



Murdoch University Submission

on

The Technical Review of the Gene Technology Regulations 2001 discussion paper:

Options for regulating new technologies October 2016

by the

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Introduction

Murdoch University hosts the Western Australian State Agricultural Biotechnology Centre (SABC), which is the major centre for agricultural biotechnology in the State. (Murdoch is the only university in Australia to have achieved the top ranking of 5 in the Excellence in Research Australia's last two ranking exercises - ERA 2012, ERA2015).

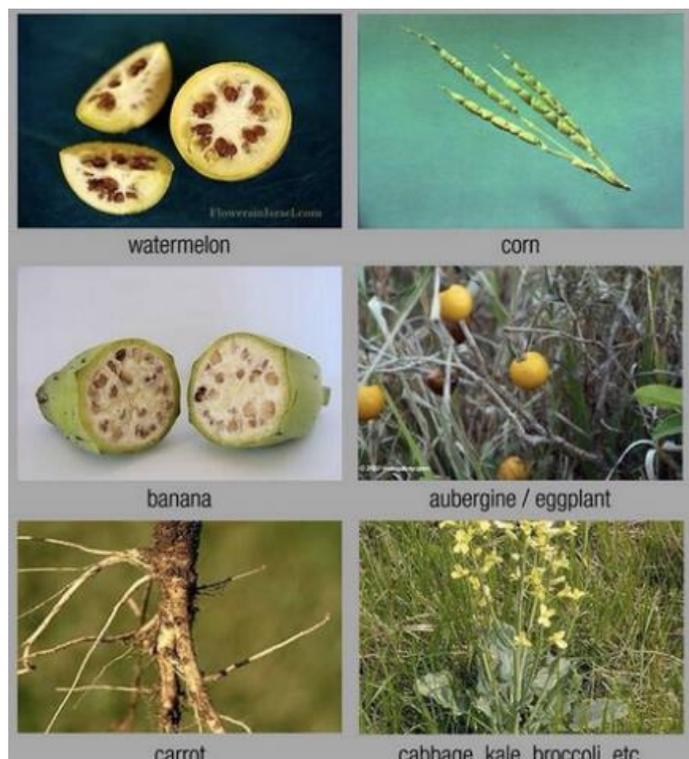
Researchers at the SABC routinely undertake R&D under OGTR Regulations in the areas of crop improvement, pest and disease control, crop biosecurity and other fields of research. These activities include cutting edge 'New Breeding Technologies', such as Gene Silencing Technology (RNAi), and Genome Editing.

