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Final Report

New breeding technologies and opportunities for the Australian vegetable industry

Michael Jones
Murdoch University

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Level 8, 1 Chifley Square
Sydney NSW 2000
Tel: (02) 8295 2300
Fax: (02) 8295 2399

Content

Summary

The plant breeding technologies available for the improvement of vegetable and other crop plants are advancing rapidly. The cost of DNA sequencing has dropped dramatically, and now complete genome sequences are available for most important crop plants. There is a twenty-year history of safe use of genetically modified crops worldwide. Coupled with advances in genetic technologies, there are exciting new opportunities for the vegetable industry in Australia and elsewhere. The focus here is on New Breeding Technologies (NBTs), which includes various means by which new plant varieties can be bred. The definition of NBT includes the transgenic plants that have become a major component of some agricultural systems internationally, but it also includes the much newer technology of genome editing, which offers a paradigm shift in the ability to generate new varieties. We are on the verge of a new plant breeding revolution [1]. Today's molecular plant breeders are armed with the technologies to make specific changes to genes in living cells, in some cases without the presence of any introduced DNA. As a result of an emerging array of NBTs, gene technology regulations need to be updated, taking on board all the NBTs, and taking into consideration the long and safe track record of use of GM crops. It is hoped that the revised regulations will make the actual traits the primary consideration when assessing new varieties produced by NBTs, and not solely the means by which they were produced.

In relation to this study, the current status of the Australian vegetable industry in relation to NBTs is outlined, and a wide range of industry and researcher consultations were undertaken.

Leading Australian researchers working on NBTs for plant improvement are, in general, strongly positive regarding the potential of NBTs to contribute to Australian vegetable production. The technological capacity for NBTs to contribute to genetic gain is seen as very high.

Vegetable seed merchants and breeders held a more conservative but open stance toward NBTs. Their concerns centred on the question of whether or not a product would be classified as genetically modified, a major consideration for market access. Breeders believed that growers were much more focused on the performance of a variety in the field than on the technology used to develop it. Leading growers were less positive on the potential for NBTs to contribute to the Australian vegetable industry. The major concern was consumer acceptance, followed by the cost of applying NBTs to the relatively small vegetable market in Australia.

There was consistent recognition that the critical hurdle for the implementation of NBTs in the Australian vegetable industry will be public/consumer acceptance.

The current regulatory systems covering NBTs as defined by Gene Technology Act 2000 and implemented by the Office of the Gene Technology Regulator (OGTR) and Food Standards Australia New Zealand (FSANZ) are outlined, and regulatory systems of major vegetable importing countries are summarised.

A series of recommendations are presented relating to the applications of NBTs to the Australian vegetable industry. It is clear that the industry must be mindful to bring society and politicians along on this journey, since their understanding and acceptance could restrict the application of these powerful new technologies.

Keywords

Vegetables, new breeding technologies, genetic manipulation, transgenic, GMO, cisgenic, intragenic, RNAi, genome editing, CRISPR-Cas9, synthetic gene drive, OGTR, FSANZ, gene technology regulation, public acceptance.

1. Terms of Reference

The aim of this review is to assess the status of new or innovative breeding technologies, collectively known as New Breeding Technologies or NBTs, both in the public (i.e. government supported) and private (commercial) sectors, and including overseas data of relevance to Australia. This information will help inform decisions around investments by Horticulture Innovation Australia (HIA) in NBTs for the vegetable industry, taking into account the economic, social and environmental outcomes flowing from the use of NBTs. These will vary depending on specific applications and strategies that are selected for future development. In addition, their potential application to export growth could be important in maximising economic benefits for the industry. This review comprises four major components:

- a scientific review of the current status of NBTs and their potential application in crop plants,
- an evaluation of industry and market attitudes toward the use of NBTs to develop improved vegetable cultivars,
- an assessment of consumer attitudes to NBTs,
- an assessment of the regulatory environment in Australia and countries that are key destinations for the export of vegetables from Australia.

It also incorporates lessons learned from other industries relating to the above components, and campaigns that have driven or hindered acceptance or access to NBTs, and a scan of relevant legislation in key trading countries.

2. Methodology

To provide data for this project, information was gathered from relevant parties using the following methods:

I. a study of the scientific literature on NBTs and their applications,
II. telephone interviews with representatives of growers, plant breeders, commodity organisations, peak bodies and State Government staff (definitions of different forms of NBTs and a list of questions relevant to the groups being interviewed were provided advance),
III. face-to-face interviews with a range of leading researchers in the field of NBTs, with regulators, including the Office of the Gene Technology Regulator, Food Standards Australia and New Zealand, and with industry representatives CropLife Australia and the Grains Research and Development Corporation,
IV. searches of foreign government web sites to assess regulatory status in key export destinations,
V. a study of the scientific literature on acceptance of NBTs in Australia and overseas, combined with interviews of social scientists working in this area.

