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

January 2024

Risk Assessment and Risk Management Plan (consultation version) for

DIR 201

Limited and controlled release of wheat and barley genetically modified for yield enhancement

Applicant: The University of Adelaide

This RARMP is open for consultation until 12 March 2024.

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

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

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

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

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

for

Licence Application No. DIR 201

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application for the intentional release of a genetically modified organism (GMO) into the environment. It qualifies as a limited and controlled release application under the *Gene Technology Act 2000* (the Act). The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed field trial poses negligible risks to human health and safety and the environment. Licence conditions have been drafted for the proposed field trial. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether or not to issue a licence.

The application

Project Title	Limited and controlled release of wheat and barley genetically modified for yield enhancement
Parent organism	Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.)
Genetic modifications	
Introduced genes and modified traits	Wheat: • Expression of three genes involved in yield enhancement (expressed both individually and in combination) • Expression of five genes involved in yield enhancement and water use efficiency (expressed individually) • Knockout of two endogenous genes involved in yield enhancement • Expression of three selectable marker genes and one reporter gene (expressed both individually and in combination) Barley: • Knockout of eight endogenous genes involved in yield, architecture, and nutrient use efficiency • Expression of one selectable marker gene (expressed individually)
Genetic modification method	Biolistic or *Agrobacterium*-mediated transformation; gene editing
Number of lines	Up to 103 lines[1] in total
Principal purpose	To assess agronomic performance of the GM wheat and barley lines under field conditions

[1] The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.

Proposed limits	
Proposed use of GM plants	No use in commercial food or animal feed proposed
Proposed location/s	The trial is proposed to take place at one site in South Australia (Light Regional Council)
Proposed release size	Up to a total of 2 ha per year
Proposed period of release	From May 2024 to January 2029
Previous releases	• Wheat lines containing all or some of the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128, DIR 152 and DIR 186. • Wheat lines containing the five genes involved in yield enhancement and water use efficiency have previously been released under DIR 186.

Risk assessment

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 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 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. Draft licence conditions are detailed in Chapter 1 of the RARMP.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the draft 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 the GMOs at the end of the trial and to conduct post-harvest monitoring at the trial site to ensure the GMOs are destroyed.

Table of contents

Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
CRISPR	Clustered regularly interspaced short palindromic repeats
DAFF	Department of Agriculture, Fisheries and Forestry
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
FSANZ	Food Standards Australia New Zealand
GFP	Green fluorescent protein
GM	Genetically modified
GMO	Genetically modified organism
HGT	Horizontal gene transfer
HPH/HPT	Hygromycin phosphotransferase
IBC	Institutional Biosafety Committee
LBO	LATERAL BRANCHING OXIDOREDUCTASE
MAP	Mitogen activated protein
MAPKKK	Mitogen activated protein kinase kinase kinase
MAX1	MORE AUXILLARY GROWTH 1
NHEJ	Non-homologous end joining
NLRD	Notifiable Low Risk Dealings
NPTII	Neomycin phosphotransferase II
OGTR	Office of the Gene Technology Regulator
PAT	Phosphinothricin N-acetyltransferase
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
SDN	Site-directed nuclease
sgRNA	Single guide ribonucleic acid
spp.	species
TGA	Therapeutic Goods Administration
the Act	*Gene Technology Act 2000*

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. 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.

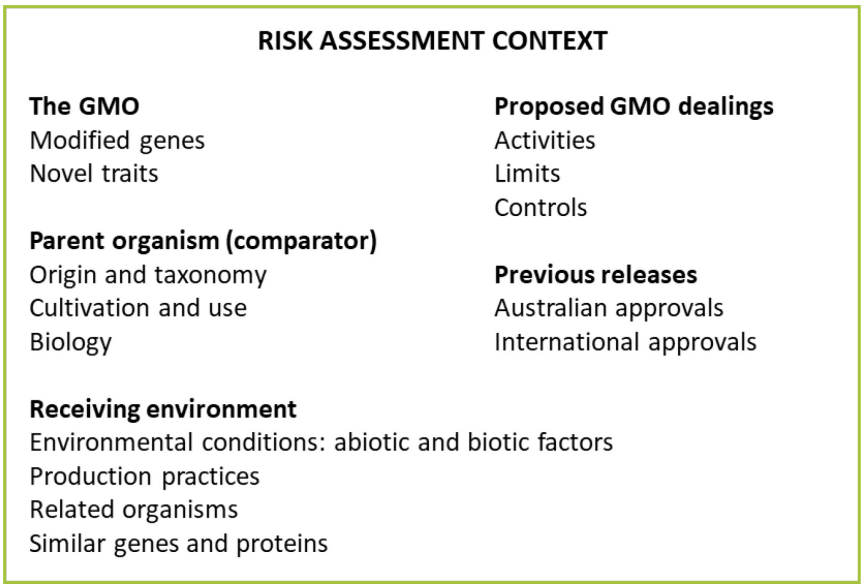

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the Risk Analysis Framework.

6. In accordance with Section 50A of the Act, this application is considered to be a limited and controlled release application, as the Regulator was satisfied that it meets the criteria prescribed by the Act. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

1.1 Interface with other regulatory schemes

7. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority, the Therapeutic Goods Administration, the Australian Industrial Chemicals Introduction Scheme and the Department of Agriculture, Fisheries and Forestry (DAFF). These dealings may also be subject to the operation of State legislation recognising an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.

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

Section 2 The proposed dealings

9. The University of Adelaide proposes to release up to 103 lines of wheat and barley genetically modified (GM) for yield enhancement.

10. The purpose of the trial is to evaluate the agronomic performance 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.

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

- conducting experiments with the GMOs
- making the GMOs
- breeding the GMOs
- propagating the GMOs
- growing or culturing 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 trial (duration, size, location and people)

12. The release is proposed to take place at one site in South Australia (Light Regional Council). The release is proposed to take place between May 2024 and January 2029, on a total of 2 ha in any year.

13. 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

14. 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
- 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.

15. 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.

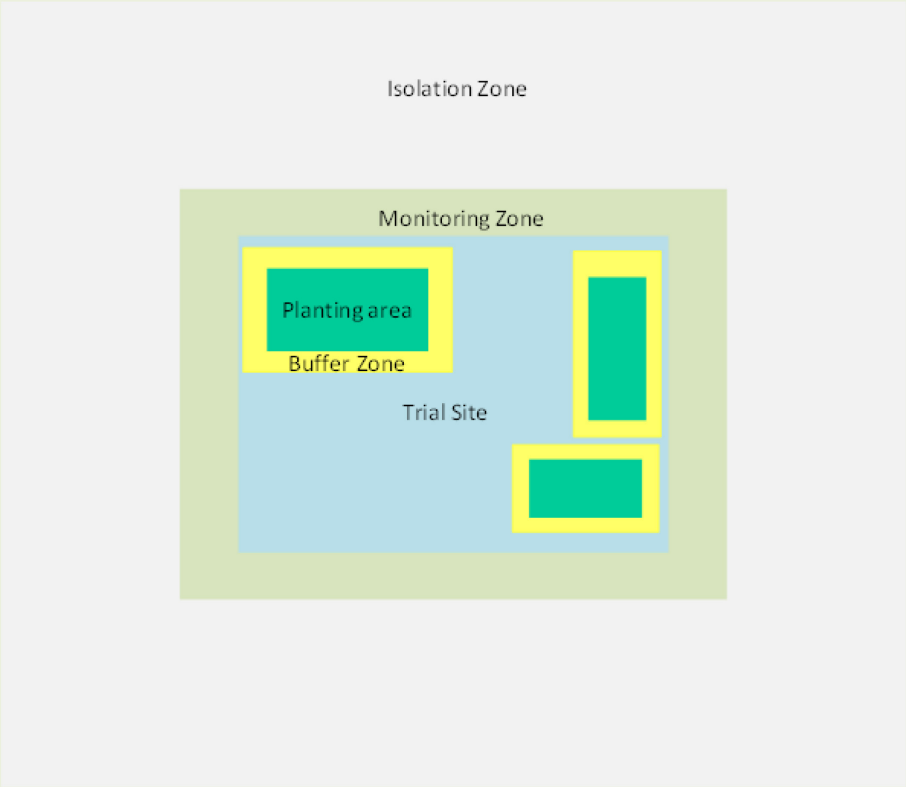

Figure 2. Schematic diagram (not to scale) of trial setup proposed by applicant: Trial site with multiple planting areas.

16. 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 organisms

17. 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 south-eastern Queensland through New South Wales (NSW), Victoria, Tasmania, southern South Australia (SA) and southern Western Australia (WA).

18. 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). Both of these documents are available from the Resources page on the OGTR website. Baseline information from these documents will be used and referred to throughout the RARMP.

19. 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.

20. Neither wheat nor barley is regarded as a weed of national significance (National Weeds List, accessed 11 December 2023), 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).

21. Weed risk assessments are included in the biology documents for wheat and barley. 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. Cultivated barley is highly domesticated, so has reduced fitness outside of agricultural environments. However, it does have a medium weed rating in parts of WA and in Victoria as it is more tolerant to drought and salinity. However, it has no weed rating in the other States where it occurs and 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

22. The applicant proposes to release up to 103 lines of wheat and barley lines genetically modified for yield enhancement. The introduced or knocked-out genes are divided into four groups based on how they alter yield (

23. Table **1**). Field trials of GM wheat containing the introduced genes for direct yield enhancement (Group 1) have previously been evaluated and licensed under DIR 102, DIR 128, DIR 152 and DIR 186. A field trial of GM wheat containing the introduced genes for yield enhancement via water use efficiency (Group 2) has also been evaluated and licensed previously under DIR 186. The applicant has indicated that the GM wheat lines with Group 1 and 2 genes proposed for release include some of the same plants released under DIR 152 and DIR 186.

Table 1. Groups of introduced or knockout genes in the GM wheat and barley

Group	Altered trait	Parent organism	Type of genetic modification	Previous DIRs
1	Direct yield enhancement	Wheat	Gene introduction	DIRs 102, 128, 152, & 186
2	Yield enhancement via water use efficiency	Wheat	Gene introduction	DIR 186
3	Yield enhancement via altered spikelet development and flowering time	Wheat	Gene knockout	-
4	Yield enhancement via altered plant architecture and nutrient use efficiency	Barley	Gene knockout	-

24. The genes *AtAVP1*, *OsNAS2*, *OsPSTOL1*, *TaMUTE*, *TaYDA1*, *TaYDA2*, *TaOST1* or *TaSLAC1* will be introduced into wheat (Table 2). For the lines containing the *AtAVP1*, *OsNAS2*, *OsPSTOL1* genes (Group 1), the applicant intends to release lines containing one of these introduced genes, or combinations of two or all three of the genes.

25. The knockout GM wheat and barley would have small insertions or deletions in endogenous wheat or barley genes making these genetic sequences non-functional (Table 2). These knockout lines have been generated using clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene editing. The *Cas9* gene and sgRNAs used CRISPR/Cas9 gene editing may also be included in these lines.

Table 2. List of introduced or knocked out genes in the GM wheat and barley

Group	Element	Source organism	Function
1	*AtAVP1*	*Arabidopsis thaliana*	Increased shoot and root biomass, photosynthetic capacity, yield and nutrient use efficiency; increased salinity tolerance
	OsNas2	*O. sativa*	Increase in shoot biomass, higher numbers of tillers and grain
	OsPSTOL1	*O. sativa*	Enhanced growth vigour and earlier heading, high yield
2	*TaMUTE*	*T. aestivum*	Stomatal development, symmetrical division of guard mother cells
	TaYDA1	*T. aestivum*	Negatively regulates stomatal development
	TaYDA2	*T. aestivum*	Negatively regulates stomatal development
	TaOST1	*T. aestivum*	Regulates stomatal aperture
	TaSLAC1	*T. aestivum*	Guard cell anion channel
3	*ALOG-1*	*T. aestivum*	Spikelet development and flowering time
	PDB-1	*T. aestivum*	Spikelet development and flowering time
4	*HvLBO*	*H. vulgare*	Strigolactone biosynthesis
	HvMAX1a	*H. vulgare*	Strigolactone biosynthesis
	HvMAX1b	*H. vulgare*	Strigolactone biosynthesis
	HvMAX1c	*H. vulgare*	Strigolactone biosynthesis
	HvMAX1d	*H. vulgare*	Strigolactone biosynthesis
	HvMAX1e	*H. vulgare*	Strigolactone biosynthesis
	HvD53a	*H. vulgare*	Strigolactone signalling
	HvD53b	*H. vulgare*	Strigolactone signalling
Marker	*hptII*	*Escherichia coli*	Hygromycin resistance gene encoding hygromycin phosphotransferase
	nptII	*E. coli K12*	Neomycin phosphotransferase gene for resistance against geneticin or kanamycin

	bar *pporRFP*	*Streptomyces hygroscopicus* *Porites porites*	Bialaphos resistance gene encoding phosphinothricin N-acetyltransferase (PAT) protein that confers tolerance to glufosinate Red fluorescent protein
CRISPR/Cas9 genetic element (Group 3 and 4 GMOs)	*Cas9*	*Streptococcus pyogenes*	RNA-guided nuclease
Single guide RNA (Group 3 and 4 GMOs)	sgRNA	*T. aestivum* *H. vulgare*	RNA-guide for genes in Group 3 and 4

*Note: *Triticum aestivum* is a hexaploid plant with three genomes, known as the A, B and D genomes. The genes for Groups 1 and 2 from each of the three *T. aestivum* genomes may be used to modify the GM wheat 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.

