



**Australian Government**

**Department of Health**

Office of the Gene Technology Regulator

# Risk Assessment and Risk Management Plan for

## **DIR 186**

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

Applicant: The University of Adelaide

February 2022

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# Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 186

## **Decision**

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

## **The application<sup>1</sup>**

<b>Project Title</b>	Limited and controlled release of wheat and barley genetically modified for yield enhancement and improved abiotic stress tolerance
<b>Parent organisms</b>	Wheat ( <i>Triticum aestivum</i> L.) and barley ( <i>Hordeum vulgare</i> L.)
<b>Genetically modified organisms</b>	
Genetic modifications	<ul style="list-style-type: none"> <li>• Expression of three genes involved in yield enhancement (expressed both individually and in combination)</li> <li>• Expression of five genes involved in yield and abiotic stress tolerance (water use efficiency)</li> <li>• Expression of three selectable marker genes (expressed both individually and in combination)</li> </ul>
Number of lines	Up to 70 lines <sup>2</sup> in total
<b>Principal purpose</b>	To assess agronomic performance of the GM wheat and barley lines under field conditions
<b>Proposed limits</b>	
Proposed use of GM plants	No use in human food or animal feed is proposed
Proposed locations	The trial is proposed to take place at one site in South Australia (Light Regional Council) <sup>3</sup>
Proposed release size	Up to a total of 2 ha per year
Proposed period of release	From April 2022 to January 2027
<b>Previous releases</b>	Wheat and barley lines containing all or some of the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128 and DIR 152.

<sup>1</sup> The applicant amended their application to remove GM wheat and barley knockout/knockdown lines which had been generated by CRISPR/Cas9 genome editing of endogenous *TaMUTE*, *TaYDA1*, *TaYDA2*, *TaOST1* and *TaSLAC1* genes.

<sup>2</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>3</sup> The applicant amended their application to remove the Shire of Merredin, in Western Australia, as one of the trial sites for the proposed release.

***Risk assessment***

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

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

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

The principal reasons for the conclusion of negligible risks are that the proposed limits and controls, such as the small trial size and not using GM plant material in food or animal feed, will effectively minimise exposure to the GMOs. In addition, there is no evidence to suggest the introduced genetic modifications would lead to harm to people or the environment.

***Risk management***

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

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

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## Abbreviations

Act	<i>Gene Technology Act 2000</i>
APHIS	Animal and Plant Health Inspection Service
APVMA	Australian Pesticides and Veterinary Medicines Authority
<i>bar</i>	Glufosinate tolerance gene from <i>Streptomyces hygroscopicus</i>
CaMV	Cauliflower mosaic virus
DAWE	Department of Agriculture, Water and the Environment
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
DPIRD	Department of Primary Industries and Regional Development (Western Australia)
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
g	Gram
GM	Genetically modified
GMO	Genetically modified organism
ha	Hectare
HGT	Horizontal gene transfer
<i>hptII</i>	Hygromycin phosphotransferase gene
LGA	Local Government Area
m	Metre
µg	Microgram
mg	Milligram
NLRD	Notifiable Low Risk Dealing
<i>nptII</i>	Neomycin phosphotransferase II gene
OGTR	Office of the Gene Technology Regulator
OECD	Organisation for Economic Co-operation and Development
PC2	Physical Containment level 2
<i>pporRFP</i>	Red fluorescent protein gene from <i>Porites porites</i>
RAF	Risk Analysis Framework
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RFP	Red fluorescent protein
SA	South Australia
SARDI	South Australian Research and Development Institute
TGA	Therapeutic Goods Administration
USDA	United States Department of Agriculture
WHO	World Health Organisation
WRA	Weed risk assessment

# Chapter 1 Risk assessment context

## Section 1 Background

1. An application has been made under *the Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.
2. The Act and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.
3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.
4. The *Risk Analysis Framework* (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR) [website](#).
5. The GMOs and any proposed dealings may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA) and the Department of Agriculture and Water Resources (DAWE). Proposed dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.
6. Figure 1 shows the information that is considered, within the regulatory framework, in establishing the risk assessment context. This information is specific for each application. Risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

RISK ASSESSMENT CONTEXT	
<p><b>The GMO</b> Modified genes Novel traits</p>	<p><b>Proposed GMO dealings</b> Activities Limits Controls</p>
<p><b>Parent organism (comparator)</b> Origin and taxonomy Cultivation and use Biology</p>	<p><b>Previous releases</b> Australian approvals International approvals</p>
<p><b>Receiving environment</b> Environmental conditions: abiotic and biotic factors Production practices Related organisms Similar genes and proteins</p>	

**Figure 1 Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the Regulator and the Risk Analysis Framework.**

7. Section 52 of the Act requires the Regulator to seek comment on the RARMP from agencies - the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils and the Minister for the Environment - and from the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. One public submission was received, and its consideration is summarised in Appendix B.

## Section 2 The proposed dealings

8. The University of Adelaide proposes to release up to 70 genetically modified (GM) wheat and barley lines into the environment under limited and controlled conditions. The GM lines have been genetically modified for yield enhancement and improved abiotic stress tolerance.

9. The purpose of the trial is to evaluate the agronomic performances of the GM wheat and barley under Australian field conditions. The GM lines will be assessed for yield enhancement under field conditions in a water-limited environment. The proposed release would also be used to produce sufficient grain for further replicated trials. The GM wheat and barley lines would not be used for human food or animal feed.

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

- conducting experiments with the GMOs
- make, develop, produce or manufacture the GMOs
- breeding the GMOs
- propagating the GMOs
- growing the GMOs
- importing the GMOs
- transporting the GMOs
- disposing of the GMOs

and the possession, supply or use of the GMOs in the course of any of these dealings.

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

11. The release is proposed to take place at one site in South Australia (Light Regional Council)<sup>1</sup>. The release is proposed to take place between April 2022 and January 2027, on a total of 2 ha in any year.

12. Only trained and authorised staff would be permitted to deal with the GM wheat and barley.

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

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

- locating the proposed trial site at least 50 m away from the nearest natural waterway
- surrounding each planting area with a 2 m buffer zone, within which plant growth and rodent activity will be controlled
- surrounding the buffer zones with a 50 m monitoring zone, in which the 10 m adjacent to the buffer zone will have plant growth controlled
- surrounding the monitoring zone with a 140 m isolation zone in which no sexually compatible crops will be grown during the cultivation of GM wheat and barley
- only permitting trained and authorised staff to access the site
- restricting access by surrounding the trial site with a fence to a height of 1.5 m, with lockable gates
- treating non-GM plants used in the trial as if they were GM

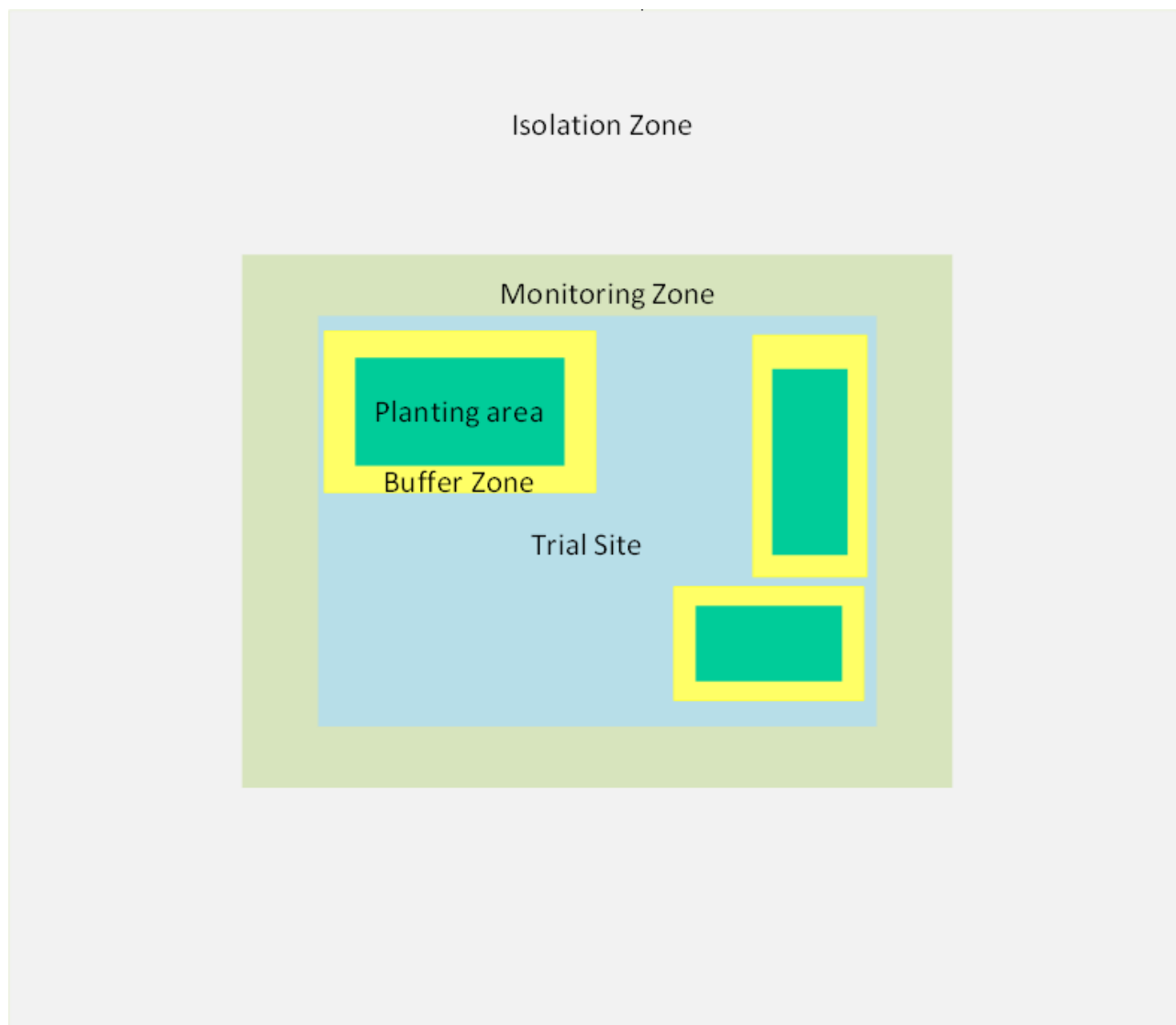
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<sup>1</sup> The applicant amended their application to remove the Shire of Merredin, in Western Australia, as one of the trial sites for the proposed release.



- inspecting all equipment for GM plant material, and cleaning as required prior to equipment leaving the site or being used for any other purpose
- transporting and storing GM plant material in accordance with the current Regulator's Guidelines for the Transport, Storage and Disposal of GMOs
- destroying all plant material from the trial not required for testing or future trials
- post-harvest monitoring of the trial site at least once every 35 days for 2 years, with any wheat or barley volunteers or related species destroyed prior to flowering
- promoting germination of any residual seed post-harvest by tillage and irrigation.

14. Figure 2 shows the layout proposed by the applicant, including some of the proposed controls. The figure shows a trial site with multiple planting areas (with associated buffer zones). The trial site would be surrounded by a monitoring zone and an isolation zone.



**Figure 2 Schematic diagram (not to scale) of trial setup proposed by applicant: Trial site with multiple planting areas.**

15. The proposed limits and controls are taken into account in the risk assessment (Chapter 2) and their suitability for containing the release will be evaluated in the risk management plan (Chapter 3).

### Section 3 The parent organism

16. The parent organisms are bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), which are exotic to Australia. Commercial wheat and barley are cultivated in the 'wheat belt' from southeastern Queensland through NSW, Victoria, Tasmania, southern SA and southern WA.

17. Detailed information about the parent organisms is contained in the reference documents produced to inform the risk analysis process for licence applications involving GM crops: *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR, 2021b) and *The Biology of Hordeum vulgare L. (barley)* (OGTR, 2021a). Baseline information from these documents will be used and referred to throughout the RARMP. Key points from those discussions are summarised as necessary in this RARMP.

18. There are a number of factors, both biotic and abiotic, which limit the growth and survival of wheat and barley, with both species grown in similar areas and conditions. Water stress (drought or waterlogging), heat and cold stress as well as nutrient deficiencies are limiting factors for both species. However, barley is generally regarded as being better adapted to salinity and to drought stress than wheat. Both are limited by a number of pests and diseases.

19. Neither wheat nor barley is regarded as a weed of national significance ([National Weeds List](#)) and both are regarded as naturalised non-native species present in all Australian states and territories with the exception of the Northern Territory (Groves et al. 2003). The weed risk assessments included in the [biology document for wheat](#) concludes that wheat possesses few attributes which would make it weedy and this is supported by the observation that there are few weedy populations of wheat in the Australian environment. In the case of barley, it also is a highly domesticated crop. Mutations conferring non-shattering seed heads were selected to enable effective harvesting of barley (Pourkheirandish et al., 2015). Losing the primary seed dispersal system of wild barley reduces the fitness of cultivated barley outside agricultural environments. Many barley cultivars have also been bred to have low seed dormancy. However, as it is more tolerant to drought and salinity, it is somewhat better able to establish in the environment which is confirmed by a medium weed rating in parts of Western Australia and in Victoria. However, it has no weed rating in the other States where it occurs. It also does not cause major or significant harm anywhere in Australia.

## Section 4 The GMOs, nature and effect of the genetic modification

### 4.1 Introduction to the GMOs

20. The applicant proposes to release up to 70 lines in total of wheat and barley lines genetically modified for yield enhancement and/or improved abiotic stress tolerance (water use efficiency). Details of the introduced genes are listed in Table 1. The applicant has stated that most lines would be GM wheat.

21. The genes *AtAVP1*, *OsNAS2*, *OsPSTOL1*, *TaMUTE*, *TaYDA1*, *TaYDA2*, *TaOST1* or *TaSLAC1* will be introduced into wheat or barley. Lines containing more than one of these genes will also be examined.

22. Short regulatory sequences that control expression of the genes are also present in the GM wheat and barley lines. All of the promoters used to drive expression of the introduced genes are constitutive promoters. Information on the introduced regulatory elements is shown in Table 1.

23. The GM wheat and barley plants may also contain selectable marker genes that confer resistance to antibiotics (*hptII* and *nptII*) or to a herbicide (*bar*). The GM wheat and barley plants modified for yield enhancement and water use efficiency may also contain the introduced *pporRFP* gene, which encodes a red fluorescent protein used to visually identify GM plant cells. The selectable marker genes and reporter gene are listed in Table 1.