3. Definitions – what are New Breeding Technologies?

New Breeding Technologies is a term used to describe a series of new technologies that are introducing dramatic advances in plant production, including in the horticultural industries. Although they are not yet widely applied to vegetable breeding in Australia, they are beginning to be used in other countries with spectacular success. Examples are provided below. NBTs are already being used to introduce exciting new traits to varieties. These traits include greater yield with fewer inputs; improved tolerance to external stresses such as water deficit, saline soils, frost, pests and diseases; altered plant maturity and harvest management; new post-harvest qualities including longer shelf-life and improved processing qualities; improved nutritional and health-related traits; and new colours, shapes and textures.

A comparison of simplified conventional breeding procedures and transgenic technologies is provided in Figure 1.

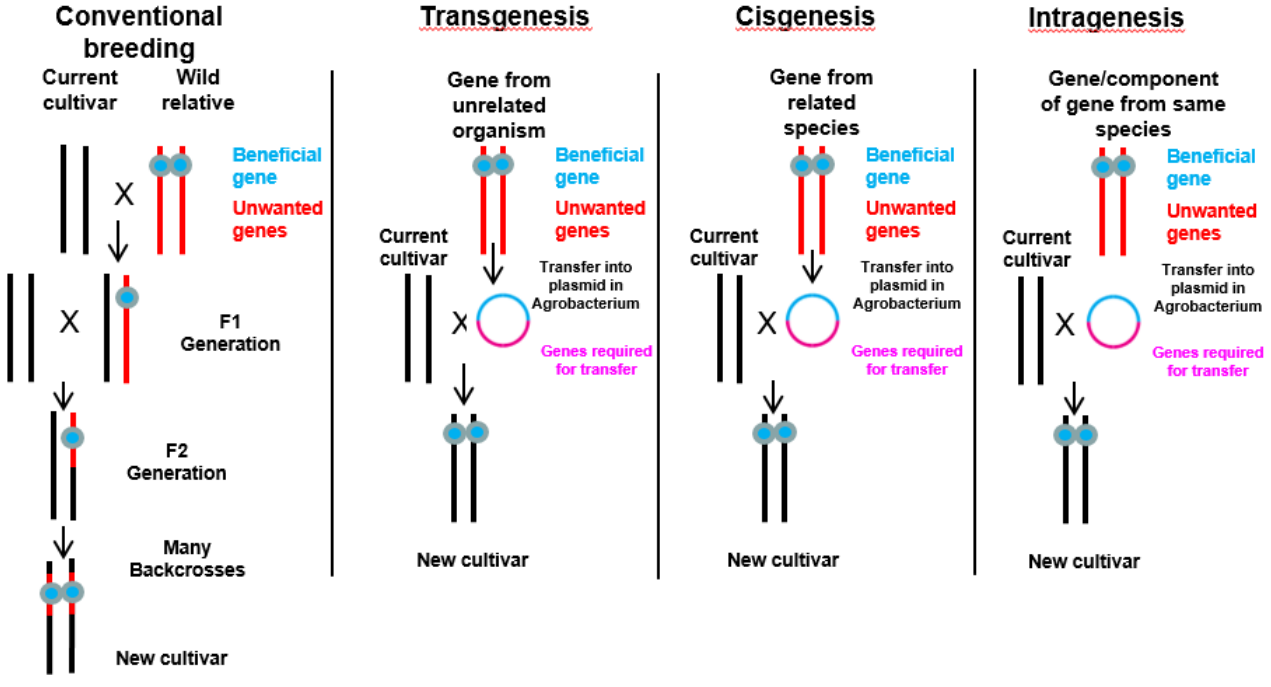

Figure 1. A comparison of conventional breeding processes (sexual crossing) with transgenic approaches where the origin of the introduced genetic material is from an unrelated organism, from a related species for which a sexual cross may be possible ('cisgenic') or from the same species ('intragenic') (see Section 5 for Agrobacterium transformation methodology). 'Linkage drag' which occurs in conventional breeding is indicated by the red colour in the new cultivar).

At present, NBTs are defined by the method used to generate the genetic changes, that is, recombinant DNA technology, rather than by the genetic characteristics or phenotypic properties of the end-products. As a result, they may, or may not generate what are general known as genetically modified organisms (GMOs), and regulators are currently assessing how to take into account and manage the products of NBTs.