26. 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 lines modified for yield enhancement via water use efficiency may also contain the introduced *pporRFP* gene, which encodes a red fluorescent protein (RFP) used to visually identify GM plant cells. The selectable marker genes and reporter gene are listed in Table 2.

27. Short regulatory sequences that control expression of the genes are also present in the GM wheat and barley proposed for release. CRISPR/Cas9 regulatory elements are also listed. 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 3.

Table 3. Introduced regulatory sequences in the GM wheat and barley

Element function	Genetic element	Source organism
Constitutive promoter	*CaMV35S* *OsUbi* *OsAct1* *PvUbi1+3*	Cauliflower mosaic virus *Zea mays* *O. sativa* *Panicum virgatum*
RNA promoter	*TaU6a* *OsU6a* *OsU6b* *OsU6c* *OsU3*	*T. aestivum* *O. sativa* *O. sativa* *O. sativa* *O. sativa*
Amplification promoting sequence	*Ubi1 Intron* *Ubi 5' UTR*	*Z. mays* *Z. mays*
Guide RNA scaffold		*S. pyogenes*
Termination sequence	*CaMV35S* *nos*	Cauliflower mosaic virus *Agrobacterium tumefaciens*

4.2 Methods of genetic modification

GM wheat

28. The GM wheat lines with introduced genes for yield enhancement (*AtAVP1*, *OsNAS2* and *OsPSTOL1*) are expressed on their own, or as combinations of genes. Wheat 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.

Gene edited wheat and barley

29. The GM wheat and barley lines with endogenous gene knockout would be generated by CRISPR/Cas9 gene editing. In the proposed CRISPR/Cas9 technique, a plasmid DNA is generated encoding the Cas9 protein and a single guide RNA (sgRNA) designed to target a specific endogenous gene. Once wheat and barley are transformed with the plasmid, the expressed Cas9-sgRNA complex creates a double-stranded break in the target DNA sequence. Imperfect natural repair of these breaks most often leads to short insertions or deletions (one or a few base pairs) in the target plant DNA sequence, although it can sometimes produce larger deletions (Soyars et al., 2018). The result of this imperfect repair is gene knockout as the target genetic sequences are non-functional.

30. Schedule 1 of the Regulations lists organisms that are not GMOs for the purposes of the Act. Items on this list exclude organisms modified through unguided repair of site-directed nuclease (SDN) activity as no nucleic acid template was added to cells to guide genome repair following SDN application. These organisms are also known as SDN-1 organisms and include CRISPR/Cas9 gene editing. However, some of these methods generate GMOs in the intermediate steps due to the presence of a transgene or expressed products. The gene edited wheat and barley proposed for release are SDN-1 organisms, but may still contain CRISPR/Cas9 genetic elements so they are still classed as GMOs under the Act (for more details see the *Overview of the status of organisms modified using gene editing and other new technologies* document, which is available on the OGTR website). The applicant has advised that the gene edited barley has been backcrossed to a wildtype parent, resulting in the segregation of the CRISPR/Cas9 cassette, which includes the *Cas9* gene, sgRNAs and the marker gene, from the barley knockout lines proposed for release. This was confirmed by PCR but not genome sequencing so the CRISPR/Cas9 genetic elements may still be present. The applicant has also stated that residual T-DNA may still be present in the gene edited barley. The gene edited wheat has not been backcrossed to parental lines so the introduced CRISPR/Cas9 genetic elements are still present in the knockout wheat lines proposed for release.

31. The plasmid DNA used to transform the gene edited wheat and barley proposed for release were produced using a hierarchical cloning system, where sgRNAs are first inserted into plasmids, which are then assembled into a multigene binary vector construct along with Cas9 and a selection cassette (*hptII*). Gene edited wheat was produced using the pGGG vector system described in Smedley et al. (2021), while gene edited barley was produced using the pYLCRISPR/Cas9 binary vectors described in Ma et al. (2015). The resulting Cas9-sgRNA constructs were transformed into wheat and barley using *Agrobacterium*-mediated transformation (Tingay et al., 1997; Matthews et al., 2001; Hayta et al., 2021). The sgRNA for the target endogenous wheat genes (Table 2) were designed to knock out or modify all three copies of the genes within each of the wheat genomes (A, B and D).

32. The gene edited wheat and barley would be grown in the glasshouse and seed from these plants will be used for planting in the field.

4.3 The introduced or knockout genes for yield enhancement, encoded proteins and associated effects

33. The genes and their encoded proteins are summarised in Table 2, with a description of their expected function in the GM wheat and gene edited wheat and barley. Both yield, water use efficiency, plant architecture, and nutrient use efficiency are multigenic traits, involving the interaction of genes where the protein products constitute different biochemical pathways.

4.3.1 Direct yield enhancement

34. The yield enhancement genes proposed for release are *AtAVP1*, *OsNAS2* and *OsPSTOL1*. As mentioned in Section 4.1, field trials of GM wheat with these genes have been evaluated and licensed previously for DIR 102, DIR 128, DIR 152 and DIR 186, so only a summary regarding these genes is presented here.

AtAVP1

35. The *Arabidopsis thaliana* vacuolar H^+-pyrophosphatase (*AtAVP1*) gene encodes an H^+-translocating pyrophosphatase (H^+-PPase) (Gaxiola et al., 1999; Khadilkar et al., 2016). H^+-PPases in wheat have been shown to be localised in sink tissues (e.g roots, leaves, kernals) and transport phloem (Regmi et al., 2020).

36. Overexpression of genes encoding the protein 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). 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 the gene in wheat also resulted in a significant yield improvement, by producing higher grain yield, an increase in the number of seeds per plant, and an increase in root biomass compared to null segregants (Regmi et al., 2020).

OsNAS2

37. The *OsNAS2* gene encodes a rice nicotianamine synthase (NAS), an enzyme that catalyses the last step in the production of nicotianamine (NA). 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).

38. Constitutive overexpression of *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). Constitutive overexpression of *OsNAS2* in GM bread wheat resulted in increased concentrations of iron and zinc in wholemeal flour, white flour and white bread and higher bioavailability of iron in white flour milled from the GM wheat (Beasley et al., 2019; Beasley et al., 2022). Unpublished results from field trials conducted under licence DIR 152 found that several of the GM wheat lines overexpressing OsNAS2 showed a 20 – 30% increase in shoot biomass due to a higher tiller number. These GM lines also produced approximately 20 – 30% more grain compared to control non-GM wheat.

OsPSTOL1

39. The rice Phosphorous Starvation Tolerance 1 (Os*PSTOL1*) gene encodes a functional serine/threonine protein kinase (Gamuyao et al., 2012). The gene has been shown to improve tolerance to low phosphorous growth conditions in rice (Milner et al., 2023). Overexpression of Os*PSTOL1* in phosphorus-starvation-intolerant rice varieties enhanced 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). GM wheat lines overexpressing *OsPSTOL1* had enhanced growth, crown root number, and overall root plasticity under low phosphorus conditions, while shoot biomass and grain yield were increased when phosphorous was well supplied (Kettenburg et al., 2023).

4.3.2 Yield enhancement via water use efficiency

40. 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. Genetic modification of stomatal development and aperture may result in a number of changes, including:

- protecting plants against drought, allowing them to continue to grow in water-limited environments (Franks et al., 2015; Hepworth et al., 2018)
- 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)
- influence flowering time (Kinoshita et al., 2011).

41. Field trials of GM wheat involving all of these genes have been evaluated and licensed previously for DIR 186. Preliminary data indicates that GM wheat lines grown in the glasshouse have either improved or poorer water use efficiency compared to control non-GM wheat. GM wheat plants with altered stomata aperture and number have also been observed (unpublished data). More detailed information regarding the genes can be found in the DIR 186 RARMP.

TaMUTE

42. MUTE is one of three transcription factors that have been shown to positively regulate stomatal development in *Arabidopsis* (Liu et al., 2019).

43. 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

44. 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 two basic helix-loop-helix domain transcription factors involved in stomatal development (Qi and Torii, 2018; Dunn et al., 2019).

45. 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). 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

46. 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).

4.3.3 Yield enhancement via altered spikelet development and flowering time

47. The applicant has stated that the knockout of the *ALOG-1* and *PDB-1* endogenous genes in wheat aims to alter spikelet development and flowering time. This may result in a change to the amount of grain in the spikelet, thereby altering yield. Both genes have been shown to regulate the Photoperiod-1 (*Ppd-1*) gene, which is an important regulator of flowering time in wheat (Gaughley, 2020). Wheat lines that contain *Ppd-1* photoperiod insensitive alleles have been shown to promote flowering, regardless of the amount of light available (Flohr, 2018). The ability of wheat to flower independently of available light could help to adapt wheat to more varied growth conditions, allowing further optimisation of yield (Hunt, 2015).

48. The *Arabidopsis* LSH1 and *Oryza* G1 (ALOG) protein is a transcription factor that is plant-specific and highly conserved among land plants (Yoshida et al., 2009). It has been shown to regulate reproductive growth in flowering plants, including floral and spikelet development, and also the transition from indeterminate to determinate growth in flowering plants (Takeda et al., 2011; Nan et al., 2018; Naramoto et al., 2020). Expression profiles of ALOG-1 shows that it is negatively regulated during the floral transition (Gaughley, 2020).

49. *PDB-1* is a bZIP transcription factor involved in wheat spike development, including flowering time and spikelet architecture (Gaughley, 2020; Cao et al., 2021). A recent study in rice proposes that complexes of bZIP proteins function together to regulate inflorescence development by forming a florigen repressor or activation complexes (Kaneko-Suzuki et al., 2018; Cerise et al., 2021).

50. The applicant has indicated that the activity of *ALOG-1* and *PDB-1* is disrupted in wheat lines that contain null or overexpression of the *Ppd-1* gene (unpublished data). The applicant has predicted that the absence of the ALOG-1 and PDB-1 proteins will modify spikelet architecture and flowering time, leading to changes in the number of grains produced and therefore yield.

4.3.4 Yield enhancement via altered plant architecture and nutrient use efficiency

51. The gene edited barley lines proposed for release have knockout of endogenous genes involved in the striglactone (SL) biosynthesis pathway. SLs are a novel class of phytohormones that control plant architecture by modulating shoot and root branching (Brewer et al., 2016; Yoneyama and Brewer, 2021). SLs negatively regulate branching, allowing them to alter plant architecture to optimise growth depending on the conditions (Kelly et al., 2023). When growing conditions are poor, the production of SL increases, which reduces the number of branches a plant can make. Conversely, SL production decreases when growing conditions are optimal, which then increases the number of branches a plant can make (Figure 3)(Kelly et al., 2023). Fewer branches result in less plant biomass, which is a desired phenotype in low rainfall or nutrient conditions, while more branching will result in more biomass and grain heads which is desirable when water and nutrient supply is plentiful.

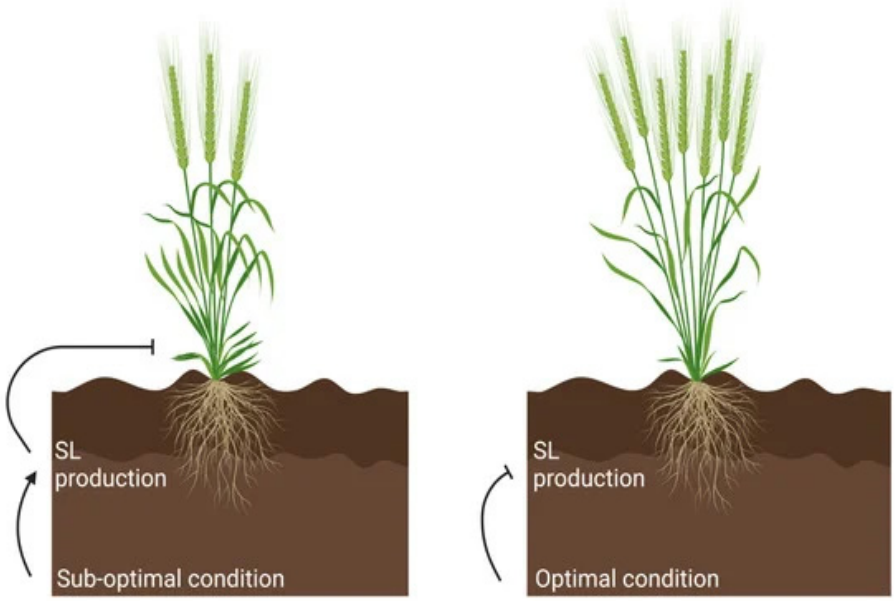

Figure 3. Strigolactone production in optimal and sub-optimal conditions (Kelly et al., 2023)

52. The genes involved in SL biosynthesis that have been targeted in the proposed release are *LBO*, *MAX1* and *D53*. The *LBO* gene encodes LATERAL BRANCHING OXIDOREDUCTASE (LBO), a 2-oxoglutarate and Fe (II)-dependent dioxygenase that has been shown to act in the final stages of strigolactone biosynthesis in *Arabidopsis* (Brewer et al., 2016). The *MAX1* gene encodes MORE AUXILLARY GROWTH 1 (MAX1), a cytochrome P450 enzyme that produces most of the structural diversity of SLs during the final stages of their biosynthesis in rice (Marzec et al., 2020). The DWARF53 (D53) protein was first identified in rice, where it acted as a negative regulator in the SL signalling pathway to promote shoot branching (Jiang et al., 2013). The applicant predicts that the D53 gene in barley encodes key transcriptional repressors that are degraded during SL signalling.