**Table 1: Genes and regulatory elements introduced in GM wheat and barley lines**

Element	Gene Source	Function
		Yield enhancement
<i>AtAVP1</i>	<i>A. thaliana</i>	Increased shoot and root biomass, photosynthetic capacity, yield and nutrient use efficiency; increased salinity tolerance
<i>OsNAS2</i>	<i>O. sativa</i>	Increase in shoot biomass, higher numbers of tillers and grain
<i>OsPSTOL1</i>	<i>O. sativa</i>	Enhanced growth vigour and earlier heading, high yield

Element	Gene Source	Function
Yield enhancement and water use efficiency*		
<i>TaMUTE</i>	<i>T. aestivum</i>	Stomatal development, symmetrical division of guard mother cells
<i>TaYDA1</i>	<i>T. aestivum</i>	Negatively regulates stomatal development
<i>TaYDA2</i>	<i>T. aestivum</i>	Negatively regulates stomatal development
<i>TaOST1</i>	<i>T. aestivum</i>	Regulates stomatal aperture
<i>TaSLAC1</i>	<i>T. aestivum</i>	Guard cell anion channel
Promoters		
<i>CaMV35S</i>	Cauliflower mosaic virus	Constitutive
<i>Ubi</i>	<i>Z. mays</i>	Constitutive, polyubiquitin
<i>OsAct1</i>	<i>O. sativa</i>	Constitutive, rice actin 1
<i>PvUbi1+3</i>	<i>Panicum virgatum</i>	Constitutive, ubiquitin
Amplification promoting sequences		
<i>Ubi1 5' UTR</i>	<i>Z. mays</i>	Translational modifier
<i>Ubi1 intron</i>	<i>Z. mays</i>	Translational modifier
Selectable Marker Genes		
<i>hptII</i>	<i>E. coli</i>	Plant selectable marker – hygromycin resistance gene encoding hygromycin phosphotransferase
<i>nptII</i>	<i>E. coli K12</i>	Plant selectable marker – neomycin phosphotransferase gene for resistance against geneticin or kanamycin
<i>bar</i>	<i>Streptomyces hygroscopicus</i>	Plant selectable marker – bialaphos resistance gene encoding phosphinothricin N-acetyltransferase (PAT) protein that confers tolerance to glufosinate
<i>pporRFP</i>	<i>Porites porites</i>	Visual selectable marker gene – red fluorescent protein
Terminator		
<i>CaMV35S</i>	<i>Cauliflower mosaic virus</i>	Viral terminator
<i>nos</i>	<i>A. tumefaciens</i>	Terminator of the nopaline synthase gene and polyadenylation signal
<i>OCS</i>	<i>A. tumefaciens</i>	Terminator sequence of the octopine synthase gene

\*Note: *Triticum aestivum* is a hexaploid plant with three genomes, known as the A, B and D genomes. The genes for yield and water use efficiency from each of the three *T. aestivum* genomes may be used to modify the GM wheat and barley lines. The genes have the same name except for the final letter (A, B or D), and are homologs derived from the different wheat genomes.

## 4.2 Methods of genetic modification

24. The genes for yield enhancement are expressed on their own, or as combinations of genes. Wheat and barley plants with single genes were transformed either with biolistic transformation or *Agrobacterium*-mediated transformation. Information about these methods can be found in the document *Methods of plant genetic modification*, available from the [OGTR Risk Assessment References](#) page. Lines containing more than one introduced gene were generated using either controlled crossing of the GM plants containing single gene insertions, or by direct transformation of GM plants with single gene insertions.

## 4.3 The introduced genes, encoded proteins and associated effects

25. The genes and their encoded proteins are summarised in Table 1, with a description of their expected function in the GM wheat and barley lines. Both yield enhancement and water use efficiency are

multigenic traits, involving the interaction of genes where the protein products constitute different biochemical pathways.

### **Gene stacked lines**

26. The introduction of each of the genes for yield enhancement or abiotic stress tolerance individually has the potential to improve the yield of wheat and barley. At this stage, there is little information on the phenotypic effect of combined overexpression of the genes. However, as each of the genes is involved in a different aspect of yield enhancement or water use efficiency, a combination of genes may have the potential to produce wheat and barley plants with increased grain yield under optimal growing conditions. Some of the GM wheat and barley proposed for release contain more than one of the introduced genes. The applicant intends that in future trials, gene stacking between some or all of the GM lines to evaluate if particular gene combinations can enhance yield and water use efficiency to be undertaken (subject of a new application).

#### **4.3.1 Yield enhancement**

27. The yield enhancement genes proposed for release are *AtAVP1*, *OsNAS2* and *OsPSTOL1*. Field trials of GM wheat and barley with these genes have been evaluated and licensed previously for DIR 102, DIR 128 and DIR 152, so only a summary and more recent material regarding these genes is presented here.

#### **AtAVP1**

28. The *Arabidopsis thaliana* vacuolar H<sup>+</sup>-pyrophosphatase (*AtAVP1*) gene encodes an H<sup>+</sup>-translocating pyrophosphatase (H<sup>+</sup>-PPase) that appears to be localised to the tonoplast and plasma membrane (Gaxiola et al. 1999; Khadilkar et al. 2016). H<sup>+</sup>-PPase proteins are proton pumps that use the energy gained from the breakdown of pyrophosphate to pump protons into the vacuoles of plant cells (Khadilkar et al. 2016). However, *AtAVP1* can function as both a pyrophosphatase and as PPI-synthase, depending on the electrical gradients across membranes (Pizzio et al., 2017; Scholz-Starke et al., 2019). In addition, other functions have been ascribed to *AtAVP1*, including a role in auxin distribution within the plant, synthesis of ascorbic acid, acidification of the rhizosphere leading to improved nutrient (e.g. iron, magnesium and potassium) uptake, and sucrose loading of the phloem (Menadue, 2018).

29. Overexpression of *AtAVP1* in *A. thaliana* increased tolerance of the plants to both drought and salt stress (Gaxiola et al. 2001), and overexpression of *AtAVP1* and its homologs in plants increased proliferation of roots and shoots (Li et al. 2005; Lv et al. 2008; Pei et al. 2012). Overexpression of H<sup>+</sup>-PPases has also been shown to significantly increase photosynthetic capacity, yield and nutrient use efficiencies in a number of crops grown under normal or stress conditions (Gaxiola et al. 2001; Park et al. 2005; Yang et al. 2007; Li et al. 2008; Lv et al. 2008). Constitutive expression of H<sup>+</sup>-PPase proteins in different plant species has produced varying phenotypes. This may indicate that the roles of the protein vary in the different plant species (Menadue, 2018).

#### **OsNAS2**

30. Although soils often have a high iron content, plants may not be able to take up the iron as it is not bioavailable (Wang et al., 2019). In crop plants under iron stress, yields suffer as iron is involved in a number of important functions, e.g. chlorophyll synthesis. If iron availability is low, plants respond by expressing various genes that help with iron uptake (Wang et al., 2019). For example, the *OsNAS2* gene encodes a rice nicotianamine synthase (NAS), an enzyme that catalyses the last step in the production of nicotianamine. Nicotianamine is a molecule made by all higher plants that chelates and transports transition metals including iron and zinc (von Wiren et al., 1999). In grasses, nicotianamine is also a precursor for biosynthesis of phytosiderophores, which are molecules that are secreted from roots to facilitate solubilisation and uptake of iron from the soil (Inoue et al., 2003).

31. Constitutive overexpression of a barley NAS gene, *HvNAS1*, in tobacco led to increased concentrations of iron, zinc, copper, manganese and nickel in shoots and/or seeds, demonstrating enhanced transport of these metals following root uptake (Kim et al., 2005). Constitutive overexpression of

rice *OsNAS1*, *OsNAS2* or *OsNAS3* genes in rice led to increased levels of iron and zinc in the grain, but no significant differences in copper, manganese or nickel content compared to non-GM control rice plants (Johnson et al., 2011). Constitutive overexpression of rice *OsNAS2* in GM wheat increased iron, zinc and copper levels in grain for all GM lines, and increased manganese and magnesium levels for most GM lines, compared to control non-GM wheat (Singh et al., 2017).

32. Several GM crops overexpressing NAS genes have demonstrated tolerance to low iron availability in alkaline soils, which causes leaf chlorosis and poor yield in control non-GM plants (Nozoye, 2018 and references cited therein). In addition, GM tobacco and *Arabidopsis* overexpressing a NAS gene have shown increased tolerance to high levels of heavy metals, particularly nickel, which cause toxicity to non-GM plants (Kim et al., 2005).

### ***OsPSTOL1***

33. The Phosphorous Starvation Tolerance 1 (*PSTOL1*) gene occurs within a major quantitative trait locus (QTL) for phosphorus-deficiency tolerance identified in the aus-type rice variety Kasalath. This gene is absent in the genome of phosphorus-starvation-intolerant rice varieties. Overexpression of *PSTOL1* in these varieties enhances grain yield in phosphorus deficient soil, putatively by promoting early crown root development and root growth, which facilitates the uptake of phosphorus and other nutrients like nitrogen and potassium (Gamuyao et al. 2012). A survey of sorghum identified six genes with high sequence similarity to rice *PSTOL1*, two of which were associated with an increased root surface and grain yield under low phosphorus field conditions (Hufnagel et al. 2014).

34. *OsPSTOL1* encodes a functional serine/threonine protein kinase (Gamuyao et al. 2012). Protein kinases are mediators of cellular signalling: they accept input information from receptors that sense environmental conditions, phytohormones and other external factors, and convert it into appropriate outputs such as changes in metabolism, gene expression, and cell growth and division (Hardie 1999). They interact with target proteins and phosphorylate them, resulting in protein activation or deactivation to effect a wide array of processes ranging from disease resistance and developmental regulation to reproduction (Hardie 1999). *OsPSTOL1* shows highest amino acid sequence similarity with serine/threonine receptor-like kinases of the LRK10L-2 family, and may be a receptor-like cytoplasmic kinase (Gamuyao et al. 2012). The molecular mechanism of *OsPSTOL1* that translates into enhanced root growth is not yet fully elucidated.

#### **4.3.2 Yield enhancement and abiotic stress tolerance (water-use efficiency)**

35. The applicant has stated that the genetic modifications involving MUTE, YDA1, YDA2, OST1 and SLAC1 aim to alter stomatal distribution, density, size and/or regulation. Stomata are central to plant drought responses because they modulate transpiration and the uptake of carbon dioxide (Lawson and Blatt, 2014). Plants can adjust stomatal aperture to maximise carbon assimilation while also limiting water loss (Raissig et al., 2017).

36. Genetic modification of stomatal development and aperture may protect plants against drought, allowing them to continue to grow in water-limited environments (Franks et al., 2015; Hepworth et al., 2018). The reduction of stomatal density in GM *Arabidopsis*, maize and barley has been shown to improve water-use efficiency and/or drought tolerance (Buckley et al., 2019). However, modification of stomates may also reduce carbon dioxide assimilation, which in turn may reduce sugar production by photosynthesis, resulting in a negative impact on plant yield (Dunn et al., 2019).

37. Genetic modification of stomatal development and aperture may also influence flowering time. Stomatal opening has been shown to be regulated by the FLOWERING LOCUS T (FT), a gene involved in early flowering in *Arabidopsis thaliana* (Kinoshita et al., 2011). FT-like genes have been identified in dicots and monocots, including wheat and barley (Guo et al., 2015; Haiyang et al., 2019). Under short day conditions, GM *Arabidopsis* plants containing a cotton homolog of this gene were found to flower 24 days after sowing when this gene was overexpressed, compared to around 39 days to flowering in wildtype *Arabidopsis* plants (Guo et al., 2015).

### **TaMUTE gene**

38. MUTE is one of three transcription factors that have been shown to positively regulate stomatal development in *Arabidopsis* (Liu et al., 2019). These three regulators are closely related basic helix-loop-helix (bHLH) domain transcription factors that control stomatal development at the initiation, meristemoid differentiation and guard cell morphogenesis (Pillitteri et al., 2007). In *Arabidopsis*, MUTE is highly expressed in meristemoids and acts as a molecular switch for meristemoid fate transition (Pillitteri et al., 2007; Liu et al., 2009).

39. Knockout of the MUTE gene in *Arabidopsis* resulted in the complete absence of stomata, while overexpression of MUTE led to the entire epidermis covered in stomata (Pillitteri et al., 2007). Orthologs of these transcription factors are found in other flowering plants, as well as in grasses and other monocots (Liu et al., 2009; Peterson et al., 2010; Raissig et al., 2017).

### **TaYDA1 and TaYDA2 genes**

40. The YDA gene encodes a mitogen activated protein (MAP) kinase kinase kinase (MAPKKK) known as YODA, which is an important negative regulator of stomatal development (Gray and Hetherington, 2004). The MAPKKK signal transduction pathway controls the activity of MUTE and the other two bHLH transcription factors involved in stomatal development (Qi and Torii, 2018; Dunn et al., 2019). In turn, the MAPKKK pathway is regulated by environmental factors, such as light and carbon dioxide, as well as endogenous peptide factors (Dunn et al., 2019).

41. In *Arabidopsis*, loss-of-function mutations in the YDA gene lead to the massive over-proliferation of stomata in the epidermis (Le et al., 2014). Abrash et al. (2018) found that the YDA gene promoted normal stomatal spacing patterns in both *Arabidopsis* and *Brachypodium* (a monocot grass model plant). A mutant copy of the YDA gene in *Brachypodium* leaves produced excess stomata arranged in clusters, along with a stunted growth phenotype (Abrash et al., 2018).

### **TaSLAC1 and TaOST1 genes**

42. The SLAC1 gene encodes a guard cell anion channel protein (SLOW ANION CHANNEL-ASSOCIATED 1) that is essential for stomatal closure in response to a number of environmental factors, including carbon dioxide, light/dark transitions, humidity and ozone (Vahisalu et al., 2008; Hedrich and Geiger, 2017). The SLAC1 protein is localised to the plasma membrane of guard cells in *Arabidopsis* (Negi et al., 2008). *Arabidopsis* SLAC1 gene mutants exhibit both impaired stomatal closing and slow stomatal opening induced by light, low carbon dioxide and elevated air humidity (Laanemets et al., 2013).

43. In *Arabidopsis* guard cells, the SLAC1 anion channel is activated by the protein kinase OST1 (Geiger et al., 2009). The OST1 gene encodes a protein kinase (OPEN STOMATA 1) that is expressed in stomatal guard cells and vascular tissue (Mustilli et al., 2002; Acharya et al., 2013). *Arabidopsis* plants lacking the OST1 gene showed a reduction in their ability to limit transpiration in water-limited environments (Mustilli et al., 2002).

44. The OST1 gene is activated by the plant hormone abscisic acid (ABA), which is synthesised in response to abiotic stress (Mustilli et al., 2002; Geiger et al., 2009).

### **4.3.3 Marker Genes**

45. The GM wheat and barley plants contain selectable marker genes that confer resistance to different classes of antibiotics or to a herbicide (Table 1). Selectable markers are used in the laboratory to select transformed GM plants or plasmids during early stages of development. The selectable marker genes are *hptII*, which codes for hygromycin phosphotransferase enzymes (HPH or HPT; confers resistance to hygromycin; (Stogios et al., 2011); *nptII* (neomycin phosphotransferase II) which encodes an aminoglycoside 3'-phosphotransferase II enzyme that is also known as neomycin phosphotransferase II (NPTII; confers resistance to kanamycin and related antibiotics) and the *bar* gene which encodes the phosphinothricin N-acetyltransferase (PAT) protein (confers tolerance to glufosinate herbicides).