The following is a list of some of the main new breeding technologies. (Note: a plant variety may contain multiple transgenes, cisgenes and/or intragenes, or genetic material derived from other NBTs. This is referred to as 'stacking'. Stacked genes may have been manipulated through the application of different NBTs, in which case the distinctions between these classifications as a way of describing a variety becomes blurred).

3.1 Transgenic plants - these are GMOs that contain one or more beneficial genes from *unrelated organisms,* for example, insect resistance in crops resulting from expression of a protein from the bacterium *Bacillus thuringiensis* (Bt) in the plant. More than 10% of the world's broadacre crops (mainly soybean, cotton, maize and canola) fall into the category of transgenic plants, with maize varieties commercially available that include up to eight stacked genes [2].

3.2 Cisgenic plants – these differ from transgenic plants only in the source of the transgene(s). Here, the plant contains a beneficial gene or genes from *closely related, but different species*. Alternatively, the cisgene could been introduced to the new variety by conventional plant breeding processes (e.g., crossing, embryo rescue, cytogenetic methods), but the artificial insertion of a single gene using recombinant technology avoids 'linkage drag', where many unknown and unwanted genes are introduced incidentally into the new variety. Cisgenic potatoes have been generated that a resistant to *Phytophthora infestans* [3].

3.3 Intragenic plants – these differ from transgenic and cisgenic plants in that the genetic sequences transferred are from the *same species*, and re-introduced back into that same species, and which could well have been similarly introduced or re-arranged in evolutionary time or the same change achieved by conventional breeding practices [4].

3.4 Non-transgenic scions can be grafted onto transgenic rootstock. In this form of NBT the produce from the non-transgenic scion may or may not be regarded as genetically modified, however under the current Australian regulations, the whole plant and its harvested products are considered as a GMO [5].

3.5 RNA interference, RNAi (also known as gene silencing), is a technology which can be used to reduce or switch off the expression of target genes. A relatively short sequence of double-stranded RNA (dsRNA) with a sequence complementary to that of a target gene is incorporated in the plant in order to down-regulate (or silence) its expression. This uses natural pathways present in plants (and other organisms). RNAi has been used to generate non-browning apples, and non-bruising and reduced cold sweetening in potatoes [6].

3.6 Host-Induced Gene Silencing (HIGS) is a variant of RNAi in which dsRNA is designed to target and down-regulate expression of a vital gene in a pest or pathogen, by expression of the dsRNA incorporated into the host plant genome. Uptake by the pest or pathogen of the dsRNA expressed from the plant triggers silence the vital gene in the pest or pathogen. Thus, the process confers enhanced host-plant resistance [7,8].

3.7 Spray-Induced Gene Silencing (SIGS). Although strictly not an NBT, development of SIGS usually employs HIGS to confirm gene targets. It is a variant of RNAi in which dsRNA complementary to a target gene is sprayed onto a plant. Although strictly this is not a breeding technology, SIGSs can be used to alter the expression of genes which alter the growth or characteristics of the host plant itself, or to interfere with the growth and reproduction of a pest or pathogen. In this technology of use the dsRNA must be stabilised against degradation whilst exposed on the surface of the plant [9].

3.8 Reverse breeding is a process in which a superior hybrid plant is transformed with a gene that prevents gene recombination during meiosis. This allows the parental genotypes of the hybrids to be identified and re-isolated. The transgene is then removed and the hybrid can be generated commercially [10].

3.9 Genome editing (also known as gene editing) includes a set of powerful new and emerging technologies, especially those based on targeted mutagenesis (CRISPR-Cas9), which are described in detail the next sections Essentially they enable precise targeting of individual genes and their regulators to alter gene expression [11].

3.10 Oligonucleotide-directed mutagenesis (also known as gene editing technology), is a tool to generate targeted mutagenesis. A specific oligonucleotide, typically 20-100 bp in length is used to produce a single DNA base change in the plant genome, thereby lowering or preventing the expression of the target gene [12].

3.11 TALENS (transcriptional activator-like effector nucleases) and **Zinc Finger Nucleases** (ZFNs) are early forms of genome editing which are based on the interaction of specifically developed peptide sequences usually linked to a double stranded DNA nuclease (dsDNAse, usually Fok1). The peptide amino acid sequences are designed to bind to a specific DNA sequence. The linked dsDNAse then cleaves the target sequence and can result in altered expression of the targeted gene [13].

