat (DI) Wheat (DI)	nos	144	144
Other genes					
<i>Gene 32</i>	2x35S	CaMV (Constitutive)	nos	3	
<i>Gene 33</i>	2x35S actin	CaMV (Constitutive) Rice (Constitutive)	nos	5	
<i>Gene 34</i>	2x35S	CaMV (Constitutive)	nos	8	
<i>Gene 35</i>	2x35S	CaMV (Constitutive)	nos	2	
<b>Total lines</b>				<b>1179</b>	<b>1161</b>

\*Inducible expression: DI: drought inducible; CI cold inducible; SI: salt inducible

<sup>†</sup>Identities of some of the promoters have been declared CCI. The applicant has designated these promoters *PROMOTER1* – *PROMOTER7*.

## 5.2 The introduced genes, encoded proteins and their end products

27. Of the 35 genes proposed for use in the genetically modified wheat and barley, all except two are thought to be involved in mediating responses to primary abiotic stresses including cold, salinity and/or drought. For the purposes of the following discussion, the genes have been grouped according to protein type and/or expected effect as shown in Table 1. The names and origins of a number of the introduced genes have been declared CCI, so have been assigned alternative designations by the applicant to protect that information (see Table 1). Most of the introduced genes are transcription factors and the classification of each gene to a specific family of transcription factors is also CCI. Therefore, these genes are discussed under the groupings Group 3A – 3G.

### *Plant molecular responses to abiotic stress*

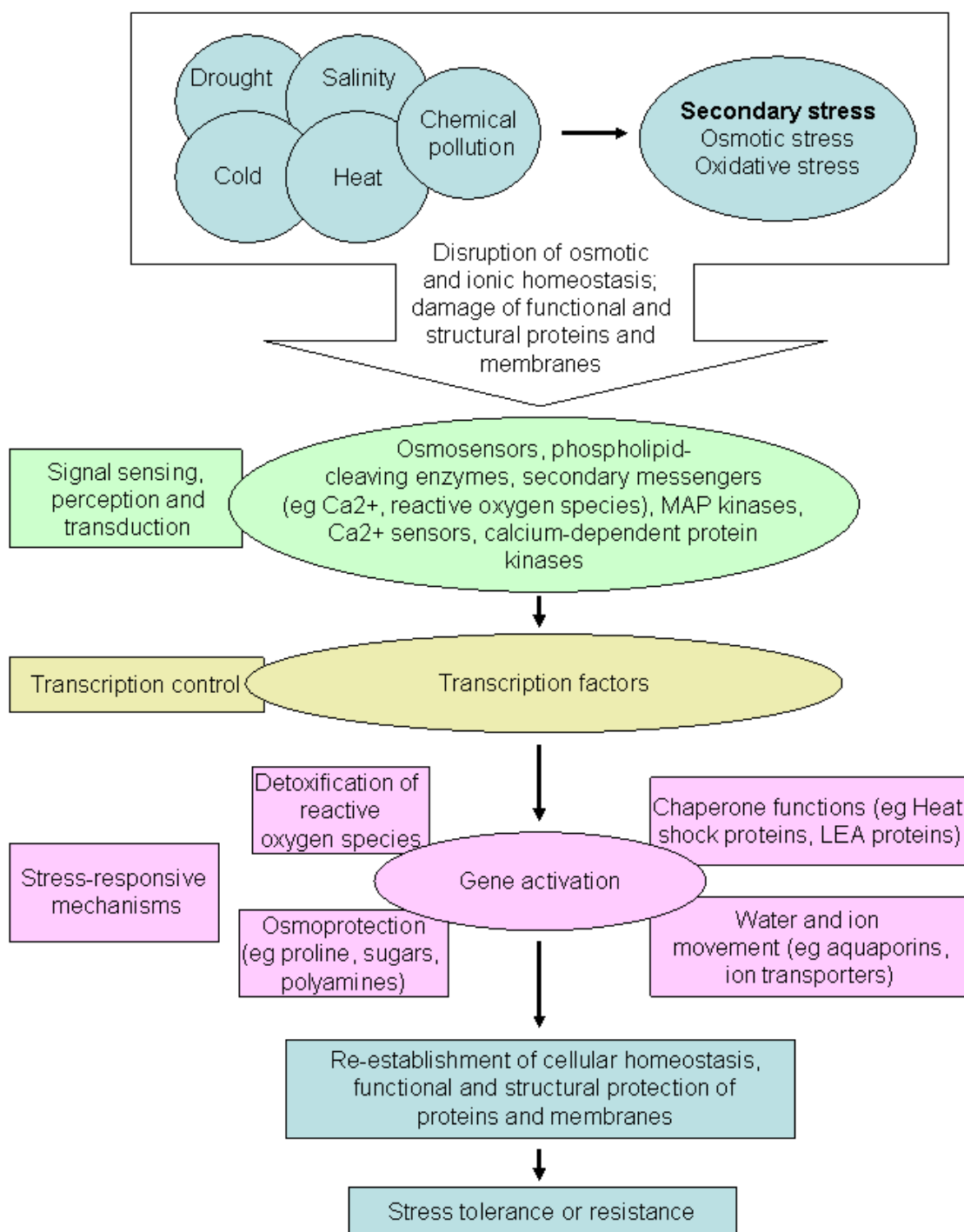
28. Primary abiotic stresses include salinity, cold, heat and chemical pollution. Plants respond to such stresses through an interconnecting series of signalling and transcription controls that ultimately serve to increase the plants' ability to tolerate the initial stress. Such response mechanisms include both biochemical and physiological processes (Figure 3). An

introduction to abiotic stress responses in plants, specifically drought stress, can be found in the RARMP for DIR 071 (OGTR 2007 and references therein ) and will be outlined only briefly here.

29. At a molecular level, there are three broad categories into which plant genes can be classified depending on their role in abiotic stress responses (Wang et al. 2003; Vinocur & Altman 2005). These are:

- Signal sensing, perception and transduction (eg Ca<sup>+</sup> signalling)
- Transcriptional control ( eg transcription factors)
- Stress tolerance response mechanisms in terms of end functions including detoxification, osmoprotection, chaperone function and water and ion movement.

30. Evidence of cross tolerance to different abiotic stresses has led researchers to conclude that the signalling pathways for abiotic stress tolerance are not strictly isolated (Yamaguchi-Shinozaki & Shinozaki 2006). This is supported by the finding that transcript levels of some genes are altered by several different abiotic stressors, with some transcript levels altered by three different abiotic stressors (drought, cold and salinity) (Mantri et al. 2007). Cross tolerance between drought and highly saline soils has been reported to be greater than cross tolerance between cold and highly saline soils (Seki et al. 2002). The DREB2 family of proteins, for example, provides tolerance to both saline soils and drought (Vij & Tyagi 2007).



**Figure 3. The abiotic stress tolerance response process in plants.**

Redrawn and modified from Current Opinion in Biotechnology, Volume 16, Vinocur B. and Altman A., Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations, Page no. 124, Copyright (2005), with permission from Elsevier. Abbreviations: MAP, mitogen activated protein; LEA, late embryogenesis abundant

### **5.2.1 The introduced *AtAVP1* gene, for improved salinity, drought and low phosphorus tolerance, and its encoded protein**

31. Twelve GM wheat lines and twelve GM barley lines contain the *AtAVP1* gene derived from *Arabidopsis* (Group 1). *AtAVP1* encodes an H<sup>+</sup>-translocating pyrophosphatase which appears to be primarily localised to the tonoplast in *Arabidopsis thaliana* (Gaxiola et al. 1999). The *AtAVP1* protein is responsible for the movement of protons (H<sup>+</sup>) into the vacuoles of plant cells from the cytoplasm. This movement of protons provides an electrochemical potential difference in H<sup>+</sup>, which can be used by other tonoplast proteins, such as the Na<sup>+</sup>/H<sup>+</sup> antiporter AtNHX1, to transport Na<sup>+</sup> across the vacuolar membrane. In the case of AtNHX1, the movement of Na<sup>+</sup> into the vacuole appears to be an important strategy which plants employ to prevent toxic build-up of Na<sup>+</sup> in the cytoplasm.

32. In *Arabidopsis*, over-expression of *AtAVP1* has led to improved salinity and drought tolerance (Gaxiola et al. 2001) and in tomato, expression of *AtAVP1* resulted in increased root biomass and enhanced recovery of plants from an episode of soil water deficit stress (Park et al. 2005). More recently, it has been shown that when *AtAVP1* was expressed in *Arabidopsis*, tomato and rice plants, the GM plants significantly outperformed non-GM plants when challenged with limited phosphorus (Yang et al. 2007). Subsequently, *AtAVP1* has been found to play a role in auxin transport and hence in auxin-dependent development (Li et al. 2005). Over-expression of *AtAVP1* resulted in increased cell division at the onset of organ formation, hyperplasia, and increased auxin transport, which resulted in larger and more robust plants.

### **5.2.2 The introduced aminotransferase gene, for enhanced nitrogen utilisation efficiency and its encoded protein**

33. Nitrogen use efficiency (NUE) is an important factor in crop plant productivity and nitrogen based fertilizers are used extensively in modern agriculture, including wheat and barley. A general outline of this topic is provided in the RARMP for DIR 094 and will not be discussed further here (OGTR 2009b).

34. Five lines of GM wheat and five lines of GM barley containing an aminotransferase gene for NUE from barley are proposed for release (Group 2). The identity of this gene and associated reference material has been declared CCI. When the gene was expressed in canola and rice under the control of root specific promoters *btg26* or *Ant1*, an increase in both plant biomass and yield upon application of exogenous nitrogen was observed. The phenotype has been shown to be consistent in both dicots and monocots, so a similar phenotype would be expected in the GM wheat and barley lines.

### **5.2.3 The introduced transcription factor genes, for improved salinity, drought and cold tolerance, and the encoded proteins**

35. The genes in group 3 have been cloned from cDNA libraries that were prepared from flower parts or early grain collected from maize, wheat and barley plants subjected to drought, drought/heat or cold/frost stresses. They belong to a number of different families of transcription factor and are potentially involved in responses to drought and/or cold stress by modulating expression of genes involved in downstream mechanisms such as protection of cellular membranes, correct protein folding, chloroplast integrity, stomata opening and drought induced sterility (Figure 3).

36. Research has shown that constitutive or drought inducible up-regulation of genes encoding members of these families of transcription factor can increase the drought as well as cold/frost tolerance of different plants (DREBs/CBFs: Chen et al. 2007; Zhao et al. 2007; Chen et al. 2008; Gutha & Reddy 2008; Wang et al. 2008).

### **Group 3A transcription factors**

37. Five of the genes used to transform wheat and barley belong to a class of transcription factors whose members are found across a range of plants and are generally involved in responses to abiotic stressors such as water and light. Genes belonging to this family are reported to be drought inducible and possibly confer protection from pathogens under drought stress. Further details about the introduced genes have been declared CCI.

#### **DREB**

38. GM wheat and barley lines will contain one of three *DREB* (Dehydration Responsive Element Binding) transcription factor genes isolated from wheat or corn: *TaDREB2*, *TaDREB3* and *ZmDREB2*. The DREB genes encode drought responsive transcription factor proteins that contain an APETALA2 DNA binding domain (AP2) (Lopato et al. 2006) and play a major role in abiotic and biotic stress tolerance (Yamaguchi-Shinozaki & Shinozaki 2006; Agarwal et al. 2006). DREBs belong to the ERF (Ethylene Response Factor) family of transcription factors (see below) consisting of two subclasses, DREB1/CBF and DREB2 that are induced by cold and dehydration, respectively (see review by Agarwal et al. 2006).

39. *TaDREB3* has 77.7% amino acid sequence identity to a cold induced CBF (C-repeat binding factor) transcription factor (Lopato et al. 2006), while DREB2 proteins have no significant sequence similarity to CBF/DREB1 proteins, except for the presence of NLS (nuclear localisation signal) and AP2 domains. DREB2 genes are induced by dehydration and salt stress, but not cold stress.

40. Both the *TaDREB2* and *TaDREB3* genes are expressed at low levels in wheat plants grown under normal culture conditions, with a higher level of *TaDREB2* expression in seedlings. However, the *TaDREB2* gene was expressed at the highest levels in wounded leaves (Lopato et al. 2006).

41. *Arabidopsis* plants modified to over-express a constitutively active form of their *DREB2A* gene were drought tolerant (unpublished data of Y. Sakuma, cited in Yamaguchi-Shinozaki & Shinozaki 2006).