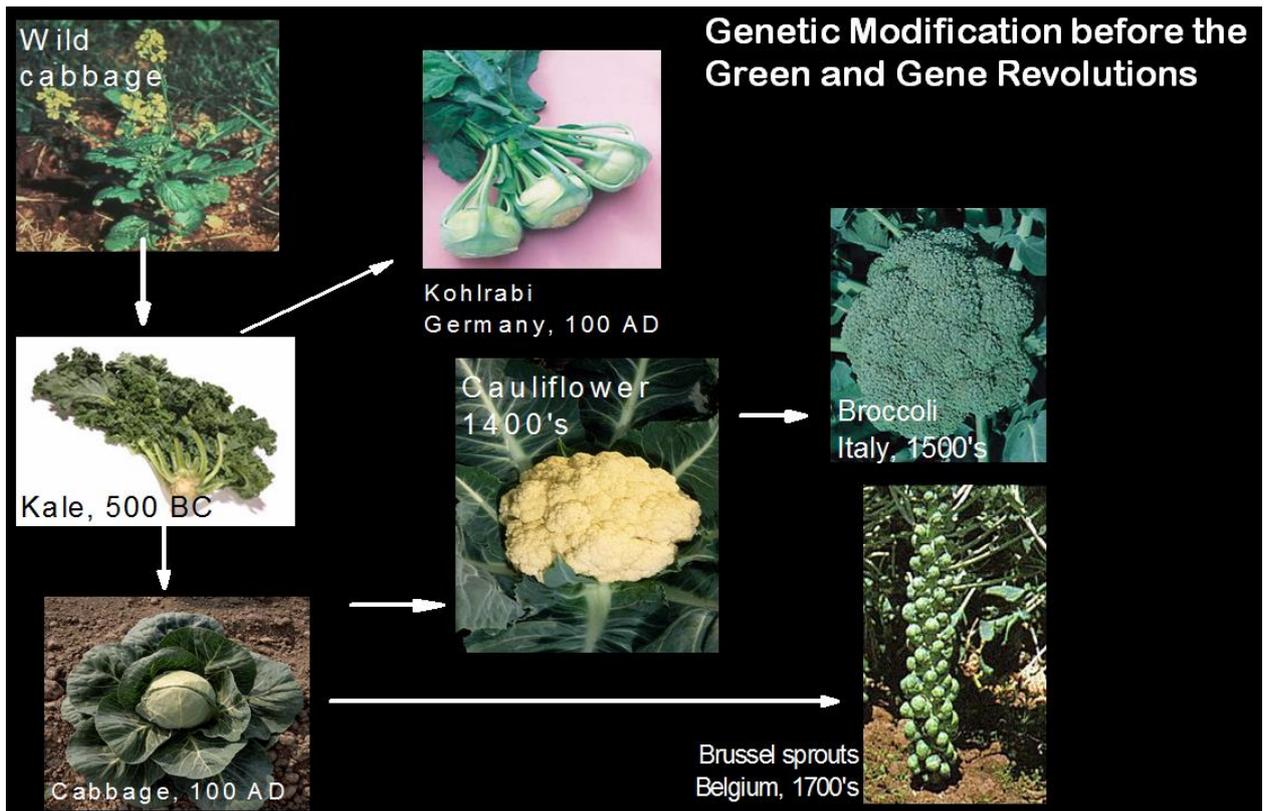
The general view held by researchers at the SABC is that future food security requires two things: the application and dissemination of the best science and technology, and the implementation of sensible, evidence-based government policies that will enable additional food to be grown on currently cropped land.

The university is therefore please to be able to make this submission to help ensure that the OGTR makes its decisions based on sensible, evidence-based policies, and not on a political basis, or as a result of pressure groups with agendas that are not evidence-based.

Current status of GM crops

All the food we eat (with the exception of 'bush tucker') has been genetically modified over time, first by early farmers then more systematically using Mendelian Genetics and then transgenic technologies: the following two figures illustrate what progenitors of some crop plants looked like:

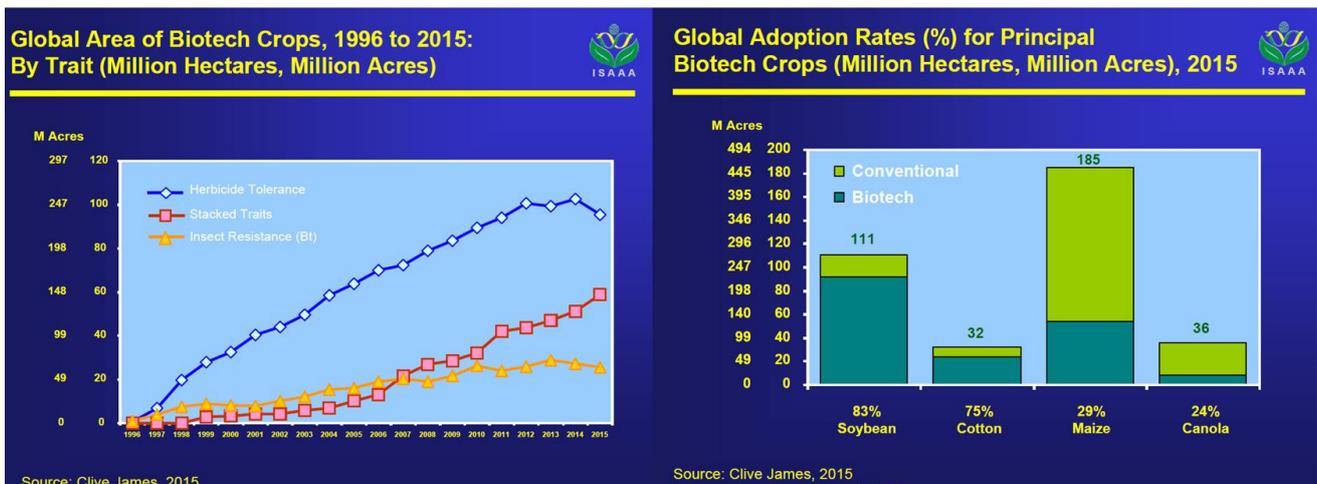




(Original source: Dr Bruce Chassey)

These figures support the view that genetic modification of crop plants *per se* is not new, and the human diet has varied during human evolution, indeed it has contributed to and enabled the human race to increase in numbers and prosper.

The current status of GM crops grown worldwide (now 10% of total; world crops) is provided below, and data from these crops now provides an extensive record of safe use for humans as a variety of foodstuffs, in clothing and the environment:



(Source: ISAAA)

Aims of the OGTR review

It is noted that the primary aim of the review is to provide clarity about whether organisms developed using a range of new breeding technologies (NBTs) are subject to regulation as genetically modified organisms (GMOs), to ensure that new technologies are regulated in a manner commensurate with the risks they pose, and that it will focus on new technologies and examine:

- cases where the capture or exclusion of these techniques is not clear, and whether those new technologies should be regulated, and
- scientific evidence relating to risks posed as a result of using new technologies.

We note that OGTR is seeks views the following options:

Option 1: no amendment to the GT Regulations

Option 2: regulate certain new technologies

Option 3: regulate some new technologies based on the process used

Option 4: exclude certain new technologies from regulation on the basis of the outcomes they produce

Which Options do you support, and why?

Of these Options, **Option 1** fails to capture the new breeding technologies and would continue to put the Australian agricultural industry at a disadvantage on the world stage. The science and technology has advanced since the GT regulations were originally enacted.

Option 2 would create an entirely new class of GMOs, for example for targeted mutagenesis. This would create additional uncertainties and is not warranted. It would result in a level of regulation not commensurate with risks posed by the technology. Regulations should be based on good science, taking into account the relative degree of risk posed to human health or the environment, and not simply by the process used to develop the improved product.

Option 3 would continue the process-based approach to regulation of GMOs, which is a major source of contention, because it is at odds with how the types of genetic manipulation (ie plant breeding) which are excluded from the regulations are judged. In any event, it is the end product and not the process used that should be regulated. The current situation presents a scientific paradox – the less that is known about how genetic modification occurs (eg by conventional breeding, mutagenesis, cytogenetic transfer of large tracts of sequence from one species to another, fusion of complete genomes of different cells) the less it is regulated – conversely, the more that is known about genes that underlie traits, and the more precise the technique of genetic modification used, the more it is regulated. Common sense would suggest that these levels of

regulatory control (in terms of risk assessment to human health or the environment) should be the reverse! The lack or regulation of accepted breeding technologies is mainly based on a history of safe usage: now, after 20 years of GM crops, with about 10% of all world crops currently classified as GMOs, a similar history of safe usage has been demonstrated, and this history of safe usage of GMOs should be reflected in an easing of the regulatory burden as appropriate.