3.12 Site-directed nucleases (SDNs) are a set of tools, which include TALENS and ZFNs, which can also generate targeted mutagenesis without any introduced DNA, or with the addition of or one or more bases at the target site. They make use of specific enzymes (dsDNA nucleases, usually 'Cas9') which can be directed to make precise cuts in both strands of a DNA sequence as defined by a 20 base RNA oligonucleotide sequence [14]. Variants of SDN applications can be sub-divided as SDN-1, SDN-2 and SDN-3, depending on whether the nuclease is used alone or in combination with a DNA-repair template (https://croplife.org/wp-content/uploads/pdf_files/Technical-Summary-of-NBTs_final.pdf). In SDN-1, mutations of the target gene result simply from errors in repairing the break. In SDN-2, with the additional of a short nucleotide 'repair' sequence, one or a few additional DNA bases can be added to the site, and in SDN-3 the introduced DNA repair sequence is longer, up to the size of a complete gene.

In the future, products of SDN-1 and SDN-2 mutations may not be regarded as GMOs by the OGTR and FSANZ, whereas those classified as SDN-3 will still be regarded as GMOs (although the latter may be considered differently if the introduced nucleotides are intragenic or possibly cisgenic sequences).

3.13 Removal of a genome editing cassette. Because genome editing occurs at a different site in the genome from where the genome editing cassette (which usually expresses a guide RNA, a Cas 9 ds DNAse protein and a selectable marker gene) is integrated, if a selectable marker gene and genome editing cassette have been used, these may be removed by crossing genome edited lines with parental lines, and selecting for plants which contain only the targeted mutation/change, and not the genome editing casette [15].

3.14 Virus-Induced Genome Silencing (VIGS) and Editing (VIGE). VIGS is a variant of RNAi in which dsRNA complementary to a target gene is integrated into a virus genome such that during virus infection of cells and subsequent replication, the target gene mRNA is down-regulated or silenced by RNAi. VIGE is a variant in which a genome editing cassette is integrated into the virus genome to edit a particular target gene in the host plant, without integration of the viral genome into the host plant chromosomes [16].

3.15 Agroinfiltration is a method in which an *Agrobacterium* containing a genome editing cassette in the 'T-DNA' (i.e. transfer DNA, the component of the Agrobacterium DNA that is transferred to the host DNA) is infiltrated into leaf spaces, allowing expression of the genome editing cassette in a few cells. Plants must then be regenerated from the cells of the infiltrated tissues, and lines with edited target genes identified [17].

3.16 Ribonucleoprotein genome editing is a strategy to generate genome-edited plants without any chance of introducing exogneous DNA. This contrasts with the more widely used approach of introducing a DNA plasmid with a guide RNA and dsDNAse in which DNA integration might occur. When ribonucleoprotein editing is undertaken, the guide RNA and dsDNAse are first made externally and then combined together, and the combined guide RNA and dsDNASe are introduced into cells (e.g., by particle bombardment, infiltration in leaf spaces or into protoplasts). Plants are then regenerated from the treated tissues and rare individuals in which the target genes have

been edited are then identified [18].

3.17 Synthetic Epigenetics. Epigenetics is the study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself. This usually results from methylation or similar modifications to the DNA bases. Synthetic epigenetic modifications can be used to change the expression of a target gene by controlling transcription and chromatin remodelling. For example, this can be achieved using a nuclease-null (dCas9) protein core linked to enzymes such as demethylases, methyltransferases or deacetylases and a specific RNA guide sequence. This strategy provides a new set of tools to edit the epigenome at precise sites without changing DNA sequences [19].

3.18 Synthetic 'Gene Drive' is a mechanism for spreading genome-edited traits through a population by inserting a gene cassette to express both Cas9 and a specific guide RNA as transgenes in a plant (or other organism) [20], such that transgenic products edit their own genomes and that of progeny. Plants with synthetic gene drives combine both transgenic technology and genome editing.

3.19 Intellectual Property. It is beyond the scope to this project to cover the Intellectual Property (IP) issues that relate to NBTs. The IP landscape surrounding NBTs can be complex, and professional advice is recommended when initiating R&D in this area. In general, there are core enabling technologies which have been patented, such as RNAi technology, and other genetic elements used to control gene expression. Many of the original patents in the area of GM crops are now lapsing so there may be increased freedom to operate. However, property rights for genome editing are still being contested. A recent court ruling in favour of the Broad Institute in the US has been challenged by UC-Berkeley with the aim of establishing that the teams led by Doudna and Charpentier were the first to engineer CRISPR/Cas9 in all cell types. The uncertainty may complicate commercialisation of products based on this editing technology.