### **Group 3C transcription factors**

42. GM wheat and barley lines will contain one of three genes belonging to a major class of transcription factors involved in abiotic and biotic stress. *DROUGHT4* was isolated from a drought/high temperature gene library and is expected to be a regulator of drought/frost tolerance. *COLD 1* and *COLD2* were isolated from a cold/frost gene library and *COLD2* is strongly induced by cold. *COLD1* is not induced by cold but can activate the cold/drought/salt inducible *PROMOTER2* (Table 2). Further details about the introduced genes have been declared CCI.

### **Group 3D transcription factors**

43. GM wheat and barley lines will contain one of two genes which have homology to a transcription factor that is thought to be involved in regulating the metabolism of lipid and/or cell wall components. When over-expressed in *Arabidopsis*, an increase in cuticular wax and enhanced drought tolerance and recovery was observed, probably related to reduced stomatal density. Further details about the introduced genes have been declared CCI.

#### **CBF**

44. GM wheat and barley lines will contain one of two CBF transcription factors isolated from maize. CBF transcription factors belong to the AP2/EREBP (APETALA2/Ethylene-Responsive Element Binding Protein) family of proteins, and bind to the C-repeat or dehydration response element (DRE) in the promoters of genes that are turned on in response to low temperatures and/or water deficit. Tomato plants expressing the *Arabidopsis* CBF1

gene show enhanced resistance to cold and oxidative stresses (Hsieh et al. 2002) and when the DREB1A (CBF3) gene from *Arabidopsis* was over-expressed in wheat under the control of a stress-inducible promoter, the plants demonstrated substantial resistance to water stress (Pellegrineschi et al. 2004). This suggests a conserved signalling and response mechanism between dicots and monocots.

45. ZmCBF1 and ZmCBF2 belong to the DREB1 class of transcription factors which are induced early upon exposure to abiotic stresses such as cold, drought, and salt. Both genes have been shown to be rapidly induced by cold (U.S. Pat. No. 7,317,141), with peak expression occurring 4 hours after imposition of cold stress. ZmCBF1 was also shown to be induced within 24 hours of withholding water. In both tests, no ZmCBF1 expression was observed prior to the stress treatment.

46. It has been observed that constitutive expression of CBF1 in tomato (Hsieh et al. 2002) or DREB1A in *Arabidopsis* (Kasuga et al. 1999) is associated with a dwarf phenotype. Expression under an inducible promoter overcomes this dwarfing, which is thought to result from an effect on gibberellic acid biosynthesis.

### **Group 3F transcription factors**

47. GM wheat and barley lines will contain one of five drought inducible genes encoding Group 3F transcription factors. *DROUGHT7 – DROUGHT11* bind to, and strongly activate, *PROMOTER2*, a relatively strong drought, cold and salt-inducible promoter (Table 2). There is evidence that Group 3F transcription factors may also be involved in biotic stress tolerance including resistance to pathogens. Further details about the introduced genes have been declared CCI.

### **Group 3G transcription factors**

48. GM wheat and barley lines will contain one of five genes that encode Group 3G transcription factors, proteins which control numerous physiological and developmental processes. Members of this family have been shown to be inducible by low temperatures, salt or drought stress and may also be involved in pathogen defence. Further details about the introduced genes have been declared CCI.

## **5.2.4 The introduced protein kinase genes, for improved drought and cold tolerance, and associated proteins**

49. Plants have evolved many interconnected strategies that enable them to survive environmental stresses such as drought (Figure 3). One of these strategies is signalling pathways that cause a change in phosphorylation status of transcription factors and other stress related proteins, which in turn switch on the expression of different genes that encode stress response proteins.

50. The kinases listed in Table 1 (Group 4) are involved or potentially involved in signalling pathways in plants in response to drought, salt and/or cold stress. These genes can regulate or potentially regulate a co-ordinated response to drought/salt/cold stress by modulation of activity of transcription factors, which in turn regulate expression of downstream genes that are involved in protection of cellular membranes, correct protein folding, chloroplast integrity, stomata opening, drought induced sterility, etc.

51. Research has shown that constitutive or drought inducible up-regulation of some stress related protein kinases can increase the drought tolerance of different plants (Kovtun et al. 2000; Xiong & Yang 2003; Umezawa et al. 2004; Ma & Wu 2007; Wohlbach et al. 2008).

### **5.2.5 The introduced *HvZIP7* gene, for zinc accumulation, and the encoded protein**

52. Up to 3 lines of GM barley will contain *HvZIP7*. *HvZIP7* was isolated from barley, and transcript profiles and sub-cellular localisation suggest a role in Zn translocation (Tiong et al. 2009).

53. Zinc is an essential micronutrient for plants and humans, and regions of the world having zinc deficient soils are also generally characterised by widespread Zn deficiency in humans (Cakmak 2008). Strategies to alleviate micronutrient deficiencies in humans include agronomic biofortification via soil fertilisation and genetic biofortification, whereby plants are bred with increased concentrations of Fe and Zn in the edible parts, particularly grain.

54. Transgenic approaches to improving Zn content of grain have included targeting the cation uptake and transport systems (see review by Palmgren et al. 2008). Metal transporters of the ZIP (Zinc Responsive Transporter/Iron Responsive Transporter related Protein) family are thought to be the key uptake systems controlling zinc influx into the cytoplasm (reviewed by Gueriot 2000). ZIP transporters were originally isolated from *Arabidopsis* and since then representatives of the family have also been identified in other plant species (eg soybean, rice and barley) and from the other eukaryotic kingdoms – animals, protists and fungi (Gueriot 2000). Members of the ZIP gene family identified in plants are capable of transporting a number of metal cations including Cd, Fe, Mn and Zn (Yang et al. 2009). Some cations such as Cd lack their own specific transport systems and will compete for transport with other ions eg Zn. Thus, under conditions of high soil zinc, there is evidence that Cd translocation (and Cd grain content) will decrease (Akay & Koleli 2007).

55. A number of the cation-transporting ZIP proteins are potentially involved in zinc transport, and may or may not be induced in response to deficiency. In *Arabidopsis*, for example, a number of ZIP proteins have been identified: *ZIP1* and *ZIP3* are expressed in roots in response to Zn deficiency, suggesting that they transport Zn from soil to plant, while *ZIP4* is expressed in both roots and shoots, suggesting that it transports Zn intracellularly or between plant tissues (Grotz et al. 1998; Gueriot 2000). Functional transporters of Zn in rice have also been reported (Ishimaru et al. 2005; Ishimaru et al. 2007); *OsZIP4* is localised to the plasma membrane and regulated by the zinc status of the plant, being highly induced by zinc deficiency.

### **5.2.6 The introduced *PpENA1*, *AtCIPK16* and *ScNHA1* genes, for salt tolerance, and the encoded proteins**

#### ***PpENA1***

56. Up to five barley lines will contain *PpENA1*, a gene encoding a Na<sup>+</sup> pumping ATPase derived from moss (*Physcomitrella patens*).

57. High cytosolic concentrations of Na<sup>+</sup> inhibit plant growth and development and higher plants use membrane bound transporters, which drive the efflux of Na<sup>+</sup> or partition Na<sup>+</sup> ions from the cytosol, to maintain low cytosolic concentrations of Na<sup>+</sup>. In the moss *Physcomitrella patens* the *PpENA1* gene encodes a Na<sup>+</sup> efflux ATPase (ENA) which has been shown to confer salinity tolerance under conditions of moderate (300 mM) salt stress (Lunde et al. 2007). Heterologous expression in yeast shows that *PpENA1* acts as a Na<sup>+</sup> pump, rescuing salt-sensitive yeast strains deficient in Na<sup>+</sup> and K<sup>+</sup> efflux (Benito & Rodriguez-Navarro 2003).

#### ***AtCIPK16***

58. Up to eight barley lines will contain *AtCIPK16*, a gene belonging to a family of calcineurin B-like interacting protein kinases (CIPKs) which play a role in regulating plant cell responses to abiotic stress (Luan 2009).

59. During abiotic stress, calcium ( $\text{Ca}^{2+}$ ) is released as an internal messenger and calcineurin B-like proteins (CBLs) interpret the  $\text{Ca}^{2+}$  signal. CBLs recruit the required CIPK to the cell membrane, where the kinase activates necessary transporters and proteins involved in the stress response (Batistic & Kudla 2004; Luan 2009). There are 25 *AtCIPK* genes in *Arabidopsis thaliana* (Batistic & Kudla 2004) and much is known about the function of AtCIPK24 protein, also known as AtSOS2. Under salt stress, AtCBL4 (AtSOS3) recruits AtCIPK24 to the plasma membrane, where it activates the  $\text{Na}^+/\text{H}^+$  antiporter AtSOS1 to remove  $\text{Na}^+$  from the cell (Qiu et al. 2002; Qiu et al. 2003). Orthologs of CIPK24 have been identified in many plant species (Wang et al. 2004; Martinez-Atienza et al. 2007; Yu et al. 2007).

60. To date, there is little published information on *AtCIPK16*, but there is some evidence that the AtCIPK16 protein interacts with CBL1 and CBL3 (Lee et al. 2007), which have been identified as being up regulated under salt stress (Zimmermann et al. 2004).

### ***ScNHA1***

61. Up to two barley lines will contain the *ScNHA1* gene encoding a  $\text{Na}^+/\text{H}^+$  antiporter (*NHA*) derived from yeast. The gene belongs to the *CPAI* (cation/proton antiporter) family of  $\text{Na}^+/\text{H}^+$  antiporters in eukaryotes and resides in the subfamily *NHA* which has no plant members (Brett et al. 2005).

62. Deletion of the *ScNHA1* gene was found to cause a loss of salt tolerance in yeast cells, and high sodium and potassium conditions increase the cytoplasmic pH in an *ScNHA1*-dependent manner (Sychrova et al. 1999). From these observations, *ScNHA1* has been suggested to function as a  $\text{Na}^+$ ,  $\text{K}^+/\text{H}^+$  antiporter and to regulate the intracellular pH, and the  $\text{Na}^+$  and  $\text{K}^+$  concentration (Banuelos et al. 1998; Banuelos et al. 2002).

### **5.2.7 The antibiotic resistance marker gene hpt and the encoded protein**

63. The GM wheat lines from Categories 3 and 4 and all of the GM barley lines contain the *hpt* gene from *E.coli*, which confers resistance to the antibiotic hygromycin B.

64. The *hpt* gene encodes the hygromycin phosphotransferase (HPT) enzyme which catalyses the phosphorylation of the 4-hydroxy group on the hyosamine moiety, thereby inactivating hygromycin (Rao et al. 1983) and preventing it from killing cells producing HPT. The *hpt* gene was used as a selectable marker gene in the early laboratory stages of development of the plants to enable selection of plant cells containing the desired genetic modification.

65. The *hpt* gene has been used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004). As discussed in the RARMP for DIR 073/2007 and more recently DIR 077/2007 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1>), the use of *hpt*, or other HPT encoding genes, as marker genes in GM plants has been assessed as not posing a risk to human health and safety or the environment. The HPT protein is easily digested by simulated gastric juices and the amino acid sequence contains no similarities to known allergens (Lu et al. 2007). The European Food Safety Authority concluded that inclusion of the *hpt* gene in GM plants would not significantly affect the health of humans or animals (EFSA 2004).

### **5.2.8 Toxicity/allergenicity of the protein/end products encoded by the introduced genes**

66. All of the genes introduced into the GM plants were isolated from wheat, barley or maize, with the exception of four genes isolated from moss, yeast, and thale cress. Although wheat and barley contain a number of anti-nutritional factors and allergens that, in extreme cases, may have a toxic effect (OGTR 2008a), the proteins encoded by the introduced genes

are not expected to have any toxic or allergenic effects as they are widely consumed as both human food and animal feed without any adverse effects. The majority of the genes are derived from wheat and barley, so for these genes the encoded proteins are already present in the GM plants, albeit at possibly higher levels and with varied expression patterns. Homologues of all of the genes and encoded proteins also occur naturally in a wide range of organisms, including animals, bacteria, yeast and plants consumed by people and animals (see discussion in Section 5.2). On this basis, people and other organisms have a long history of exposure to the introduced genes.

67. It is possible that the GM wheat and barley plants containing the aminotransferase gene will produce altered levels of some metabolites in both below and above ground tissues. These metabolites are ubiquitous in nature and consumed widely by humans.