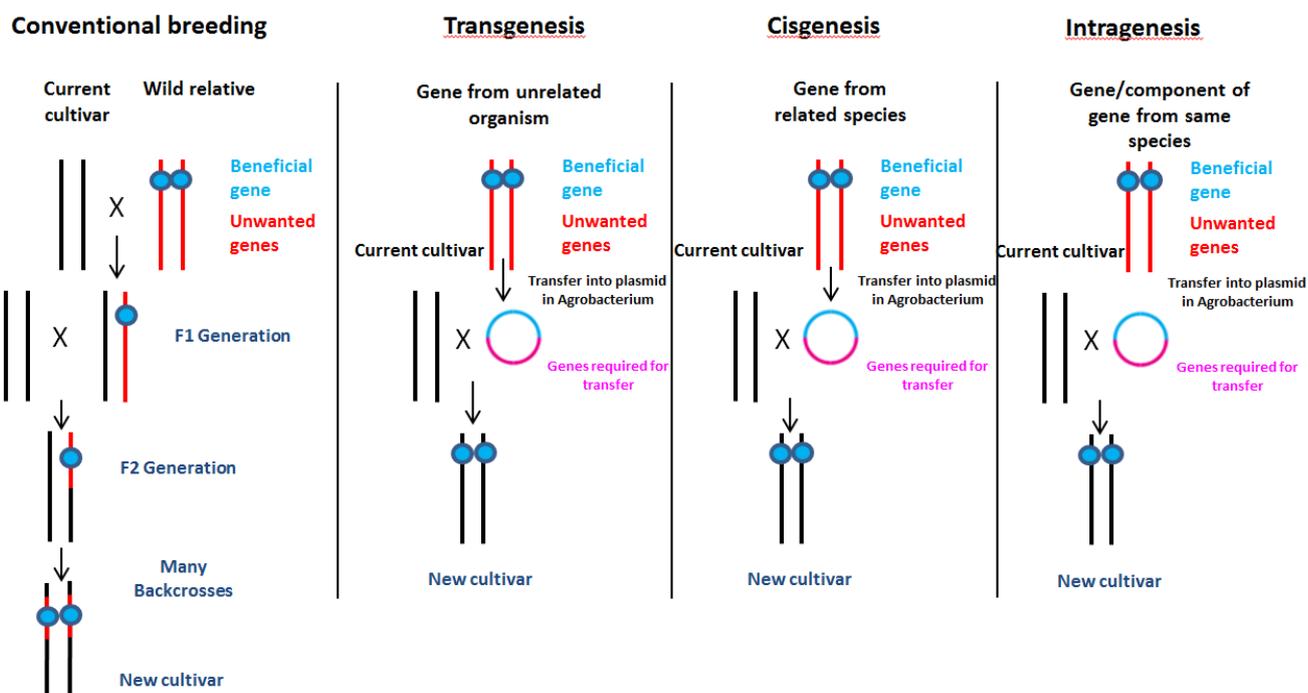
Favoured option: Option 4

Given that the review is restricted to a review of the Gene Technology Regulations 2001, of the Options above, **Murdoch University clearly supports Option 4**: that OGTR should exclude certain new technologies from regulation, on the basis of the outcomes they produce.

Option 4 proposes to exclude organisms from regulation as GMOs if the genetic changes they carry are similar to or indistinguishable from the products of conventional breeding (eg chemical and radiation mutagenesis methods, natural mutations, the natural variations present in nucleic acid sequences between genotypes, **or conventional breeding technologies**, including wide crosses, selection of genotypes with particular properties, cytogenetic transfer of blocks of genes between species, and genome wide screening of progeny to find desired genotypes after crossing).

Plant breeding comparisons

The following diagram provides a comparison of breeding technologies, comparing a simplified conventional plant breeding protocol, with transgenesis (gene from an unrelated organism), cisgenesis (gene from a related or sexually compatible species) and intragenesis (gene or gene component for the same species).