4. Introduction: challenges and opportunities

In the field of crop agriculture, and biology in general, we are now living in one of the most exciting and challenging times. There are unprecedented global challenges; the world is changing rapidly, and many believe that global sustainability of food production is under serious threat. There are increasingly more mouths to feed from a decreasing land area, with a changing climate and increasingly restricted resources. According to Nobel Laureate Professor Brian Schmidt, Vice-Chancellor of the Australian National University, gene technology will enable all humanity to live well on this planet and ensure food security, human health and protection of the environment (OGTR 7th National Institutional Biosafety Committee Forum, May 2017). Importantly, Schmidt also emphasised that we must be mindful to bring society and politicians along on this journey, or they will block the application of these new technologies.

Modern primary food production is at the forefront of addressing the challenges outlined above of a burgeoning human population, predicted to be 9-12 billion by 2100, and the uncertainty of the full impacts that climate change and increased climate variability will have on food production. These changes will not only influence crop productivity directly (e.g. [21,22]), they will also influence other factors that relate to food production, for instance, changing the relative importance of pests and diseases [23,24].

Australian vegetable growers play a critical role in maintaining the physical health of Australians by supplying fresh and affordable produce. It is well established that regular consumption of vegetables is essential to maintaining health and reducing incidence of the most common causes of premature death in the developed world—cardiovascular disease and cancer [25]. However, vegetable breeders and producers must also be prepared to adapt to changing consumer demands. Consumers in local and export markets are becoming more discerning about the species and varieties of vegetables they consume. Societies are becoming wealthier, and consequently, consumers are demanding high quality produce all year round. Tastes in food are becoming more globalised, and cuisines are changing. Food fashion mimics other popular fashion trends in that products and dishes may become very popular for a limited period, and then demand drops away equally quickly. Some of the so-called 'superfoods' are cases in point. The horticulture industry must be flexible enough to capitalise on such trends.

At the same time, a range of new biotechnologies is emerging that have the potential to change how vegetables are grown, which cultivars are grown, how produce is marketed, and why consumers will buy it. New cultivars developed using NBTs promise growers and consumers advantages over older cultivars. Advantages to growers will include lower input costs, higher yields and greater profits. Advantages to consumers could include produce that remains fresher longer, possesses enhanced health qualities, or has better taste and colour.

This review introduces the most important of the NBTs that will impact vegetable production and the new products that may become available. Case studies of NBT-generated products already on the market are presented. The various legislative requirements of the new technologies are also addressed, and perhaps most importantly, an assessment is provided of current public attitudes towards genetically modified food, and how the mood might shift over time as new products become available.

4.1 The phenomenal advances in DNA sequencing technology

One of the main factors contributing to the advances in NBTs is that there have been phenomenal advances in DNA sequencing technology over the past 15 years. The first human genome published in 2001 (consisting of 3 billion bases) cost US$3 billion and took 15 years to sequence. By 2014, with the introduction of the Illumina HiSeqXTen, it became possible to sequence more than 45 human genomes per day, at the cost of about $1,000 each (Figure 2) [26].

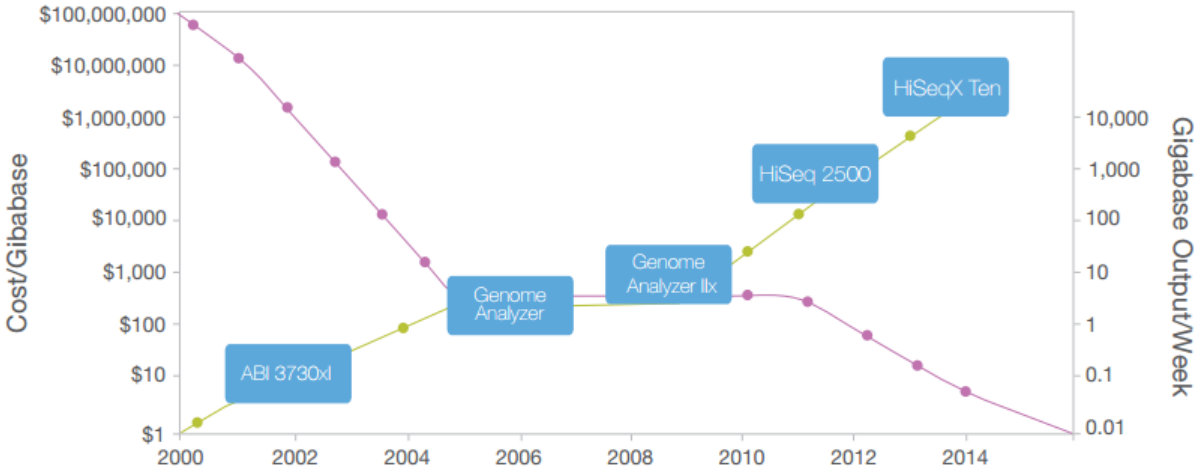

Figure 2. The considerable advances in new DNA sequencing technologies have reduced the cost of genome sequencing projects to enable seqeuncing of vegetable crop genomes: note the logarithmic scales of the axes (Source: Illumina).