68. For GM barley plants containing the *HvZIP* gene, the aim of the modification is to increase Zn content in the grain for the purpose of supplementing zinc deficient diets. The levels measured by the applicant in GM barley grain range from 40 mg/kg in low zinc soil to 118mg/kg in high zinc soils. The applicant has also measured levels of other metal cations including Fe, Mn and Cu in grains of the GM barley and found there was no increase relative to non-GM barley grains. Cd was not detected in the grain when GM barley was grown in non contaminated soils (information supplied by applicant).

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

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

### 5.3 The regulatory sequences

#### 5.3.1 Regulatory sequences for expression of the introduced gene for enhanced nutrient utilisation efficiency

71. Promoters are DNA sequences that are required to allow RNA polymerase to bind and initiate correct transcription. Table 2 shows the regulatory sequences used to control expression of the introduced genes in the GM wheat and barley lines. Two of the promoters are constitutive (*35S* and *Ubi1*) and direct the genes to be expressed in most plant tissues and throughout the plant lifecycle. The remaining promoters are root specific or inducible by abiotic stress such as drought, cold or salt.

72. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. The mRNA termination region for the introduced genes in the GM wheat and barley is derived from the *nos* gene mRNA termination region from *A. tumefaciens*.

#### 5.3.2 Regulatory sequences for the expression of the selectable marker gene

73. Expression of the *hpt* gene in GM wheat and barley plants is controlled by the *35S* gene promoter from cauliflower mosaic virus (CaMV) (Odell et al. 1985) and the *35S* mRNA termination region from CaMV.

### 5.4 Method of genetic modification

74. Two different methods were used to generate the GM wheat and barley lines for the proposed release – biolistic transformation (wheat) or *A. tumefaciens*-mediated transformation (barley). Biolistic transformation (Pellegrineschi et al. 2002) involved coating very small gold

particles with two transformation constructs, one containing a plant selectable marker and a second containing the gene of interest. The particles were then ‘shot’ into intact immature embryos from *T. aestivum* cultivar Bobwhite, Drysdale or Frame. Genetically modified plant tissues were recovered by survival on tissue culture media containing the selective agent hygromycin.

75. *A. tumefaciens*-mediated transformation was used to generate the GM barley lines. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants (Van Larebeke et al. 1974), through transfer of DNA (transfer-DNA or T-DNA, located between specific border sequences on a resident plasmid) from *A. tumefaciens*.

76. Disarmed *Agrobacterium* strains have been constructed specifically to facilitate genetic modification of plants with desired genes without causing disease. The disarmed strains used for genetic modification do not contain the genes responsible for the overproduction of auxin and cytokinin (*iaaM*, *iaaH* and *ipt*), which are required for tumour induction and rapid callus growth (Klee & Rogers 1989). *Agrobacterium* plasmid vectors used to transfer T-DNAs contain well characterised DNA segments required for their replication and selection in bacteria, and for transfer of T-DNA from *Agrobacterium* and its integration into the plant cell genome (Bevan 1984; Wang et al. 1984).

77. To generate the GM barley lines in the current application, immature barley embryos were infected with *A. tumefaciens* carrying the gene construct (Tingay et al. 1997; Matthews et al. 2001). Following the co-cultivation step of the transformation, the barley calli were cultured on media containing the antibiotic Timentin to limit the growth of *A. tumefaciens*.

78. Both biolistic and *Agrobacterium*-mediated transformation have been widely used in Australia and overseas for introducing new genes into plants and are not known to cause any adverse effects on human health and safety or the environment.

## 5.5 Characterisation of the GMOs

### 5.5.1 Stability and molecular characterisation

79. The applicant states that all genes to be introduced into wheat and barley have been sequenced. As the project is in its early stages, further molecular characterisation of the different GM wheat and barley lines has been carried out only to a limited extent:

- *AtAVP1*: the number of copies of the introduced gene present in each line is unknown, but the applicant intends, when possible, to characterise the lines using Southern blot hybridisation.
- The aminotransferase gene for NUE: The copy number and insertion site(s) are not known, but the inserted genes were inherited over three generations of selfing as dominant Mendelian traits.
- *Transcription factors and protein kinases*: Levels of expression in selected wheat and barley lines were analysed by northern blot hybridisation as well as quantitative PCR (qPCR). Insertion of multiple copies has been verified for some genes by Southern blot analysis of several generations, but not for all.
- *HvZIP*: barley lines carrying a single copy have been taken through to T3 or T4 generations. Levels of expression have been analysed by qPCR.
- *PpENAI*: All lines have a single insert. Expression levels of the introduced gene have been established by qPCR.
- *AtCIPK16* and *ScNHA1*: no information is currently available relating to copy number or expression levels and these experiments are underway.

80. The number of gene copies integrated into a plant genome varies depending on the method of introduction. Copy number of an introduced gene following biolistic transformation usually varies from 1 to more than 20 (Pawlowski & Somers 1996), whereas 1-3 copies of introduced genes are commonly seen in GM lines obtained through *Agrobacterium*-mediated transformation (Arencibia et al. 1998). The genomic locations of the introduced DNA has not been characterised for any of the introduced genes.

81. The parent wheat and barley lines used for transformation have stable genotypes. Prior to the proposed release, the transgenes will have been inherited over two to three generations of selfing and over the course of the trial the applicant proposes to confirm the stability of the genotype in each generation using PCR

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

#### *AtAVPI*

82. The aim of the modification is to increase the salt tolerance of the GM wheat and barley lines. Results of glasshouse experiments suggest that the GM wheat and barley plants are more tolerant to salinity stress than non-GM plants and are consequently larger and more robust. The applicant has over-expressed the *AtAVPI* gene in barley ('Golden Promise') via the CaMV 35S promoter. For the three independent lines tested, the plants were shown to have increased fresh weight and greater tolerance than control plants when grown in hydroponic solution containing 200mM NaCl for 14 days. Following this, further transgenic lines were produced in barley and wheat under constitutive (maize Ubiquitin) and inducible (Rab17) promoters. As yet, these lines have not been characterised.

#### *The aminotransferase gene for NUE:*

83. The purpose of the modification is to increase biomass and yield in the GM wheat and barley plants compared with controls when grown under field conditions. Traits that will be measured include; heading date, plant height and other growth characteristics as well as yield traits. The lines under development by the applicant are at an early stage and have not yet been assayed for the expected phenotype under controlled conditions. However, as the phenotype has been shown to be consistent in dicots and monocots, the phenotype demonstrated for canola and rice (see section 5.2.2) would also be expected for the GM wheat and barley.

#### *Transcription factors*

84. The aim of the modification in each case is to increase the drought and/or cold tolerance of the GM wheat and barley lines. The applicants have not yet characterised the phenotype for all the lines in this category. Preliminary experiments in the glasshouse using GM wheat and barley lines containing *TaDREB2* and *TaDREB3* under control of a constitutive promoter indicated that some of the lines had increased water use efficiency (WUE). However, under well-watered conditions, many of these GM plants showed a semi-dwarfed or dwarfed phenotype and a delay in flowering compared with non-GM lines. When drought inducible promoters (eg ZmRab17) were used to control expression of the introduced genes, the development of such phenotypes was suppressed in barley and abolished in wheat. Constitutive expression of *CELLWALL1* gave a potentially useful developmental phenotype, but no pronounced effect was observed in lines containing *DROUGHT7*.

85. Glasshouse experiments were also conducted investigating the effect of limited water availability on growth of GM wheat plants over-expressing *TaDREB3* under a drought inducible promoter. For a number of lines, recovery after drought stress was improved in the GM plants, though no difference was observed before and during stress. The applicants also reported an improvement in cold/frost tolerance in GM barley plants constitutively expressing *DREB2* or *DREB3* under both mild and stringent frost conditions.

### *Protein kinases*

86. The aim of the modification is to increase the drought tolerance of the GM wheat and barley lines. Phenotypic characterisation of the GM plants is limited, but preliminary experiments in the growth room indicated that GM wheat plants with constitutively upregulated expression of *KINASE1* have improved recovery from prolonged drought. GM wheat lines with constitutively upregulated *KINASE1* or *KINASE2* did not show any pronounced developmental phenotype under well-watered conditions, but *KINASE3* plants had a semi-dwarfed or dwarfed phenotype with a short delay in flowering and darker green leaves compared with non-GM plants.

### *ZIP7*

87. The aim of the modification is to increase the zinc content in grain for GM barley plants over-expressing the barley *ZIP7* gene. Zinc content of vegetative plant parts and grain was measured by Inductive Coupled Plasma Emission Spectrometer (ICP-OES) analysis and showed that the Zn content of the GM barley grain was increased by up to 50% at low Zn supply and doubled under high zinc supply. There was no effect on Fe, Mn or Cu content.

### *PpENAI*

88. The aim of the genetic modification is to increase the salinity tolerance of the GM barley plants. There is currently no information regarding the salinity tolerance status of the GM barley lines expressing *PpENAI*.

### *AtCIPK16*

89. The aim of the genetic modification is to increase the salinity tolerance of the GM barley plants. The phenotype for GM barley expressing *AtCIPK16* has not been described but glasshouse experiments are currently underway (information provided by applicant).

## **Section 6 The receiving environment**

90. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs 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 2009a).

91. The size, locations and duration of the proposed release are outlined in Section 3.2. The proposed dealings involve planting at two sites in South Australia and one site in Western Australia. Each trial will be conducted within a fenced area of 50 m x 50 m with a lockable gate.

92. Site 1 is located close to University of Adelaide's Waite Campus at O'Halloran Hill. The site is fully fenced and there is a caretaker living on the site, which is accessible via a single locked gate.

93. Site 2 is part of a commercial farming operation located in a dryland agricultural area in the Pinery region, approximately 70 km north of Adelaide. Access to the site is via a private road and a gate.

94. Site 3 is located near Corrigin in a key wheat growing area of the WA wheat belt but is currently not used for broad acre cropping due to salinity problems. The site is in a single paddock accessible only via a farm road running past the owner's house.

## 6.1 Relevant abiotic factors

95. The abiotic factors relevant to the growth and distribution of commercial wheat and barley can be found in *The Biology of Triticum aestivum L. (bread wheat)* and *The Biology of Hordeum vulgare L. (barley)* (OGTR 2008a; OGTR 2008b).

96. The proposed release site 1 is situated within a farm owned by the University of Adelaide and is typical of rain-fed, wheat production environments in South Australia.

97. Release site 2 is in a dryland agricultural area expected to be useful for assessment of GM lines that are expected to show increased tolerance to drought.

98. Release site 3 is an area where dryland salinity is a problem and plants at this site are likely to grow more slowly and develop less biomass than under more benign conditions. The site receives reliable rainfall and is located in a relatively flat low lying area not subject to flooding. The site is occasionally subject to frost.

99. The three sites have a typical temperate climate (as defined by the Koeppen classification system used by the Australian Bureau of Meteorology, <http://www.bom.gov.au/lam/climate/levelthree/ausclim/koepen2.htm>). The rainfall and temperature statistics for the nearest weather station relevant to each site are given in Table 3.

**Table 3. Climatic data for South Australia (Sites 1 and 2) and Western Australia (Site 3)**

	Adelaide (Waite)	Adelaide (Kapunda)	WA (Corrigin)
Average daily max/min temperature (winter)	14.8°C /8.1 °C	14.2 °C /5.8 °C	16.0 °C /5.2 °C
Average daily max/min temperature (summer)	27.0 °C /15.8 °C	28.9 °C /14.1°C	31.5 °C /15.2 °C
Average monthly rainfall (winter)	79.9 mm	59.8 mm	56.5 mm
Average monthly rainfall (summer)	25.6 mm	22.0 mm	15.2 mm

**Source:** <<http://www.bom.gov.au>>. The means for monthly mean temperatures and rainfall were collected over 70 years and were averaged for all months of a season to obtain the reported data (winter: June – August ; summer: December-February).