46. The *nptII* and *hptII* genes are derived from *Escherichia coli*, a common gut bacterium that is widespread in human and animal digestive systems and in the environment. The *bar* gene is derived from *Streptomyces hygroscopicus* (Thompson et al., 1987), a common saprophytic, soil-borne microorganism that is not considered to be a pathogen of plants, humans, or other animals (OECD, 2002). More information on marker genes in general may be found in the document [Marker Genes in GM Plants](#).
47. Some GM wheat and barley plants contain the introduced *pporRFP* gene, which encodes a novel DsRed-type red fluorescent protein (RFP) derived from the coral *Porites porites* (Alieva et al., 2008). Coral fluorescent proteins like *pporRFP* and DsRed are homologous to green fluorescent proteins (GFP) from the jellyfish *Aequorea victoria*, which have been widely used as reporter genes in GM plants (Jach et al., 2001; Alieva et al., 2008; Mann et al., 2012a). More information on the use of reporter genes in general may be found in the document [Marker Genes in GM Plants](#).
48. The *pporRFP* gene may be used as a real-time visual marker gene in the GM wheat and barley plants. In humans and animals, vision is based on being able to perceive certain wavelengths of light. Different physical and chemical systems are in play that manipulate wavelengths and are important in vision (reviewed in Marshall and Johnsen, 2017). Of note, fluorescence is weak compared to reflected light, and therefore filters to remove reflected light must be employed in order to perceive fluorescence.
49. The visual marker used in the current proposal is a fluorescent marker, and the expression of the *pporRFP* gene can be monitored in the GM plant tissue using *in vivo* fluorescence microscopy with an RFP filter, avoiding the need to destroy the tissue (Mann et al., 2012b). The maximal excitation and emission wavelength of the *pporRFP* protein are in the visible spectrum at 578 nm and 595 nm, respectively. Neither its excitation nor emission spectrum is in the UV region. While the protein is often referred to as a red fluorescent protein, its emission is closer to orange (Mann et al., 2012a).
50. Autofluorescent molecules are common in plant tissues (Marshall and Johnsen, 2017). Other plant molecules that are fluorescent in the orange and red visible spectrum include chlorophyll, alkaloids, anthocyanins and tannins (Donaldson, 2020). Fluorescent systems in budgerigars and jumping spiders have been shown to be used in mate choice (Marshall and Johnsen, 2017). However, the functional significance of fluorescence in plants under natural conditions is unclear. A recent review by van der Kooi et al. (2019) concluded that there was no clear experimental evidence showing that fluorescence increased the visibility of flower nectar and pollen to pollinators. Furthermore, the ratio of photons absorbed to photons emitted (quantum efficiency) of the majority of natural pigments is low, meaning that the use of fluorescence as visual signal is rare (Lagorio et al., 2015; Marshall and Johnsen, 2017; van der Kooi et al., 2019). Studies of intact flowers from a range of species, including petunias and bougainvillea, found that photons emitted as fluorescence were negligible compared to photons reflected from the petals, suggesting that it is highly unlikely that fluorescence is involved in visual signalling for pollinators in these plants (Lagorio et al., 2015). For example, *Citrus aurantium* white petals emit only 1.4% of the absorbed light in the blue spectrum as fluorescence, whereas 45% of the incident light is reflected in this region (Iriel and Lagorio, 2010). Expression of the red fluorescent protein in the GM wheat and barley proposed for release will be visible with appropriate filters in the laboratory, but will be difficult to observe when planted in the field due to the reflected visible light swamping the orange/red fluorescence.
51. Expression of the *pporRFP* visual marker gene has been shown to be very effective in GM rice (*Oryza sativa*) and GM switchgrass (*Panicum virgatum*) (Mann et al., 2010; Ondzighi-Assoume et al., 2019). The reporter gene has also been used as a phytosensor in GM plants to enable the detection of plant pathogens. In these studies, the *pporRFP* gene was fused to plant pathogen inducible promoters, which were transformed into tobacco and *A. thaliana*. The red fluorescent protein was expressed in the presence of plant pathogens, allowing real-time monitoring, using appropriate filters, and early warning of plant infection (Liu et al., 2011; Fethe et al., 2014). GM maize containing the DsRed protein as a visual reporter gene have been approved for release in the US and Brazil (Wu et al., 2016).

#### 4.4 Toxicity/allergenicity of the proteins associated with the introduced genes

52. Non-GM wheat and barley contain a number of anti-nutritional factors and allergens that, in extreme cases, may have a toxic effect (OGTR, 2021a, b).

53. The applicant has not yet performed any toxicity or allergenicity studies on the GM wheat and barley lines proposed for release. Apart from *pporRFP*, all of the genes introduced into the GM plants were isolated from common sources, thus people and other organisms have a long history of exposure to them.

54. A comprehensive search of the scientific literature yielded no information to suggest that the genes themselves, their protein products, or any associated products or effects were toxic or allergenic to people, or toxic to other organisms, except for *OsNAS2* as discussed below. This includes homologues isolated from other species, apart from the *pporRFP* homologue *DsRed*. However, toxicity/allergenicity tests have only been performed on the introduced HPH, NPTII and PAT proteins.

55. In the current application, the introduction of the *OsNAS2* gene is being examined for its role in yield enhancement as a result of increased iron uptake. This gene has been studied by other research groups with the aim of increasing levels of iron in plant tissues and biofortification. Iron content in whole wheat plants is approximately 30 µg/g plant material, with a biofortification target of 52 µg/g (Bouis et al. 2011). People must obtain iron from their diet as it is involved in several essential processes in the body. However, excessive iron in the diet can result in toxicity, i.e. more than 20 mg/kg of body weight (Balmadrid & Bono 2009). Even in research aimed at producing biofortified wheat lines, the targeted concentrations of iron are such that these levels are unlikely to occur as a result of typical consumption. Certain conditions such as thalassemia (Tanno et al. 2007; Nemeth 2010) and hereditary haemochromatosis (Barlow-Stewart et al. 2007) may be further complicated by iron overload.

56. For dogs, no clinical signs of iron toxicity are expected after oral ingestion of less than 20 mg iron/kg of body weight. In all companion animals, oral doses between 100 and 200 mg iron/kg of body weight are potentially lethal (Albretsen, 2006). This suggests that susceptibility to excess iron toxicity is similar in people and other mammals.

57. *OsNAS2* introduction or overexpression could lead to accumulation of metals other than iron. Several heavy metals are toxic to humans and animals, e.g. arsenic, lead, mercury and cadmium (Flora et al., 2008; Jaishankar et al., 2014; Clemens and Ma, 2016). Of these, cadmium is of interest because: (a) more than 80% of human cadmium exposure is from consumption of cereals and vegetables; (b) many populations around the world already have cadmium intake above recommended levels, so moderate increases in cadmium exposure could have toxic effects; and (c) in terms of chemical characteristics, cadmium mimics iron and zinc, so may be taken up by biological pathways that are used for biofortification (Khan et al., 2014; Clemens and Ma, 2016). It is recommended to monitor levels of cadmium in agricultural produce in general in Australia, as it is naturally present in Australian soils from less than 0.1 to 0.5 mg/kg in the top 10 cm of soil. It can be present at higher levels in the vicinity of, e.g. smelters (Horticulture Australia, 2003).

58. However, GM rice overexpressing *OsNAS2* and soybean ferritin genes had grain cadmium, lead and arsenic levels below detection limits when grown in normal soil, and when grown in cadmium-contaminated soil there was no difference between grain cadmium levels in the GM and non-GM rice (Trijatmiko et al., 2016).

59. There have been no adverse effects reported from similar GM lines planted under DIR 102, DIR 128 or DIR 152. It should be noted that neither licence permitted use of the GM lines in human food or animal feed.

60. There is no evidence that the *nptII* or *hptII* genes or the proteins they encode are toxic or allergenic (OGTR Risk Assessment documents and references therein). GM foods containing the *nptII* and *hptII* genes have been assessed and approved for sale in Australia (FSANZ website, accessed 20 September 2021).

61. The *bar* gene and the protein it encodes (phosphinothricin N-acetyl transferase or PAT) has been extensively assessed in other RARMPs most recently in DIR 178, and in scientific literature. The PAT protein has been assessed to lack toxicity to humans or animals, or allergenicity in humans on the following basis:

- the *bar* gene was derived from the common soil bacterium *S. hygroscopicus*, which is not considered a pathogen of humans or other animals
- no sequence homology has been found between PAT and any known toxic or allergenic proteins



- the PAT protein does not possess any of the characteristics associated with food allergens
- the PAT protein is inactivated by heat, e.g. through cooking, and by low pH, e.g. in the human stomach
- purified PAT protein was not toxic to mice and rats when administered at high doses in acute toxicity studies.

62. FSANZ has approved food derived from a number of GM crops expressing the PAT protein as safe for human consumption. This includes GM canola (ANZFA, 2001; FSANZ, 2017), cotton (FSANZ, 2005b, 2010a, b, 2013), corn (FSANZ, 2005a) and rice (FSANZ, 2008).

63. The *pporRFP* gene and its encoded protein have not been previously assessed by the OGTR. Like other red fluorescent proteins, pporRFP is a tetramer, which can lead to cytotoxicity when expressed as a fusion protein in GM plants (Campbell et al., 2002; Shemiakina et al., 2012). However, the GM wheat and barley do not contain pporRFP as a fusion protein, and there is no information in the literature to suggest that this introduced gene or its product is toxic or allergenic to people or toxic to other desirable organisms.

#### 4.5 Characterisation of the GMOs

64. Although these lines are at an early stage of development, the applicant has provided some preliminary information on expected phenotypes for some of the genes.

65. Some GM wheat lines constitutively overexpressing *OsNAS2* have increased iron concentration in grains (Beasley et al., 2017). The applicant stated that the lines also show a 20 - 30 % increase in shoot biomass due to a higher tiller number and produce approximately 20 - 30 % more grain than wild-type plants.

66. The applicant also claims that overexpression of *OsPSTOL1* in GM wheat resulted in enhanced plant vigour and earlier heading. In GM rice, *OsPSTOL1* conferred enhanced root growth, thus increasing uptake of phosphorous as well as nitrogen and potassium (data not supplied). Recently, six genes with sequence similarity to *OsPSTOL1* have been identified in sorghum. Two of these genes were associated with an increased root surface and grain yield under low phosphorous conditions in the field (Hufnagel et al., 2014). The applicant also stated that data from DIR 152 shows field grown GM wheat expressing *OsPSTOL1* has enhanced grain yield.

67. The genetic modification of *MUTE*, *YDA1*, *YDA2*, *OST1* and *SLAC1* aims to alter stomatal distribution, density, size and/or regulation. As discussed in Section 4.3.2, published data indicates that overexpression, or mutant copies of these genes, do alter the presence and function of stomata in studied plants. The applicant anticipates the same phenotypes in the GM wheat and barley proposed for release, however there is no information on phenotype changes in the GM wheat and barley proposed for release.

68. Genetic modification of the *OST1* and *SLAC1* genes in the GM wheat and barley may also affect the abiotic stress tolerance of the plant, as *SLAC1* (under the control of *OST1*) has been shown to be involved with anion transport (Geiger et al., 2009).

69. The applicant has stated that one of the unintended changes from the proposed genetic modification may be reduced time to flowering. Some of the GM lines approved for release under DIR 152, have been shown to flower 5-10 days earlier than non-GM plants within the same cultivar in the glasshouse and under field conditions.

70. The GM wheat lines for yield enhancement (*AtAVP1*, *OsNAS2* or *OsPSTOL1*, individually and in combination) were grown under DIR 152. According to the applicant, these lines had up to 60% increased biomass under greenhouse conditions, however this phenotype was not observed in the field under increased nutrient and water-limited conditions. Only yield was successfully increased by up to 30% under these field conditions.

## Section 5 The receiving environment

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

72. Detailed information about non-GM wheat in the Australian environment is presented in the document *The Biology of Triticum aestivum L. (Bread Wheat)* (OGTR, 2021b). Detailed information relevant to the commercial cultivation and distribution of non-GM barley in Australia is available in *The Biology of Hordeum vulgare L. (barley)* (OGTR, 2021a).

### 5.1 Relevant biotic factors

73. A number of biotic factors are important in the cultivation of both wheat and barley. There are several weeds that impact on wheat production, while barley is generally regarded as being more competitive with weeds. A number of vertebrate pests, which are discussed further in Chapters 2 and 3, affect both wheat and barley. Insect pests are generally regarded as more of a concern for wheat than for barley, although barley can also be damaged under conditions where insect populations build up. Both wheat and barley are affected by a number of invertebrate pests and pathogens including nematodes, fungal diseases, bacteria and viruses. Both species also interact with potentially beneficial endophytic bacteria and fungi.

### 5.2 Relevant abiotic factors

74. It is proposed that the GMOs will be grown at a field trial facility at Rosedale in SA. The applicant intends to plant the GMOs in more than one planting area per site, which allows for the analysis of seasonal and environmental stress variation. The total planting area would be up to 2 ha per year. GM plants approved under other DIR licences, including DIR 152 (in SA) and future DIR licences, if approved, would also be grown at the site, although in different planting areas.

75. The site in Rosedale is located in Light Regional Council, a local government area north of Adelaide. The proposed trial site is on land leased by The University of Adelaide from the South Australian Research and Development Institute (SARDI). Light Regional Council is located in commercial wheat and barley growing regions of South Australia, based on information discussed in the [OGTR Biology documents](#) for these plants. The proposed Rosedale site has a climate typical of rain-fed wheat production areas for South Australia based on [Bureau of Meteorology climate data](#), which shows a concentration of rainfall during the winter months and drier summer months.

76. Nutrient stress, particularly nitrogen, potassium and phosphorus, affects both species. Both crop species are affected by drought, although barley is generally regarded as more tolerant to drought than wheat with better water use efficiency. However, barley is susceptible to waterlogging. Heat stress impacts on wheat and barley production, and barley is generally regarded as less cold tolerant than wheat, although both can be affected by frost. Wheat is susceptible to salinity, while barley is generally regarded as the most salinity tolerant cereal crop. Barley is also sensitive to acidic soils and to aluminium and boron toxicity.

### 5.3 Relevant agricultural practices

77. The limits and controls of the proposed release are outlined in Section 2.1 and Section 2.2 of this Chapter. It is anticipated that the agronomic practices for the cultivation of the GM wheat and barley by the applicant will not differ significantly from industry best practices used in Australia.

78. Seeds would be harvested either by hand or with a machine (e.g. plot harvester) which can be cleaned within the planting area. Threshing would occur within the same planting area or heads transported to approved facilities for threshing, analysis or other processing.