(Source: unpublished, Murdoch University, extended from original from the University of Reading)

It can be seen that there is no difference in the mechanism of transgenesis, cisgenesis and intragenesis, the differences lie in the source of the introduced genetic material. In cisgenesis and intragenesis the transferred genes or parts thereof already exist in the gene pool for that genus or species, and could be introgressed in evolutionary time or by conventional breeding. What these approaches do is to widen the gene pool available for conventional breeding, akin to making crosses from wild relatives or land races in conventional breeding. The benefit of the gene transfer approach is its precision, which enables exclusion of unwanted sequences of unknown and possibly undesirable function, and so could well be regarded as less risky than conventional breeding using wide crosses.

Categories of 'New Breeding Technologies' - Oligonucleotide-Directed Mutagenesis (ODM) and Site –Directed Nucleases (SDN)

CropLife International (CropLife International aisbl, 326 avenue Louise, Box 35, B-1050 Brussels Belgium, 'Technical Summaries of New Plant Breeding Techniques-NBTs June 2014') has defined a series of types of New Breeding Technologies. They emphasise that classical mutagenic approaches (chemical/radiation) have been used for many years to develop a range of crop varieties (FAO/IAEA mutant variety database at <http://mvgs.iaea.org/AboutMutantVarities.aspx>). Classical mutagenesis generates mutations at random in many sites in plants, from which undesirable genotypes are excluded and plants with desired characteristics may be selected. Plants selected in this way are grown widely, and include seedless oranges and ruby red grapefruit.

ODM makes use of a specific oligonucleotide to produce a single DNA base change in the plant genome. It contains no introduced DNA.

SDNs make use of specific dsDNAses (eg *Fok1*) and peptides (eg ZFNs, TALENs) or more recently oligonucleotides (eg CRISPR/Cas9) that guide DNA cleavage at exact sites in the host DNA, and therefore can generate site specific mutagenesis, in a much more precise way than the random breaks cause by classical mutagenesis. This is because the natural process of DNA repair makes mistakes in repairing dsDNA breaks. There are variants of SDN technology, in which in the presence of oligonucleotides with ends homologous to each side of the dsDNA break, one or more bases may be inserted at the repair site. These are sub-classified as:

SDN-1 – non-homologous end joining (NHEJ), in which natural repair mechanisms can result in small nucleotide deletions, additions or substitutions

SDN-2 – in the presence of an oligonucleotide template with ends homologous to each side of the double-stranded break, homologous end joining (HEJ) can occur, such that one or more bases can be included in the repaired sequence

SDN-3 – as for SDN-2, but with a longer DNA insert, for example up to a full gene expression cassette

The question which arises is where to draw the line in terms of defining what is and what is not a GMO. This question can be illustrated as follows:

Where to draw the line (there is a 'genome editing continuum')?



Targeted deletion mutants – allele silencing	Targeted gene editing – recreate an allele	Targeted gene insertion - <u>cisgenic</u>	Targeted gene insertion – targeted transgenic
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This consideration argues that 'dealings' with organisms produced by oligonucleotide-directed mutagenesis and those categorised as SDN-1 and SDN-2 would be excluded from regulation.

Proposed changes/exclusions from the Regulations

- Organisms that do not contain nucleic acids from a foreign/exogenous source integrated into the genome. These may be developed using techniques for targeted mutagenesis, eg ODM, SDN-1, SDN-2.
 - The changes to endogenous gene sequence and function induced by these methods are in principle possible to be created using conventional breeding methods (eg mutagenesis, plant breeding).
 - Plants developed with these methods are considered as safe as conventionally bred plants.
- Organisms developed using cisgenesis and intragenesis. These may be developed using transgenesis or SDN-3 methods.
 - The DNA integrated into the genome originates from the same or a cross-compatible species and the product could in principle be developed using conventional breeding techniques.
- Organisms derived from GMOs that through normal segregation have not inherited the transgenic event (ie negative segregants for a selectable marker or genome editing cassette).
 - These should be excluded from the regulations

In summary, changes need to be made to the GeneTechnology Act 2000 (meaning of a genetically modified organism and Section 10 Definitions) and the Gene Technology Regulations 2001 (Schedule 1 and Schedule 1A) to take into account New Breeding Technologies, suggested changes as follows:

GT Act 2000: Genetically modified organism means as defined in (a), (b), (c) and (d), but does not include: *an organism that, as a consequence of normal segregation, has not inherited genes or other genetic material from an organism (the initial organism) that were introduced to the initial organism because of gene technology*

Section 10 Definitions

Additional exclusion: *other techniques that do not result in the insertion of 'foreign' nucleic acids into the genome*

Gene Technology Regulations 2001

Schedule 1, included as exclusions that are not genetically modified organisms:

- *An organism modified by a technique that modifies the sequence or expression of genes or genetic material at targeted sites in the genome that does not result in introduction of foreign nucleic acids in the genome.*
- *An organism that, as a consequence of normal segregation, has not inherited genes or other genetic material from an organism (the initial organism) that were introduced to the initial organism using gene technology.*

Schedule 1A – Techniques that are not gene technology

Include:

- *Cisgenesis and intragenesis.*
- *Techniques that modify the sequence or expression of genes or genetic material at targeted sites in the genome but do not result in the insertion of foreign nucleic acids into the genome.*

Overseas precedents

Over the past 5 years, the USDA has decided that ~30 types of genetically modified plants do not need to be regulated. These include CRISPR/cas9 waxy corn with high amylopectin starch, corn modified for reduced phytate content (ZFN technology), non-browning white button mushrooms (Penn State U, using CRISPR/Cas9 technology), and powdery mildew-resistant wheat in which the final product contained no introduced DNA (Calyxt, TALENs technology).

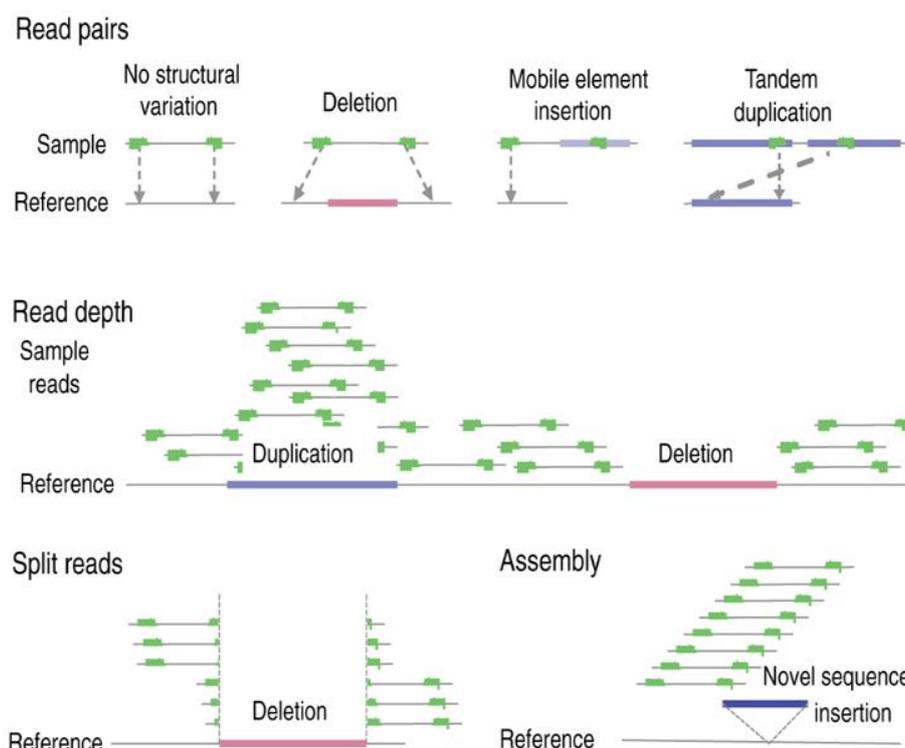
OGTR should recognise where regulators in other advanced jurisdictions, such as in the USA, have assessed the risks to human health and the environment to be the same as currently excluded technologies, and determined that no regulation is required, that Australian regulations should (after scientific consideration) follow suit. It is in the public interest that international regulations and terminologies are harmonised, and this will also help promote international trade, and could potential lead to the enhancement of agricultural exports from Australia as a major exporting nation.