53. The applicant has stated that the knockout of endogenous LBO, MAX1 and D53 barley genes may be a way to maintain plant growth under low nutrient conditions and therefore maintain or enhance yield.

4.3.5 Marker genes

54. The GM wheat and barley plants contain selectable marker genes that confer resistance to different classes of antibiotics or to a herbicide (Table 2). 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).

55. 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*, available on the OGTR website.

56. Some of the GM wheat plants that have introduced genes for yield enhancement via water use efficiency may contain the introduced *pporRFP* gene as a visual marker. This gene encodes a novel DsRed-type 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., 2012). More information on the *pporRFP* gene can be found in the DIR 186 RARMP on the OGTR website. General information on the use of reporter genes may be found in the document *Marker Genes in GM Plants*, also available on the OGTR website.

4.4 Toxicity/allergenicity of the proteins associated with the introduced and knockout genes

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

58. The applicant has not yet performed any toxicity or allergenicity studies on the GM wheat and barley plants proposed for release.

59. Apart from *pporRFP*, all of the genes introduced into the GM wheat were isolated from common sources, thus people and other organisms have a long history of exposure to them. A comprehensive search of the scientific literature yielded no information to suggest that the introduced 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.

60. The CRISPR/Cas9 genetic elements are still present in the knockout wheat lines proposed for release and may also be present in the knockout barley lines (Chapter 1, Section 4.2). Cas9 is an RNA-guided nuclease for genome editing derived from *Streptococcus pyogenes*, a human-specific bacterial pathogen (Ibrahim et al., 2016). Comparisons of amino acid sequences revealed that the Cas9 protein from *S. pyogenes* was similar to Cas9 proteins found in food and the environment, indicating that people and animals are widely exposed to this protein (El-Mounadi et al., 2020). A recent bioinformatic and literature assessment of a human codon-optimized version of the Cas9 protein derived from *S. pyogenes* Cas9 found that, while Cas9 nuclease activity can be toxic to some cell types *in vitro*, there was no evidence from previous studies of a risk of toxicity to humans and other animals from the *Cas9* gene. Also, the full amino acid sequence of this Cas9 protein was not homologous to any known allergens (Qureshi and Connolly, 2023).

61. 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 also been studied by other research groups with the aim of increasing levels of iron in plant tissues and biofortification. Excessive iron in the diet can result in toxicity (Balmadrid and Bono, 2009). Studies have indicated that susceptibility to excess iron toxicity is similar in people and other mammals (Albretsen, 2006). *OsNAS2* introduction or overexpression could lead to accumulation of metals other than iron, such as cadmium, that are also toxic to humans and animals (Flora et al., 2008; Jaishankar et al., 2014; Clemens and Ma, 2016). 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). There have been no adverse effects reported from similar GM lines overexpressing *OsNAS2* planted under DIR 102, DIR 128, DIR 152 and DIR 186. It should be noted that none of these licences permitted use of the GM lines in human food or animal feed, and this use is also not proposed in the current application. Further details on the potential toxicity of the *OsNAS2* gene is detailed in the DIR 186 RARMP.

62. 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 17 December 2023).

63. The *bar* gene and the protein it encodes (phosphinothricin N-acetyl transferase or PAT) has been extensively assessed in other RARMPs, and in scientific literature. The PAT protein has been assessed to lack toxicity to humans or animals, or allergenicity in humans. Further details are available in the DIR 186 RARMP. 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).

64. The *pporRFP* gene and its encoded protein has been recently assessed by the OGTR in the DIR 186 RARMP. 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 organisms.

4.5 Characterisation of the GMOs

65. Although the GM wheat lines are at an early stage of development, the applicant has provided some preliminary information on expected phenotypes for some of the genes introduced into the GM wheat. There is no characterisation data available for the gene edited wheat and barley proposed for release.

66. Some GM wheat lines constitutively overexpressing *OsNAS2* have increased iron concentration in grains (Beasley et al., 2019; Beasley et al., 2022). 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 (data not provided).

67. The applicant also claims that overexpression of *OsPSTOL1* in GM wheat resulted in enhanced plant vigour and earlier heading. Data from DIR 152 and DIR 186 shows field grown GM wheat expressing *OsPSTOL1* has enhanced grain yield (Kettenburg et al., 2023). In GM rice, *OsPSTOL1* conferred enhanced root growth, thus increasing uptake of phosphorous as well as nitrogen and potassium (data not supplied). 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).

68. The 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 proposed for release. GM wheat lines expressing these genes were released under DIR 186. The applicant has stated that preliminary data from glasshouse trials indicate that GM wheat lines expressing these genes have either improved or reduced water use efficiency compared to non-GM controls. GM wheat lines with altered stomata aperture and number have also been observed.

69. The applicant has stated that one of the unintended changes in the GM wheat lines is reduced time to flowering. Some of the GM wheat lines approved for release under DIR 152 and DIR 186 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. Genetic modification of the *OST1* and *SLAC1* genes in the GM wheat and barley may also alter 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).

71. The GM wheat lines for yield enhancement (*AtAVP1*, *OsNAS2* or *OsPSTOL1*, individually and in combination) were grown under DIR 152 and DIR 186. 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. The applicant stated that only yield was successfully increased by up to 30% under these field conditions (no data or further information supplied).

Section 5 The receiving environment

72. 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).

73. 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

74. 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

75. 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 at the 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 186 and future DIR licences, if approved, would also be grown at the site. The applicant has indicated that although DIR 186 allows planting of GM barley, only GM wheat has been planted at the site to date.

76. The site in Rosedale is located in Light Regional Council, a local government area (LGA) 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 SA, 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 SA based on Bureau of Meteorology climate data, which shows a concentration of rainfall during the winter months and drier summer months.

77. 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 due to 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

78. 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.

79. Seeds would be harvested either by hand or with a machine (e.g. plot harvester) that 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.

80. 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

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

82. 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 2026/2027 growing season under the DIR 186 licence, so planting could occur under DIR 186 concurrently with that proposed under DIR 201.

83. Cultivated wheat and barley are not known to hybridise with one another naturally, but each can hybridise with other species. 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. 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. Further details are given in the biology documents for these species and briefly summarised in the RARMP for DIR 186.

5.5 Presence of similar genes and encoded proteins in the environment

84. The introduced genes listed in Table 2 were originally isolated from naturally occurring organisms, most of which are already widespread and prevalent in the environment. The edited genes listed in Table 2 are endogenous wheat and barley genes. Thus, humans and animals have been exposed to the introduced genes and their encoded proteins, and the edited genes, 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.

85. The CRISPR/Cas9 mechanism is an adaptive immune system that occurs naturally in many bacteria and archaea, where it provides protection against invading pathogens and any toxic molecules (Ran et al., 2013; Modrzejewski et al., 2020). A recent review found that CRISPR and genes coding for their associated proteins were present in a diverse range of bacteria, including those used in food production (El-Mounadi et al., 2020). The *Cas9* gene used in the gene edited wheat and barley proposed for release has been isolated from *Streptococcus pyogenes*, a human-specific bacterial pathogen that causes a wide array of infections ranging from mild to life-threatening (Ibrahim et al., 2016). Comparisons of amino acid sequences revealed that the Cas9 protein from *S. pyogenes* was similar to Cas9 proteins found in food and the environment, indicating that people and animals are widely exposed to this protein (El-Mounadi et al., 2020).

86. 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.

87. The *bar* gene was isolated from the common bacterium *S. hygroscopicus*, which is a saprophytic, soilborne 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).

88. 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.

89. All promoters used to drive expression of the introduced genes, including the CRISPR/Cas9 genetic elements, 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.

90. While some of the source organisms can cause allergies (e.g. wheat, Section 4.4), the introduced proteins are not known to cause harm.

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

91. 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), DIR 152 and DIR 186 (*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.

92. Wheat and barley lines containing the five genes for water use efficiency (*MUTE*, *YDA1*, *YDA2*, *OST1* and *SLAC1*) proposed for release under the current application have been approved in Australia for limited and controlled release under licence DIR 186. There have been no reports of adverse effects on human health and safety or the environment resulting from this release.

93. The GM wheat and barley knock-out lines (Table 1, Groups 3 and 4) have not been grown in the field.

94. Information on previous DIR licences for GM wheat and barley is available from the OGTR GMO Record. The Regulator has previously approved 23 field trial releases of GM wheat, of which eleven are licences for both wheat and barley. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

95. There have been no approvals for the commercial release of GM wheat or barley in Australia.

6.2 International approvals

96. 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 December 2023).

97. On a commercial scale, drought tolerant HB4 GM wheat has been approved for cultivation in Argentina, Paraguay and Brazil, and for food and feed in a number of countries including Australia and New Zealand (ISAAA website; accessed 17 December 2023; FSANZ website; accessed 17 December 2023, BioTrack Product database; accessed 14 January 2024).

98. 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

99. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 4). 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.

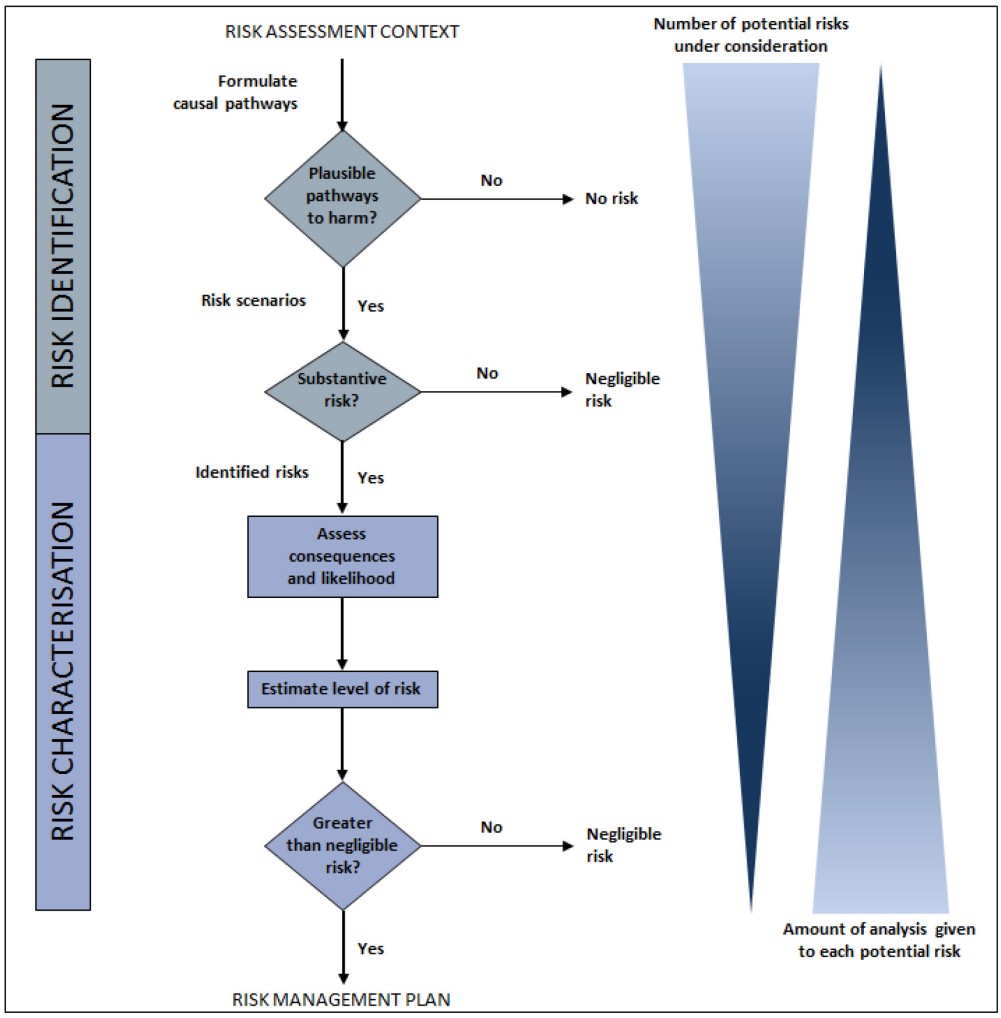

Figure 4. The risk assessment process

100. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, previous agency experience, 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 considered in postulating risk scenarios (Keese et al., 2014). Risk scenarios postulated in previous RARMPs prepared for licence applications for the same or similar GMO are also considered.