## 6.2 Relevant biotic factors

100. The biotic factors relating to the growth and distribution of commercial wheat and barley in Australia are discussed in the reference documents, *The Biology of Triticum aestivum L.em Thell. (bread wheat)* and *The Biology of Hordeum vulgare L. (barley)* (OGTR 2008a; OGTR 2008b). In addition, the following points are of particular relevance to this release:

Site 1:

- The University of Adelaide has used ‘Glenthorne Farm’ in the past for wheat and barley production and for small scale field trials as part of the South Australian Barley Improvement Program. A GM wheat and barley trial (DIR 077/2007) was previously located within the 50m x 50 m fenced area of the proposed release and is approved for release from June 2008 and June 2011.<sup>9</sup> However, due to adverse weather conditions the GM wheat and barley from the first year of the release were removed before flowering. Only GM barley was planted at the same location in June 2009 and harvested in January 2010. The applicant has indicated there will be no further planting of GMOs at this site

<sup>9</sup> New information provided by the applicant. The consultation RARMP originally stated that the DIR077 trial site was located 1.5 km from the proposed release.

under licence DIR077. Apart from these trials, no other wheat or barley has been planted at this site since 2000.

- There are two small scale farming operations in the immediate area; one is located 2.5km away and has previously been planted to wheat and barley for hay production. The other is located 1.5km away and barley was grown there for seed in 2005, but no crops were planted between 2007 and 2009.

Site 2:

- ‘Karawatha’ is part of a commercial farming operation and cropping includes wheat and barley. In 2008 the 42 ha area of the paddock was planted with barley and in 2007 with wheat.
- The proposed trial location has most recently been sown to oaten hay and treated with herbicide after the hay was cropped in late 2009. Subsequent plantings in 2010 are expected to be lentils or chick pea.
- The farm will be used in 2010 for field trials under the South Australian Barley Improvement Program, The location of these trials expected to be at least 1.1 km away from the proposed GM release site.

Site 3:

- In 2008 the land was fallow and in 2009 the land was sown to salt bush with barley planted between the rows. The barley did not progress past tillering due to salt and drought.
- Intergrain (a WA wheat breeding company) will be conducting non-GM wheat trials in 2010 on a site previously used for GM wheat trials under DIR 053 and 1.5 km distant from the proposed location.

All sites:

- Invertebrates, vertebrates and microorganisms could be exposed to the introduced genes, their encoded proteins and end products. In particular, rodents and native birds may visit the proposed release sites.

### 6.3 Relevant agricultural practices

101. It is anticipated that the agronomic practices used by the applicant for the cultivation of the GM wheat and barley will not differ significantly from conventional practices, with the exception that the applicant proposes to harvest by hand or using a machine such as a custom-built plot harvester. Conventional cultivation practices for wheat and barley are discussed in more detail in *The Biology of Triticum aestivum L. em Thell. (bread wheat)* and *The Biology of Hordeum vulgare L. (barley)* (OGTR 2008a; OGTR 2008b).

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

103. The parental wheat and barley cultivars are spring cultivars. In Australia, spring wheat and barley varieties are commonly grown as a winter crop and are usually planted in late autumn or early winter, depending on variety and location. Harvest of the mature grain generally occurs in early summer.

104. The applicant anticipates planting the trial in June 2010 and the trial is proposed to take place over five growing seasons.

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 to encourage germination of any fallen seed, then treated with herbicide to destroy volunteers.

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

106. The GM wheat and barley lines proposed for release will be grown together at the field trial sites. Barley and wheat are not known to hybridise with each other under natural conditions (OGTR 2008a; OGTR 2008b).

107. The applicant proposes to maintain a 500 m zone in which there is no cultivation of wheat or barley breeding lines around the site of the trial for the full duration of the trial. A 200 m zone clear of all other wheat and barley cultivation will also be maintained.

108. 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* are known to occur in Australia. In addition, wild barley, *H. vulgare* ssp. *spontaneum*, is not known to be present in Australia.

109. Wheat is sexually compatible with many species within the genus *Triticum*. Wheat can hybridise with *Hordeum marinum* but only with substantial human intervention (Pershina 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 2008b).

110. *Hordeum vulgare* ssp. *spontaneum* (wild barley) is the only species that can cross with cultivated barley under natural conditions (Nevo 1992; OGTR 2008a). As mentioned above, wild barley is not found in Australia (OGTR 2008a).

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

111. The majority of the introduced genes for abiotic stress tolerance and increased zinc uptake were isolated from barley, wheat and corn, all of which are already widespread and prevalent in the environment and consumed by humans and animals. The remaining genes were isolated from moss, yeast and thale cress, which are also widespread in the environment. In addition, homologues of most of the genes and encoded proteins occur naturally in animals, plants, yeast and bacteria.

112. The *hpt* gene is derived from the common gut bacteria *E. coli* which is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997). As such, it is expected humans routinely encounter the encoded protein through contact with plants and food.

113. Apart from the 35S promoter, all the promoters used to drive expression of the introduced genes have been obtained from plants (rice, maize, wheat or barley). These crops have been safely consumed by humans and animals for centuries.

114. The 35S promoter and expression termination sequences were originally isolated from CaMV and the soil bacterium *A. tumefaciens*. Although CaMV and *A. tumefaciens* are plant pathogens, the regulatory sequences comprise only a small part of their total genomes and are not capable in themselves of causing disease. CaMV is a virus which infects many human food crops and would be commonly consumed in food. *A. tumefaciens* is also widespread in the environment. No proteins are encoded by the introduced regulatory elements.

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

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

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

115. There has been no release of these GM wheat and barley lines in Australia.

116. The work in the present application has developed from: NLRD 1038/2003 Functional analysis of genes in cereals; NLRD 584/2003 Embryo and endosperm development in wheat and barley; NLRD 1403/2004 Molecular Analysis of zinc deficiency responsive genes in plants; NLRD 2367/2007 Large scale analyses of gene function; NLRD 3017/2009 Improving nitrogen use efficiency in cereal crops.

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

- DIR 053/2004 was issued to Grain Biotech for GM salt tolerant wheat on an area of 0.45 ha in WA
- DIR 054/2004 was issued to CSIRO for GM wheat with altered starch content on 0.412 ha in the ACT
- DIR 071/2006 was issued to Department of Primary Industries – Victoria 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 SA
- DIR 080/2007 was issued to Department of Primary Industries – Victoria 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 1.0 ha in the ACT
- DIR 093 was issued to CSIRO for GM wheat and barley with altered grain starch composition on 1.0 ha in the ACT
- DIR 094 was issued to CSIRO for GM wheat and barley with enhanced nutrient utilisation efficiency on 1 ha 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 New South Wales and Western Australia.
- DIR 100 was issued to CSIRO for GM wheat with enhanced carbon assimilation in drought and heat prone environments on 0.1 ha in Queensland.

118. 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). Five field trials of different types of GM barley also occurred under GMAC. They ranged in size from 400-2940 plants: PR88 (1998), PR92 (1998), PR106 (1998), PR88X (1999) and PR139 (2000).

119. 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**

120. 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.

121. The applicant does not intend any material from the GM wheat or barley lines proposed for release to be used in animal feed or human food. All genetically modified foods intended for sale in Australia must undergo a safety evaluation by FSANZ. Accordingly, the applicant has not applied to FSANZ to evaluate the GM wheat or barley lines. FSANZ approval would be required before materials or products derived from the GM wheat or barley lines could be sold for human consumption.

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

122. There have been no releases of these GM wheat and barley lines internationally. However, there have been releases of other GM wheat and barley plants in field trials. The traits which have been modified include novel protein production, disease resistance, altered grain properties and herbicide tolerance<sup>10</sup>.

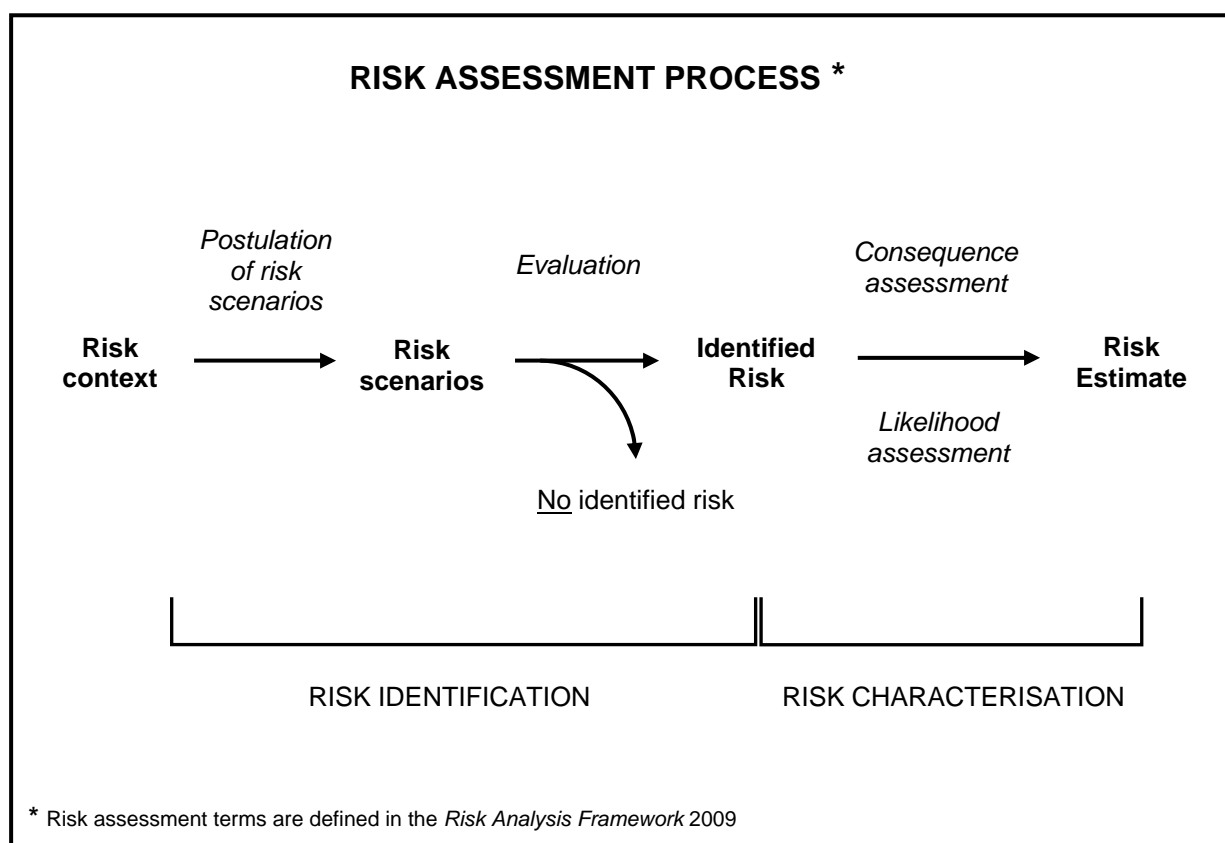
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<sup>10</sup>< <http://www.aphis.usda.gov/brs/status/relday.html>>, <<http://gmoinfo.jrc.ec.europa.eu/>> accessed 10 February 2010.

## Chapter 2 Risk assessment

### Section 1 Introduction

123. 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 4. The risk assessment process**

124. 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).

125. 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.

126. 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 2009a). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

127. 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**

128. 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)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the biotic and abiotic environment at the site(s) of release
- agronomic management practices for the GMOs.

129. Eight risk scenarios were identified and evaluated. These are summarised in Table 4, 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.

130. As discussed in Chapter 1, Section 5.2.7, the GM wheat and barley lines contain the selectable marker gene *hpt*, encoding the HPT protein which confers tolerance to the antibiotic hygromycin. The prevalence of the *hpt* gene in the environment and the lack of evidence for toxicity or allergenicity of the HPT protein to humans and animals have been discussed previously (see Chapter 1, Section 5.2.7). The use of *hpt* has been assessed as not posing a risk to human health or the environment (EFSA 2004; EFSA 2007). Therefore, the potential effects of the *hpt* gene will not be further assessed for this application.