79. Waste material derived from the harvest would be left on the trial area and ploughed back into the soil along with any stubble remaining after harvest. Cultivation would be to the depth of seeding so that grain is not transferred any deeper into the soil profile. If not ploughed back into the soil, the waste may be burnt or buried elsewhere on site.

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

80. The proposed location is within a cereal-producing region.

81. The Rosedale site has previously been used for sheep grazing for over 10 years. No wheat or barley has been sown in surrounding fields. However, planting of GM wheat and barley can occur at the site until (and including) the 2022/2023 growing season under the DIR 152 licence, so planting could occur under DIR 152 concurrently with that proposed under DIR 186.

82. Cultivated wheat and barley are not known to hybridise with one another naturally, but each can hybridise with other species. Details are given in the [biology documents](#) for these species and briefly summarised below.

##### 5.4.1 Wheat

83. Bread wheat (*Triticum aestivum* L.) is sexually compatible with other bread wheat or durum plants. Bread wheat is cultivated in the LGA where proposed field trial site may be located.

84. *Triticum aestivum* can spontaneously hybridise with a number of closely related species from the *Triticum-Aegilops* genera complex (Zaharieva and Monneveux, 2006). The only other *Triticum* species present in Australia is *T. turgidum* (durum wheat), which is cultivated for pasta production ([Atlas of Living Australia](#), accessed 22 September 2021). No *Aegilops* species (goatgrasses) are cultivated or naturalised in Australia ([Weeds Australia](#), accessed 22 September 2021).

85. There have been occasional reports of natural hybridisation of wheat with rye (*Secale cereal*) or triticale (*xTriticosecale*), which are minor crops in Australia. However, these hybridisation events are rare and progeny are usually sterile (Hegde and Waines, 2004; Kavanagh et al., 2010).

86. A European study of gene flow from wheat to *Hordeum marinum* found no first-generation hybrids, however one *H. marinum* plant contained a low level of introgressed genetic material from wheat (Guadagnuolo et al., 2001). It is unclear whether this gene flow occurred directly from wheat to *H. marinum* or via one or more bridge species.

##### 5.4.2 Barley

87. Barley has a primary gene pool consisting of *H. vulgare* and *H. vulgare ssp. spontaneum*, which produce completely fertile offspring following crossing. The secondary gene pool consists of *H. bulbosum* L. where mating can occur but often hybrids are sterile, and a tertiary gene pool containing all other *Hordeum* species (Pickering & Johnston 2005). There are strict isolation barriers to gene flow between *Hordeum* species. It is therefore highly unlikely that barley would outcross to other species to produce fertile progeny and *H. vulgare ssp. spontaneum*, with which it may outcross, is not known to be present in Australia.

88. Although there have been interspecific crosses within the *Hordeum* genus and intergeneric crosses across a number of genera, all have been under experimental conditions and successful hybrids have not been observed under natural conditions. Details of experimental crosses are provided in the barley [biology document](#).

#### 5.5 Presence of similar genes and their products in the environment

89. The introduced genes listed in Table 1 were originally isolated from naturally occurring organisms most of which are already widespread and prevalent in the environment. Thus, humans and animals have been exposed to these genes and their encoded proteins either through consumption of the parent organisms or through other exposures in the environment. In addition, homologues of the genes and encoded proteins occur naturally in animals, plants, yeast and bacteria.

90. The *hptII* and *nptII* genes are derived from *E. coli*, a common gut bacterium that is widespread in human and animal digestive systems and in the environment. Both humans and animals are routinely exposed to the genes and their encoded proteins through contact with plants or food.
91. The *bar* gene was isolated from the common bacterium *S. hygrosopicus*, which is a saprophytic, soil-borne microorganism that is not considered a pathogen of plants, humans or other animals (OECD, 1999). Genes encoding PAT and similar acetyltransferase enzymes are present in a range of common soil bacteria, and are not known to be toxic or allergenic (Hérouet et al., 2005).
92. The *pporRFP* gene was isolated from *P. porites*, a finger-like coral that is distributed in a variety of coral reefs environments across the Caribbean, in the western Atlantic Ocean and also along the coast of West Africa (Aronson et al., 2008). *Porites spp.*, including *P. porites*, are collected and traded for use as decorative objects (e.g. ornaments, jewellery and aquarium decoration) (Kinch et al., 2010; Taylor, 2016). Sources of the *pporRFP* protein in the terrestrial environment would be minimal.
93. All promoters used to drive expression of the introduced genes are derived from plant species (maize, rice and switchgrass), with the exception of the *CaMV35S* promoter from a plant virus. Humans and animals have been exposed to these plants and the plant virus for centuries. Other regulatory sequences are from common organisms including maize (*Z. mays*) and *A. tumefaciens*, a common bacterium that can cause galls in various plants.
94. While some of the source organisms can cause allergies (e.g. wheat), the introduced proteins are not known to cause harm.

## Section 6 Relevant Australian and international approvals

### 6.1 Australian approvals

95. Wheat and barley lines containing the three genes for yield enhancement (*AtAVP1*, *OsNAS2* and *OsPSTOL1*) proposed for release under the current application have been approved in Australia for limited and controlled release under licences including DIR 102 (*AtAVP1*), DIR 128 (*AtAVP1* and *OsNAS2*, individually) and DIR 152 (*AtAVP1*, *OsNAS2* and *OsPSTOL1*, individually and in combination). There have been no reports of adverse effects on human health and safety or the environment resulting from these releases.
96. Information on previous DIR licences for GM wheat and barley is available from the [OGTR GMO Record](#). The Regulator has previously approved 22 field trial releases of GM wheat, of which ten are licences for both wheat and barley. There have been no credible reports of adverse effects on human health or the environment resulting from any of these releases.
97. There have been no approvals for the commercial release of GM wheat or barley in Australia.

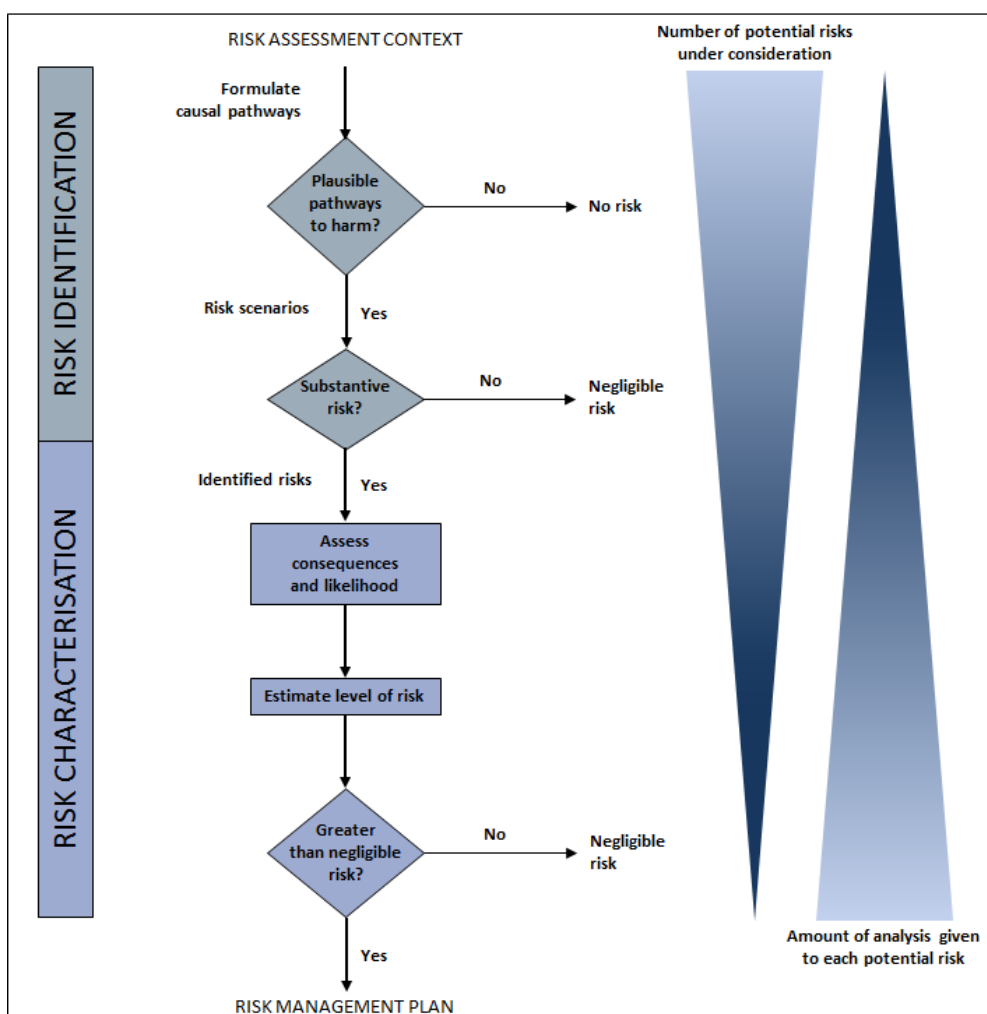
### 6.2 International approvals

98. Field trials of other GM wheat and barley have been approved in a number of countries including the United States, Canada, the United Kingdom and a number of European countries, for a range of modified traits, including improved yield and tolerance to abiotic stresses ([USDA APHIS Biotechnology Permits, EU GMO Register](#); accessed 17 September 2021).
99. On a commercial scale, drought tolerant HB4 GM wheat has been approved in Argentina.
100. None of the GM wheat and barley in the current application have been approved for release in any other country.

## Chapter 2 Risk assessment

### Section 1 Introduction

101. 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 2). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.



**Figure 3 The risk assessment process**

102. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, reported international experience and consultation (OGTR, 2013). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants, as this approach addresses the full range of potential adverse outcomes associated with plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are used to postulate risk scenarios (Keese et al., 2014). Risk scenarios examined in RARMPs prepared for licence applications for the same or similar GMOs, are also considered.

103. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating plausible causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are risk scenarios. These risk scenarios are screened to identify those that are considered to have a reasonable chance of causing harm in the short or long term. Pathways that do not lead to harm, or

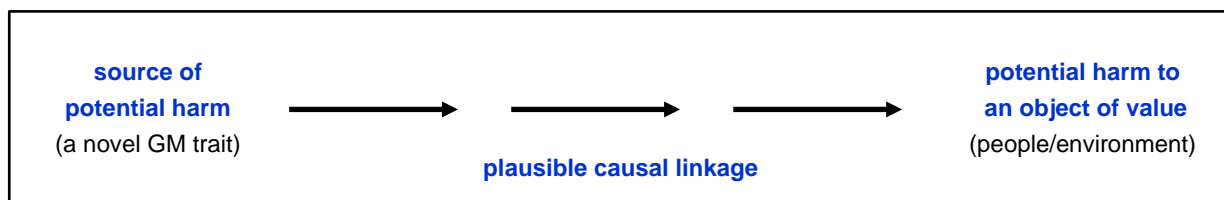
those that could not plausibly occur, do not advance in the risk assessment process (Figure 3) i.e. the risk is considered to be no greater than negligible.

104. Risks identified as being potentially greater than negligible are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). Risk evaluation then combines the Consequence and Likelihood assessments to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

## Section 2 Risk Identification

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

- i. the source of potential harm (risk source)
- ii. a plausible causal linkage to potential harm (causal pathway)
- iii. potential harm to people or the environment.



**Figure 4 Risk scenario**

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

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

### 2.1 Risk source

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

108. As discussed in Chapter 1, the GM wheat and barley lines have been modified by overexpression of genes. The intended effect of insertion of the genes is yield enhancement or yield enhancement and water use efficiency. Also, a red colour marker gene, *pporRFP*, has been introduced into some of the GM wheat and barley lines, the effect of which has not been risk assessed in previous DIR RARMPs. These introduced genes will be considered further as a potential source of risk.

109. The GM wheat and barley also contains other marker genes, *nptII* and *hptII* from *E. coli* that confer antibiotic resistance, and the *bar* gene that confers herbicide tolerance. These genes were used as selectable markers during development of the GM plants. The *nptII*, *hptII* and *bar* genes and their products have been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator, as well as by other regulatory agencies in Australia and overseas. Further information about the antibiotic resistance genes can be found in the document [Marker genes in GM plants](#) on the OGTR website. The *bar* gene and its protein product, PAT, have been assessed in other RARMPs as well as in scientific literature, as detailed in Chapter 1 (4.3). The environmental safety of the PAT protein present in biotechnology-derived crops has also been extensively assessed worldwide (CERA,

2011). As the marker genes have not been found to pose a substantive risk to either people or the environment, their potential effects will not be further considered for this application.

110. The introduced genes are controlled by introduced regulatory sequences. These were originally derived from viruses, bacteria and plants. Regulatory sequences, such as promoters, enhancer sequences and terminators, are naturally present in all plants and the introduced sequences are expected to operate in similar ways to endogenous sequences. These sequences are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary DNA has no toxicity (Society of Toxicology, 2003). Hence, potential harms from the regulatory sequences will not be further assessed for this application.

111. The genetic modifications involving introduction of genes have the potential to cause unintended effects in several ways. These include insertional effects such as interruptions, deletions, duplications or rearrangements of the genome, which can lead to altered expression of endogenous genes. There could also be increased metabolic burden due to expression of the introduced proteins, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, these types of effects also occur spontaneously and in plants generated by conventional breeding. Accepted conventional breeding techniques such as hybridisation, mutagenesis and somaclonal variation can have a much larger impact on the plant genome than genetic engineering (Schnell et al., 2015). Plants generated by conventional breeding have a long history of safe use, and there are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al., 2013). Therefore, the potential for the processes of genetic modification to result in unintended effects will not be considered further.

## 2.2 Causal pathway

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

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

113. Although all of these factors are taken into account, some are not included in risk scenarios because they have been considered in previous RARMPs.

114. The potential for horizontal gene transfer (HGT) from GMOs to species that are not sexually compatible, and any possible adverse outcomes, have been reviewed in the literature (Keese, 2008) and assessed in many previous RARMPs. HGT was most recently considered in the RARMP for [DIR 108](#). Although the DIR 108 RARMP is for GM canola, the HGT considerations are the same for the current RARMP: HGT events rarely occur and the wild-type gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, no substantive risk was identified in previous assessments and HGT will not be further considered for this application.

115. The potential for unauthorised activities to lead to an adverse outcome has been considered in many previous RARMPs, most recently in the RARMP for [DIR 117](#). In previous assessments of unauthorised activities, no substantive risk was identified. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

### 2.3 Potential harm

116. Potential harms from GM plants are based on those used to assess risk from weeds (Virtue, 2008; Keese et al., 2014) including:

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

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

### 2.4 Postulated risk scenarios

118. Four risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 2 and examined in detail in Sections 2.4.1– 2.4.4.

119. In the context of the activities proposed by the applicant and considering both the short and long term, none of the risk scenarios gave rise to any substantive risks.