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

102. 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 4), i.e. the risk is considered to be no greater than negligible.

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

Section 2 Risk identification

104. Postulated risk scenarios are comprised of three components (Figure 5):

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.

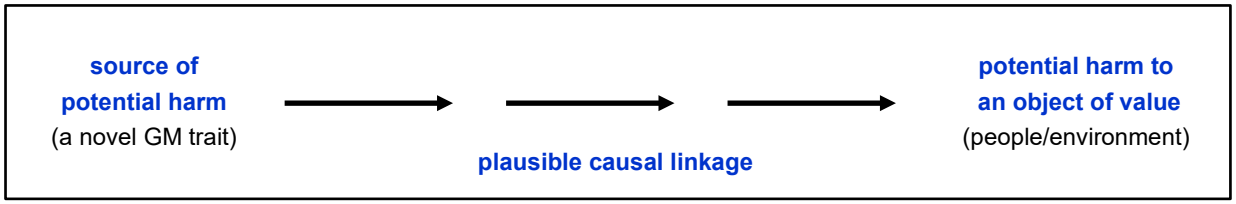

Figure 5. Components of a risk scenario

105. 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

106. 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.

107. As discussed in Chapter 1 (Table 2 and 3), the GM wheat and barley have been modified by introduction (wheat) or knockout of genes (wheat and barley) conferring yield enhancement. These introduced genes will be considered further as a potential source of risk.

108. 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 (Section 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.

109. A red colour marker gene, *pporRFP*, has also been introduced into some of the GM wheat lines. As discussed in Chapter 1 (Section 4.3.5), the gene was isolated from the coral *P. porites* and encodes a novel DsRed-like red fluorescent protein pporRFP (Alieva et al., 2008). The *pporRFP* gene and its encoded protein has been previously assessed in the RARMP for DIR 186 and was not found to pose a substantive risk to either people or the environment. Therefore, this visual marker gene will not be considered further for this application.

110. The introduced genes for yield enhancement, including the genes used for CRISPR/Cas9 gene editing of knockout wheat and barley lines (Chapter 1, Section 4.2), are controlled by introduced regulatory sequences 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.

112. The genetic modifications involving knockout of genes by CRISPR/Cas9 have the potential to cause two classes of unintended effects. The first class of unintended effects are significant genomic deletions or rearrangements at the intended site of gene editing (Hahn and Nekrasov, 2018), leading to altered expression of endogenous genes. The applicant will use CRISPR/Cas to generate double-stranded breaks in DNA sequences that will be randomly repaired by non-homologous end joining (NHEJ). The conventional plant breeding technique of mutagenesis also generates double-strand breaks repaired by NHEJ and can also produce significant genomic deletions or rearrangements (Shirley et al., 1992). As discussed in the previous paragraph, conventional breeding using mutagenesis has a long history of safe use. The second class of unintended effects is off-target gene editing, leading to inadvertent knockout of additional genes with sequences that closely match the intended site of gene editing. A recent review of CRISPR/Cas off-target edits in plants found that most of the observed off-target changes were small insertions or deletions or nucleotide substitutions, and large deletions were rare (Sturme et al., 2022). Off-target sites have few mismatches with the target sequence and were often located in homologues of the target gene (Modrzejewski et al., 2020; Sturme et al., 2022). Other studies have observed that off-target CRISPR/Cas9 gene editing is rare in plants (Hahn and Nekrasov, 2018; Soyars et al., 2018; Tang et al., 2018; Wang et al., 2021). It is also noted that all DNA breaks generated by conventional mutagenesis are untargeted. CRISPR/Cas9 edited plants show lower off-target mutation frequencies than conventionally bred plants (Sturme et al., 2022). Therefore, unintended effects arising from genome editing will not be further assessed for this application.

2.2 Causal pathway

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

- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential 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.

114. Although all of these factors are taken into account, some are not included in the risk scenarios because they have been considered in previous RARMPs and a plausible pathway to harm could not be identified.

115. The potential for HGT from GMOs, including GM plants, to species that are not sexually compatible, and any possible adverse outcomes, have been reviewed in the literature (Keese, 2008; Philips et al., 2022) 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.

116. Previous RARMPs have considered the potential for unauthorised activities to lead to an adverse outcome. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires the Regulator to have regard to the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities, and no risk greater than negligible was identified in previous RARMPs. Therefore, unauthorised activities will not be considered further.

2.3 Potential harm

117. 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).

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

2.4 Postulated risk scenarios

119. Four risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 4 and examined in detail in Sections 2.4.1 - 2.4.4 (this Chapter).

120. The CRISPR/Cas9 genetic elements are still present in the knockout wheat lines proposed for release and may also be present in the knockout barley lines (Chapter 1, Section 4.2). Therefore, these gene editing elements are included in the Risk scenarios.

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

Table 4. Summary of risk scenarios from the proposed dealings with the GMOs

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements	Growing GM wheat and barley at the trial site ⬇ GM wheat and barley composition is different from non-GM wheat and barley ⬇ Exposure of people who deal with the GM plants or of people in the vicinity of the trial sites OR Exposure of animals eating the GM wheat or barley	Increased toxicity or allergenicity to people OR Increased toxicity to desirable animals	No	• GM plant material would not be used as human food or animal feed. • Proposed limits and controls would further minimise the exposure of people and animals to GM plant material. • The GM wheat lines with introduced genes have previously been assessed under DIRs 102, 128, 152 and 186, and no substantive risks were identified. There have been no reports of adverse effects from these releases. • The Cas9 gene is widespread in the environment.
2	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements	Growing GM wheat and barley at the trial site ⬇ Pollen flow to other GM wheat or barley grown at the trial site ⬇ GM wheat and barley composition is different from non-GM wheat and barley ⬇ Exposure of people who deal with the GM plants or of people in the vicinity of the trial sites OR Exposure of animals eating the GM wheat or barley	Increased toxicity or allergenicity to people OR Increased toxicity to desirable animals	No	• Wheat and barley are mostly self-pollinating, and outcrossing occurs at low levels. • 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, site monitoring and post-harvest monitoring.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
3	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements	Growing GM wheat and barley at the trial site ⬇ Presence of GM wheat and barley outside the trial limits ⬇ Spread and persistence of GM wheat and barley in the environment	Increased toxicity or allergenicity for people OR Increased toxicity to desirable animals 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	• The proposed limits and controls minimise the likelihood of seed dispersal or persistence outside the trial limits. • There is no expectation the introduced genes confer characteristics in the GM wheat and barley that may lead to environmental harms. • Dispersal by natural means, and ability to establish outside agriculture is limited in wheat and barley. • The GM wheat and barley is susceptible to most standard weed control measures.
4	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements	Pollen flow from GM wheat and barley to sexually compatible plants outside the trial site ⬇ GM hybrid seed grows into volunteer plants ⬇ Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas ⬇ Increased exposure of people and desirable animals 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 desirable animals OR Reduced establishment and yield of desirable plants OR Reduced utility or quality of the environment	No	• The proposed limits and controls minimise the likelihood of pollen flow from the trial site to sexually compatible plants. • Wheat and barley have limited ability to outcross. • Risk scenarios 1, 2 and 3 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks.

2.4.1 Risk scenario 1

Risk Source	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements
Causal Pathway	Growing GM wheat and barley grows at the trial site ⬇ GM wheat and barley composition is different from non-GM wheat and barley ⬇ Exposure of people who deal with the GM plants or of people in the vicinity of the trial sites OR Exposure of animals eating the GM wheat or barley
Potential Harm	Increased toxicity or allergenicity to people OR Increased toxicity to desirable animals

Risk source

122. The source of potential harm for this postulated risk scenario are the introduced or knocked-out genes conferring yield enhancement and the CRISPR/Cas9 genetic elements in the GM wheat and barley plants.

Causal Pathway

123. The inserted genes and the genes used for CRISPR/Cas9 gene editing are under the transcriptional control of constitutive promoters and so the encoded proteins could potentially be produced in all plant tissues throughout plant development. However, this has not been determined and therefore the level of exposure is an area of uncertainty for this risk assessment.

Exposure of people to the GM wheat and barley

124. The GM wheat and barley would be grown at the trial site. People could be exposed to the GM plant material through inadvertent ingestion, skin contact or inhalation.

125. The applicant proposes that the GM wheat and barley would not be used as human food. There is little potential for accidental ingestion of wheat and barley grown on the trial site. Therefore, it is not expected that people would be exposed to the GM wheat and barley by consumption.

126. The applicant proposes that only trained and authorised persons would be permitted to deal with the GM wheat and barley, or to access the trial site. Due to the small scale of the proposed trial, few people would handle the GM wheat and barley. These authorised staff could have direct skin contact with GM plant material or could inhale GM pollen during cultivation, transportation or analysis. 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.

127. 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 and 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 the 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 proposed trial is of a small size and limited duration, 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.

Exposure of animals eating the GM wheat and barley

128. The GM wheat and barley would not be used as animal feed. However, animals, including birds and insects, entering the trial site could consume the GM wheat and barley. A range of animals consume cereals (Hill et al., 1988; AGRI-FACTS, 2002; OGTR, 2021b, a) 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 some large animals such as livestock. However, other animals such as insects, birds and small animals 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 animals that could be exposed to the GM wheat and barley.

Potential harm

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

130. GM wheat containing the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128, DIR 152 and DIR 186. GM wheat containing the five introduced genes for yield enhancement via water use efficiency have also been previously released under DIR 186. 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. Consistent with the assessment in the DIR 186 RARMP and as no further data has been provided, uncertainty still remains about the potential for increased toxicity or allergenicity of the GM plants expressing *OsNAS2* relative to non-GM wheat and barley, including the potential for uptake of heavy metals. More information can be found in the DIR 186 RARMP on the OGTR website.

131. No toxicity or allergenicity studies have been performed on the GM plant material with the knockout of endogenous genes and this is an area of uncertainty for this risk assessment. As discussed in Chapter 1 (Section 4.4), a recent review found that CRISPR and genes coding for their associated proteins were present in a diverse range of bacteria (El-Mounadi et al., 2020). Amino acid sequence comparisons revealed that the Cas9 protein from *S. pyogenes* was similar to Cas9 proteins found in food and the environment, indicating that people and animals are already widely exposed to this protein (El-Mounadi et al., 2020). The applicant has indicated that the purpose of this trial is to evaluate the candidate genes for yield enhancement under field conditions. Gene edited lines that are shown to have a beneficial impact on yield will have the CRISPR/Cas9 genetic elements segregated out by backcrossing to a wildtype parent. Segregation will be confirmed by genome sequencing before the wheat and barley lines are progressed to the next stage of assessment by the applicant.

132. 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, 2021b, a). The genes knocked-out in the GM plants proposed for release are involved in spikelet development and flowering time (wheat) and altered plant architecture and nutrient use efficiency (barley) (Chapter 1, Section 4.3.3 and 4.3.4). Altering spikelet development, flowering and plant architecture may lead to an increase in the amount of pollen produced per plant compared to non-GM wheat and barley, but there is no information to suggest that pollen characteristics that facilitate dispersal would be changes. Furthermore, there is no reasonable expectation that the knockout of the endogenous wheat and barley genes proposed for this trial would influence the pathways producing known allergens in wheat or barley or lead to the production of a novel toxin.

Conclusion

133. Risk scenario 1 is not identified as a substantive risk because the GM wheat and barley would not be used for human food or animal feed, and other proposed limits and controls would minimise exposure of people and animals to the GM wheat and barley. In addition, there is no reasonable expectation that the gene knockouts and the presence of the Cas9 protein could lead to increased toxicity or allergenicity in people or to increased toxicity in animals. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk Source	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements
Causal Pathway	Growing GM wheat and barley at the trial site ⬇ Pollen flow to other GM wheat or barley grown at the trial site ⬇ GM wheat and barley composition is different from non-GM wheat and barley ⬇ Exposure of people who deal with the GM plants or of people in the vicinity of the trial sites OR Exposure of animals eating the GM wheat or barley
Potential Harm	Increased toxicity or allergenicity to people OR Increased toxicity to desirable animals

Risk source

134. The source of potential harm for this postulated risk scenario are the introduced or knocked-out genes conferring yield enhancement and the CRISPR/Cas9 genetic elements in the GM wheat and barley plants.

Causal Pathway

135. The GM wheat and barley would be grown at the trial site and would produce pollen. 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 GM 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.

136. It is possible that the different lines proposed for release would be planted in close proximity to one another during the trial. In addition, the GM wheat and barley may be grown in close proximity to other GM wheat or barley planted under licence DIR 186. Given that the different GM lines are sexually compatible and that they may have similar flowering times, pollen flow between plants with different introduced or knocked-out genes may occur. This may result in hybrid GM wheat and barley seeds with additional – 'stacked' – introduced or knocked-out genes for yield enhancement.

137. 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.

138. 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, 2021b, a).