**Table 4. Summary of risk scenarios from dealings with GM wheat and barley.**

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
<b>Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms</b>	1. Exposure to GM plant material containing the introduced genes, encoded proteins 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 encoded proteins and their end products are widespread in the environment and are unlikely to be toxic/allergenic to people or toxic to other organisms.</li> <li>None of the GM wheat material would be used for human food or animal feed as part of this release.</li> <li>The limited scale, short duration and other proposed limits and controls, minimise exposure of people and other organisms to the GM plant material.</li> </ul>
<b>Section 2.2 Spread and persistence of the GM wheat and/or barley plants in the environment</b>	2. Expression of the introduced genes improving the survival of the GM wheat and/or barley plants	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>Many abiotic and biotic factors are expected to restrict the spread and persistence of wheat and barley in the areas proposed for release, for example low intrinsic competitive ability, nutrient availability, pests and diseases.</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; increased 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, including: locating the field trial sites at least 50 m from natural waterways, measures to exclude livestock and control rodent numbers, and transporting materials according to the Regulator's guidelines.</li> </ul>
<b>Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants</b>	4. Expression of the introduced genes or regulatory sequences in other wheat and/or barley plants	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>Pollen-mediated gene transfer in wheat and barley occurs at low rates, and generally over short distances.</li> <li>A 200 m separation between the GM lines proposed for release and other non-GM wheat and barley will restrict pollen-mediated gene flow.</li> <li>The other proposed limits and controls would also minimise gene flow.</li> <li>The toxicity, allergenicity and weediness potential of the GM wheat and barley lines were assessed in Risk scenarios 1-3 and no risks were identified.</li> </ul>

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
	5. Expression of the introduced genes or regulatory sequences in other sexually compatible plants	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>• Pollen-mediated gene transfer in wheat and barley occurs at low rates, and generally over short distances.</li> <li>• The proposed limits and controls (eg the monitoring and inspection zones surrounding the location) would also minimise gene flow.</li> <li>• The toxicity, allergenicity and weediness potential of the GM wheat and barley lines were assessed in Risk scenarios 1-3 and no risks were identified.</li> </ul>
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	6. Expression of the introduced genes or regulatory sequences in other organisms as a result of gene transfer	Weediness; increased 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 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 and/or barley plants resulting from expression, or random insertion, of the introduced genes	Weediness; increased allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>• Unintended, adverse effects, if any, would be minimised by the proposed limits and controls.</li> <li>• Obvious unexpected alterations are likely to have been detected and eliminated during the production and laboratory screening of the GM wheat and barley lines.</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

131. 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).

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

133. A range of organisms may be exposed directly or indirectly to the proteins (and end products) encoded by the introduced genes, and their associated effects. Workers cultivating the wheat and barley would be exposed to all plant parts. Organisms may be exposed directly

to the end products of the introduced proteins through biotic interactions with GM wheat and barley 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 and barley plant parts or degrade them (vertebrates, invertebrates, fungi and/or bacteria).

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

134. Expression of any one of the introduced genes could potentially result in the production of novel toxic or allergenic compounds in the GM wheat and barley lines, or alter the expression of endogenous wheat and barley 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.

135. Non-GM wheat and barley are not known to be toxic to humans or other organisms. However, non-GM wheat and barley flour can produce allergic and autoimmune responses in susceptible individuals on inhalation or ingestion. Ingestion of wheat and barley flour by coeliac disease sufferers will trigger a sensitivity response caused by the prolamin fraction of the storage protein complex, gluten (reviewed in OGTR 2008a; OGTR 2008b). These properties are not expected to be altered in the GM wheat and barley lines proposed for release.

136. No toxicity studies have been performed on the GM wheat and barley plant material or the encoded proteins. However, the majority of the genes were isolated from barley, wheat, and maize, which are already widespread and prevalent in the environment and consumed by humans and animals. The remainder of the genes were isolated from moss, yeast and thale cress, which are also widespread and prevalent in the environment, and encode proteins (or members of classes of proteins) that occur widely in eukaryotes. No information has been found to suggest that the proteins encoded by the introduced genes or their end products are toxic or allergenic to people or toxic to other organisms (Chapter 1, Section 5.2.8), or could affect the production of endogenous wheat and barley toxins and allergens. Therefore, exposure to the expressed proteins in GM plant materials from these lines is not expected to adversely affect the health of humans or other organisms.

137. GM barley plants expressing the HvZIP7 protein are expected to produce grain with higher zinc content than non-GM barley. While excessive zinc can be toxic to plants and animals, the levels of zinc reported by the applicant in grain from GM plants grown on low and high zinc soils (40 mg/kg and 118 mg/kg, respectively) are comparable to the 43 mg/kg to 101 mg/kg range found for grain from non-GM barley plants (McDonald et al. 2001). In addition, plantings of the HvZIP7 lines constitute only a small percentage of the total proposed release. Thus, although animals could be exposed to GM barley grain with elevated zinc content, the levels of zinc ingested overall would not be likely to be outside the normal range of exposure for animals consuming non-GM grain.

138. Australian soils tend to be low in zinc and cadmium, and zinc deficiency may enhance absorption and transport of cadmium in crop plants (Akay & Koleli 2007). However, accumulation of cadmium in the grain is unlikely, as cadmium tends to be retained in the roots after uptake (though some may be transported to the grain) and the applicant has stated that there was no detectable cadmium in grain from GM barley plants grown in glasshouse trials. The applicant has stated that they will continue to monitor the grain for cadmium content during the trial.

139. The proposed limits and controls for 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. Each of the trial sites will be surrounded by a stock-proof fence, around which rodent baiting and trapping will be carried out. The area of each trial will be accessible by gate via private road, and only approved staff with appropriate training will have access to the site. These measures will reduce inadvertent access by humans and prevent grazing livestock from entering the site, which minimises exposure of the public and animals to the GM plant material. Livestock and other animals would not be intentionally exposed as the GM plant material will not be used as feed as part of this release.

140. Contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff. There is little potential for exposure of the public to GM plant material via ingestion, skin contact or inhalation as no GM plant material will be used as human food, animal feed or plant products. The short duration (2010-2015) and small size (0.75 ha) of the proposed trial would also limit the potential for exposure to the GM plant material.

141. **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 the protein encoded by any one of the introduced genes for abiotic stress tolerance, or its end product, is **not an identified risk** and will not be assessed further.

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

142. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM wheat and barley plants in particular, is given in *The biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008b) and *The Biology of Hordeum vulgare L. (Barley)* (OGTR 2008a). In summary, wheat and barley share some characteristics with known weeds, such as wind-pollination (although both species are predominantly self-pollinating) and the ability to germinate or to produce some seed in a range of environmental conditions. However, both species lack 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 seed dispersal and long-distance seed dispersal (Keeler 1989). In addition, wheat and barley have been bred to avoid seed shattering, and white wheats and modern barley cultivars have little seed dormancy (OGTR 2008a; OGTR 2008b).

143. Scenarios that could lead to increased spread and persistence of the GM wheat and barley lines include expression of the introduced genes conferring tolerance to abiotic or biotic stress, 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.

### ***Risk scenario 2. Expression of the introduced genetic material improving the survival of the GM wheat and/or barley plants***

144. If the GM wheat or barley plants 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.

145. If the expression of the introduced genes for abiotic stress tolerance were to provide the GM wheat and barley plants with a significant selective advantage over non-GM wheat and barley plants and if they were able to establish and persist in favourable non-agricultural

environments, this may give rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species composition. Similarly, the GM wheat and barley plants could adversely affect agricultural environments if they exhibited a greater ability to establish and persist than non-GM wheat and barley.

146. The impact of the genetic modifications on survival of the GM wheat and barley lines is uncharacterised. However, a number of predictions can be made based on knowledge of the individual gene functions and their predicted effects, as well on observed phenotypes of other GM plants expressing the same gene (Chapter 1, Section 5.2).

147. The aim of the proposed release is to improve abiotic stress tolerance in the GM wheat and barley lines. In addition, some of the introduced genes may also confer tolerance to infection by some pathogens and improve nitrogen or zinc uptake and utilisation. The predicted phenotype conferred by each group of genes has been discussed in Chapter 1, Section 5.2 and, where characterised, the phenotype for the GM wheat or barley lines proposed for release is also summarised. Depending on the introduced gene, GM plants may display the following: improved salinity and/or drought tolerance, tolerance to limited phosphorus, increased nitrogen use efficiency, cross-tolerance to cold, drought and/or pathogen resistance or salt tolerance. Thus, if the introduced genes have the intended effect(s), it is possible that the genetic modifications could confer a competitive advantage on the GM wheat and barley plants under certain conditions.

148. Modern wheat and barley cultivars, some of which are bred for high vigour, are not recognised as a significant weed risk in Australia, and there have been no reports of bread wheat or barley becoming an invasive pest in Australia or overseas. The ‘Bob White’ wheat and ‘Golden Promise’ barley cultivars used for the proposed release are poorly adapted to the Australian cropping environment and consequently neither is used in commercial plantings. Similarly, the barley line ‘WI4330’ is a former breeding line developed by the SA Barley Improvement Program at the University of Adelaide that performed poorly under drought conditions. While it is likely that the transgenic lines will perform better than these parental lines under drought conditions, resulting in improved grain yields, the introduced genes for drought tolerance are unlikely to lift yield and vigour to the level of modern Australian varieties.

149. Neither wheat nor barley is able to survive over the summer months in southern Australia. Even if the genetic modifications resulted in considerable improvement in drought tolerance, it is unlikely that the lines would be able to grow for more than a few weeks or days longer than the non-GM plants. Additionally, the spread and persistence of the GM wheat and barley plants would still be limited by lack of seed shattering and low intrinsic competitive ability

150. The GM wheat and barley would not be expected to have any greater weediness characteristics than non-GM plants, but if dispersed may have greater capacity than non-GM wheat and barley plants to survive a number of abiotic stresses, since single genes may confer cross-tolerance. However, it is likely that the degree of stress tolerance will vary between individual lines and no single gene would confer a high level of tolerance to all stresses. Therefore, the GM plants would still be limited by other environmental factors that normally limit the spread and persistence of wheat and barley plants in Australia such as nutrient availability (other than nitrogen), pests and diseases (Slee 2003; Condon 2004).

151. In addition, if there were any significant advantages conferred to the GM wheat and barley lines as a result of the genetic modification, the proposed limits and controls of the trial (Chapter 1, Sections 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 proposed site.

152. **Conclusion:** The potential for increased weediness, allergenicity or toxicity due to expression of the introduced genes for abiotic stress tolerance improving the survival of the GM wheat and barley lines 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***

153. If the GM wheat and barley plants were to be dispersed from the release sites there could be increased exposure of humans and other organisms to the GM plant material and/or the GM plants may establish and persist in the environment. The effects of contact, inhalation or ingestion of the GM wheat and barley plants have been assessed in Risk scenario 1 and were not an identified risk. The potential for the introduced genes to result in improved survival of the GM wheat and barley plants in the environment was considered in Risk scenario 2 and was not an identified risk.

154. Dispersal of reproductive GM plant materials, for example viable grain, could occur in 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. It is possible that seed yield may be increased in the GM wheat and barley lines. Other seed production and dispersal characteristics, such as grain number per spike, may also be altered compared to non-GM parental cultivars.

155. Seed dispersal for wheat or barley through endozoochory has not been reported, however it is possible that wheat or barley seeds could germinate after passage through the digestive system of some mammals. For example, viable wheat and barley 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. Barley also produces large seeds and the parental cultivar, Golden Promise, is a malting barley, which typically have low levels of dormancy (Briggs 1978).

156. Kangaroos, rabbits and mice are known pests of wheat and barley crops. 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). Other studies have shown that generally very few viable seed are obtained from rabbit dung (Wicklow & Zak 1983; Welch 1985).

157. 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. The applicant proposes to

place baits and traps around the perimeter of each site, which will further limit seed dispersal by rodents.

158. Wheat lacks seed dispersal characteristics such as stickiness, burrs, and hooks, which can contribute to seed dispersal via animal fur (Howe & Smallwood 1982). Barley seeds, however, have special bristles on the spikelet structures and seeds could potentially adhere to animals and the clothing of people, thus facilitating dispersal (OGTR 2008a). Each of the proposed release sites will be surrounded by a fence with a gate, which would reduce the possibility of livestock or unauthorised people accessing the site, and minimise the potential for seed dispersal via this route. Dispersal by authorised people entering the proposed trial sites would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing. 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. Extremes of weather may cause dispersal of plant parts. However, control measures have been proposed by the applicant to minimise dispersal outside the trial site (Chapter 1, Section 3.3). These include locating the proposed release away from natural waterways to prevent dispersal in the event of flooding.

160. **Conclusion:** The potential for increased allergenicity, toxicity or weediness due to dispersal of reproductive 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**

161. 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).

162. Baseline information on vertical gene transfer associated with non-GM wheat and barley plants can be found in the *The Biology of Triticum aestivum L. em Thell (Bread Wheat)* (OGTR 2008b) and *The Biology of Hordeum vulgare L. (Barley)* (OGTR 2008a). Plant genotypes and environmental context and conditions, such as wind direction and humidity, can influence gene flow. In summary, wheat and barley plants are predominantly self-pollinating and the chances of natural hybridisation occurring with commercial crops or other sexually compatible plants are low.

