



Australian Government

Department of Health and Ageing

Office of the Gene Technology Regulator

APPLICATION FOR LICENCE FOR INTENTIONAL RELEASE OF GMOs INTO THE ENVIRONMENT: Application No. DIR 077/2007

SUMMARY INFORMATION

Project Title:	Limited and controlled release of wheat and barley genetically modified for enhanced tolerance to abiotic stresses or increased beta glucan. ¹
Applicant:	The University of Adelaide
Common name of the parent organisms:	Wheat and barley
Scientific name of the parent organisms:	<i>Triticum aestivum</i> L., <i>Hordeum vulgare</i> L.
Modified trait(s):	Enhanced tolerance to abiotic stressors, including soil boron and drought, and increased beta glucan levels.
Identity of the gene(s) responsible for the modified trait(s):	<ul style="list-style-type: none">• three <i>cellulose synthase-like F</i> genes from barley; <i>HvCslF4</i>, <i>HvCslF6</i> and <i>HvCslF8</i> (increased beta glucan levels)• two drought responsive transcription factors from wheat; <i>TaDREB2</i> and <i>TaDREB3</i> (drought tolerance)• a boron tolerance gene from barley; <i>Bot1</i> (soil boron tolerance)• a transcription factor isolated from wheat (abiotic stress tolerance)²• <i>hpt</i> gene (hygromycin resistance marker)• <i>sacB</i> gene (selectable marker)
Proposed Location(s)	One site in the Marion local council (Adelaide), South Australia
Proposed Release Size:	0.04 ha
Proposed Release Dates:	May 2008 to June 2009

Introduction

The *Gene Technology Act 2000* (the Act) in conjunction with the *Gene Technology Regulations 2001*, an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, comprise Australia's nationally consistent regulatory system for gene technology. Its objective is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs).

The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), an Australian Government regulatory agency located within the Health and Ageing portfolio.

¹ The title of the licence application submitted by the University of Adelaide is *Intentional release of modified wheat and barley*.

² Some details of the genetic modifications have been declared CCI under section 185 of the Act.

The legislation sets out the requirements for considering applications for licences for dealings with GMOs and the matters that the Regulator must take into account before deciding whether, or not, to issue a licence. The Regulator's *Risk Analysis Framework*³ outlines the assessment process that will be followed.

The application and the proposed dealings

The Regulator has received a licence application from The University of Adelaide for a licence for dealings involving the intentional release of genetically modified (GM) wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) into the Australian environment on a limited scale under controlled conditions.

Up to thirty GM wheat and barley lines⁴ are proposed for release. They contain genes derived from wheat and barley that are expected to enhance tolerance to abiotic stressors⁵ including soil boron and drought, or to increase the level of beta glucan⁶ in the grain and leaves. The purpose of the proposed release is to conduct proof of concept experiments to assess the agronomic performance of the lines under field conditions, and to obtain tissue samples for subsequent analysis of characteristics such as gene and protein expression levels and metabolite profiles. Some seed will be saved for possible future trials of promising lines, subsequent to further approval(s). The GM wheat and barley plants will not be used for human food or animal feed.

The applicant proposes to limit the release to one site in the shire of Marion, South Australia (SA) on a total area of 0.04 ha between May 2008 and June 2009.

The applicant has also proposed a number of controls to restrict the dissemination or persistence of the GM plants and their introduced genetic material, that will be considered in the assessment of this application, including:

- locating the proposed trial site over 1000 m from the closest wheat or barley breeding sites and 50 km from the closest commercial wheat or barley crop
- surrounding the 0.04 ha trial site with a 1 metre border of non GM barley, a 5 metre plant free zone and a 200 m isolation zone free of wheat and barley plants
- surrounding the site with a 1 m high, 50 m by 50 m, cyclone fence and placing rodent baits within the fence
- keeping a 10 m zone surrounding the 1 m high cyclone fence and the remaining area within the fence (apart from the 0.04 ha trial site) mown to below 10 cm
- hand harvest plants to minimise GM seed spillage
- analysing GM plant materials from the trial in a certified PC2 facility and then destroying the materials (except saved seed)
- destroying all (GM and non-GM) plant materials from the field trial by ploughing, irrigating the site one month after harvest and killing any volunteers with herbicide
- post harvest monitoring of the trial site for 24 months or until the site has been clear of volunteers from one growing season and destroying any volunteers

³ Available on the Office of the Gene Technology Regulator (OGTR) website at <<http://www.ogtr.gov.au/pubform/riskassessments.htm>>. Information on the assessment of licence applications is also available at <<http://www.ogtr.gov.au/ir/process.htm>> or Freecall 1800 181 030.