139. Outcrossing rates in both wheat and barley are very low, and decrease as distance from the pollen source increases (DIR 186 RARMP). Outcrossing rates are also influenced by the genotype of the variety, and environmental conditions, such as wind direction and humidity (OGTR, 2021b, a). The GM wheat containing the introduced genes for yield enhancement and water use efficiency have been previously released under DIR 186. The RARMP for DIR 186 did not identify any information that would indicate an effect of these introduced genes on pollen characteristics leading to an increase in the likelihood of outcrossing. There have also been no reports of changes to phenotypic characteristics that may influence outcrossing rates from this previous release. Similarly, there is no current information to indicate that knockout of genes involved in spikelet development and flowering time (wheat) and altered plant architecture and nutrient use efficiency (barley) would influence pollen characteristics leading to an increase in the likelihood of outcrossing.

140. 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 186 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, site monitoring and post-harvest monitoring requirements have been imposed under licence DIR 186. In addition, seeds, including any possible hybrid seeds, obtained from the trial authorised under DIR 186 must not be used for breeding or propagation to produce cultivars for future commercial release. Taken together, exposure of people or animals to any hybrid GM wheat and barley would be highly unlikely.

Potential harm

141. If pollen flow occurred between the GM wheat or the GM barley grown under DIR 201, or between lines from DIR 186 and DIR 201, it is possible that some GM hybrid seed may be produced. If this occurs, hybrid seeds and any resulting plants may express new combinations of introduced or knocked-out genes which may be harmful to people or other organisms. It is noted that all of the GM wheat lines with introduced genes are the same as those released under DIR 186. Therefore, if hybridisation were to occur between these GM wheat lines and those released under DIR 186, then the same hybrid plants with the same gene combinations could occur. Therefore, only hybridisation between GM barley lines of DIR 201 and DIR 186 would result in new combinations of genes.

142. It is unlikely that any plants grown from the hybrid GM seeds would persist, due to post-harvest control measures to ensure removal of GM volunteers (Chapter 1, Section 2.2). Thus, exposure of people or other desirable animals to hybrid GM wheat and barley would be minimal.

143. No substantive risks for toxicity or allergenicity of the introduced or knocked-out genes were identified in Risk Scenario 1 (above) and the RARMP for DIR 186. Likewise, there is no expectation that combinations of introduced or knocked-out 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.

Conclusion

144. Risk scenario 2 is not identified as a substantive risk because of the proposed limits and controls for this application and also those imposed by the DIR-186 licence, and because wheat and barley are mainly self-pollinating with low levels of outcrossing. There is also no reasonable expectation that expression of the introduced genes or the knockout of genes could lead to increased toxicity or allergenicity in people or to increased toxicity in animals. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk Source	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements
Causal Pathway	Growing GM wheat and barley at the trial site ⬇ Presence of GM wheat and barley outside the trial limits ⬇ Spread and persistence of GM wheat and barley in the environment
Potential Harm	Increased toxicity or allergenicity for people OR Increased toxicity to desirable animals 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

Risk source

145. The source of potential harm for this postulated risk scenario are the introduced or knocked-out genes conferring yield enhancement and the CRISPR/Cas9 genetic elements in the GM wheat and barley plants.

Causal Pathway

146. GM wheat and barley would be grown at the trial site and would produce seed. 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 desirable animals to the GM wheat and barley.

147. As discussed in Risk scenario 2, the different GM lines proposed for release would be planted close to one another, and to other GM wheat or barley planted under licence DIR 186. Pollen flow between these GM plants may result in hybrid GM wheat and barley seed with stacked traits for yield enhancement, including CRISPR/Cas9 genetic elements, which could also be dispersed from the trial site.

148. GM wheat containing the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128, DIR 152 and DIR 186. GM wheat containing the five introduced genes for yield enhancement via water use efficiency have also been previously released under DIR 186. No substantive risks for spread and persistence of the proteins were identified (See the respective RARMPs for details). There have not been any reports of changes in dispersal, establishment and survival from these earlier releases and the same containment measures as required under the DIR 186 licence have been proposed for this release. Therefore, the likelihood of dispersal of Group 1 and 2 GM lines will not be discussed further here.

Persistence of GM wheat and barley on the trial site

149. 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 during sowing and/or near maturity 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 (OGTR, 2021b, a). The knocked-out genes have not been linked to alterations in this trait in the GM wheat and barley proposed for release under the current application.

150. The applicant has proposed hand harvesting of seeds or use of a plot harvester, which would reduce the likelihood of seeds ending up on, or in the ground, when compared to the use of commercial harvesting equipment.

151. 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. The knocked-out genes are not expected to alter seed dormancy in the GM wheat and barley.

152. 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

153. Seeds of the GM wheat and barley could be dispersed outside the trial site through the activity of people or through natural means, such as animals, wind and water. There is no reasonable expectation that the knocked-out genes would affect any of the seed characteristics important for dispersal.

154. Human activity is the most important dispersal pathway for non-GM wheat and barley seed (OGTR, 2021b, a). 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; using 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 human activity.

155. Animals can potentially spread plant seed by movement of seeds adhering to fur, feathers or feet, consumption and excretion of whole seeds, or by removing and hoarding seed (Chambers and MacMahon, 1994). Ingestion and excretion can affect seed viability and reduce the likelihood of germination (Cummings et al., 2008; Oveisi et al., 2021). Further details are available in the DIR 186 RARMP (Risk scenario 3).

156. The applicant proposes controls that would reduce the likelihood of seed dispersal by animals, including: 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.

157. 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, 2021b, a). 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 plants or the

soil surface. Wheat and barley seeds on the soil surface could also be transported 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.

Ability of the GM wheat and barley to establish populations in the environment

158. Wheat and barley are domesticated plants that have limited ability to survive outside cultivation (OGTR, 2021b, a). During domestication, both wheat and barley lost their natural seed dispersal mechanism of seed shattering and lost seed dormancy traits that allow seeds to delay germination until environmental conditions are favourable (OGTR, 2021b, a). This limited ability to survive outside cultivation is reflected in the weed risk ratings for wheat and barley (OGTR, 2021b, a). Although both crops have a long history of cultivation in Australia, neither is listed as a weed of national significance (National Weeds List, accessed 11 December 2023), nor as a significant weed in Australian ecosystems (Groves et al., 2003). Large weedy populations of wheat and barley are not observed in the agricultural or natural environment. There is no reasonable expectation that any of the knocked-out genes will alter characteristics such as seed shattering, other seed dispersal characteristics or seed dormancy which would alter the GMOs' ability to disperse and establish outside an agricultural setting.

159. The knocked-out genes are likely to be pleiotropic (that is, they have effects on several traits) thus potentially enhancing their ability to thrive in sub-optimal conditions. For example, a gene involved in abiotic stress tolerance may impart tolerance to a number of abiotic stresses or to biotic stresses (Howles and Smith, 2013). This may increase the ability of the GM wheat and barley to establish in agricultural, natural and intensive use areas, and may provide the GM wheat and barley with an advantage over non-GM wheat and barley. No studies have been conducted and this is an area of uncertainty for this risk assessment. However, tolerance to abiotic stress(es) or enhanced yield in an agricultural setting will not in isolation increase the invasiveness and persistence of the plants, due to the complexity of environmental conditions.

160. If the wheat and barley knockout plants have improved abiotic stress tolerance compared to non-GM wheat and barley, this could increase their ability to spread and persist in the environment. Knockout of the *ALOG*-1 and *PDB-1* genes in the GM wheat is predicted to alter spikelet development and flowering time (Chapter 1, Section 4.3.3). The applicant has stated that these gene knockouts could increase or decrease the amount of grain from the wheat spikelet. Increased seed production is a factor that contributes to the invasiveness of plants (Keese et al., 2014). A recent laboratory study has shown that *ALOG-1* wheat knockout lines flowered faster and produced fewer spikelets compared to non-GM controls, but there is no information on the length of flowering or the number of grains produced (Gaughley, 2020). Knockout of genes involved in the strigolactone biosynthesis pathway in the GM barley may improve yield under nutrient limited conditions (Chapter 1, Section 4.3.4). Therefore, it might be expected that their competitive ability may be increased under poor nutrient conditions compared to non-GM barley. However, in order to increase weediness these characteristics would need to be coupled with other mechanisms that increase invasiveness through increased spread and persistence in the environment, through changes in dispersal, establishment and survival. Furthermore, proposed controls, including site monitoring and post-harvest requirements, would reduce the likelihood of spread and persistence of any GM wheat and barley seed outside the trial site.

Potential harm

161. If GM plants were able to establish outside the trial site, they could cause increased toxicity to people or animals, 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.

162. GM wheat containing the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128, DIR 152 and DIR 186. GM wheat containing the five introduced genes for yield enhancement via water use efficiency have also been previously released under DIR 186. No substantive risks for spread and persistence of the GM wheat containing the expressed proteins were identified (see the respective RARMPs for details) and therefore will not be discussed further here.

163. If the GM wheat or barley with the knocked-out genes were able to establish outside the trial site, the quality of the biotic environment could be potentially reduced. This could occur through reduced establishment or yield of desirable plants in agricultural or natural land uses; reduced utility of intensive use areas, such as roadsides, drains or channels; or increased ability to provide a reservoir for pathogens or shelter for pests. However, none of the knocked-out genes have 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 knocked-out 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 knocked-out genes are involved in relevant pathways, there is no reasonable expectation this may occur.

164. The ability of volunteer GM wheat and barley to compete with desirable plants is restricted because the genetic modifications are not expected to change the susceptibility of the GM wheat and barley to conventional weed management. Thus, GM wheat and barley volunteers could be controlled by standard weed management measures, such as cultivation or the use of appropriate herbicides, if required.

165. As discussed in risk scenarios 1 and 2, knocking out the genes are unlikely to change the GM wheat or barley composition such that they would more toxic or allergenic than the non-GM parents.

Conclusion

166. 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 the GM wheat or barley to spread and persist outside the trial site and their susceptibility to 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

Risk Source	Introduced or knocked-out genes conferring yield enhancement, and CRISPR/Cas9 genetic elements
Causal Pathway	Pollen flow from GM wheat and barley to sexually compatible plants outside the trial site ⬇ GM hybrid seed grows into volunteer plants ⬇ Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas ⬇ Increased exposure of people and desirable animals 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
Potential Harm	Increased toxicity or allergenicity to people or toxicity to desirable animals OR Other environmental harms (see risk scenario 3)

Risk source

167. The source of potential harm for this postulated risk scenario are the introduced or knocked-out genes conferring yield enhancement and the CRISPR/Cas9 genetic elements in the GM wheat and barley plants.

Causal Pathway

168. The GM wheat and barley would be grown at the trial site and would produce pollen. 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 could form the basis for establishment, spread and dispersal of the knocked-out genes in other varieties of wheat or barley, or other sexually compatible plant species. This could increase the likelihood of exposure of people or animals to the GM wheat and barley.

169. Baseline information on vertical gene transfer associated with non-GM wheat and barley plants can be found in the wheat and barley biology documents. Relevant details have also been provided in the DIR 186 RARMP and Risk scenario 2.

170. 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).

171. 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 GM wheat and barley volunteers would be destroyed before flowering during post-harvest monitoring.

172. Any hybrid seed resulting from vertical gene flow would need a suitable environment for germination, plant establishment and persistence (see Risk scenario 3). Volunteers can be controlled with integrated weed management practices.

Potential harm

173. If GM hybrid plants spread and persisted in the environment, this may lead to increased toxicity to people or other desirable animals, or allergenicity to people. Hybrids containing the knocked-out genes could also reduce the establishment and yield of desired plants and cause other environmental harms as per Risk scenario 3.

174. GM wheat containing the three introduced genes for yield enhancement have previously been released under DIR 102, DIR 128, DIR 152 and DIR 186. GM wheat containing the five introduced genes for yield enhancement via water use efficiency have also been previously released under DIR 186. No substantive risks for hybrid GM wheat containing the expressed proteins were identified (see the respective RARMPs for details) and therefore will not be discussed further here.

175. The knocked-out genes could be introduced, via vertical gene transfer, into other non-GM wheat, barley or other sexually compatible species. The properties that the knocked-out 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 knocked-out genes in any subsequent hybrids would confer the same properties as the GM parent.

176. As discussed in Risk scenarios 1-3, knocking out the genes are unlikely to change the GM wheat or barley characteristics such that they would more toxic or allergenic than the non-GM parents.

Conclusion

177. Risk scenario 4 is not identified as a substantive risk due to the limited occurrence of long distance pollen flow for wheat and barley, the limited reproductive capacity of barley, and the proposed controls. In addition, Risk scenarios 1, 2 and 3 did not identify toxicity, allergenicity or weediness of the GMOs or their hybrids as substantive risks. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

178. Uncertainty is an intrinsic part of risk and is present in all aspects of risk analysis. This is discussed in detail in the Regulator's Risk Analysis Framework document.

179. 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.

180. 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.