**Table 2: Summary of risk scenarios from the proposed dealings with the GM wheat and barley**

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker	GM wheat and barley grows at the field trial site ↓ Expression of the introduced genes results in the GM wheat and barley composition being different from non-GM wheat and barley ↓ Exposure of people and other desirable organisms by ingestion of, or contact with, the GM wheat or barley	Increased toxicity or allergenicity for people or increased toxicity to other desirable organisms	No	<ul style="list-style-type: none"> <li>• GM plant material would not be used as human food or animal feed.</li> <li>• Other proposed limits and controls would further minimise the exposure of people to GM plant material.</li> <li>• The source organisms for the introduced genes, other than <i>pporRFP</i>, are routinely used for food or feed or are commonly found in the environment.</li> </ul>



Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
2	Introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker	GM wheat and barley grows at the field site ↓ Hybridisation with other GM wheat or barley grown under a different licence at the trial site ↓ Expression in the GM hybrids of the introduced genes from both parental GM lines ↓ Exposure of people and other desirable organisms at the trial site by ingestion of, or contact with, the GM hybrid wheat or barley	Increased toxicity or allergenicity to people or increased toxicity to other desirable organisms	No	<ul style="list-style-type: none"> <li>The limited time, small scale and other proposed limits and controls minimise exposure of people and other desirable organisms to the GM hybrid seeds and other plant material, including buffer zones, site monitoring and post-harvest monitoring.</li> <li>Wheat and barley are mostly self-pollinating, and outcrossing occurs at low levels.</li> <li>The introduced genes, except the visual marker gene, were sourced from common food plants and the encoded proteins are not known to be toxic or allergenic.</li> </ul>
3	Introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker	Presence of GM seed outside the trial limits ↓ GM seed germinates ↓ Increased exposure of people and other desirable organisms by ingestion of, or contact with, the GM wheat or barley OR Establishment of GM wheat or barley in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity for people or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants OR Reduced utility or quality of the environment OR Increased ability to provide a reservoir for pathogens or shelter for pests	No	<ul style="list-style-type: none"> <li>The proposed limits and controls minimise the likelihood of seed dispersal or persistence outside the trial limits.</li> <li>There is no expectation the introduced genes confer characteristics in the GM wheat and barley that may lead to environmental harms.</li> <li>Dispersal by natural means, and ability to establish outside agriculture is limited in wheat and barley.</li> <li>The GM wheat and barley is susceptible to most standard weed control measures.</li> </ul>

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
4	Introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker	Fertilisation of sexually compatible plants outside the trial area by pollen from GM wheat or barley ↓ Germination of GM hybrid seed ↓ Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas ↓ Increased exposure of people and other desirable organisms by ingestion of, or contact with, the GM hybrid plant material OR Establishment of GM wheat or barley in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity for people or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants OR Reduced utility or quality of the environment	No	<ul style="list-style-type: none"> <li>• The proposed limits and controls minimise the likelihood of pollen flow from the trial site to sexually compatible plants.</li> <li>• Wheat and barley have limited ability to outcross.</li> <li>• Risk scenarios 1, 2 and 3 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks.</li> </ul>

**2.4.1 Risk scenario 1**

<i>Risk Source</i>	Introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker
<i>Causal Pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">GM wheat and barley grows at the field trial site</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Expression of the introduced genes results in the GM wheat and barley composition being different from non-GM wheat and barley</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Exposure of people and other desirable organisms by ingestion of, or contact with, the GM wheat or barley</p> <p style="text-align: center;">↓</p>
<i>Potential Harm</i>	Increased toxicity or allergenicity to people or increased toxicity to other desirable organisms

**Risk source**

120. The source of potential harm for this postulated risk scenario is the introduced genes for yield enhancement, improved abiotic stress tolerance and a visual marker in GM wheat and barley plants.

**Causal pathway**

121. The GM wheat and barley would be planted at the trial site. The aim of the introduced genes is to enhance yield and improve abiotic stress tolerance in the GM wheat and barley plants. The purpose of the visual marker is to identify GM wheat and barley plants in the laboratory. The encoded proteins could potentially be produced in all plant tissues throughout plant development, but this has not yet been determined. This is an area of uncertainty for this risk assessment.

122. The GM wheat and barley would not be used as human food, so people would not be exposed to plant material from the trial through ingestion. People may be exposed to GM plant material through inhalation of pollen when the GMOs flower, or through direct skin contact with GM plant material. This contact is most likely at the trial site. Transport and storage of the GM plant material would be conducted according to the Regulator's [Guidelines for the Transport, Storage and Disposal of GMOs](#), thus limiting exposure of people during transport and storage of the GMOs.

123. The applicant proposes that only authorised persons would be permitted to deal with the GM wheat and barley, or to access the trial site. These authorised staff could have direct skin contact with GM plant material or could inhale GM pollen.

124. Wheat pollen is wind dispersed, and although most pollen falls within 3 m of the source plant, some travels up to 60 m (reviewed in Hegde and Waines, 2004). Similarly, barley pollen is predominantly dispersed over short distances by wind (Wagner & Allard 1991), but some has been detected at distances of up to 50 m from the pollinator source (Ritala et al. 2002). Therefore, people who are not involved with the trial but who pass within 60 m of a trial site could be exposed to low levels of GM pollen, if the GM wheat or barley were flowering at the time. However, as the small size and limited duration of the proposed trial, and the fact that the proposed trial site is located in agricultural areas, only a very limited number of people not involved with the trial could be exposed to small amounts of GM pollen during flowering.

125. The GM wheat and barley would not be used as animal feed. However, animals, including mammals, birds and invertebrates, and other desirable organisms, may have direct contact with the GM wheat and barley at the trial site. A range of animals consume cereals (Hill et al. 1988; AGRI-FACTS 2002; OGTR 2021a; OGTR 2021b) and may be attracted to the GM plant material. The applicant proposes to surround the trial site with a fence and locked gates that would restrict access to large animals. Desirable animals such as small native mammals or birds could enter the trial site and feed on the GM wheat and barley. The small size and short duration of the proposed field trial and the proposed controls (Chapter 1, Section 2.1 and 2.2) would restrict the numbers of desirable organisms that would be exposed to the GM plant material.

### **Potential harm**

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

127. Potentially, people exposed to the proteins expressed by the introduced genes may show increased toxic reactions or increased allergic reactions. From consideration of the causal pathway, exposure would be mostly limited to staff involved in handling and harvesting the GM wheat and barley plants during the field trial. Similarly, exposure to the proteins expressed by the introduced genes may lead to increased toxicity to other desirable organisms.

128. No toxicity or allergenicity studies have been performed on the GM plant material and this is an area of uncertainty for this risk assessment. Most of the introduced genes were isolated from naturally occurring organisms that are widespread and prevalent in the environment, including common food sources such as rice and wheat. The fluorescent protein marker gene, *pporRFP*, was sourced from a coral, *Porites porites*, which is distributed in a variety of coral reefs environments and used for aquarium and home decoration (Chapter 1, Section 5.5). Thus, people and other organisms are exposed to the same or similar proteins through their diet and/or in the environment. There is no information in the literature to suggest that the introduced genes or their products are toxic or allergenic to people or toxic to other desirable organisms.

129. GM wheat or barley containing the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128 and DIR 152. No substantive risks for toxicity or allergenicity of the proteins were identified in the respective RARMPs nor have there been any reports of adverse effects from these earlier releases to people or animals. As noted in Chapter 1, section 4.3 and in the RARMPs for DIR 128 and DIR 152, the *OsNAS2* gene is associated with increased iron uptake in plant tissues and excessively high dietary iron can have toxic effects. Preliminary data suggest that the iron levels in these plants will not be in a range of concern for iron toxicity.

130. A further uncertainty is whether the *OsNAS2* modified plants increase uptake of cadmium or other heavy metals, which are toxic in people and other desirable animals. The level of cadmium accumulation in the GM wheat and barley will depend on soil cadmium concentration as is the case in non-GM wheat and barley. It is noted that some plants naturally accumulate higher levels of cadmium than wheat (Brennan and Bolland, 2004). In people, absorption of cadmium through the skin is negligible ([Agency for Toxic Substances and Disease Registry](#), accessed 9 November 2021). Absorption of cadmium through inhalation of plant material is known to occur, as smokers on average accumulate twice the cadmium burden of non-smokers, due to high cadmium levels in tobacco leaves. However, even heavy smokers receive only about 10% of the FAO/WHO Provisional Tolerable Weekly Intake for cadmium from smoking (EFSA, 2009). In addition, there is uncertainty regarding potential harm because sensitivity to cadmium toxicity varies between animal species and between different developmental stages in the same species (Furness, 1996; EFSA, 2004). Glasshouse trials involving other *OsNAS2* GM wheat lines found no difference in cadmium, lead or arsenic levels between the GM wheat plants and their non-GM counterpart (see paragraph 61 in the RARMP to [DIR 165](#)). Before the GM wheat and barley could be sold for use in food in Australia, assessment and approval by FSANZ would be required.

131. Five genes that have not been assessed in previous RARMPs are derived from wheat, and people and other desirable organisms have been exposed to these genes and their products through the consumption of, or other exposure to, wheat products. However, since the GM wheat and barley lines overexpressing these genes have not been studied in detail, this is an area of uncertainty for this assessment.

132. The visual marker gene *pporRFP* was isolated from the coral *P. porites* and encodes a DsRed-like red fluorescent protein pporRFP (Alieva et al., 2008). DsRed and its derivatives have been used in the genetic modification of a variety of organisms, including plants (Jach et al., 2001; Nishizawa et al., 2006; Sun et al., 2018). The original DsRed protein sourced from *Discosoma* sp. has demonstrated cytotoxic effects (Strack

et al., 2008; Shemiakina et al., 2012). Low or non-cytotoxic versions of DsRed have been generated and are currently in use (Bevis and Glick, 2002; Clontech Laboratories, 2003; Shemiakina et al., 2012). If the protein encoded by *pporRFP* were also cytotoxic, then the cells of the GM wheat and barley plants could be affected by this introduced protein. However, even if the *pporRFP* protein were cytotoxic, this does not necessarily mean that it would cause toxicity in people or other desirable organisms after ingestion as most proteins are digested before the resulting protein pieces or amino acids can be taken up by interstitial cells. Only proteins with (some) resistance to digestion may cause toxicity upon ingestion. The DsRed-like fluorescent protein encoded by the visual reporter gene, *pporRFP*, has not been characterised with regard to its digestibility, or toxicity or allergenicity in general, and this is an uncertainty for this assessment.

133. Non-GM wheat and barley are not regarded as toxic to humans or animals. However, both can produce allergic responses in susceptible individuals via inhalation of pollen or inhalation of flour (Astwood et al., 1995; Pahr et al., 2012). Common symptoms of respiratory allergy to wheat include rhinitis, conjunctivitis and asthma (Houba et al., 1998). Both wheat and barley can produce allergic and autoimmune responses in susceptible individuals by inhalation of flour (for example baker's asthma) or ingestion (coeliac disease). Barley pollen may also cause allergic reactions in susceptible individuals (OGTR, 2021a, b). There is no reasonable expectation that any of the genes introduced into the GM wheat or barley proposed for this trial would be allergenic or influence the pathways producing known allergens in wheat or barley.

### Conclusion

134. Risk scenario 1 is not identified as a substantive risk due to the proposed limits and controls, including the small size of the trial and not permitting the GM plant material in food or feed, and the fact that most of the introduced genes are already present in wheat or rice and lack toxicity or allergenicity in people as well as toxicity in other organisms. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

#### 2.4.2 Risk scenario 2

<i>Risk Source</i>	Introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker
<i>Causal Pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">GM wheat and barley grows at the field site</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Hybridisation between GM lines or with other GM wheat or barley grown under a different licence at the trial site</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Expression in the GM hybrids of the introduced genes from both parental GM lines</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Exposure of people and other desirable organisms at the trial site by ingestion of, or contact with, the GM hybrid wheat or barley</p> <p style="text-align: center;">↓</p>
<i>Potential Harm</i>	Increased toxicity or allergenicity to people or increased toxicity to other desirable organisms

### Risk source

135. The source of potential harm for this postulated risk scenario are the introduced genes for yield enhancement, improved abiotic stress tolerance and a visual marker in GM wheat and barley plants.

### Causal pathway

136. GM wheat and barley would be planted at the trial site. When the GM wheat and barley flowers, GM pollen could be carried by wind to other GM wheat and barley plants nearby. If these are also flowering, the GM pollen could fertilise some flowers, producing hybrid GM plant material. People or animals could be exposed to the hybrid plant material if it is used for human food or animal feed, or by coming into contact with the hybrid GM plant material at the trial site.

137. During this field trial, it is possible that different lines grown under DIR 186 would be planted in close proximity to one another. In addition, the GM wheat and barley proposed for release may be grown in close proximity to other GM wheat or barley planted under licence DIR 152. Given that the different GM lines are sexually compatible and that they may have similar flowering times, pollen flow between plants with different introduced genes may occur. This may result in hybrid GM wheat and barley seeds with additional – ‘stacked’ – introduced genes for yield enhancement and/or abiotic stress tolerance. People and other organisms may be exposed to this hybrid GM wheat or barley.

138. It should be noted that vertical gene flow *per se* is not considered an adverse outcome but may be a link in a chain of events that may lead to an adverse outcome.

139. Both the current DIR 186 proposal and the licence for DIR 152 include several lines in which either one, two or all three of the genes for yield enhancement, i.e. *AtAVP1*, *OsNAS2* and *OsPSTL1*, are present. The proposed DIR 186 trial also includes another five genes for abiotic stress tolerance. In addition, lines containing one of seven individual genes for frost tolerance is also approved under DIR 152.

140. Wheat and barley are mainly self-pollinating and where pollen dispersal does occur, the main method is wind. The chances of natural hybridisation occurring with sexually compatible plants are low and decline significantly over distance, with most pollen falling within the first few metres (OGTR, 2021a, b).

141. A Canadian study of gene flow in wheat detected trace rates ( $\leq 0.01\%$ ) up to 300 m (for a 16 ha pollinator block) or 2.75 km (for a 30 ha pollinator block) away from the pollen source (Matus-Cádiz et al., 2004; Matus-Cádiz et al., 2007). Another highly relevant study for the current application was conducted in south-eastern Australia and has shown much lower rates of intraspecific gene flow than that observed overseas (Gatford et al., 2006). Using three blocks of 1.2 m x 2.5 m planted to GM wheat surrounded by 27 blocks of the same size planted to non-GM wheat, the authors measured gene flow rates far lower than those observed in similar conditions overseas, i.e. up to 0.055% at 8 m from the pollen source (Gatford et al., 2006).