#### **Risk scenario 4. Expression of the introduced genes in other wheat and/or barley plants**

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

164. Many of the introduced genes were originally isolated from wheat and barley, so transfer of these genes to other wheat or barley does not introduce new proteins, though may result in altered protein levels and/or protein localisation. Similarly, most of the promoters have been isolated from wheat or other plants and all of the introduced regulatory sequences are expected to operate in the same manner as regulatory elements endogenous to the wheat and barley plants. While the transfer of either endogenous or introduced regulatory sequences

could result in unpredictable effects, the impacts from the introduced regulatory elements are likely to be equivalent to, and no greater than, those from endogenous regulatory elements.

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 and barley plants by the introduced genes for abiotic stress tolerance, or introduced regulatory sequences. This will be the same if any one of the introduced genes is transferred to other wheat or barley plants.

166. Both wheat and barley are predominantly self-pollinating (94-99%) and any outcrossing occurs through wind pollination (reviewed in OGTR 2008a; OGTR 2008b). Intraspecific gene flow generally occurs over much shorter distances for small scale experimental releases compared to commercial scale, although gene flow levels are highly variable. The majority of gene flow from small scale fields of wheat 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). Gene flow in barley rapidly decreases at distances beyond a few metres (Gatford et al. 2006). However, cross fertilisation with very low frequencies has been observed at distances of up to 60 m (Wagner & Allard 1991).

167. Studies under Australian field conditions (South Australia and the ACT), indicate that gene flow occurs at extremely low frequencies and over very short distances. Wheat gene flow occurred at less than 12 m; 0.012% and 0.0037% in the ACT and South Australia, respectively (Gatford et al. 2006). Pollen flow from GM barley was found to be 0.005% over a distance of less than 10 m at a site in South Australia that was part of the same small scale study (Gatford et al. 2006). For some of the GM barley lines proposed for release, the likelihood of gene flow is even less likely, since the plants are predicted to have delayed flowering (up to one month) and reduced growth (See Chapter 1, Section 5.5.2), so are unlikely to be able to cross with non-GM barley.

168. The survival of the GM wheat and barley plants proposed for release would be restricted by a diverse range of environmental factors that normally limit the spread and persistence of wheat and barley plants in Australia (see Risk scenario 2). Expression of genes for abiotic stress tolerance in other wheat and barley plants would result in plants that may have some competitive ability under very specific conditions, but as for the GM lines proposed for release, these plants would also be limited by other environmental factors. Further, expression of some of the genes may reduce survival under some conditions. The gene for Na<sup>+</sup> translocation, for example, could reduce yield under low salt soil conditions.

169. The applicant proposes to prevent cultivation of non-GM wheat and barley breeding lines within 500 m of the trial site, and prevent cultivation of other non-GM lines of wheat and barley within 200 m of the site. These measures are further discussed in Chapter 3, Section 4.1.1. Isolation from other wheat and barley cultivation will greatly restrict the potential for pollen flow and gene transfer.

170. Cross-pollination between the different GM wheat lines or between the GM barley lines proposed for release at each site must also be considered in relation to combining ('stacking') of GM traits, possibly contributing to weediness of the resultant GM wheat and barley lines. As discussed in Risk scenario 2, the GM wheat and barley lines proposed for release may show increased seed yield and enhanced growth under some environmental conditions, such as low nitrogen, phosphorus or zinc, and greater survival or recovery under abiotic stress conditions such as cold, drought or saline soils. If individual lines were to cross pollinate, these characteristics may combine and contribute to the spread and persistence of the GM lines.

171. The combination of these traits with those in the other GM wheat and barley 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 restricted by measures proposed by the applicant to restrict the persistence of the GM lines at the release sites (see Risk Scenario 2 and Chapter 1, Sections 3.1 and 3.3)

172. As outlined in Chapter 1, Section 6.2, there has been a previous trial of GM wheat and barley (DIR 077) at site 1, in the same 50 m x 50 m fenced area proposed for DIR 102. Therefore, the possibility of stacking between the GM wheat and barley lines of DIR 102 and any volunteers arising as a consequence of DIR 077/2007 is also considered. During the first year of the release for DIR077/2007, the GM plants were destroyed before flowering and, thus, did not produce seed which may germinate as volunteers. The site was planted again, to GM barley only, in June 2009 and harvested in January 2010. The applicant has stated that there will be no further planting at this site under DIR 077/2007. Since the first planting in 2008, the site was subject to licence conditions requiring inspection and removal of volunteers. Currently it is subject to post-harvest licence conditions, which also require inspection and removal of volunteers. Therefore, crossing between the GM wheat and barley plants of the proposed release and volunteers resulting from DIR077/2007 is not expected to occur.

173. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the potential for gene transfer to non-GM wheat and barley plants. In particular, the applicant proposes to isolate the trial site from other plantings of wheat and barley, 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 and to destroy any volunteer plants found at the site. These latter measures would ensure any remaining GM wheat and barley seeds, or plants that were potentially the product of gene flow, in these areas would be destroyed.

174. **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 and barley 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 or regulatory sequences in other sexually compatible plants***

175. Transfer and expression of the introduced genes for abiotic stress tolerance to other sexually compatible plants could increase the weediness potential, or alter the allergenicity and/or toxic potential of the resulting plants.

176. 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 and barley plants by the introduced genes for abiotic stress tolerance. Similarly, if the introduced genes for abiotic stress tolerance are expressed in other sexually compatible species, allergenicity and toxicity are also not expected to be altered.

177. Expression of the introduced genes for abiotic stress tolerance in other sexually compatible plants may give these plants some selective advantage. However, many of the conditions that normally restrict the spread and persistence of hybrids between non-GM wheat or barley and other sexually compatible plants would also be expected to restrict the spread

and persistence of any hybrids between the GM wheat or barley and other sexually compatible species.

178. *Hordeum vulgare* ssp. *spontaneum* (wild barley) is the only species that can cross with cultivated barley under natural conditions (Nevo 1992; OGTR 2008a). Wild barley is not found in Australia (OGTR 2008a).

179. As discussed in *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008b), 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). 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.

180. Information in addition to that discussed in the *The Biology of Triticum aestivum L. em Thell. (Bread Wheat)* (OGTR 2008b) 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).

181. 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 quarantine weed species but are not known to be present naturally (see Chapter 1, Section 6.4).

182. The applicant has indicated that all three sites are located within established agricultural areas. It is therefore possible that wheat and barley will be grown near the proposed trial sites. In addition, the GM wheat and barley lines proposed for release will be grown together at the field trial site. Barley and wheat are not known to hybridise with each other under natural conditions (OGTR 2008a; OGTR 2008b).

183. 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 sites from other sexually compatible species, and the majority of the pollen is expected to fall within the trial site or the 10 m area directly surrounding the trial site.

184. **Conclusion:** The potential for allergenicity in people, or toxicity in people and other organisms or increased weediness due to the expression of the introduced genetic material 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

185. 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.

186. Risks that might arise from horizontal gene transfer have been considered in previous RARMPs (eg 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 events, relative to those HGT events that occur in nature, and the limited chance of providing a selective advantage to the recipient organism.

187. 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. Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer**

188. 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.

189. HGT could result in the presence of the introduced gene in bacteria, plants, animals or other eukaryotes. The probability of transfer of the introduced gene sequences and regulatory sequences contained in the GM wheat and barley plants is no greater than transfer of any of the native wheat and barley genes. The majority of the introduced genes were isolated from wheat, barley and maize (the remaining genes from thale cress, moss or yeast) and homologues of the encoded proteins occur naturally in animals, plants, yeast and bacteria. The regulatory sequences are also widespread in the environment (see Chapter 1, Section 6.5). Therefore, the introduced genes and regulatory sequences are already available for transfer via demonstrated natural mechanisms.

190. 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 HGT *per se* (Thomson 2000). If the introduced genes, the encoded proteins or the 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 - 5 associated with the expression of the introduced genes did not represent an identified risk. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 6.5. Most of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment. 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.

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

## 2.5 Unintended changes in biochemistry, physiology or ecology

192. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy<sup>11</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 encoded protein of the introduced gene changing chromatin structure, affecting methylation patterns, or regulating signal transduction and transcription
- increased metabolic burden associated with high level expression of the introduced gene
- novel traits arising from interactions of the protein encoded by the introduced gene product with endogenous non-target molecules
- secondary effects arising from altered substrate or product levels in biochemical pathways incorporating the protein encoded by the introduced gene.

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

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

194. The applicant indicates that the GM wheat and barley lines have undergone only very limited phenotypic characterisation in the glasshouse, as the project is in early stages.

195. For most of the GM wheat and barley lines, the intention of the genetic modification is to confer enhanced tolerance to a range of abiotic stresses and preliminary characterisation suggests the GM wheat and barley lines display these characters to differing extents. No observable secondary effects are apparent for the GM wheat and barley lines, with the exception of barley plants expressing DREB TFs under constitutive promoters, which display a delayed flowering phenotype and stunting. The applicant indicates that any plants showing a

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<sup>11</sup> Pleiotropy is the effect of one particular gene on other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

delay in flowering of more than a month relative to non-GM plants will be uprooted and destroyed.

196. The outcome of random insertion of an introduced gene is impossible to predict. Such outcomes may include, for example, alteration to reproductive capacity, altered responses to environmental stress, production of novel substances, and changes to levels of endogenous substances. This could include higher levels of endogenous toxins, allergens or anti-nutritional compounds. Non-GM wheat can be toxic to animals if consumed in large quantities (due to nitrate poisoning), and flour from both wheat and barley is allergenic to some people and may also trigger coeliac disease. For further discussion regarding the toxicity and allergenicity of non-GM wheat and barley see *The Biology of Triticum aestivum L. em Thell. (bread wheat)* (OGTR 2008b) and *The Biology of Hordeum vulgare L. (barley)* (OGTR 2008a).

197. Unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003). While the GM wheat lines have not undergone thorough phenotypic analysis, it is expected that substantial changes in these parameters would have been detected in the time these lines have been under development in the glasshouse.

198. The likelihood of any unintended 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. Access to the proposed trial sites would be by private road, which limits exposure of the public to the GM plant material. The public and livestock would not be intentionally exposed as the GM plant material will not be used as food or animal feed as part of this release.

199. **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)***

200. If a licence were to be issued, non-compliance with the conditions of the licence could lead to spread and persistence of the GM wheat and barley 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 has 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.

201. **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***

202. 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.

203. Eight risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression

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

204. A **risk** is only identified 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 represent an identified risk and do not advance any further in the risk assessment process.

205. 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, locations and duration of the release proposed by the University of Adelaide
- suitability of controls proposed by the University of Adelaide to restrict the spread and persistence of the GM wheat and barley plants and their genetic material
- limited ability and opportunity for the GM wheat and barley plants to transfer the introduced genetic material to commercial wheat and barley crops or other sexually related species
- none of the GM plant materials or products will be used human food or animal feed as part of this release
- widespread presence of the same genes or sequences in the environment and lack of known toxicity or evidence of harm from them.

206. Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM wheat and barley 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.

## **Section 4 Uncertainty**

207. 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.

208. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability<sup>12</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.

209. For DIR 102, which involves early stage research, uncertainty is noted particularly in relation to the characterisation of:

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<sup>12</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.

- Risk scenario 1, regarding potential increases in allergenicity or toxicity through ingestion or contact with plant material containing the introduced gene(s) or encoded proteins
- Risk scenario 2, associated with a potential for increased survival of the GMOs, particularly in relation to cross-tolerance to other abiotic stresses
- Risk scenario 7, due to incomplete molecular characterisation of the GMOs.

210. 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 and barley lines if they are selected for further development.

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

## Chapter 3 Risk management plan

212. 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. 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**

213. 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.

214. 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.

215. 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**

216. 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>13</sup>.

217. 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.

218. 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 and barley plants to be used for human food. Accordingly, the applicant has not applied

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<sup>13</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/riskassessments-1>>.

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

219. No other approvals are required.

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

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

221. These risk scenarios were considered in the context of the scale of the proposed release (a maximum area of 0.75 ha per growing season on three sites, two in South Australia and one in Western Australia, between June 2010 and December 2015), the proposed containment measures (Chapter 1, Section 3), and the receiving environment (Chapter 1, Section 6).

