



8 January 2010

**APPLICATION FOR LICENCE FOR INTENTIONAL RELEASE OF GMOs INTO THE ENVIRONMENT: Application No. DIR 099**

**SUMMARY INFORMATION**

Project Title:	Limited and controlled release of wheat and barley genetically modified for altered grain composition or nutrient utilisation efficiency <sup>1</sup>
Applicant:	CSIRO
Common name of the parent organism:	Wheat and Barley
Scientific name of the parent organism:	<i>Triticum aestivum</i> L. and <i>Hordeum vulgare</i> L.
Modified trait(s):	Altered grain composition, nutrient utilisation efficiency, antibiotic resistance
Identity of the gene(s) responsible for the modified trait(s):	<ul style="list-style-type: none"><li>• a synthetic gene designed to reduce expression of an endogenous gene in wheat (referred to as <i>Starch Metabolic Enzyme B<sup>2</sup></i>; <i>SMEB</i>) (altered grain composition)</li><li>• a metabolic enzyme gene (referred to as <i>Me1</i>) from barley (nutrient utilisation efficiency)</li><li>• <i>nptII</i> gene from the bacterium <i>Escherichia coli</i> (antibiotic resistance)</li><li>• <i>hpt</i> gene from the bacterium <i>Escherichia coli</i> (antibiotic resistance)</li></ul>
Proposed Location(s):	Two sites in the shires of Narrabri (NSW) and Corrigan (WA)
Proposed Release Size:	Up to 2 ha per growing season
Proposed Release Dates:	May 2010 - June 2013

**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 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.

The legislation sets out requirements for considering applications for licences for dealings with GMOs, including matters that the Regulator must take into account before deciding whether or not

---

<sup>1</sup> The title of the licence application submitted by CSIRO is 'Limited and controlled release of wheat and barley with altered grain composition or nutrient utilisation efficiency'.

<sup>2</sup> The precise identity of some genes has been declared as Confidential Commercial Information.

to issue a licence. The Regulator's *Risk Analysis Framework*<sup>3</sup> outlines the assessment process that will be followed.

## The application and the proposed dealings

The Regulator has received an application from CSIRO 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.

Eleven lines<sup>4</sup> of GM wheat and three lines of GM barley are proposed for release. Four of the GM wheat lines contain a gene designed to reduce the expression of a starch metabolic enzyme gene involved in determining grain qualities important for dough making and nutritional purposes. The other seven GM wheat lines and three GM barley lines contain a metabolic enzyme gene derived from barley, which is expected to improve the plants' ability to utilise nutrients from the soil. All of the GM wheat and barley lines contain a selectable marker gene.

The purpose of the trial is to:

- assess whether expression of the genes results in altered growth, biomass and yield characteristics in the GM plants, compared to non-GM wheat and barley plants, when grown under field conditions
- assess the impact of expression of the genes on grain characteristics and evaluate how such changes may affect performance of flour from the GM grain in foods.

The applicant proposes to limit the release to two sites between May 2010 and June 2013. One site is proposed in the shire of Narrabri, New South Wales, and the other site in the shire of Corrigan, Western Australia. Each site will be up to 1 ha in size per growing season.

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

- locating the trial sites at least 1 km away from natural waterways
- restricting animal access by surrounding the trial site in the shire of Corrigan with a fence and lockable gates
- locating the trial sites at least 200 m from all other wheat and barley plantings
- surrounding the GM wheat and barley with a 2 m wide buffer of sexually incompatible species and a 10 m isolation zone in which growth of related species is controlled
- post harvest monitoring of the trial site on a monthly basis for 24 months and destroying any volunteer wheat and/or barley before they flower or set seed
- destroying all plant material from the trial not required for testing or future trials
- transporting and storing of the GMOs in accordance with the Regulator's guidelines
- not allowing the GM plant material or products to be used for human food or animal feed.

---

<sup>3</sup> The *Risk Analysis Framework* guides the risk assessment and risk management process. This document and further information on the assessment of licence applications is available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au>>.

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

## Confidential Commercial Information

Some details, including names and sequences of genes, 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, wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), are exotic to Australia. Commercial wheat and barley cultivation occurs in the wheat belt from south eastern Queensland through New South Wales, Victoria, southern South Australia and southern Western Australia. A small amount of barley is also grown in Tasmania.