142. In a study using male sterile barley at a distance of 1 m as the recipient, viable pollen flow resulted in an average of less than half a seed to one seed per head, and seed set diminished with distance (Ritala et al. 2002). In normal fertile barley, the cross-pollination frequency was between 0 and 7% at a distance of 1 m. This study used open flowered barley as the recipient, so outcrossing would be expected to be lower in most cultivated barley varieties as many of these are closed-flowering for part or all of their flowering period (Ritala et al. 2002). In observations of pollen migration between commercial barley fields, outcrossing rates were 0.05% and 0.01% for distances of 1 m and 10 m, respectively. No pollen migrants were observed in these studies at distances of 20 m or 50 m (Allard unpublished, cited in Wagner & Allard 1991; Ritala et al. 2002). However, in other studies, cross fertilisation with very low frequencies has been observed at distances of up to 50 m (Ritala et al. 2002) and 60 m (Wagner & Allard 1991), although cross pollination at such distances is rare.

143. Outcrossing rates in both wheat and barley are also influenced by the genotype of the variety, and environmental conditions, such as wind direction and humidity (OGTR, 2021a, b). There is currently no information that would indicate an effect of the introduced genes on pollen characteristics leading to an increase in the likelihood of outcrossing.

144. The low likelihood of cross pollination between GM wheat and barley grown under different licences is further reduced by the limits and controls imposed under the DIR 152 licence as well as those proposed for the current application. These would reduce the likelihood of presence and persistence of any hybrid GM wheat and barley at the trial site. For example, buffer zones, site monitoring and post-harvest monitoring requirements have been imposed under licence DIR 152. In addition, seeds, including any possible hybrid seeds, obtained from the trial authorised under DIR 152 must not be used for breeding or propagation to produce cultivars for future commercial release. Taken together, exposure of people or other desirable organisms to any hybrid GM wheat and barley would be highly unlikely.

145. The fluorescent visual marker gene and its product are not expected to alter characteristics that may affect pollen dispersal or outcrossing rates in the GM wheat and barley. As discussed in Chapter 1, Section 4.3.3, the level of fluorescence emitted by the GM plants is expected to be low in comparison to reflected

light. Therefore, expression of the RFP protein in the GM plants is not expected to impact on insect and other animal behaviour, such as changes to levels of pollen dispersal or outcrossing with sexually compatible plants. Furthermore, as mentioned above, wheat and barley are mainly self-pollinated, with any other dispersal occurring by wind.

**Potential Harm**

146. If pollen flow occurred between the GM wheat or the GM barley lines grown under DIR 186, or between lines from DIR 152 and DIR 186, it is possible that some GM hybrid seed may occur. For example, these plants could contain additional yield and water use efficiency genes, or a yield enhancement gene and a yield and water use efficiency gene. If this occurs, hybrid seeds and any resulting plants may express a combination of introduced genes. These proteins may be toxic or allergenic to people or toxic to other organisms.

147. Risk Scenario 1 (above) and the RARMP for DIR 152, did not identify toxicity or allergenicity of any of the individual genes as a substantive risk. Likewise, there is no expectation that combinations of genes will result in the production of novel proteins, or that their expression will be altered in a hybrid background, thus production of novel allergens or toxins is highly unlikely. The genes are sourced from organisms already present in the environment, most of the genes from dominant food crops, suggesting that people and other organisms have a long history of exposure to them.

**Conclusion**

148. Risk scenario 2 is not identified as a substantive risk due to the proposed limits and controls, wheat and barley being mainly self-pollinating with low levels of outcrossing, and the fact that most introduced genes were sourced from common food plants and the encoded proteins are not known to be toxic or allergenic. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

**2.4.3 Risk scenario 3**

<i>Risk Source</i>	Introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker
<i>Causal Pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Presence of GM seed outside the trial limits</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">GM seed germinates</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Increased exposure of people and other desirable organisms by ingestion of, or contact with, the GM wheat or barley</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Establishment of GM wheat or barley in nature reserves, roadside areas or intensive use areas</p> <p style="text-align: center;">↓</p>
<i>Potential Harm</i>	<p style="text-align: center;">Increased toxicity or allergenicity for people or increased toxicity to other desirable organisms</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Reduced establishment and yield of desirable plants</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Reduced utility or quality of the environment</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Increased ability to provide a reservoir for pathogens or shelter for pests</p>

**Risk source**

149. The source of potential harm for this postulated risk scenario is the introduced genes for yield enhancement, improved abiotic stress tolerance and a visual marker in GM wheat or barley.

### ***Causal pathway***

150. GM wheat and barley would be grown at the trial site, and viable seeds would be available for dispersal when the trial is sown and as the GM wheat and barley reaches maturity. If viable GM wheat and barley seeds remained at the trial site after completion of the trial, or if GM seed dispersed outside the trial site, volunteer GM wheat and barley may establish populations in the environment. These hybrids could then spread further and persist in the environment. This could increase the likelihood of exposure of people or animals to the GM wheat and barley, and the introduced genes and their products.

151. Similarly, if GM hybrids were formed between the GM wheat and barley in this application and those authorised for release at the same site under DIR 152, then GM hybrid seed with combined traits could also be available for this scenario.

152. The features of both non-GM wheat and barley relevant to their spread and persistence in the environment are summarised in the [biology documents](#).

### ***Persistence of GM wheat and barley on the trial site***

153. For GM wheat and barley seeds to be available to persist at the proposed trial site, seeds from any GM wheat or barley would need to drop to the ground either near maturity or during sowing and harvest. During domestication, both non-GM wheat and barley have been selected for reduced shattering of seed heads – a mechanism for seed dispersal in ancestral wheat and barley plants. The introduced genes have not been linked to alterations in this trait in GM wheat and barley authorised proposed for release under the current application or under DIR 152.

154. While in a commercial setting loss of seeds during harvest is common (e.g. up to 10% in the case of non-GM barley; (OGTR, 2021a), the applicant proposes hand harvesting of seeds or use of a plot harvester. Both proposed methods of harvest would reduce the likelihood of seeds ending up on, or in the ground, when compared to commercial equipment.

155. GM wheat or barley at the trial site could persist through dormant seeds in the seed bank. This could increase the number of volunteers at the site after the trial and provide seeds for spread to other areas. Although a range of factors in the environment can influence seed dormancy in both wheat and barley, neither species shows a high degree of dormancy or a persistent seed bank under Australian conditions (for details, see the [biology documents](#)). Importantly, both wheat and barley seeds germinate easily under favourable conditions which includes appropriate temperature while sufficient soil moisture is present.

156. The applicant proposes to remove or destroy all GM wheat and barley plants at the trial site after each harvest, but some seeds may remain. The applicant also proposes post-harvest monitoring for at least two years after the final harvest, as well as tillage and irrigation to encourage seed germination. Any wheat and barley volunteers found would be destroyed prior to flowering. In previous GM wheat and barley field trials in Australia, these control measures to minimise the persistence of GM wheat and barley at trial sites were considered appropriate.

### ***Dispersal of GM seed outside the trial site***

157. Dispersal of GMOs outside the trial site could occur through the activity of people or through natural means, such as animals, wind and water. There is no reasonable expectation that the introduced genes would affect any of the seed characteristics important for dispersal. In addition, the applicant has not reported any such changes in the GM wheat and barley already planted under DIR 152 and proposed for further release under the current application.

158. The main means of dispersal in non-GM wheat and barley is deliberate and inadvertent dispersal by people (OGTR 2021a; OGTR 2021b). Important mechanisms for inadvertent seed dispersal by people include dispersal via equipment such as harvesters, and grain loss during transport. The applicant has proposed controls to reduce the likelihood of this occurring during the trial, including restricting access to the site; trained staff; cleaning all equipment before removing it from the site or using it for any other purpose; and transporting and storing all GM wheat and barley in accordance with the [Regulator's](#)



Transport, Storage and Disposal of GMOs guidelines. These control measures would minimise dispersal of GM wheat and barley seed outside the trial site by people.

159. Animals, including birds, could spread viable mature seeds:

- through seeds adhering to fur, feathers or feet, e.g. wheat seeds in sheep wool (Ryves 1988) or on muddy feet or legs of birds (Cummings et al. 2008), or barley seeds in fur and feathers (Von Bothmer 1992; Von Bothmer et al. 1995)
- by removing and hoarding seed, e.g. by ants (up to a few metres; (Gómez and Espadaler, 1998)), rodents (up to 50 metres; (Andersson and deVicente, 2010) or birds (Chambers and MacMahon, 1994)
- by consuming and excreting whole seeds, e.g. wheat seeds by birds, including emus (Calvino-Cancela et al. 2006).

160. While seed dispersed using the first two mechanisms mentioned above would retain viability, ingestion and excretion can affect seed viability and reduce the likelihood of germination:

- physically intact seed may make up to 30% (wheat) or 15% (barley) of dry matter in the faeces of cattle fed grain (Beauchemin et al. 1994), noting that germination rates were not measured. Viable wheat seeds have not been found in rabbit dung (Malo & Suárez 1995), and viable barley seeds have not been found in sheep and goat manure (Oveisi et al., 2021).
- after consumption by birds, a small proportion of intact wheat seed can be excreted by corellas (0.25%) and galahs (0.1%), with varying germination rates (Woodgate et al. 2011). Wheat seed may be dispersed by emus, however germination rates were very low (Rogers et al. 1993; McGrath & Bass 1999). Viable barley seed is not excreted by a range of birds (Cummings et al. 2008).

161. The applicant proposes controls that would reduce the likelihood of seed dispersal by animals, e.g. fencing the site to limit access by large animals, a 10 metre wide monitoring zone where the vegetation is controlled which would also deter rodent activity; and using rodent bait or traps. The limited time frame during which viable seed would be available in each growing season and the small size of the trial would further reduce the likelihood of seed dispersal by animals.

162. Wheat and barley seeds are not usually dispersed by wind as domesticated wheat and barley have non-shattering seed heads, the seeds are heavy and they lack specialised structures to aid windborne dispersal (OGTR, 2021a, b). It is possible that some viable GM wheat or barley seeds could be dispersed by high winds if a severe storm occurred while mature seed was present on the trial site.

163. Seeds could also be transported off a site by water during heavy runoff or flooding. Proposed controls, including locating the trial site at least 50 m from any natural waterway in areas not prone to flooding, would minimise the potential for seed dispersal through flooding.

#### *Establishment of GM volunteer populations in the environment*

164. If GM wheat and barley seed were dispersed beyond the trial, they require an environment conducive to germination and establishment. Non-GM wheat and barley are domesticated plants that have limited ability to survive outside cultivation. This is also evident from the weed risk ratings for wheat and barley, respectively (OGTR, 2021a, b).

165. The introduced genes for yield enhancement and improved abiotic stress tolerance are likely to be pleiotropic (that is, they have effects on several traits) thus potentially enhancing their ability to thrive in sub-optimal conditions. A gene involved in abiotic stress tolerance may impart tolerance to a number of abiotic stresses or to biotic stresses (Howles & Smith 2013). This may increase the ability to establish the GM wheat and barley in agricultural, natural and intensive use areas, and may provide the GM wheat and barley with an advantage over non-GM wheat and barley. However, no studies have been conducted and this is an area of uncertainty for this risk assessment.

166. The fluorescent visual marker gene and its product are not expected to alter characteristics that may affect the establishment of the GM wheat and barley as they are not involved in a relevant biochemical pathway. Additionally, as discussed in Chapter 1, Section 4.3.3, the level of fluorescence emitted by the GM plants is expected to be low in comparison to reflected light. Therefore, expression of the RFP protein in the GM plants is not expected to impact on insect and other animal behaviour, such as changes to levels of predation of the GM plants which may increase their ability to spread and persist.

### **Potential Harm**

167. If GM plants were able to establish outside the trial site, they could cause increased toxicity to people or desirable organisms, or increased allergenicity for people through increased exposure. As discussed in risk scenarios 1 and 2, no substantive risk was identified for increased toxicity or allergenicity of the GM wheat and barley, or any of their hybrids with other GM wheat or barley.

168. Establishment of the GM wheat or barley outside the trial limits could also reduce the establishment or yield of desirable plants in agricultural or natural land uses; reduce the utility of intensive use areas, such as roadsides, drains or channels; or increase its ability to provide a reservoir for pathogens or shelter for pests. However, none of the introduced genes has been reported to affect characteristics that would lead to an increase in these harms in the GM wheat or barley. For example, none of the genes are known to make the GM wheat or barley susceptible to pathogens non-GM wheat or barley are resistant to; or to enable the GM wheat or barley to produce allelopathic substances which would negatively affect plant establishment around them. As none of the introduced genes or their products are involved in relevant pathways, there is no reasonable expectation this may occur.

### **Conclusion**

169. Risk scenario 3 is not identified as a substantive risk due to the proposed limits and controls designed to restrict dispersal, the limited ability of wheat or barley to spread and persist outside the trial site and their susceptibility to a number of standard weed control measures. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

#### **2.4.4 Risk scenario 4**

<i>Risk Source</i>	Introduced or modified genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker
<i>Causal Pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Fertilisation of sexually compatible plants outside the trial area by pollen from GM wheat or barley</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Germination of GM hybrid seed</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Increased exposure of people and other desirable organisms by ingestion of, or contact with, the GM hybrid plant material</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Establishment of GM wheat or barley in nature reserves, roadside areas or intensive use areas</p> <p style="text-align: center;">↓</p>
<i>Potential Harm</i>	<p style="text-align: center;">Increased toxicity or allergenicity to people or toxicity to other organisms</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Other environmental harms (see risk scenario 3)</p>

### **Risk source**

170. The source of potential harm for this postulated risk scenario is the introduced genes conferring yield enhancement, improved abiotic stress tolerance and a visual marker in the GM wheat and barley.

### **Causal pathway**

171. GM wheat and barley would be planted at the trial site. When these plants flower, their pollen could be carried by wind to sexually compatible crops growing in the vicinity of the trial site. If these related crops are also flowering, the GM pollen could fertilise some flowers, producing hybrid GM seed. Hybrid GM plants carrying the introduced genes could form the basis for establishment, spread and dispersal of the introduced genes in other varieties of wheat or barley, or other sexually compatible plant species. Exposure of people and other organisms could increase to the proteins expressed by the introduced genes through ingestion, contact with plant material or inhalation of pollen.

172. Baseline information on vertical gene transfer associated with non-GM wheat and barley plants can be found in the wheat and barley [biology documents](#) (see Chapter 1, Section 5.4), and relevant details have also been provided in the pathway section for risk scenario 2.

173. Interspecific cross-pollination from bread wheat to durum wheat occurs at lower levels than intraspecific cross-pollination between bread wheat plants (Matus-Cádiz et al., 2004). Crossing of bread wheat to *Hordeum marinum* and other close relatives rarely occurs (OGTR, 2021b). Barley has a primary gene pool containing only one *H. vulgare* subspecies – which is not known to be present in Australia. Interspecific crosses within the *Hordeum* genus and intergeneric crosses have not been observed under natural conditions (OGTR, 2021a). The proposed limits and controls for this trial would minimise the likelihood of pollen flow from the trial to related species. For example, no wheat or barley crops may be planted within at least 200 m of a planting area while GM wheat or barley are being cultivated, any sexually compatible species would be controlled within at least 50 m of a planting area during flowering, and destruction of GM wheat and barley before flowering during post-harvest monitoring.