The same advances in technology are being applied to sequence vegetable and other crop plant genomes, so that now the genomes of most of the major vegetable crop plants have been sequenced (including those of broccoli, cabbage, capsicum, carrot, Chinese cabbage, cucumber, lettuce, mushrooms, potato and tomato) [27]. This huge increase in genetic information needs to be analysed and understood, but it provides unprecedented new information on the basis of commercial traits in crop plants and is being used to increase the efficiency and precision of crop improvement. The new information has also provided the impetus for the development of NBTs, increasingly using a knowledge-based approach to crop improvement.

5. Overview of the status of new breeding technologies

Over the past 20 years since their introduction in 1996, transgenic plants have been the most rapidly adopted new technology in agriculture The most common process used to generate transgenic plants is outlined in Figure 3. A desired gene is cloned into the circular Ti plasmid of the common soil bacterium *Agrobacterium tumefaciens* to replace the T-DNA (transferred DNA) in the Ti plasmid. Co-cultivation of the bacterium with plant tissues results in transfer of the T-DNA into plant cells, which can be cultured to grow complete plants containing the introduced gene.

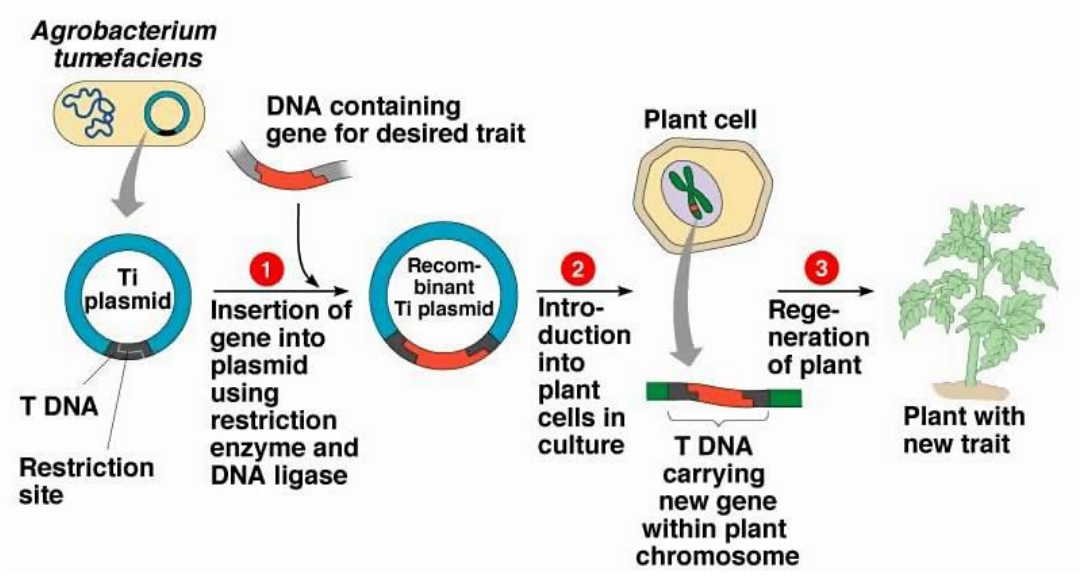

Figure 3. The process of generating transgenic plants using Agrobacterium tumefaciens as a gene vector. The transferred DNA (T-DNA) of the Agrobacterium is replaced by a cassette with the gene encoding the desired trait (1), which is transferred and incorporated into chromosomes of plant cells (2). Regeneration of whole plants (3) from these cells results in a transgenic plant (Source: Creative Commons).

Transgenic crops now occupy 185.1 million hectares (ISAAA, 2017) or about 10% of the total cropping area of the world (Figures 4, 5). However, because of the cost of regulatory compliance, their implementation has been restricted mainly to four major broadacre crops: soybean, maize, cotton and canola. The most widely adopted technologies confer herbicide and insect tolerance, but there are many other crops, including vegetable crops, either released for commercial growth or advancing towards deregulation.

Transgenic herbicide tolerance is based on one of three principles: expression of a gene encoding an enzyme that is insensitive to the herbicide, over-expression of a sensitive wild-type gene so that there is still enough of the active enzyme after herbicide treatment, or expression of a gene whose product detoxifies the herbicide. Most insect resistance is based on expression of specific '*Cry*' proteins originating from *Bacillus thuringienesis* (Bt), which disrupt gut functioning of target insects [28].