⁴ The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

⁵ An abiotic stressor is a nonliving factor that causes harmful effects to plants. Examples include drought, salinity, aberrant temperature and chemical pollution.

⁶ Beta glucan is a plant polysaccharide (carbohydrate) which forms part of the soluble fibre in cereal grains.

- transporting GM plant materials to and from the proposed trial site in accordance with OGTR transportation guidelines
- not using the GMO in human food or animal feed

Confidential Commercial Information

Some details, including the name of an introduced gene expected to enhance abiotic stress tolerance, the precise function of the gene product and its application, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information will be made available to the prescribed experts and agencies that will be consulted on the Risk Assessment and Risk Management Plan (RARMP) for this application.

Parent organism

The parent organisms are wheat (*Triticum aestivum* L.) cultivar ‘Bob White’ and barley (*Hordeum vulgare* L.) cultivar ‘Golden Promise’ or ‘Flagship’, which are exotic to Australia. The wheat cultivar ‘Bobwhite’ and the barley cultivar ‘Golden Promise’ are not grown commercially in Australia but are commonly used in genetic modification work because they are relatively easy to transform. The barley cultivar ‘Flagship’ was a new commercial cultivar released in Australia in 2006.

Commercial wheat cultivation occurs in Australia’s wheat belt which extends from south eastern Queensland through New South Wales, Victoria, southern South Australia and southern Western Australia. Commercial barley cultivation in Australia occurs in the wheat production areas in New South Wales, Victoria, Queensland, Western Australia and South Australia. A small amount of barley is also grown in Tasmania.

The genetic modifications and their effect

Thirty lines of GM wheat and barley are proposed for release. The lines contain one of seven genes encoding proteins expected to enhance their tolerance to different abiotic stressors, such as soil boron or drought, or to increase beta glucan levels. The introduced genes are derived from either wheat or barley and include:

- Two drought responsive transcription factors (*TaDREB2* and *TaDREB3*) from wheat that have been introduced into both wheat (cv. Bob White) and barley (cv. Golden Promise). Plants from these lines are expected to have enhanced drought tolerance;
- A boron tolerance gene (*Bot1*) from barley that has been introduced into barley (cv. Flagship). Plants from these lines are expected to display enhanced soil boron tolerance;
- An abiotic stress tolerance transcription factor isolated from wheat that has been introduced into barley (cv. Golden Promise);
- Three cellulose synthase-like F genes (*HvCslF4*, *HvCslF6* and *HvCslF8*) isolated from barley that have been introduced into barley (cv. Golden Promise). In glass house trials, plants from these lines the plants have increased levels of beta glucan in the leaves and grain.

The expression of six of the seven genes is regulated by a constitutive promoter. The expression of the seventh gene is regulated by the promoter endogenous to the introduced gene⁷.

The GM wheat and barley lines also contain an antibiotic resistance marker gene, *hpt*, from the bacterium, *Escherichia coli*. The *hpt* gene encodes hygromycin phosphotransferase which was used to select for modified plants in the laboratory. Hygromycin will not be applied to the plants during the proposed field trial. While the *hpt* gene is derived from a bacterium capable of causing illness in

⁷ A promoter is a short regulatory sequence that controls the level, timing and location of expression of the protein encoded by a gene.

humans the *hpt* gene comprises only a small part of the *E. coli* genome and is not capable of causing disease.

Additionally, three of the GM barley lines contain a selective marker gene, *SacB*, from the bacterium *Bacillus amyloliquefaciens* which encodes an enzyme (levansucrase) involved in sucrose metabolism. This gene was used to select for bacteria containing plasmids with the desired genes in the laboratory, prior to the production of the GM plants. The *SacB* gene is not expected to be expressed in the GM barley lines as its bacterial promoter is prevented from functioning by the insert.