174. Any hybrid seed resulting from vertical gene flow would need a suitable environment for germination, plant establishment and persistence. Volunteers can be controlled with integrated weed management practices.

### **Potential harm**

175. If GM hybrid plants spread and persisted in the environment, this may lead to increased toxicity to people or other desirable organisms, or allergenicity to people. Hybrids expressing the introduced genes could also reduce the establishment and yield of desired plants and cause other environmental harms as per risk scenario 3.

176. The introduced genes could combine, via vertical gene transfer, with those of other non-GM wheat, barley or other sexually compatible species. The properties that the introduced genes confer are not expected to differ in a hybrid background. Therefore, in the event of vertical transfer from the GM wheat or barley lines to non-GM wheat or barley plants or sexually compatible species, it is expected that the introduced genes in any subsequent hybrids would confer the same properties as the GM parent.

177. As discussed in risk scenarios 1-3, the introduced genes are unlikely to change the GM wheat or barley characteristics such that they would cause more harm than the non-GM parents.

### **Conclusion**

178. Risk scenario 4 is not identified as a substantive risk due to the proposed limits and controls, the limited ability of long distance pollen flow in wheat and barley, and the limited ability of wheat and barley to survive outside of cultivation. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

## **Section 3 Uncertainty**

179. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis. This is discussed in detail in the Regulator's [Risk Analysis Framework](#) document.

180. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to

lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

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

182. For DIR 186, uncertainty is noted particularly in relation to:

- expression patterns of the introduced genes in the GM plants
- potential for increase in toxicity or allergenicity, including potential for uptake of heavy metals by the GM plants
- potential for the introduced genes to increase spread and persistence of the GM plants.

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

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

## Section 4 Risk evaluation

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

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

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

187. Four risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the control measures proposed by the applicant, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 2 and include:

- no GM plant material would enter human food or animal feed
- limits on the size and duration of the proposed release
- controls proposed by the applicant to restrict the spread and persistence of the GM wheat and barley plants and their genetic material (see Chapter 3 for their suitability)
- GM wheat and barley have limited ability to survive outside of cultivation
- GM wheat and barley volunteers could be controlled by various standard weed management methods.

188. Therefore, risks 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. The *Risk Analysis Framework* (OGTR 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no additional controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

## Chapter 3 Risk management plan

### Section 1 Background

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

190. 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 can be managed in a way that protects the health and safety of people and the environment.

191. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: section 64 requires the licence holder to provide access to premises to OGTR inspectors and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder must also be reported to the Regulator.

192. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and to manage risk to people or the environment. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

### Section 2 Risk treatment measures for substantive risks

193. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed field trial of GM wheat and barley. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 2.1), the proposed controls (Chapter 1, Section 2.2), and the receiving environment (Chapter 1, Section 5), and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

### Section 3 General risk management

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

#### 3.1 Licence conditions to limit and control the release

##### 3.1.1 *Consideration of limits proposed by The University of Adelaide*

195. Sections 2.1 and 2.2 of Chapter 1 provide details of the limits and controls proposed by The University of Adelaide in their application. Many of these are discussed in the four risk scenarios considered for the proposed release in Chapter 2. The appropriateness of these limits and controls is considered further in the following sections.

196. The applicant proposes that the release would take place at one site in Rosedale (SA). The field trial would run between April 2022 and January 2027, inclusive. A total of 2 hectares in any year can be used for planting of the GM plants. The applicant has stated that more than one planting area may be used at the site. The small size and short duration of the trial restricts the potential exposure of people and desirable animals to the GMOs (Risk Scenario 1), and limit the opportunity for presence of the GM outside or after the trial (remaining risk scenarios).

197. The applicant proposes that only trained and authorised staff would be permitted to deal with the GMOs. Standard licence conditions require that only people authorised by the licence holder are covered by the licence and that the licence holder must inform all people dealing with the GMOs of applicable licence conditions. These measures limit the exposure of people to potential harm from the GM wheat and barley (Risk Scenario 1).

198. The Rosedale site is also approved for planting of GM wheat and barley under licence DIR 152. The licence for DIR 152 permits planting until the end of the 2022/23 growing season (inclusive), so GMOs from both DIR 152 and DIR 186 (if approved) could be grown concurrently at the same site. Pollen transfer between individual GM lines proposed to be grown under DIR 186 has been considered, as has the risk of gene flow between GM lines from DIR 152 and DIR 186 (Risk Scenario 2). Like the DIR 152 licence, the DIR 186 licence include requirements to treat any collected seeds as GMOs, i.e. the seeds cannot be released into the environment unless an appropriate authorisation has been obtained. Thus, even if outcrossing between GM plants from both trials were to occur, exposure of humans or other desirable organisms to GM hybrid plants (Risk Scenario 2) or the spread of any GM hybrid plants outside the trial site (Risk Scenario 3) would be highly unlikely.

### **3.1.2 Consideration of proposed controls regarding exposure to the GMOs**

199. The applicant proposes not allowing the GMOs or GM products to be used for human food or animal feed. A licence condition requires that GM plant material must not be used as food for humans or feed for animals. This condition restricts the exposure of people and desirable animals to the GMOs (Risk Scenario 1).

200. The applicant has indicated that all properties will have lockable gates on perimeter fences. Since restricting the dealings to only authorised personnel is considered appropriate for limiting exposure of humans to the GMOs, it is not considered necessary to have fences with lockable gates and hence this is not a licence condition. In addition, there is no evidence that the GM wheat and GM barley lines or hybrid GM wheat or barley lines would be more toxic or allergenic to people than the non-GM parental wheat or barley lines (Risk Scenarios 1 and 2).

### **3.1.3 Consideration of proposed controls regarding pollen flow from the GMOs**

201. The applicant proposes surrounding each GM wheat and barley planting area with a 2 m buffer zone, where plant growth will be controlled. A 10 - 20 cm border of non-GM wheat will be planted as a pollen trap around each planting area, inside the buffer zone. The buffer zone is surrounded by a 10 m monitoring zone and a 50 m inspection zone. The monitoring and inspection zones would be inspected while the GMOs are flowering to destroy any wheat, barley, or sexually compatible plants. The inspection zone would be surrounded by a 140 m isolation zone where no wheat, barley, or sexually compatible plants would be deliberately grown. The combination of a 10 m monitoring zone, the 50 m inspection zone and a 140 m isolation zone were considered in Risk Scenarios 3 and 4 and in previous RARMPs (e.g. [DIR 152](#)). These isolation distances are expected to minimise pollen flow from the GMOs to non-GM plants outside the trial site, so are included in the licence. The 2 m buffer zone and pollen trap are not required to minimise pollen flow in addition to these isolation distances, so they are not proposed under the conditions of the licence.

202. The applicant proposes that the monitoring and inspection zones would be inspected at least every 14 days from 14 days prior to the expected flowering of the GMOs until all GMOs in the planting area have finished flowering. It is desirable to have one inspection after the completion of flowering of the GMOs, in case any plants were missed in the previous inspection, but no further inspections are necessary. Therefore, a licence condition requires the monitoring and inspection zones to be inspected at least every

14 days from 14 days prior to the expected flowering of the GMOs until 14 days after all GMOs in the planting area have finished flowering.

203. The applicant has stated that, under field conditions, the GM lines for yield enhancement flower 5-10 days earlier than non-GM plants within the same cultivar. The introduced genes for yield enhancement may also influence tillering in the GM wheat lines (Chapter 1, Section 4.5). Additionally, genetic modification of stomatal development and aperture may also reduce time to flowering in the GM wheat and barley plants (Chapter 1, Section 4.3.2). Earlier flowering in the GM lines could potentially alter the flowering period for the different GM lines, such that pollen would be present for a longer period, thus increasing the time during which gene flow could occur. A monitoring zone of at least 10 m, kept free of volunteers and related species and maintained in a manner that facilitates the detection of such plants, would help to minimise the likelihood of gene flow from the planting area (Risk Scenarios 2 and 4). Gene flow is further minimised by licence conditions requiring the monitoring and inspection zones to be inspected at least every 14 days from 14 days prior to the expected flowering of the GMOs until 14 days after all GMOs in the planting area have finished flowering. Any volunteers or related species are to be destroyed or prevented from flowering.

204. The applicant proposes that more than one planting area could be established at the trial site. Under the conditions in the licence, where more than one planting area is established at a field trial site, all planting areas must be inside a 10 m monitoring zone surrounding the whole trial site (see Figure 1 in licence). Any land between planting areas is also considered part of the monitoring zone and would need to be maintained and inspected as such.

#### **3.1.4 Consideration of proposed controls regarding persistence of the GMOs**

205. After harvest of each trial site, the applicant proposes to destroy all plant material from the trial not required for testing or future plantings. It is only necessary to destroy viable plant material, i.e. live GM plants or viable GM seed, to limit persistence of the GMOs. Licence conditions require that the trial site must be cleaned (which would destroy any surviving GM plants) within 35 days after harvest, and that harvested GM seed not required to conduct experiments or for future planting must be destroyed as soon as practicable. In addition, to deal with the case of failed crops that are not harvested, licence conditions require that GMOs must be harvested or destroyed within ten months after planting, and that if all GMOs in a planting area have been destroyed, then the area is considered to have been cleaned.

206. The applicant has proposed that all waste material generated from harvest of the GM wheat and barley would be left in the planting area and either ploughed into the soil with crop stubble to the depth of seeding or burned/buried on site. They have also proposed that any waste material collected during cleaning would be destroyed using a method approved by the Regulator. These methods may include, but are not limited to, autoclaving, hammer-milling, incineration or burial to a depth of 1 m. Autoclaving, crushing and milling are considered effective for destruction, as they render seed non-viable, therefore minimising the likelihood of germination and/or spread. Deep burial of seed is also considered an effective method of destruction, therefore conditions allowing deep burial have been included in the licence. To ensure the effectiveness of destruction by seed burial, a licence condition specifies how this must be carried out, including a requirement that seeds must be sufficiently irrigated at time of burial to encourage decomposition.

207. The applicant has proposed that areas used for destruction of plant material by burial, burning or incineration would take place in a clearly marked area, immediately adjacent to the trial site. The applicant proposes to inspect these areas for the presence of volunteers at least once every 35 days for two years, and until the site is free of volunteer plants for at least 6 months. A licence condition has been included where the burial site must not be intentionally disturbed for 12 months from the date of burial. If seed is dispersed during burial, this area would be considered an area in which the GMOs have been dispersed in the course of dealings under the licence, and post-cleaning conditions would apply.

208. The applicant has proposed that any non-GM wheat or barley planted as part of the field trial would be treated as if it were GM. Non-GM wheat or barley grown at the trial site may be cross-pollinated by the GM wheat and barley, resulting in hybrid seeds. It is therefore appropriate to require non-GM wheat and

barley to be destroyed in the same manner as GM wheat and barley, to manage persistence of the GMOs, and this measure is included in the licence.

209. At the trial site, where GMOs from DIR 152 could be planted in close proximity, the GM lines from each licence could hybridise with one another, or with future trials approved at the site, resulting in hybrid lines containing additional introduced genes and/or traits. Therefore, if seed from the DIR 186 trial was used to develop future GM wheat or barley lines, there is a possibility that other genes could be unintentionally present. Therefore, as in the licence for DIR 152, a licence condition for DIR 186 has been imposed to prevent seed from trials where such gene flow could have occurred being used for development of cultivars for potential future commercial release (Risk Scenarios 2 and 4). On sites where no other GM trials have been planted, the seed could be used for future variety development, subject to appropriate approvals from the Regulator.

210. The applicant has proposed that any equipment used during the trials, including for seeding, harvesting, and threshing on site, will be inspected for seeds and cleaned as soon as practical after use and before it is used for other purposes. Cleaning would take place either in the trial area or in dedicated washdown facilities at the exit point for each trial site. Dedicated equipment would be used for the GM trial, where possible. A licence condition requires that any area used to clean equipment used in connection with the GMOs, and any area where GMOs have dispersed in the course of dealings under this licence, must be cleaned as soon as practicable, and then monitored in the same way as the planting areas after cleaning.

211. After harvest, the applicant proposes to inspect the planting areas and monitoring zone at least once every 35 days for two years, and until the site is free of volunteer plants for at least 6 months. Any wheat or barley volunteers found would be destroyed prior to flowering, to prevent pollen flow to non-GM plants outside the trial site. Wheat typically requires 1275 degree-days to grow from emergence to flowering (Bowden et al., 2008), which in hot weather (average daily temperature 26°C), would be about 49 days. Flowering in many barley varieties responds to day length as well as temperature, so development patterns can vary with latitude. Many varieties of barley pollinate while still in the head, so no physical flowering occurs. Sowing of most barley varieties grown in Australia occurs between early May and early June, depending on variety and location, so that flowering occurs from September to early October (OGTR, 2021a). Allowing for variation between cultivars and between individual plants, as well as early flowering of the GM wheat and barley lines (Chapter 1, Section 4.3.2), monitoring the trial site at least every 35 days would be sufficient to detect volunteers before flowering. The total monitoring period of at least two years, with at least the last six months volunteer-free is expected to minimise persistence of GM wheat and barley at the trial site, so is included in a licence condition.

212. The applicant proposes at least one tillage to the depth of seeding within the planting areas, and three irrigations for each trial site during the post-harvest monitoring period. This will encourage germination of any remaining seed. There is a difference in germination rates between buried grain and grain lying on the surface; grains remaining near the surface, e.g. following shallow tillage after harvest, can generally easily germinate and become established (Ogg & Parker 2000). An Australian field trial found that wheat seed banks were most persistent during dry seasons in no-tillage plots (Wicks et al., 2000). Shallow tillage after harvest, combined with irrigation, will germinate much of the seed lying on the surface (Ogg & Parker 2000). However, deep cultivation in certain soil types can reduce seed viability, but can also encourage prolonged dormancy in seeds as a result of a cool, moist low oxygen environment (Pickett 1989; Ogg & Parker 2000).

213. The Regulator considers that under Australian conditions, a post-harvest monitoring period of at least two years, with monthly inspections, and with no volunteers detected for a minimum of 6 months prior to the end of the time period, would effectively manage survival and persistence of viable wheat and barley seeds in the soil. Therefore, these measures are included in the licence. The licence contains conditions requiring that after harvest, the trial site should receive at least three irrigations, at intervals of at least 28 days, with the last required irrigation occurring at a time that would promote germination of volunteers within the final volunteer-free period. These measures will minimise the persistence of the GMOs in the environment (Risk Scenarios 3 and 4).



214. The applicant proposes that rainfall events of greater than 10 mm in a 24 h period would be deemed to be equivalent to an irrigation event. A licence condition states that a period of natural rainfall may be taken as irrigation if it meets specified rainfall totals or is agreed to by the Regulator. Evidence (such as rainfall measurements, photos etc.) that the rainfall has been sufficient to promote germination may need to be provided. Additionally, prior to the last irrigation, the area must be tilled to a depth no greater than the depth of sowing. These treatments would ensure that seeds are exposed to sufficient moisture and placed at an appropriate depth for germination, as well as encouraging the microbial decomposition of any residual seed (Risk Scenarios 3 and 4).