181. For DIR-201, uncertainty is noted particularly in relation to:

- expression patterns of the introduced genes in the GM plants
- potential for increased toxicity or allergenicity of the GM plants
- potential for the introduced or knockout genes to increase weediness of the GM plants.

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

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 limits and controls 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 4 and include:

- none of the GM plant material would be used for human food or animal feed
- limits on the size and duration of the proposed release

- suitability of controls proposed by the applicant to restrict the spread and persistence of the GM wheat and barley plants and their genetic material
- 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 [2].

[2] As none of the proposed dealings are considered to pose a significant risk to people or the environment, Section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP.

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 are able to 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, draft 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 draft licence.

3.1 Limits and controls on the release

195. Sections 2.1 and 2.2 in Chapter 1 list the limits and controls proposed by the University of Adelaide. Many of these are discussed in the four risk scenarios considered in Chapter 2. The appropriateness of the limits and controls is considered further in the following sections. Furthermore, many of the control measures replicate licence conditions as issued for DIR 186.

3.1.1 Consideration of limits and controls proposed by The University of Adelaide

196. The applicant proposes that the release would take place at one site in Rosedale (SA). The field trial would run between May 2024 and January 2029, 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 animals to the GMOs (Risk Scenario 1) and limits the opportunity for presence of the GMOs 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 all people dealing with the GMOs to be informed of relevant licence conditions. These measures would limit the potential exposure of people to the GMOs (Risk scenario 1).

3.1.2 Consideration of proposed controls regarding exposure to the GMOs

198. The applicant states that the GM wheat and barley and its material would not be used for human food or animal feed. A licence condition prohibits the use of GM plant material in human food or animal feed. This measure would minimise exposure of people or animals to the GM wheat and barley by consumption (Risk scenario 1).

199. The applicant has indicated that the Rosedale property 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 harmful 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

200. Figure 2 in Chapter 1 shows a schematic diagram of the trial setup proposed by applicant. Each GM wheat and barley planting area is proposed to be surrounded 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 186). 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 outside the trial site, so they are not proposed under the conditions of the licence.

201. 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.

202. The applicant has stated that, under field conditions, the GM wheat lines expressing *PSTOL1* and *AVP1* (direct yield enhancement) flower 5-10 days earlier than non-GM plants within the same cultivar. The introduced genes for direct yield enhancement may also influence tillering in the GM wheat lines (Chapter 1, Section 4.5). Genetic modification of stomatal development and aperture and gene editing of *ALOG-1* and *PDB-1* may also reduce time to flowering in the GM wheat and barley plants (Chapter 1, Section 4.3 and 4.5). 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.

203. 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

204. 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.

205. Consistent with the DIR 186 licence, the draft licence for DIR 201 does not require the monitoring zone to be cleaned post-harvest. Experience of both the applicant and the OGTR is that there has been negligible dispersal of GMOs into the monitoring zone for similar previous trials of this type and size. If any GMOs are dispersed into areas within the monitoring zone, the draft licence specifies that the area would need to be cleaned and inspected for any volunteers.

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. The Rosedale site is currently approved for planting of GM wheat and barley under licence DIR 186. The licence for DIR 186 permits planting until the end of the 2026/27 growing season (inclusive), so GMOs

from both DIR 186 and DIR 201 (if approved) could be grown concurrently at the same site in different planting areas. The applicant has proposed buffer zones and pollen traps that may reduce the amount of pollen flow between different planting areas at the site, but these measures will not eliminate crossing and so a buffer zone and pollen trap are not proposed in the licence. The applicant has also indicated that, if needed, they may sow DIR 201 GMOs over planting areas that have been previously planted with DIR 186 GMOs that are in post-harvest monitoring. Plots may be sown directly over previous DIR 186 plots or offset to previous DIR 186 planting areas. As discussed in Chapter 1 (Section 4.1) and assessed in Risk scenario 2, the GM wheat lines released under DIR 186 contain the same introduced genes as those proposed for release under DIR 201. Therefore, no new gene combinations would occur if there was hybridisation between DIR 186 and DIR 201 GM wheat lines, as the same hybridisation could occur between DIR 201 GM lines. Hybridisation between DIR 186 and DIR 201 GM barley lines could result in new combinations of genes. Therefore, if a DIR 186 site is overplanted or if DIR 201 and DIR 186 sexually compatible lines are grown concurrently, a licence condition has been proposed whereby seed produced from the GMOs grown under DIR 201 must not be used for development of cultivars for potential future commercial release, unless it has been determined that the GM seed only contains the expected genetic modifications. The proposed licence conditions also include requirements to notify the OGTR of planting area details, including the GPS coordinates, identity of the GMOs planted, and a history of how the area has been used for the previous two years. These conditions are expected to manage untended mixing of seed or production of unexpected hybrids.

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 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, until the site is free of volunteer plants for the last 6 months of the post-harvest inspection period. 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[3] 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 lines (Chapter 1, Section 4.5), 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 and 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

[3] The physiological development of a plant can be measured in degree-days, which is a means of combining time and temperature into a single number. Degree-days in wheat have been calculated as the sum of the average daily temperature, minus the minimum temperature at which the plant grows, over consecutive days (Bowden et al., 2008).

(Ogg and 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 and 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 and 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 and 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 barley 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 trial 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 with 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 May 2024 and January 2029
- 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 future wheat and barley cultivars if there is the potential of hybridisation/mixing with GMOs authorised under other licences
- 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 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. If a licence were issued, the conditions would include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

230. In addition, the applicant organisation must have access to an IBC and be an accredited organisation under the Act.

3.2.2 Contingency plans

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

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

3.2.4 Reporting requirements

234. If issued, the licence would require the licence holder to immediately report any of the following to the Regulator:

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

235. A number of written notices would also be required under the licence regarding dealings with the GMO, 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 with 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 would continue until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

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 the 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 the GM wheat and barley 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
- additional sequencing data to confirm that CRISPR/Cas9 genetic elements and residual T-DNA have been segregated from the gene edited wheat and barley lines.

Section 5 Conclusions of the consultation RARMP

240. The risk assessment 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. These negligible risks do not require specific risk treatment measures.

241. If a licence is issued, conditions would be 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.

Chapter 4 Draft licence conditions

Section 1 Interpretations and Definitions

1. In this licence:

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

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

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

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

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

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

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

2. In this licence:

'Act' means the *Gene Technology Act 2000* (Commonwealth) or the corresponding State law under which this licence is issued.

'Barley' means plants of the species *Hordeum vulgare* L.

'Clean' means, as the case requires:

   (a) in relation to Equipment or a facility, remove and/or Destroy the GMOs; or

   (b) in relation to an area of land specified in this licence as requiring Cleaning:

      i. Destroy wheat and barley plants, if present, to the reasonable satisfaction of the Regulator, and

      ii. remove wheat and barley seeds from the soil surface to the reasonable satisfaction of the Regulator.

Note: The intent of removing seeds from the soil surface is to minimise seed dispersal. One method of removing seeds from the soil surface is Tillage, which moves seeds to under the soil. Tillage must be in accordance with condition 42.

'Contingency Plan' means a written plan detailing measures to be taken in the event of the unintended presence of the GMOs outside an area that must be inspected. A Contingency Plan must include procedures to:

   (a) ensure the Regulator is notified immediately if the licence holder becomes aware of the event; and

   (b) recover and/or Destroy the GMOs to the reasonable satisfaction of the Regulator; and

   (c) inspect for and Destroy any Volunteers that may exist as a result of the event to the reasonable satisfaction of the Regulator.

'Destroy', (or **'Destruction'**) means, as the case requires, kill by one or more of the following methods:

(a) uprooting;

(b) root cutting and shredding/mulching;

(c) Tillage, but only in accordance with condition 42;

(d) treatment with herbicide;

(e) burning/incineration;

(f) autoclaving;

(g) milling/hammer milling;

(h) crushing or grinding of seed;

(i) burial, but only in accordance with condition 43;

(j) a method approved in writing by the Regulator.

Note: 'As the case requires' has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate. For example, treatment with herbicide would not successfully kill GM seeds.

'Equipment' includes, but is not limited to, seeders, harvesters, threshers, storage equipment, transport equipment (e.g. bags, containers, trucks), clothing, footwear and tools.

'Extreme Weather' includes, but is not limited to, fires, flooding, cyclones or torrential rain, that could disperse GMOs or affect the licence holder's ability to comply with licence conditions.

'Flowering' is taken to begin when anthers emerge from any plant of the class of plants referred to in a particular condition, and is taken to end when anthers have dried up or dropped off all plants in the class of plants.

'GM' means genetically modified.

'GMOs' means the genetically modified organisms that are the subject of the dealings authorised by this licence. GMOs include live plants and viable seed.

'Inspection Zone' means an area of land extending outwards at least 50 metres from the outer edge of a Monitoring Zone, as shown in Figure 1.

'Isolation Zone' means an area of land extending outwards at least 140 metres from the outer edge of an Inspection Zone, as shown in Figure 1.

'Logbook' means a written or electronic record containing information required to be collected and maintained by this licence and which is able to be presented to the Regulator on request.

'Monitoring Zone' means an area of land extending outwards at least 10 metres from the outer edge of a Planting Area, as shown in Figure 1. If multiple Planting Areas are present in a Site, the Monitoring Zone also includes the areas of land, of any size, between Planting Areas, as shown in Figure 1.

'OGTR' means the Office of the Gene Technology Regulator.

'Personal Information' means information or an opinion about an identified individual, or an individual who is reasonably identifiable:

(a) whether the information or opinion is true or not; and

(b) whether the information or opinion is recorded in a material form or not.

'Planting Area' means an area of land where the GMOs and non-GM wheat and barley are intentionally planted and grown pursuant to this licence.

'Plant Material' means any part of the GM or non-GM wheat and barley plants grown at a Planting Area, whether viable or not, or any product of these plants.

'Regulations' means the Gene Technology Regulations 2001 (Commonwealth) or the corresponding State law under which this licence is issued.

'Regulator' means the Gene Technology Regulator.

'Related Species' means durum wheat, rye or triticale plants.

'Sign off' means a notice in writing from the Regulator, in respect of an area, that post-Cleaning obligations no longer apply to that area.

'Site' means an area of land containing one or more Planting Areas and their joint Monitoring Zone, as shown in Figure 1.

'Tillage' means the use of any technique to disturb the soil.

Note: Tillage must be in accordance with condition 42.

'Volunteers' means GM or non-GM wheat and barley plants, which have not been intentionally grown.

'Wheat' means plants of the species *Triticum aestivum* L. em Thell.

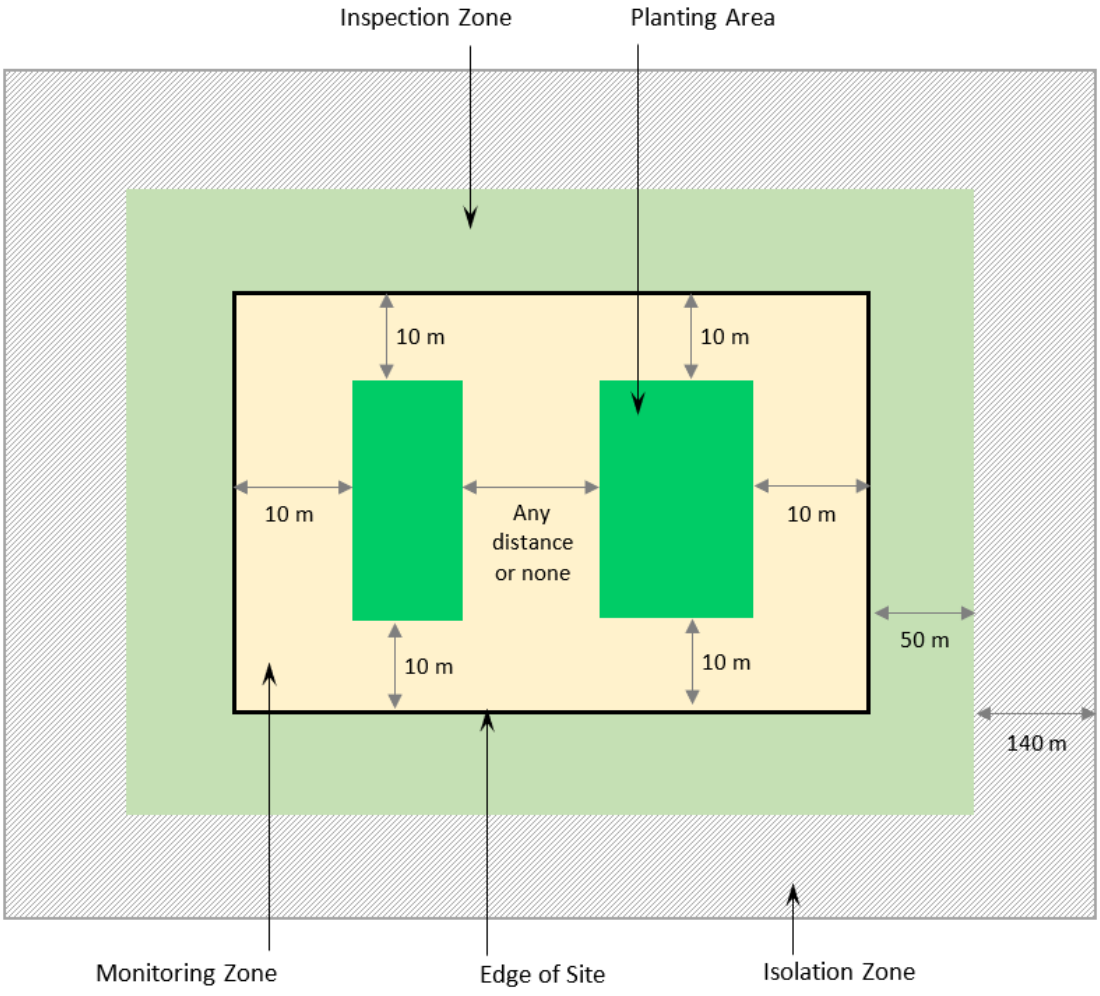

Figure 6. Diagram (not to scale) showing the relationship between Planting Area, Monitoring Zone, Site, Inspection Zone and Isolation Zone.