The International Service for the Acquisition of AgriBiotech Applications (ISAAA) provides a detailed annual summary of the global status of commercialised Biotech/GM crops: the current summary (ISAAA Brief 52) provides global data for 2016. A searchable database provided by ISAAA, includes a comprehensive list of GM crop traits, events (currently 493), a list of transgenes that have been introduced, countries in which GM crop events have been approved, GM traits

which have been approved for commercial use, a list of GM developers, and methods of trait Introduction .

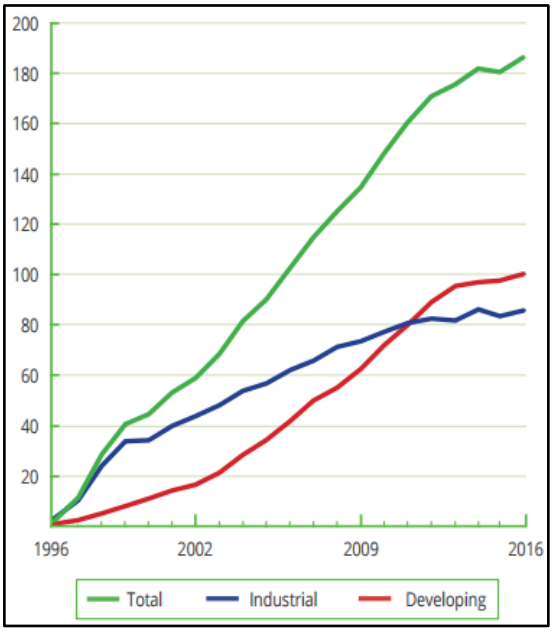

Figure 4. Global increase in growth of GM crops, X- axis Millions of hectares.
https://www.isaaa.org/resources/publications/briefs/52/download/isaaa-brief-52-2016.pdf