The wheat and barley cultivars used to produce the GMOs proposed for release were selected because they are relatively easy to genetically modify. The two wheat cultivars are 'Bobwhite' and 'Frame', and the barley cultivar is 'Golden Promise'. Of these cultivars, only 'Frame' is grown commercially in Australia.

## The genetic modifications and their effect

Four of the GM wheat lines contain an introduced synthetic gene designed to reduce or silence the expression of a specific wheat gene by a mechanism known as RNA interference (RNAi). The gene introduced is under the control of an endosperm-specific promoter and targets a *Starch metabolic enzyme B (SMEB)* gene which, when silenced, alters the composition of grain starch.

Seven of the GM wheat lines and all of the GM barley lines contain an introduced metabolic enzyme gene, referred to as *Me1*. This gene is naturally present in non-GM barley plants. Expression of the introduced gene is under the control of a tissue specific promoter derived from rice. The *Me1* gene has previously been overexpressed in rice and resulted in a phenotype of increased plant biomass and increased yield. A similar effect is expected for overexpression of *Me1* in wheat and barley.

All of the GM wheat lines contain one of two selectable marker genes derived from bacteria: either the *nptII* gene which confers resistance to aminoglycoside antibiotics related to kanamycin and neomycin, or the *hpt* gene which confers resistance to the antibiotic hygromycin. All of the GM barley lines contain the selectable marker gene *hpt*. These were used as selectable markers during early stages of development of the GM plants in the laboratory.

Other short regulatory sequences that contribute to control of expression of the introduced genes are also present in the GM wheat and barley. These are derived from wheat, rice, Cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens* (a common soil bacterium). Although some of these sequences are derived from a plant pathogen (*A. tumefaciens* and CaMV), the regulatory sequences comprise only a small part of the pathogen's total genome, and in themselves have no pathogenic properties.

## Method of genetic modification

Two different biolistic transformation methods were used to generate the GM wheat lines proposed for release. Seven of the lines were generated by transformation of the wheat cultivar Bobwhite 26. This involved 'shooting' wheat embryos with gold particles coated with two plasmids – one carrying the *nptII* selectable marker gene, the other carrying either an RNAi gene (designed to silence the *SMEB* gene) or the *Me1* gene. GM wheat plants were regenerated from the embryos using tissue culture techniques, with selection for the *nptII* marker gene. Molecular analysis was used to confirm presence of the synthetic RNAi gene or *Me1* gene.

Biolistic transformation was also used to introduce the *Me1* gene and the *hpt* selectable marker gene into wheat to generate the remaining four GM wheat lines. A co-bombardment strategy was

used in which a plasmid containing the *hpt* gene and a linearised fragment of DNA containing the *Me1* gene were 'shot' into wheat embryos from Bobwhite 26 or Frame cultivars.

All of the GM barley lines proposed for release were generated by the transformation of the barley cultivar Golden Promise using *Agrobacterium*. The *Me1* and *hpt* genes were introduced on a plasmid vector carried by *Agrobacterium tumefaciens*. The vector is 'disarmed' since it lacks the genes that encode the tumorigenic functions of *A. tumefaciens*.

### **Previous releases of the same or similar GMOs**

The GMOs have previously been released in Australia, on a limited scale under controlled conditions, under the licences for DIR 092 and DIR 094.

### **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 capacity to meet the conditions of a licence, relevant convictions and revocation of a licence or permit held under law relating to the health and safety of people.

The Regulator has determined that CSIRO 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, under 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 GMOs and their 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 consult on the assessment of this application until after a RARMP has been prepared in accordance with section 51 of the Act. In the interim, copies of the application are available on request from the OGTR. Please quote application number DIR 099.

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. 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 his decision whether or not to issue a licence.

At this stage, **the RARMP is expected to be released for comment in March 2010.** 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:

**The Office of the Gene Technology Regulator, MDP 54 GPO Box 9848 Canberra ACT 2601**

**Telephone: 1800 181 030 Facsimile: 02 6271 4202 E-mail: [ogtr@health.gov.au](mailto:ogtr@health.gov.au)**

**Website <http://www.ogtr.gov.au>**