Section 2 General conditions and obligations

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

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

Note: Although this licence has no expiry date, the period when GMOs may be grown is restricted in accordance with Condition 19.

5. The licence holder is The University of Adelaide.
6. The persons covered by this 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 this licence.
7. The GMOs with which dealings are authorised by this licence are those listed at **Attachment A**.
8. The dealings authorised by the licence are to:
   (a) conduct experiments with the GMOs;
   (b) make the GMOs;
   (c) breed the GMOs;
   (d) propagate the GMOs;
   (e) grow or culture the GMOs;
   (f) transport the GMOs;
   (g) dispose of the GMOs;

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

9. This licence does not apply to dealings with the GMOs conducted as a Notifiable Low Risk Dealing (NLRD) or pursuant to another authorisation under the Act.

Note: Dealings conducted as an NLRD must be assessed by an Institutional Biosafety Committee (IBC) before commencement and must comply with the requirements of the Regulations.

General obligations of the licence holder

10. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and must comply with its instrument of accreditation.
11. The licence holder must be able to access and control all Planting Areas, Monitoring Zones, Inspection Zones, Isolation Zones and approved facilities to the extent necessary to comply with this licence.

Note: Arrangements to access and control these areas must be notified to the Regulator as part of each planting notification (Condition 51(a)).

12. The licence holder must inform any person covered by this licence, to whom a particular condition of the licence applies, of the following:
    (a) the particular condition, including any variations of it;
    (b) the cancellation or suspension of the licence;
    (c) the surrender of the licence.
13. The licence holder must not permit a person covered by this licence to conduct any dealing with the GMOs unless:
    (a) the person has been informed of any applicable licence conditions, including any variation of them; and
    (b) the licence holder has obtained from the person a signed and dated statement that the person:
        i. has been informed by the licence holder of the licence conditions including any variation of them; and

ii. has understood and agreed to be bound by the licence conditions, or variation.

14. The licence holder must inform the persons covered by this licence that any Personal Information relevant to the administration and/or enforcement of the licence may be released to the Regulator.

General obligations of persons covered by the licence

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

Note: Under the Act, the definition of premises includes a building, area of land or vehicle.

Section 3 Limits and control measures

3.1 Limits on the release

The following licence conditions impose limits on where and when the GMOs may be grown.

16. The only plants that may be intentionally grown at a Planting Area are:
    (a) the GMOs covered by this licence; and
    (b) non-GM wheat and barley plants; and
    (c) plants approved in writing by the Regulator.
17. Subject to any conditions imposed by the DIR 186 licence, this licence does not prohibit the planting of GMOs authorised under this licence on Planting Areas previously used for DIR 186 or adjacent to Planting Areas concurrently planted under the DIR 186 licence.
18. Non-GM wheat and barley plants grown in a Planting Area must be handled as if they were the GMOs.
19. Planting and growing of the GMOs may only occur within the following limits:

Area and duration

Period	Maximum number of Sites per year	Maximum combined area of Planting Areas per year	Local Government Areas in which Sites may be located
May 2024 - January 2029	1	2 ha	Light Regional Council (SA)

3.2 Control measures

The following licence conditions restrict the spread or persistence of the GMOs and their genetic material in the environment.

GMOs must not enter food or feed

20. Plant Material must not be used, sold or otherwise disposed of for any purpose which would involve or result in its use as food for humans or feed for animals.

Conditions to restrict pollen flow

21. A Planting Area must be surrounded by a Monitoring Zone (as shown in Figure 1). Multiple Planting Areas may be contained within a single Monitoring Zone. No Planting Area may be less than 10 metres from the outer edge of the Monitoring Zone.
22. The Monitoring Zone, with the exception of areas planted in accordance with condition 23, must be maintained in a manner appropriate to allow the identification and Destruction of Volunteers and Related Species while the GMOs are growing in the Planting Areas and until the Planting Areas are Cleaned.

Note: Acceptable measures to achieve this include keeping land free of vegetation or keeping vegetation mown to a height of less than 10 centimetres. Condition 52(d) requires details of current land use and recent land management practices to be recorded upon inspection of the Monitoring Zone.

23. In the Monitoring Zone, the only sexually-compatible plants which may be grown are plants authorised under another licence issued by the Regulator, or plants approved in writing by the Regulator.

24. The Monitoring Zone must be surrounded by an Inspection Zone (as shown in Figure 1).

25. The Inspection Zone must be surrounded by an Isolation Zone (as shown in Figure 1).

26. The GMOs must not be grown in a Planting Area if any crop of wheat and barley or a Related Species is present in the Inspection Zone or Isolation Zone.

27. While the GMOs are growing in a Planting Area, associated areas must be inspected by people trained to recognise wheat and barley and Related Species, and actions must be taken as follows:

Area	Period of inspection	Inspection frequency	Inspect for	Action
Monitoring Zone and Inspection Zone	**From** 14 days prior to the expected commencement of Flowering of any GMOs* **until** 14 days after all GMOs in the Planting Area have finished Flowering	At least once every 14 days	Volunteers & Related Species	Destroy before Flowering or prevent from Flowering

**Condition 51(a) requires the licence holder to provide information to the Regulator on the expected Flowering period, however the inspection period should be based on the observed development of the GMOs, so that inspections commence prior to Flowering of any GMOs.*

Note: Details of any inspection activity must be recorded in a Logbook (Condition 52) and reported to the Regulator (Condition 51).

Conditions to restrict seed dispersal

28. Equipment used in connection with the GMOs must be Cleaned as soon as practicable after use with the GMOs and before use for any other purpose.

29. Planting Areas must be at least 50 metres away from any permanent natural watercourses or man-made drainage features that flow into natural watercourses.

Note: This includes irrigation channels or storm water drains that flow into a natural watercourse.

30. Planting Areas must not be located in flood prone areas.

31. Measures must be implemented to control rodents within each Planting Area from at least 7 days prior to planting the GMOs, while the GMOs are being grown and until the Planting Area is Cleaned.

Note: Measures for rodent control may include, but are not limited to, traps and/or poison baits within and/or surrounding the Planting Area.

32. The Monitoring Zone must be maintained in a manner that does not attract or harbour rodents while the GMOs are being grown at a Planting Area and until the Planting Area is Cleaned.

Note: Acceptable measures to achieve this include keeping land free of vegetation or keeping vegetation mown to a height of less than 10 centimetres.

33. If GM plants, other than the GMOs authorised by this licence or those that satisfy condition 16(c):

    (a) are grown under another licence within the Site at a time when the GMOs authorised by this licence are also being grown; or

    (b) were planted previously on the Planting area and the Planting Area had yet to be signed off; and

    (c) are sexually compatible with the GMOs authorised by this licence;

then seed produced from the GMOs grown under this licence in the Planting Area must not be used for breeding or propagation to produce cultivars for future commercial release, unless it has been determined that the GM seed only contains the expected genetic modifications.

Conditions relating to harvesting

34. GMOs must be harvested or Destroyed within ten months after planting.

35. If all GMOs in a Planting Area have been Destroyed, then for the purposes of this licence:

    (a) the GMOs are taken to have been harvested; and

    (b) the Planting Area is taken to have been Cleaned.

Note: Cleaning activities must be reported to the Regulator (Condition 51). Areas of land that have been Cleaned are subject to inspections (Condition 40).

36. GMOs must be harvested in a manner that minimises dispersal of GMOs outside the Planting Area.

37. The GMOs must be harvested and threshed separately from any other crop.

38. Harvested GM seed not required for experimentation or future planting must be Destroyed as soon as practicable.

Conditions to restrict persistence of GMOs on trial sites

39. Areas of land used in connection with the GMOs must be Cleaned as follows:

Areas of land to be Cleaned	**When**
Planting Area	Within 14 days after harvest of the GMOs
Any area, outside a Planting Area, used to Clean any Equipment used in connection with the GMOs	As soon as practicable
Any area, outside a Planting Area, where GMOs have dispersed, e.g. during planting, growing, harvesting or Destruction	As soon as practicable

Note: Cleaning activities must be reported to the Regulator (Condition 51). Areas of land that have been Cleaned are subject to inspections (Condition 40).

40. After Cleaning, areas of land must be inspected by people trained to recognise wheat and barley. Inspections must cover the entirety of areas to be inspected. Actions must be taken as follows:

Area	**Period of inspection**	**Inspection frequency**	**Inspect for**	**Action**
Planting Area and other areas of land that were Cleaned in accordance with Condition 39.	From the day of Cleaning until: i. the area is planted as a new Planting Area in accordance with Condition 16; or ii. the Regulator has issued a Sign off for the area.	At least once every 35 days	Volunteers	Destroy before Flowering

Note: Details of any inspection activity must be recorded in a Logbook (Condition 52) and reported to the Regulator (Condition 51).

41. While post-Cleaning inspection requirements apply to an area:

(a) the area must be maintained in a manner appropriate to allow identification of Volunteers; and

(b) no plants may intentionally be grown in the area unless:

i. the area is planted as a new Planting Area in accordance with condition 16; or

ii. the plants are planted in accordance with condition 23; or

iii. the plants are agreed to in writing by the Regulator; and

(c) the area must not be used for grazing livestock; and

(d) prior to an application for Sign off, the area must receive at least three watering events as described in **Attachment B**, at intervals of at least 28 days, with the final required watering event occurring within the six months prior to submission of the Sign off application; and

(e) within the six months prior to submission of the Sign off application, and before the final required watering event, the area must be Tilled.

Tillage

42. Any Tillage of the Planting Area must be to a depth no greater than five centimetres.

Destruction by burial

43. If Destruction of GMOs occurs by burial:

(a) the GMOs must be buried in a pit and covered by a layer of soil at least one metre in depth, the top of which is no higher than the surrounding soil surface; and

(b) seeds must be wet when buried to encourage decomposition; and

(c) the licence holder must take measures to ensure that the burial site is not disturbed for a period of at least 12 months from the date of burial.

Note: GMOs may be added to the pit over a few weeks prior to covering the pit with a layer of soil, provided measures are taken to ensure that the GMOs do not disperse from the burial pit during this time. If GMOs are dispersed on the soil surface during the process of burial, the burial site becomes an area of land that requires Cleaning under Condition 39 and is subject to post-Cleaning requirements.

Note: The date and location of burial, and measures used to ensure that the burial site is not disturbed, must be reported to the Regulator (Condition 51(f)).

Processing or experimentation with the GMOs

44. Treatment, threshing or processing of GM seed, or experimentation or analysis with the GMOs may only be undertaken within:

(a) a Planting Area before Cleaning; or

(b) a facility approved in writing by the Regulator.

Note: This condition does not apply to dealings conducted as an NLRD (see Condition 9).

45. Within a facility approved in writing by the Regulator in accordance with Condition 44, any area that is used for treatment, threshing, processing, experimentation or analysis of the GMOs must be Cleaned as soon as practicable and before use for any other purpose.

Transport or storage of the GMOs

46. Transport or storage of the GMOs must:

(a) only occur to the extent necessary to conduct the dealings permitted by this licence or other valid authorisation under the Act, or to the extent necessary to enable export of the GMOs; and

(b) be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* for PC2 GM plants as current at the time of transportation or storage; and

(c) comply with all other conditions of this licence.

Note: Activities with the GMOs within a Planting Area prior to Cleaning are not regarded as transport or storage.

Note: Condition 13 requires signed statements for persons transporting the GMOs.

Note: This condition does not apply to dealings conducted as an NLRD (see Condition 9).

47. Methods and procedures used to transport GMOs must be recorded, and must be provided to the Regulator, if requested.

Note: The Contingency Plan must be implemented if the GMOs are detected outside areas under inspection (Condition 48).

Contingency plan

48. If any unintentional presence of the GMOs is detected outside the areas requiring Cleaning, the Contingency Plan must be implemented.

Section 4 Sign off

49. The licence holder may make written application to the Regulator that planting restrictions and inspection requirements no longer apply to the Planting Area and other areas requiring Cleaning if:

(a) post-Cleaning inspection activities have been conducted for at least 24 months on the area; and

(b) conditions have been conducive for germination and detection of Volunteers; and

(c) no Volunteers have been detected in the area during the six months prior to the Sign off request.