Natural structural variation in sequence between individuals

Substantial structural variation in sequences between individuals has been revealed by genome re-sequencing projects which go well beyond single nucleotide polymorphisms (SNPs) and short insertion and deletions (indels). Structural Variations and Copy Number Variations are major sources of genomic variation which occur naturally. They include inversions, translocations, duplications and deletions, and insertion of novel sequences. Natural Structural variations can be neutral, but they can also significantly influence phenotypic properties, for example susceptibility to disease, nutrition etc. These natural structural variations in sequence between individuals therefore have phenotypic consequences.

There is therefore a strong argument that changes to DNA sequences using NBTs, which are essentially the same as those which occur naturally, should not be regulated as GMOs. Some examples of natural structural sequence variations are provided in the Figure below, which

compares the types of natural variation found between reference and sample sequences (<http://bioinformaticsonline.com/blog/view/30104/structural-variation-the-hidden-genomic-treasure>).



In addition, under **Other amendments under consideration**

Other techniques also regarded as NBTs:

- plants comprised of genetically modified (GM) parts grafted to non-GM parts are GMOs

It is the University's view that products of non-transgenic scions grafted onto transgenic rootstocks should be regarded as non-GMO. Any conditions for growth should address whether the rootstock does or does not flower or generate suckers: if the latter occurs and can be controlled by standard horticultural practices, then it will not present a significant risk.

- null segregants (offspring of GMOs that have not inherited the genetic modification or a trait from genetic modification) are not GMOs

As indicated above, it is the University's view is that segregants derived from GMO plants (eg using CRISPR/Cas9 technology and a selectable marker gene) in which the transgenic cassette is no longer present, having been crossed out, should not be regarded as GMOs

- organisms that are genetically modified in a transient manner (eg using agro-infiltration) are GMOs while the genetic modification or trait is present, and are no longer GMOs once both the trait and genetic modification are no longer present

The University agrees with the above statement.

Gene drives

The university regards the development of gene drive technology, in which organisms carrying GM gene drives, which involve stably integrated transgenes, as organisms that should be regulated under the GT Act. A case-by-case approach is needed until a track record of their behaviour has developed. Depending on the target gene and organism there will be different levels of risk – for example, if a CRISPR/cas9 guide RNA cassette is used under tight control of an inducible promoter, this would present a much lower risk, and might allow field based applications in which the gene drive is only switched on after a crop is sprayed with a chemical which activates the promoter.

RNA interference

There is now a track record that application of RNAi traits present a relatively low level of risk, as evidenced by their commercial development and release, for example in non-browning apples, non-bruising potatoes, potatoes with reduced acrylamide on cooking, and virus resistant papayas. Although under current regulations these crops will be regarded as GMOs, their passage through the regulatory system could be more rapid than some other forms of GMOs.

However, there is now the option of spray delivery of dsRNA to silence target genes in pests, and this should not be subject to OGTR regulation, since the treated plants are not transgenic.

Need for international harmonisation of GMO regulations

Australia is an agricultural exporting nation which needs to use the best science and technologies to ensure high quality produce at a competitive price. The lack of harmonisation of gene technology regulation in different countries, and especially those that are major importers of Australian produce, limits the application of the best science and technology in Australia by not having compatible regulations in place for accepting transgenic produce.

A major beneficial role of government, and the OGTR, should be to enable international harmonisation of regulation of GMOs, especially with Australia's major trading partners.

Need for harmonisation of regulations between Australian Government agencies on GMOs

Similarly, there should be harmonisation and consistency of regulations that relate to GMOs across Australian government entities.