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

222. 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, locations 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 the licence and summarised in Section 4.1.2.

#### **4.1 Licence conditions**

##### **4.1.1 Consideration of limits and controls proposed by the University of Adelaide**

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

224. The permitted dealings are confined to a maximum of 0.75 ha per growing season on three sites, two located in the LGAs of Marion and Wakefield (South Australia), and one in Corrigin (Western Australia), and the duration of the proposed release has been limited to five 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 (Chapter 2, Risk scenario 1) and the potential for the GM wheat and barley lines to disperse and establish outside the proposed release site (Chapter 2 Risk scenario 2 and 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*.

225. The release site will also be surrounded by a fence to restrict access to the site by humans and livestock, further minimising both exposure to and dispersal of GM plant material outside the proposed release site (Risk scenario 3).

226. The applicant has stated that the trial sites will be at least 2 – 20 km from the nearest waterways, which would reduce the likelihood of plant material being washed away from the sites. For site 2, there is no irrigation within 30 km and no watercourses that flow within 20

km, but the site is located at the end of a floodway with a 1 in 20 year incidence of flooding. However, based on advice from the landowner, the applicant has stated that the chance of water flowing onto the site during the growing season is closer to 1 in 50 years. It is a standard DIR licence condition that the trial site to be located at least 50 metres from a natural waterway to limit the dispersal of viable GM plant material in the event of flooding (Risk scenario 3).

227. The applicant's proposals to restrict gene flow from the GM wheat and barley (162 and Risk scenario 5) include: a 1 m buffer zone kept free of vegetation surrounding the GMO planting, a 10 m wide zone in which vegetation is controlled and related species are prevented from flowering surrounding the fenced area, and a 200 m zone in which no other wheat or barley plants are to be grown (including breeding lines).

228. As discussed in Chapter 1, Section 6.4 and Risk scenario 5, there are few species with which wheat can naturally form hybrids, and the fertility of hybrids formed is typically low. *Hordeum vulgare* ssp. *spontaneum* (wild barley) is the only species that can cross with cultivated barley under natural conditions (Nevo 1992; OGTR 2008a). Wild barley is not found in Australia (OGTR 2008a). There are no reports of barley forming hybrids with cultivated wheat under natural conditions.

229. Differences in pollen flow have been observed in different pollen flow studies in both wheat and barley. 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. In Northern America, levels of gene flow in wheat have been shown to be dependent on the size of the field (reviewed by OGTR 2008b). For an experimental scale wheat field, extremely low rates of gene flow (0.002 – 0.003%) were detected at distances up to 100 m. For commercial scale fields, outcrossing rates of 0.25% were detected at 61 m and in one instance gene flow was recorded up to 2.75 km from a commercial wheat field (Matus-Cadiz et al. 2007).

230. In barley, outcrossing rates are generally lower than those found for wheat and gene flow is mostly detected between adjacent plants (reviewed in OGTR 2008a), with very low levels of outcrossing (0.1%) occurring at up to 60 m (Wagner & Allard 1991). In an Australian study, gene flow from a small planting of GM wheat occurred at a frequency of 0.012% and 0.0037% over 8 m and for barley gene flow occurred at 0.005% over a maximum distance of 10 m (Gatford et al. 2006). However, gene flow was not measured beyond 10 m and therefore the true rate may be higher.

231. Isolation distances for GM field trials vary greatly amongst different countries. For example, field trial releases of GM wheat in Canada require a 30 m isolation distance between the GM plants and other wheat plants, while in the United States the isolation distance is reduced to 20 feet (approximately 6.1 m) (USDA-APHIS 1994; Canadian Food Inspection Agency 2006). In the European Union, various GM wheat field trials have been approved, requiring border plantings of non-GM wheat or other plants ranging up to 30 m wide and isolation distances from other wheat ranging from 2 to 50 m (European Commission Directorate General for the Environment 2009). A field trial release of GM barley in Iceland requires a separation distance of at least 300 m from other barley fields (European Commission Directorate General for the Environment 2009).

232. 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) do not specify isolation distances but stipulate maximum acceptable levels of off-types or other cultivars of the same species of 0.1% for basic wheat or barley seed and 0.3% for certified wheat or barley seed (1<sup>st</sup> generation). Seed crops of self-fertilising cereals (eg wheat and barley) 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 either wheat or barley 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).

233. The applicant has proposed to maintain a 200 m separation between the GM wheat and barley lines and other wheat and barley cultivation. If gene flow to a breeding line was to occur, and the breeding line was to eventually become commercially successful, this could lead to increased propagation and dispersal of the GMOs. However, on the basis of the scientific literature on gene flow, international containment measures for GM wheat and barley trials, and the rules for producing basic and certified seed, a 200 m isolation zone clear of sexually compatible species is considered adequate to minimise gene flow from the GM wheat and barley plants to any other wheat and barley (including breeding lines) or other sexually related species outside the release site (162 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 and must be inspected for sexually compatible species, which if found must be destroyed before flowering.

234. While the applicant proposes to maintain the 200 m separation from any other non-GM wheat and barley plantings, they propose that inspection requirements be limited to the first 50 m of this zone. This is based on data indicating that gene flow for wheat or barley is extremely low over distances of 60 m for wheat (see discussion above) and even lower for barley. In particular the Australian trial by Gatford et al (2006) and the study by Wagner and Allard (1991) suggests that, at 60 m separation, cross-fertilisation between barley plants would occur at less than 1 in every 10,000 gametes. In effect, the applicant's proposal is for the GMOs to be surrounded by a zone of 60 m, within which related species are controlled or removed, made up of: a 10 m monitoring zone which would be mown or treated with herbicide to limit growth of sexually compatible species, and a 50 m inspection zone where sexually compatible species are inspected for and destroyed by herbicide treatment or uprooting.

235. The likelihood of non-GM wheat or barley 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 applicant has stated that, for all sites, the most closely related grasses that may occur nearby are cereal rye (*Secale cereale*), Triticale and barley grass. While wheat can cross-pollinate with these species, no viable seed results (OGTR 2008b).

236. The applicant has indicated that all of the trial sites are in productive agricultural areas, so it is likely that wheat and/or barley plants will be grown in the wider area (ie beyond 200 m) during the trial. As a result, there is the possibility that non-GM volunteers may germinate in the 200 m isolation zone during the trial. However, the likelihood of this occurring differs between the three sites. Site 1 is located at least 1.5 km from any previous commercial wheat or barley crops, and those plantings occurred prior to 2007. There has been no non-GM wheat or barley planted at the site since 2000, apart from trials for the South Australian Barley Improvement Program approximately 1 km away.

237. The location for the GM trial at Site 2 was sown to oaten hay in 2009 and was treated with herbicide after the hay was cropped. Site 3 was sown to salt bush and barley in 2009, but the barley did not progress past tillering due to drought and salt. Plants died at anthesis, so there would have been negligible contribution to the seed bank in the soil. Thus, for these

three sites, cropping history is likely to have a great influence on the levels of volunteer non-GM wheat and/or barley plants within the 200 m isolation zone.

238. There is a possibility of GM wheat and barley volunteers occurring at site 1 as a consequence of the previous release of GM wheat and barley under DIR 077/2007. In 2008, 400 m<sup>2</sup> of GM wheat and barley was planted within the 50 x 50 m fenced area proposed for DIR 102 but plants were destroyed prior to flowering. In 2009, the same location was planted to GM barley only, with some non-GM barley included for purposes of comparison. The final harvest of GM barley for DIR 077/2007 occurred at this site in December 2009. During harvest, there is a possibility of GM and non-GM barley being dispersed outside the trial site. Under DIR 077/2007 licence conditions, the location and monitoring zone has undergone post-harvest cleaning and inspection and any volunteers removed. Thus, there are unlikely to be volunteers resulting from DIR 077/2007 and the likelihood of gene flow between the proposed release and any GM barley volunteers within the fenced area is very low.

239. The licence also required inspection of the 200 m isolation zone for the occurrence of GM or non-GM wheat and barley volunteers and related species while GM plants were being grown. This provides a documented history for the isolation zone. As discussed above, a 200 m isolation zone clear of sexually compatible species is considered adequate to minimise gene flow from the GM wheat and barley plants to any other wheat and barley crops (including breeding lines). Therefore, no wheat or barley may be planted within 200 m of the GM trial site.

240. Taking into consideration the cropping history of the areas and environmental/climatic contexts, it is unlikely that any non-GM wheat or barley plants will occur within the 200 m isolation zone. Furthermore, since source size has a significant effect on gene flow rates (see discussion above), the likelihood of gene flow between the small-scale trial (400 m<sup>2</sup>) and any isolated non-GM wheat or barley plants beyond 60 m is extremely low. Therefore, a licence condition has been imposed that initially requires inspection for, and destruction of, related species in the total area of the 200 m isolation zone. However, if no wheat or barley has been grown in the isolation zone for the previous two years, the University of Adelaide may request a reduction of inspection requirements within the isolation zone to 50 m after one growing season. This reduction is dependent on not finding any populations of wheat, barley or sexually compatible species during monitoring of the site or isolation zone in the previous growing season. A requirement to maintain a minimum separation distance of 200 m from other wheat and barley crops will not be affected by any reduction in inspection area.

241. 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. In the context of the release site(s) 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 species outside of *Hordeum vulgare* and *Triticum* species.

242. The proposed 10 m wide monitoring zone outside the trial site, with little or no vegetation, will deter rodent activity at the proposed release site (Risk scenario 3). The applicant also proposes to conduct mouse baiting and trapping around the perimeter of each site, which will aid in reducing the size of the mouse population which may have access to the GM wheat and barley. Whilst there are differing reports regarding the average territory size of mice, reduced vegetation has been shown to help reduce rodent numbers in agricultural settings. 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 the site has been cleaned. This will minimise the potential exposure of vertebrates to the GMOs (Risk scenario 1) and the potential dispersal of the GMOs (Risk scenario 3).

243. The applicant has stated that the trial sites will be located at least 2 km from the nearest waterway. A standard DIR licence condition is imposed requiring the trial site to be located at least 50 m from a natural waterway to minimise the dispersal of viable GM plant material in the event of flooding (Risk scenario 3).

244. 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. The applicants have proposed a 1 m buffer zone kept clear of all plants during the trial which would be subject to post-harvest inspections along with the trial site. However, to manage the possibility of mechanical dispersal of seed from the trial location, the licence conditions include a requirement to clean and inspect an area at least 2 m wide around the site after harvest.

245. The applicant has proposed a number of measures to minimise the persistence of any GM wheat or barley plants and seeds in the seed bank at the release site after harvest of the trial (Risk scenario 2). These measures include monthly monitoring of the trial site for volunteer wheat and barley plants following harvest, and destroying volunteer plants that emerge after harvest by spraying with herbicide, or pulling out volunteer plants before they flower. The applicant has also proposed to monitor the release site for 24 months after harvest. All volunteers will be destroyed before flowering.

246. 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 and barley plants either by hand or with a plot harvester. Wicks et al. (2000) reported that small plot headers are less efficient than commercial harvesters, so self-sown wheat and barley may be a greater problem under experimental conditions.

247. Cereal grains require an after-ripening period before germination can occur, which takes up to nine months depending upon genetic background and environmental conditions (Pickett 1989; Anderson & Soper 2003). The process of after-ripening is favoured by hot dry conditions, which can be facilitated in the field by retaining any seed remaining after harvest on the soil surface prior to irrigation (Pickett 1993). Although the time required for after-ripening of the GM wheat and barley lines under the expected field conditions is unknown, retention of dropped seed on the soil surface for at least 28 days is imposed as a licence condition.

248. The persistence of seed depends on several factors which contribute to seed dormancy: cultivar genetics, environmental conditions during seed formation, crop nutrition, environmental conditions after shedding, and field treatment (reviewed by Anderson & Soper 2003). Viable seeds persist in the soil for longer periods in dry than in moist conditions (Anderson & Soper 2003), and wheat seeds present as un-threshed ears have longer dormancy than loose seeds (Komatsuzaki & Endo 1996). Shallow tillage after harvest, combined with irrigation, will germinate much of the grain dropped at harvest (Ogg & Parker 2000), while 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.

249. There is high variability in volunteer wheat and barley emergence, and various field studies report volunteer emergence up to two years following harvest (reviewed by Anderson & Soper 2003). In a study in Germany, a small proportion of barley seeds were recovered after 15 months (Rauber 1988). In a Scottish survey, volunteer winter barley was reported to persist for up to five seasons, and volunteer spring barley for up to two seasons, in some rotations (Davies & Wilson 1993). 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). 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, Germany and Scotland.