Some of the regulatory sequences that control the expression of the introduced genes in the GM wheat and barley lines are derived from plant pathogens (*Agrobacterium tumefaciens* and Cauliflower mosaic virus). These regulatory sequences comprise only a small part of their total respective genome and are not capable of causing disease.

Method of genetic modification

Biolistic transformation, also known as particle bombardment, was used to produce the GM wheat lines. This technique involves coating the expression cassette containing the gene constructs onto very small gold particles that are 'shot' into wheat embryos. Particle bombardment has been widely used in Australia and overseas for introducing new genes into plants without causing any biosafety problems.

The GM barley lines were produced using *Agrobacterium tumefaciens* mediated transformation. This technique introduces the gene and associated regulatory sequences into immature barley embryos via the vector *A. tumefaciens*. The vector is 'disarmed' since it lacks the genes which encode the tumorigenic functions of *A. tumefaciens*.

Transformed plant tissues were identified using the expression of the marker genes and grown into plants in the laboratory. Molecular analysis was used to confirm the presence of the gene(s) of interest in the individual plants that were selected to produce the GM lines. Each of the 30 GM wheat and barley lines proposed for release is the result of a separate genetic modification event.

Previous releases of the same or similar GMOs

The GM wheat and barley lines proposed for release were developed in Australia have not been previously released.

The Regulator has issued licences for the conduct of three field trials involving other GM wheat lines under limited and controlled conditions: DIR 053/2004 was issued to Grain Biotech for GM salt tolerant wheat on an area of 0.45 ha in Western Australia, DIR 054/2004 was issued to CSIRO for GM wheat with altered starch content on 0.25 ha in the Australian Capital Territory and DIR 071/2006 was issued to Department of Primary Industries – Victoria for GM drought tolerant wheat on 0.315 ha in Victoria.

Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), there have been five field trials of different types of GM wheat ranging in size from 325–1500 plants: PR65 (1996), PR66 (1996), PR102 (1998), PR102X (2000), and PR107 (1999). Five field trials of different types of GM barley also occurred under GMAC. They ranged in size from 400-2940 plants: PR88 (1998), PR92 (1998), PR106 (1998), PR88X (1999) and PR139 (2000).

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

Suitability of Applicant

Section 43(2)(f) of the Act requires the Regulator to be satisfied regarding the suitability of the applicant to hold a licence as a pre-requisite for considering DIR applications. The matters to be considered are outlined in Section 58 of the Act and include relevant convictions, revocation of a

licence or permit relating to the health and safety of people, and capacity to meet the conditions of the licence.

The Regulator has determined that The University of Adelaide currently meets the suitability requirements and will verify this continues to be the case prior to making any decision regarding the issuing of a licence.

Consultation process for this DIR application

The Regulator has made an assessment of whether the application should be considered as a limited and controlled release, in accordance with Section 50A of the Act. As its principal purpose is to enable the conduct of experiments, and the applicant has proposed limits on the size and duration of the release and controls to restrict the dissemination and persistence of both the GMO and its genetic material in the environment, **the Regulator has decided that the application qualifies as a limited and controlled release.**

This means that the Regulator is not required to seek advice on the assessment of this application until after RARMP has been prepared. In the interim, copies of the application are available on request from the OGTR. Please quote application number DIR 077/2007.

The Regulator will seek comment on the consultation RARMP from the public as well as a wide range of experts, agencies and authorities including the Gene Technology Technical Advisory Committee, State and Territory Governments, Australian Government agencies and the Minister for the Environment, Heritage and the Arts and relevant local councils. The RARMP will then be finalised, taking into account matters raised relating to risks to human health and safety and the environment, and form the basis of her decision whether or not to issue a licence.

At this stage, **the RARMP is expected to be released for comment in mid March 2008.** The public will be invited to provide submissions on the RARMP via advertisements in the media and direct mail to anyone registered on the OGTR mailing list. The RARMP and other related documents will be available on the OGTR website, or in hard copy from the OGTR.

If you have any questions about the application or the assessment process, or wish to register on the mailing list, please contact the OGTR at:

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