Note: An area requires Tillage and three watering events prior to a Sign off application (Condition 41).

Note: The Regulator will take into account the management and inspection history for the Planting Area and other areas requiring Cleaning, including post-harvest crops planted (if any), Tillage, irrigation, rainfall, application of herbicide and occurrence of Volunteers, in deciding whether or not further inspections are required to manage persistence of the GMOs.

Section 5 Reporting and documentation

The following licence conditions are imposed to demonstrate compliance with other conditions and facilitate monitoring of compliance by staff of the OGTR.

50. General notifications must be sent to the Regulator as follows:

Note: Please send all correspondence related to the licence to OGTR.M&C@health.gov.au.

Notice	Content of notice	Timeframe
a. Changes to contact details	Changes to any of the contact details of the project supervisor that were notified in the licence application or subsequently	As soon as practicable
b. Ongoing suitability to hold a licence	i. any relevant conviction of the licence holder; or ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; or iii. any event or circumstances that would affect the capacity of the licence holder to meet the conditions of the licence; and	As soon as practicable after any of these events occur

	iv. any information related to the licence holder's ongoing suitability to hold a licence, that is requested by the Regulator	Within the timeframe stipulated by the Regulator
c. People covered by the licence	i. names of all organisations and persons, or functions or positions of the persons, who will be covered by the licence, with a description of their responsibilities; and *Note: Examples of functions or positions are 'project supervisor', 'site manager', 'farm labourer' etc.* ii. detail of how the persons covered by the licence will be informed of licence conditions	At least 14 days prior to conducting any dealings with the GMOs (to be updated within 14 days if the notified details change)
d. Testing methodology	A written methodology to reliably detect the genetic modifications described in this licence. The detection method/s must be capable of identifying each GM wheat and barley line planted under this licence	At least 14 days prior to conducting any dealings with the GMOs (to be updated within 14 days if the notified details change)
e. Contingency plan	A Contingency Plan to respond to inadvertent presence of the GMOs outside an area that must be inspected	At least 14 days prior to conducting any dealings with the GMOs (to be updated within 14 days if the notified details change)
f. Training records	Copies of the signed and dated statements referred to in condition 13 if requested by the Regulator	Within the timeframe stipulated by the Regulator
g. Additional information required by the Act	i. additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or ii. any contraventions of the licence by a person covered by the licence; or iii. any unintended effects of the dealings authorised by the licence *Note: The Act requires, for the purposes of the condition 50(g), that:* • *the licence holder will be taken to have become aware of additional information of a kind mentioned in Condition 50(g) if he or she was reckless as to whether such information existed; and* • *the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in Condition 50(g), if he or she was reckless as to whether such contraventions had occurred, or such unintended effects existed* *Note: Contraventions of the licence may occur through the action or inaction of a person.*	Without delay after becoming aware of any new information *Note: An example of notification without delay is contact made within a day of a contravention of the licence via the OGTR free call phone number 1800 181 030. Notification without delay will allow the OGTR to conduct a risk assessment on the incident and attend the location, if required*
h. Further details regarding additional information	Any further details requested by the Regulator in relation to information provided under condition 50(g)	Within the timeframe stipulated by the Regulator

51. Notifications relating to each trial site must be sent to the Regulator as follows:

Note: please send all correspondence related to the licence to OGTR.M&C@health.gov.au.

Notice	Content of notice	Timeframe
a. Intention to plant	i. Details of the Planting Area including size, the local government area, GPS coordinates, a street address, a diagrammatical representation of the Site (e.g. Google Maps) and any other descriptions ii. Detail of how the licence holder will access and control the Planting Area and the associated Monitoring Zone, Inspection Zone and Isolation Zone, in accordance with condition 11 *Note: this should include a description of any contracts, agreements, or other enforceable arrangements.* iii. Identity of the GMOs to be planted at the Planting Area (e.g. lines or construct details) iv. Date on which the GMOs will be planted v. Period when the GMOs are expected to Flower vi. Period when harvesting is expected to commence vii. How all areas requiring post-Cleaning inspections are intended to be used until Sign off, including proposed post-harvest crops (if any) viii. Details of how inspection activities will be managed, including strategies for the detection and Destruction of Volunteers ix. History of how the Site has been used for the previous two years	At least 7 days prior to each planting (to be updated as soon as practicable if the notified details change)
b. Planting	i. Actual date(s) of planting the GMOs ii. Any changes to the details provided under part (a) of this condition	Within 7 days of any planting
c. Extreme Weather	Any Extreme Weather event that is expected to affect or has already affected an area where the GMOs are or may be present. *Note: The Contingency Plan must be implemented if the GMOs are detected outside areas requiring Cleaning (Condition 48).*	As soon as practicable
d. Harvest	Actual date(s) of harvesting the GMOs	Within 7 days of commencement of any harvesting
e. Cleaning	i. Date(s) on which required Cleaning was performed on any areas of land ii. Method(s) of Cleaning	Within 7 days of completion of Cleaning
f. Destruction by burial	Date of burial, location of burial including GPS co-ordinates, and details of measures used to ensure that the burial site will not be disturbed for the period required by Condition 43.	Within 7 days of burial of any GMOs
g. Inspection activities	Information recorded in a Logbook as per the inspection requirements (Conditions 27, 40 and 52).	Within 35 days of inspection

Note: Additional records must be provided to the Regulator, if requested, in accordance with condition 47.

52. Details of any inspection activity must be recorded in a Logbook and must include:

(a) date of the inspections; and

(b) name of the person(s) conducting the inspections; and

(c) details of the experience, training or qualification that enables the person(s) to recognise wheat and barley and/or Related Species, if not already recorded in the Logbook; and

(d) details of areas inspected including current land use (including any post-harvest crops) and recent management practices applied; and

Note: management practices include Tillage events, spraying or maintenance measures used to facilitate inspections.

(e) details of the developmental stage of the GMOs while they are being grown; and

(f) details of any post-Cleaning rainfall events including measurements at or near the area, or any irrigation events; and

(g) details of any Volunteers and/or Related Species observed during inspections or during land-management activities, including number, developmental stage and approximate position of the Volunteers and/or Related Species within each area inspected†; and

(h) date(s) and method(s) of Destruction of or preventing Flowering of any Volunteers and/or Related Species, including destruction of Volunteers and/or Related Species during land-management activities; and

(i) details of rodent control methods used and any evidence of rodent activity, while rodent control methods are required.

† Examples of acceptable ways to record the positional information for Volunteers and/or Related Species in the Logbook include:
- descriptive text
- marking on a diagram
- indicating grid references on a corresponding map/sketch.

Note: Details of inspection activities must be provided to the Regulator (Condition 51). The Regulator has developed a standardised proforma for recording inspection activities. This can be made available on request.

ATTACHMENT A

DIR No: 201

Full Title: Limited and controlled release of wheat and barley genetically modified for yield enhancement

Organisation Details

Postal address: The University of Adelaide

SA 5005

Phone No: (08) 8313 4455

IBC Details

IBC Name: The University of Adelaide Institutional Biosafety Committee

GMO Description

GMOs covered by this licence

Wheat and Barley plants genetically modified by introduction or knockout of only the genes and genetic elements listed below.

Parent Organism

Common Name: Wheat and Barley

Scientific Name: *Triticum aestivum* L. and *Hordeum vulgare* L.

Modified traits

Category: Yield enhancement

Abiotic stress tolerance

Altered plant morphology

Selectable markers – antibiotic resistance, herbicide tolerance, visual marker

Description: The licence holder is authorised to release up to 103 lines of wheat and barley genetically modified for yield enhancement.

Altered traits, parent organism, and the type of genetic modification are listed in Table 1. Genes that have been introduced or knocked-out in the GM wheat and barley are listed in Table 2. The introduced regulatory sequences permitted in the GM wheat and barley are listed in Table 3.

Group 1 GM wheat lines may be crossed with other Group 1 GM wheat lines.

Purpose of the dealings with the GMO

The purpose of the release is to evaluate the agronomic performance of the GM wheat and barley under Australian field conditions. The GM Wheat and barley is not permitted to be used for human food or animal feed.

Table 1. Groups of introduced or knockout genes in the GM wheat and barley

Group	Altered trait	Parent organism	Type of genetic modification
1	Direct yield enhancement	Wheat	Gene introduction
2	Yield enhancement via water use efficiency	Wheat	Gene introduction
3	Yield enhancement via altered spikelet development and flowering time	Wheat	Gene knockout
4	Yield enhancement via altered plant architecture and nutrient use efficiency	Barley	Gene knockout

Table 2. List of introduced or knocked out genes in the GM wheat and barley

Group	Element	Source organism	Function
1	*AtAVP1*	*Arabidopsis thaliana*	Increased shoot and root biomass, photosynthetic capacity, yield and nutrient use efficiency; increased salinity tolerance
	OsNas2	*O. sativa*	Increase in shoot biomass, higher numbers of tillers and grain
	OsPSTOL1	*O. sativa*	Enhanced growth vigour and earlier heading, high yield
2	*TaMUTE*	*T. aestivum*	Stomatal development, symmetrical division of guard mother cells
	TaYDA1	*T. aestivum*	Negatively regulates stomatal development
	TaYDA2	*T. aestivum*	Negatively regulates stomatal development
	TaOST1	*T. aestivum*	Regulates stomatal aperture
	TaSLAC1	*T. aestivum*	Guard cell anion channel
3	*ALOG-1*	*T. aestivum*	Spikelet development and flowering time
	PDB-1	*T. aestivum*	Spikelet development and flowering time
4	*HvLBO*	*H. vulgare*	Strigolactone biosynthesis
	HvMAX1a	*H. vulgare*	Strigolactone biosynthesis
	HvMAX1b	*H. vulgare*	Strigolactone biosynthesis
	HvMAX1c	*H. vulgare*	Strigolactone biosynthesis
	HvMAX1d	*H. vulgare*	Strigolactone biosynthesis
	HvMAX1e	*H. vulgare*	Strigolactone biosynthesis
	HvD53a	*H. vulgare*	Strigolactone signalling

	HvD53b	*H. vulgare*	Strigolactone signalling
Marker	*hptII*	*Escherichia coli*	Hygromycin resistance gene encoding hygromycin phosphotransferase
	nptII	*E. coli K12*	Neomycin phosphotransferase gene for resistance against geneticin or kanamycin
	bar	*Streptomyces hygroscopicus*	Bialaphos resistance gene encoding phosphinothricin N-acetyltransferase (PAT) protein that confers tolerance to glufosinate
	pporRFP	*Porites porites*	Red fluorescent protein
CRISPR/Cas9 genetic element (Group 3 and 4 GMOs)	*Cas9*	*Streptococcus pyogenes*	RNA-guided nuclease
Single guide RNA (Group 3 and 4 GMOs)	sgRNA	*T. aestivum* *H. vulgare*	RNA-guide for genes in Group 3 and 4

Table 3. Introduced regulatory sequences in the GM wheat and barley

Element function	Genetic element	Source organism
Constitutive promoter	*CaMV35S* *OsUbi* *OsAct1* *PvUbi1+3*	Cauliflower mosaic virus *Zea mays* *O. sativa* *Panicum virgatum*
RNA promoter	*TaU6a* *OsU6a* *OsU6b* *OsU6c* *OsU3*	*T. aestivum* *O. sativa* *O. sativa* *O. sativa* *O. sativa*
Amplification promoting sequence	*Ubi1 Intron* *Ubi 5' UTR*	*Z. mays* *Z. mays*
Guide RNA scaffold		*S. pyogenes*
Termination sequence	*CaMV35S* *nos*	Cauliflower mosaic virus *Agrobacterium tumefaciens*

ATTACHMENT B

A watering event is irrigation or natural rainfall that provides sufficient soil moisture to promote germination of wheat and barley seeds on a trial site.

Examples of acceptable watering events are:

- At least 26 millimetres of rainfall over one day; or
- At least 28 millimetres of rainfall over two days; or
- At least 30 millimetres of rainfall over three days; or
- At least 32 millimetres of rainfall over four days; or
- Irrigation that provides equivalent levels of soil moisture to one of the examples of rainfall above.

Rainfall measurements must be taken on the site or within 3 km of the site. An irrigation or natural rainfall that matches one of the examples listed above, and occurs during the time period specified for a watering event in Condition 41 of the licence, is considered a valid watering event. The licence holder should keep records of the date/s and amount of water applied during the watering event, and provide this information when requesting Sign off of the relevant site.

If an irrigation or natural rainfall does not match one of the examples listed above, the licence holder may submit a request to the Regulator for it to be considered a watering event. The request should provide:

- evidence of amount of water applied, such as rainfall measurements on the site or within 3 km of the site, and
- evidence that resultant soil moisture is suitable for germination, such as photos of germinating plants on the site.

It is recommended that any requests that an irrigation or natural rainfall be considered a watering event be submitted at the time of the event, to minimise potential delays to Sign off of the site.

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