250. Following harvest, the applicant proposes to irrigate the location and surrounding unplanted border and till to the depth of the original planting. Any volunteer plants will be destroyed. It is considered that three irrigations (or sufficient rainfall), 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 and barley seeds in the soil. Therefore, the licence conditions require an initial irrigation to take place within 60 days of harvest to encourage surface seed to germinate. Two further irrigations would be required at intervals of at least 28 days, with the last irrigation occurring during the final six months of the monitoring period. These treatments will 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. Post harvest monitoring of the release site for at least 24 months after harvest, with no volunteers observed in the most recent six months, needs to be completed before an application that inspection conditions no longer apply can be made to the Regulator. These measures will minimise the persistence of the GMOs in the environment (Risk scenario 2).

251. The applicant proposes to establish interim storage for harvested GM wheat and/or barley seed at sites 2 and 3 prior to transport to the University of Adelaide for further experimentation. The applicant has stated that any plant material taken off-site for experimental analysis will be transported according to the OGTR *Guidelines for the transport of GMOs*, <<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. Standard licence conditions allow storage of GM plant material in a facility approved by the Regulator. Dealings with the GMOs in certified facilities, such as PC2 laboratories or glasshouses, may also be conducted as Notifiable Low Risk Dealings (NLRDs) under the Act, and therefore would not be subject to licence conditions.

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

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

- limit the release to a total area of up to 0.75 ha per growing season at three sites, two in the LGAs of Marion and Wakefield (SA) and the other in the LGA of Corrigin (WA), between June 2010 and December 2015
- locate the trial sites at least 50 m away from natural waterways
- establish a 10 m zone around the trial sites in which any related species are prevented from flowering and which is maintained in a manner that does not attract or harbour rodents

- surround the GM wheat and barley with an inspection zone of up to 200 m in which growth of sexually compatible species is controlled
- ensure no other crops of wheat or barley are within 200 m of the trial sites
- enclose each trial site with a livestock-proof fence with lockable gates
- harvest the GM wheat and barley plant material separately from other crops
- clean the sites and equipment used on the sites following harvest
- apply measures to promote germination of any wheat and barley seeds that may be present in the soil after harvest, including 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 and barley plants that may grow
- destroy all GM plant material not required for further analysis or future trials
- transport and store material from the GMOs in accordance with Regulator's guidelines
- not permit any GM wheat or barley plant material to be used in human food, animal feed or in the production of therapeutic goods as part of this release.

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

## **4.2 Other risk management considerations**

254. 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**

255. 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.

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

257. 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.

258. The University of Adelaide 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**

259. The University of Adelaide is required to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan will detail measures to be undertaken in the event of any unintended presence of the GM wheat and barley lines outside of the permitted areas.

260. The University of Adelaide 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**

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

#### **4.2.4 Reporting structures**

262. 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.

263. 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.

264. 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**

265. 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.

266. 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.

267. 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**

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

- additional data on the potential allergenicity or toxicity of plant materials from the GM wheat and barley lines
- additional phenotypic characterisation of the GM wheat and barley lines, in particular of characteristics indicative of weediness, including measurement of altered reproductive capacity and competitiveness, and information relating to cross tolerance to other abiotic stressors
- characterisation of the introduced genetic material in the plants, including copy number and genotypic stability.

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

269. The risk assessment concluded that this proposed limited and controlled release of up to 1161 GM wheat lines and 1179 GM barley lines on a maximum total area of 0.75 ha per growing season over five years in South Australia and Western Australia, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

270. 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, locations 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 102

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
The wording of the licence conditions should be modified to reflect that the fence at the site is to keep out 'domestic livestock' rather than 'large animals'.	The wording of the licence has been modified accordingly.
Inactive ingredients produced from grain, eg gluten and sugars, are often used in medicine capsules, tablet coating or fillings. Consequently, wherever 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'.	A statement that the GMOs should be excluded from use as ingredients in 'therapeutic goods' has been included in Chapter 3, Section 4 of the RARMP. The Licence has been amended to ensure the GMOs or products thereof will not be used in the production of therapeutic goods.
Draft RARMP could be improved by including a more detailed discussion of potential effects of gene stacking on the weediness of the GM wheat or barley lines. The introduced genes are likely to confer tolerance to a range of abiotic stresses that could enhance the growth and survival of the GM wheat and barley lines in non-agricultural environments. 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 or barley through intended and unintended effects is discussed in risk scenario 2. The combination of traits as a result of crossing of the GM wheat or barley lines is likely to contribute only incrementally to the potential weediness of the GM plants. The conditions that normally limit the spread and persistence of non-GM wheat and barley 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 and barley plants. The persistence of the GM wheat and barley would also be managed by measures proposed by the applicant and imposed in the licence. Further discussion on potential stacking of the traits has been added to the RARMP. The RARMP also outlines further data which may be required to assess an application for a larger scale release or commercial release.

<sup>14</sup> GTTAC, State and Territory Governments, Australian Government agencies, Local Governments and the Minister for Environment Protection, Heritage & the Arts.

Summary of issues raised	Comments
<p>There are a number of traits currently being trialled in wheat and barley across Australia and early consideration of possible interactions of the expanding number of traits may lead to enhanced risk assessment outcomes. Recommend inclusion of discussion of this in the RARMP</p>	<p>Stacking of the GM traits in this release with those from other limited &amp; controlled releases of GM wheat and barley was not discussed because it is highly unlikely that stacking between GM plants in different trials will occur. The proposed containment measures and licence conditions imposed for each release will effectively restrict gene flow. Site 1 has recently been planted to another limited and controlled release of GM barley (DIR 077). However, this trial is now subject to post-harvest licence conditions requiring cleaning of the site and removal of any volunteers, and the likelihood of gene flow occurring between DIR 102 and any volunteers from this release is remote. Therefore, a discussion on inter-trial stacking is not considered warranted at this stage. 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 or barley.</p>
<p>States that species belonging to the genera <i>Elytrigia</i>, <i>Elymus</i> and <i>Secale</i> are known to occur in Australia and be sexually compatible with wheat, although resulting hybrids exhibit reduced fertility. A broader definition of "Related species" to include plants of the Tribe <i>Triticeae</i> would ensure that all possible sexually compatible species are monitored and removed from the isolation one, thereby further reducing the potential for gene flow.</p>	<p>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, such as a few <i>Aegilops</i> species, do not occur naturally in Australia. In the context of the proposed release sites 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>
<p>The fence height, at 1 m, is not consistent with other trial site requirements. A standardised minimum height needs to be set.</p>	<p>The purpose of the fence is to exclude livestock. Therefore, an outcome-focused licence condition has been imposed to reflect this.</p>
<p>The risk assessment appears to be identical to other wheat and barley trials</p>	<p>All RARMPs for dealings involving the limited and controlled release of GM plants are structured similarly and may contain similar considerations for a number of reasons, including</p> <ul style="list-style-type: none"> <li>▪ the same legislation applies to all DIR licence applications</li> <li>▪ all plants have a number of common characteristics; these characteristics could potentially be involved in giving rise to a limited number of harms</li> <li>▪ the harms that could potentially arise from proposed dealings with any plant GMO fall into a limited number of categories</li> <li>▪ the limits and controls proposed in an application may be highly similar or even identical for a particular crop species. There will be further similarities between RARMPs for applications which share parent organisms.</li> </ul>
<p>Mouse plagues are not an uncommon occurrence and they would overwhelm any rodent control measures employed. Such events are not considered in the risk analysis.</p>	<p>Licence conditions for DIR 102 require the applicant to implement rodent control measures and maintain the monitoring zone in a manner that does not harbour rodents. In addition, under standard licence conditions the licence holder must inform the Regulator if they become aware of additional information as to any risks to the health and safety of people or to the environment associated with the dealings authorised by the licence. Therefore, if rodent control measures were overwhelmed by a plague of mice, the applicant would be obliged to inform the Regulator of this additional information and implement a contingency plan.</p>

Summary of issues raised	Comments
<p>Transcription factors are known to have effects on a range of biochemical and physiological mechanisms involved in growth, development, biotic and abiotic stress responses. The transcription factors may affect biological pathways other than those described in the literature and over expression may induce unintended changes to unidentified biological mechanisms.</p>	<p>This was discussed in Risk scenario 7, Chapter 2 of the consultation RARMP</p>
<p>The RARMP states that there is no identified risk from unintended changes in biochemistry, physiology and ecology (eg. Table 4, section 2.5). However, transcription factors have a range of effects and may pose a risk of unintended changes in biochemistry and physiology.</p> <p>This is particularly relevant to heterologous expression of transcription factors from maize, which are subtly different from wheat or barley transcription factors. Therefore, suggests the conclusions (eg statement 205) need to reflect a level of risk of unintended changes in biochemistry, physiology or ecology that will require further investigation prior to release of the GM wheat or barley for human or animal consumption.</p> <p>[Assessment of] agronomic performance is not sufficient to identify a potential range of unintended effects, which may require further investigation.</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, 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 further data which may be required to assess an application 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 (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 or barley could be sold as food.</p>
<p>Noted that an area of particular concern is the use of antibiotic resistance markers; there is fear in the public about how these genes might spread and threaten the effectiveness of antibiotic drugs</p>	<p>Risks to the health and safety of people and environment associated with the selectable marker gene were assessed as negligible. The <i>hpt</i> gene, which confers hygromycin resistance and was originally isolated from a soil bacterium, is widespread in bacteria in the environment. Bacteria readily exchange genetic material by natural mechanisms. In contrast, horizontal gene transfer from the GM plants to bacteria is highly unlikely. A report by the European Food Safety Authority also concluded that inclusion of <i>hpt</i> in GM plants would not significantly affect the health of humans or animals.</p>
<p>Would like further information on the indirect environmental, social, cultural, economic risks associated with growing GM crops in the Shire of Marion.</p>	<p>The consideration of social, cultural and economic effects is outside the scope of issues to which the GTR may have regard when deciding whether or not to issue a licence. Risks to the environment were assessed as negligible in the context of the proposed release.</p>
<p>States that Glenthorne Farm is an important site that forms part of the Great Southern Urban Forest (GSUF), which proposes an integrated open space framework for southern Adelaide. The preservation of the larger area is important to the GSUF project partners.</p>	<p>Noted.</p>
<p>The Friends of Glenthorne should be consulted on this trial and any future trials of GMOs.</p>	<p>All members of the public were given the opportunity to comment on the RARMP during the consultation period. Invitations to comment were placed on the OGTR website, emailed to the OGTR email list and placed in two newspapers available in Adelaide. Friends of Glenthorne are encouraged to contact our office for inclusion on the OGTR mailing list.</p>
<p>Trials of this type could lead to increased interest in the commercialisation of GM crops.</p> <p>Supportive of the South Australian State Government ban on commercialisation of</p>	<p>The current proposal is for a limited and controlled release. Additional data would be required to assess any application for a large scale or commercial release of these GM wheat and barley lines.</p> <p>Noted. The Regulator's obligations in relation to considering licence applications relate to the health and safety of people and the</p>

Summary of issues raised	Comments
genetically modified crops.	environment. However, state governments may designate non-GM areas for marketing purposes.
Further information is needed on the consequences of genetic material moving to surrounding vegetation.	Spread and persistence of the GM wheat and barley, as well as transfer of the introduced genetic material to sexually compatible species or to sexually incompatible organisms was considered in risk scenarios 2 to 5. In all of these scenarios, the potential for increased weediness, allergenicity or toxicity was not an identified risk given the context of the proposed limited and controlled release.
<p>Further information is needed on:</p> <ul style="list-style-type: none"> <li>• how the proposed release contributes to the public interest</li> <li>• how patents relating to gene technology are managed</li> <li>• potential limitations to market choices for consumers or farmers through creation of Market monopolies via patenting of plant varieties.</li> </ul> <p>Requests that the OGTR seek further information from the relevant agencies on the social, cultural, economic and environmental concerns highlighted in the submission</p>	The Regulator's obligations in relation to considering licence applications relate to the health and safety of people and the environment. Risks to the environment were assessed as negligible in the context of the proposed release. The consideration of social, cultural and economic effects, and management of intellectual property, is outside the scope of issues to which the GTR may have regard when deciding whether or not to issue a licence.