215. The applicant has proposed that a 2 m buffer zone, kept free of vegetation, surround each planting area with specific inspection and cleaning requirements. A 2 m buffer zone is not proposed under the conditions of the licence, however licence conditions do require any other areas where GM material has been dispersed, including during planting, harvest or threshing, must be inspected and volunteers and related species must be destroyed or prevented from flowering. The licence also requires harvest of GM wheat and barley to be conducted separately from other crops. These conditions are imposed to manage the likelihood for spread and persistence of the GMOs due to mechanical dispersal of grain during sowing and harvesting (Risk Scenario 3).

### **3.1.5 Consideration of proposed controls to limit dispersal of the GMOs**

216. The applicant proposes to conduct harvest by hand or a dedicated plot harvester, and that all equipment used in connection with cultivating and harvesting the GMOs, such as harvesters, seeders, storage equipment, transport equipment (bags, container, trucks etc.), tools, shoes and other clothing, would be inspected for seeds and cleaned after use on site. The Rosedale site has a dedicated washdown facilities at its exit point, which allows for cleaning to occur prior to re-use or removal from the area. The applicant has stated that, where possible, dedicated equipment would be used for the GM trials. These measures would minimise human-mediated dispersal of GM plant material (Risk Scenario 3).

217. Threshing of wheat or barley after harvest would take place in the planting area or seed heads would be packaged and transported to approved facilities for threshing, analysis or other processing. As required for previous wheat and barley field trial licences issued by the Regulator, a licence condition states that GM wheat and barley must be threshed separately from any other crop, and threshing must take place on the planting areas, monitoring zones or in a facility approved by the Regulator. Any seed heads or grain for analysis would be bagged on site and transported to approved facilities for analysis according to the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. Any grain remaining after analysis would be stored in an approved facility for subsequent use, or destroyed by autoclaving or another method approved by the Regulator. These are standard conditions for the handling of GMOs to minimise exposure of people and other organisms to the GMOs (Risk Scenario 1 and 2), dispersal into the environment and gene flow/transfer (Risk Scenario 3 and 4).

218. The applicant has proposed to fence the trial site. Whilst animals will consume wheat or barley plant material, there is negligible risk of seed spread via livestock and there is no evidence that the GM wheat and barley would be more toxic to livestock than non-GM wheat or barley. A standard licence condition has been included in the licence which prohibits the use of plant material in this trial for food or feed, thus livestock would not be allowed to feed on the GM wheat or barley (Risk Scenarios 1, 2 and 3). The applicant may achieve this requirement in a number of ways, not limited to fencing the trial site, so a fence would not be a requirement.

219. A variety of birds may feed on cereal crops, including wheat and barley, however a search of the literature found little evidence of extensive spread of seed via birds. Birds such as cockatoos do most damage to wheat during germination (Temby & Marshall 2003). Emus may feed on wheat seed, but generally prefer other foods (Davies 1978) and it is likely that germination rates of seed after digestion are low, although experimental evidence is sparse. Corellas and galahs will feed on wheat seed, but even under controlled conditions germination rates of seed were very low, ranging from 0.8 % to 2 % (Woodgate et al. 2011). The majority of wheat varieties grown in Australia are white wheat varieties (Blakeney et al. 2009) which have thin seed coats and are easily broken down during digestion (Temby & Marshall 2003; Yasar

2003). Viable barley seeds were not excreted by birds fed barley grain (Cummings et al. 2008; Woodgate et al. 2011), thus spread of barley by this route is highly unlikely. For these reasons, it is considered unnecessary to propose measures to control access of birds to the planting areas (Risk Scenario 3).

220. In addition, there is no evidence that the GM wheat and GM barley lines or hybrid GM wheat or barley lines would be more toxic to birds than the non-GM parental wheat or barley lines. Hence, there is no requirement to control access of birds to the GM wheat and barley lines with respect to Risk Scenarios 1 and 2.

221. Both wheat and barley seed may be spread through animal fur, feathers or muddy feet or hooves and barley seeds do have some structures which increase their ability to do so. However, the limited duration and size of the trials and the limited time in which viable seed is available reduces opportunities for contact with and spread of viable seed by large animals or birds. In addition, the proposed requirement that livestock not be allowed to access viable grain further limits the likelihood of spread of wheat or barley seed via these routes (Risk Scenario 3).

222. Small animals including rodents may remove seed from the planting area, providing a potential means of dispersal (Risk Scenario 3). Although the applicant has not discussed the incidence of rodent activity at the site, they have proposed rodent control by use of traps and/or baits in the planting areas and surrounding areas and keeping the 2 m buffer zone surrounding each planting area where vegetation is heavily controlled. The applicant also proposes a 10 m monitoring zone, with vegetation kept mown at a maximum height of 10 cm. It has been a requirement of previous GM wheat and barley licences that the monitoring zone is maintained in a manner that does not attract or harbour rodents, such as keeping the area either free of vegetation or planted with vegetation mown to a height of less than 10 cm. This is expected to deter rodents from transporting seed through the monitoring zone, as well as facilitate the detection of GM plant material that has been dispersed during sowing and harvesting (Risk scenario 3).

223. As discussed in Risk Scenario 3, a combination of rodent baits and/or traps in the planting area in conjunction with a monitoring zone of at least 10 m, maintained in a manner that would deter rodents, would be adequate to minimise rodent activity, thus a 2 m buffer zone would not be required. Rodent control measures such as traps and/or baits in the planting area are a requirement under the conditions of the licence.

224. Both licence conditions (keeping vegetation short and rodent controls) apply while the GMOs are being grown and until the planting area is cleaned. Cleaning of a planting area, as defined in the licence, includes removal of most of the GM seeds from the soil surface where they could be readily accessed by rodents or dispersed by other means.

225. The applicant has proposed that the trial site would be located at least 50 m from any natural waterway and in areas that are not prone to flooding. This would reduce the likelihood of plant material being washed away from the planting areas (Risk Scenario 3). It is a standard licence condition that trial sites be located at least 50 m from waterways to limit the dispersal of viable plant material in the event of flooding. There is also a condition in the licence requiring immediate notification of any extreme weather event affecting the properties during the release to allow assessment and management of any risks.

### **3.1.6 Summary of licence conditions to be implemented to limit and control the release**

226. A number of licence conditions are proposed to limit and control the release, based on the above considerations. These include requirements to:

- limit the duration of the release to the period from April 2022 to January 2027
- limit the release to one site in SA (Rosedale)
- limit the release to a combined total of 2 ha in any year
- locate trial site at least 50 m from any natural waterways
- surround the planting area(s) with a monitoring zone of at least 10 m, maintained in a manner that does not attract or harbour rodents, and in which related species must be prevented from flowering

- surround the monitoring zone with a 50 m inspection zone in which no wheat or barley may be planted and which must be inspected for volunteers and related species during flowering
- surround the inspection zone with a 140 m isolation zone in which no wheat, barley or related species may be grown
- implement measures including rodent baits and/or traps to control rodents within the planting areas
- harvest the GM wheat and barley separately from other crops
- harvest the GM wheat and barley by hand or with a dedicated plot harvester
- clean the areas after use including the planting area and any area in which seed has been dispersed
- clean any equipment used on site after use
- apply measures to promote the germination of any wheat or barley seeds that may be present in the soil after harvest, including irrigation and shallow tillage
- monitor for at least 24 months after harvest and destroy any wheat or barley plants that may grow, until no volunteers have been detected for a continuous six-months period
- destroy all GMOs not required for further analysis or future trials
- transport and store the GMOs in accordance with the Regulator’s guidelines
- not use GM seeds to develop other wheat and barley cultivars
- not allow the GM plant material to be used for human food or animal feed.

### 3.2 Other risk management considerations

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

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

#### 3.2.1 Applicant suitability

228. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under Section 58 of the Act, matters that the Regulator must take into account, for either an individual applicant or a body corporate, include:

- any relevant convictions of the applicant
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the capacity of the applicant to meet the conditions of the licence.

229. 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. The licence includes a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

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

#### 3.2.2 Contingency plan

231. The University of Adelaide is required to submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any unintended presence of the GM wheat and barley outside permitted areas.

232. Before planting the GMOs, The University of Adelaide must provide the Regulator with a method to reliably and uniquely detect the GMOs or the presence of the genetic modifications in a recipient organism.

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

233. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to growing the GMOs, The University of Adelaide are required to provide a list of people and organisations that will be covered by the licence, or the function or position where names are not known at the time.

### **3.2.4 Reporting requirements**

234. The licence requires 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.

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

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

### **3.2.5 Monitoring for compliance**

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

237. 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.

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

## **Section 4 Issues to be addressed for future releases**

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

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

## **Section 5 Conclusions of the RARMP**

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

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

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## Appendix A: Summary of submissions from prescribed experts, agencies and authorities on the consultation RARMP

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

Submission	Summary of issues raised	Comment
1	<p>Agrees with the overall conclusions of the RARMP.</p> <p>The Regulator should further consider possible risks associated with RFP expression and whether there may be changes in insect and other animal behaviours during the trial.</p>	<p>Noted.</p> <p>Additional text to address the effect of RFP expression on insect and other animal behavior has been added to Chapter 1 (Section 4.3), and Chapter 2 (Section 2.4.2 (Risk scenario 2) and Section 2.4.3 (Risk scenario 3)).</p>
2	<p>Accepts that, overall, the application has negligible risks to the health and safety of people and the environment.</p> <p>Satisfied that measures taken to manage the short- and long-term risks from the proposal are adequate.</p>	Noted.
3	<p>Agrees with the overall conclusions of the Risk Assessment and Risk Management Plan.</p> <p>Matters for consideration in the finalisation of the RARMP include:</p> <ul style="list-style-type: none"> <li>greater dispersal of seed by birds of barley seed</li> </ul>	<p>Noted.</p> <p>Risk scenario 3 (Chapter 2) and Chapter 3 discuss the potential for the spread of wheat and barley seeds by animals, including birds.</p> <p>The proposed trial site is small and the period during which viable seed is available for animal consumption or for spread of viable seeds via animal fur, feathers or muddy feet is short (during sowing and immediately prior to harvest) thus limiting the opportunity for consumption or spread of viable seed.</p> <p>The weed risk assessment for barley is provided in Appendix A of the barley biology document. This assessment is based on information in the biology document. Data to support the rating for barley seed dispersal by birds as ‘unlikely to occasional’ is based on information that there could be occasions where seed may be dispersed by bird, but that the potential and frequency of this is low (OGTR 2021).</p>

Submission	Summary of issues raised	Comment
	<ul style="list-style-type: none"> <li data-bbox="427 241 914 304">potential for higher heavy metal uptake, and impact on birds</li> </ul>	<p data-bbox="994 241 1442 365">Risk scenario 1 in Chapter 2 discusses the potential increased toxicity of the GM wheat and barley lines to desirable animals.</p> <p data-bbox="994 383 1406 568">Text was expanded to include information about GM wheat lines released under DIR 165 that show no difference between GM wheat plants containing the <i>OsNAS2</i> gene and their non-GM counterpart.</p> <p data-bbox="994 586 1426 710">Uncertainty regarding potential harm from uptake of heavy metals by the GM plants has been noted in Chapter 3, Section 3.</p> <p data-bbox="994 728 1445 1104">The small size and short duration of the proposed field trial (Chapter 1, Section 2.1) would restrict the numbers of birds that could be exposed to the GM plant material. It should be noted that galahs and corellas are undesirable pest species in the cereal growing areas, particularly the wheat belt where the GM plants will be grown. These birds are not protected under the <i>National Parks and Wildlife Act 1972</i> in South Australia, allowing landowners to use deterrence measures.</p>
	<ul style="list-style-type: none"> <li data-bbox="427 1131 927 1252">ability to survive outside cultivation and higher weediness potential of the GM barley due to the genetic modification for drought and salinity tolerance</li> </ul>	<p data-bbox="994 1131 1442 1218">Risk scenario 3 in Chapter 2 discusses the potential of spread and persistence of the GM plants outside cultivation.</p> <p data-bbox="994 1236 1442 1391">Non-GM wheat and barley are domesticated plants that have limited ability to survive outside cultivation. This is also evident from the weed risk ratings for wheat and barley, respectively.</p> <p data-bbox="994 1408 1437 1532">Uncertainty regarding the potential for the introduced genes to increase the spread and persistence of the GM plants has been noted in Chapter 3, Section 3.</p> <p data-bbox="994 1550 1426 1697">The small size and short duration of the proposed field trial (Chapter 1, Section 2.1) would limit the spread and persistence of the GM plants outside cultivation.</p>
4	Supported the Gene Technology Regulator's conclusion that DIR 186 poses negligible risk of harm to human health and the environment.	Noted.
5	<p data-bbox="376 1836 932 1991">Aware of concerns from primary producers about the potential impacts from cross-contamination by GM crops. Acknowledge that these considerations are separate from the protection of human health and safety and the environment.</p> <p data-bbox="376 2009 936 2072">Understand that any risk factors have been deemed to be acceptable, as the trial is limited, will</p>	Noted.

<b>Submission</b>	<b>Summary of issues raised</b>	<b>Comment</b>
	<p>be contained in isolation zones designed to limit pollen transmissibility and will be regularly monitored.</p> <p>Note the related conditions that are intended to be added to the licence in support of the relevant requirements.</p>	

## Appendix B: Summary of submissions from the public on the consultation RARMP

The Regulator received one submission from the public on the consultation RARMP. The issues raised in the submission are summarised in the table below. All issues that related to risks to the health and safety of people and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator’s decision to issue the licence.

Submission	Summary of issues raised	Comment
1	<p>The destruction wrought by man playing god with gain-of-function experiments (e.g. the covid virus) has occurred despite best intentions and scientific assurances. Instead, traits should be selectively bred into plants, allowing control and responsibility without causing grief down the track.</p> <p>The destruction of plants, animals and soil has been relentless over the last 100 years. Scientific discovery has largely led to new chemicals to kill insects, new herbicides to kill plants, plastic to pollute and kill fish and animals. All with good intentions and disastrous outcomes.</p>	<p>The functions of the Gene Technology Regulator (the Regulator) are defined by the Gene Technology Act 2000 (the Act) which is legislation passed by the Parliament of Australia. The Regulator must consider each application for a licence for dealings with GMOs based on criteria listed in the Act.</p> <p>In making a decision regarding a licence application, the Regulator is required to assess whether the particular GMOs and activities included in the licence application pose risks to human health and safety or the environment and whether those risks are able to be managed. In the case of licence application DIR 186, the RARMP concludes that the proposed field trial poses negligible risks to human health and safety or the environment. Licence conditions regarding limits and controls have been imposed to maintain the context in which the risks have been assessed.</p>