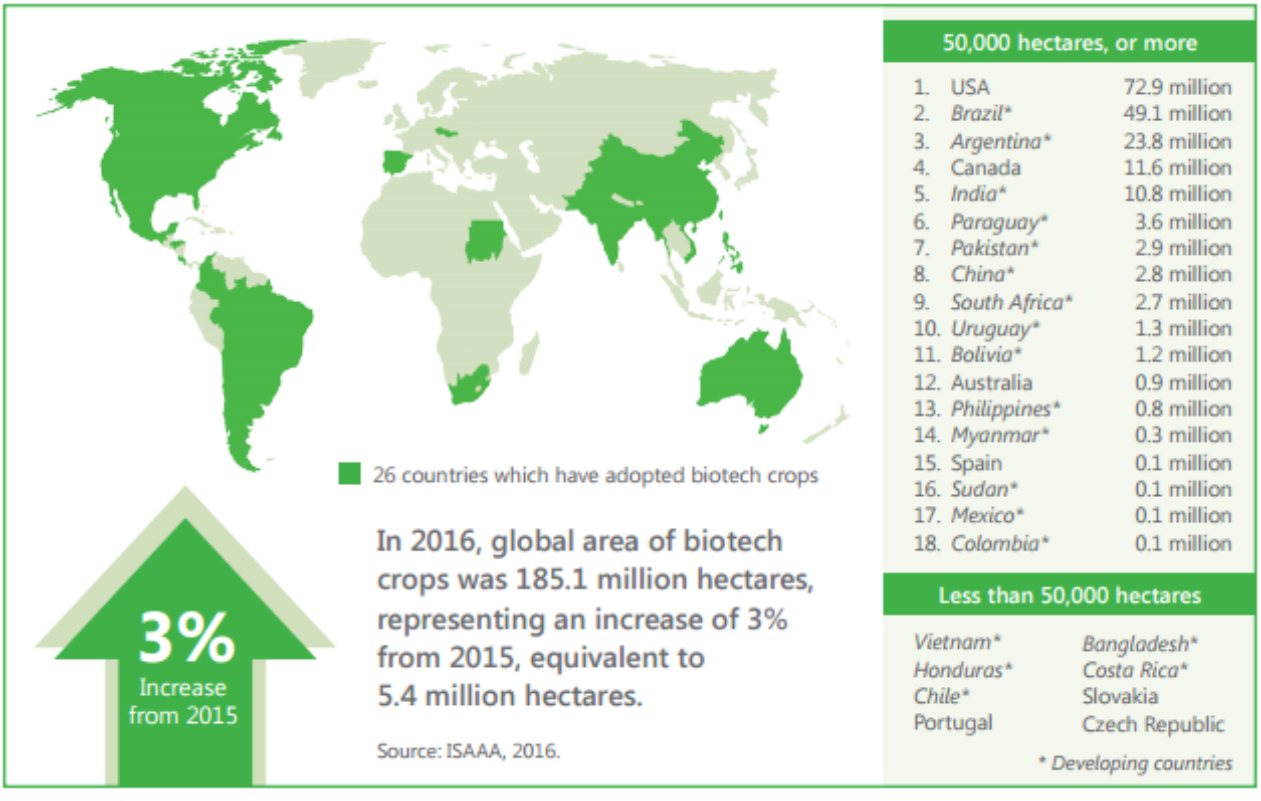

Figure 5. Global area (million Ha) of GM (Biotech) crops by country in 2016.
https://www.isaaa.org/resources/publications/briefs/52/download/isaaa-brief-52-2016.pdf

The current list of GM crops includes the following 29 crops (Table 1) (http://www.isaaa.org/gmapprovaldatabase/).

Table 1. GM crops approved for commercial growth

- Alfalfa (*Medicago sativa*)
- Apple (*Malus x Domestica*)
- Argentine Canola (*Brassica napus*)
- Bean (*Phaseolus vulgaris*)
- Carnation (*Dianthus caryophyllus*)
- Chicory (*Cichorium intybus*)
- Cotton (*Gossypium hirsutum L.*)
- Creeping Bentgrass (*Agrostis stolonifera*)
- Eggplant (*Solanum melongena*)
- Eucalyptus (*Eucalyptus sp.*)
- Flax (*Linum usitatissumum L.*)
- Maize (*Zea mays L.*)
- Melon (*Cucumis melo*)
- Papaya (*Carica papaya*)
- Petunia (*Petunia hybrida*)
- Plum (*Prunus domestica*)
- Polish canola (*Brassica rapa*)
- Poplar (*Populus sp.*)
- Potato (*Solanum tuberosum L.*)
- Rice (*Oryza sativa L.*)
- Rose (*Rosa hybrida*)
- Soybean (*Glycine max L.*)
- Squash (*Cucurbita pepo*)
- Sugar Beet (*Beta vulgaris*)
- Sugarcane (*Saccharum sp*)
- Sweet pepper (*Capsicum annuum*)
- Tobacco (*Nicotiana tabacum L.*)
- Tomato (*Solanum esculentum*)
- Wheat (*Triticum aestivum*)

The major traits and the numbers of those traits developed for field release in the USA are provided in Table 2 (http://www.isb.vt.edu/reports.aspx).

Table 2. The major GM crop traits developed for field release in the USA

Phenotype/Category	Total
Herbicide Tolerance	8012
Agronomic Properties	7750
Product Quality	5814
Insect Resistance	5599
Other	3088
Marker Gene	2821
Fungal Resistance	1612
Virus Resistance	1476
Bacterial Resistance	287
Nematode Resistance	239

Initially, single-gene transgenic events encoding production traits (e.g., Bt insect resistance, herbicide tolerance from bacterial sources) were commercialised. With time, gene traits have been combined, with up to eight stacked traits in some varieties of maize ("SmartStax"). Smartstax includes two herbicide tolerance genes (for tolerance to glyphosate and glufosinate) and six insect resistance genes. SmartStax corn provides control of a range of insect pests: larvae of the European corn borer, black cutworm, southwestern corn borer, corn earworm, fall armyworm and western bean cutworm, and below-ground larvae of the western corn rootworm and northern corn rootworm. The marketed seed includes 5% non-GM seeds to reduce the development of resistance in pests; a technology called "Refuge-in-a-bag" (RIB) (https://www.genuity.com/corn/Pages/SmartStax-RIB-Complete.aspx).

More recently, varieties with RNAi-based traits have been commercialised. In some cases new varieties also include traits which combine technologies such as an added gene and a silenced gene. RNAi-based traits are used to silence

gene expression, for example, non- browning apples and potatoes, high amylose wheat starch, prevention of toxin production.

5.1 Compositional comparisons, NBTs and traditional breeding technologies

When the composition of GM and equivalent conventional crops have been compared, based on more than 20 years of research, many published reports and hundreds of regulatory submissions, GM varieties differ only in the deliberately introduced new traits, whereas traditionally bred varieties often differ in unintended ways because of 'linkage drag' of unintended genes [29]. However, the possibility of unintended effects, for example using RNAi or genome editing, is taken seriously, and whole genome sequencing coupled with bioinformatic analysis can reduce or essentially eliminate this possibility. Thus, NBTs offer far greater precision, predictability and biosafety, and far less time in the development of new varieties than do the traditional breeding technologies.

5.2 The Economic benefits of NBT crops

NBTs are, of course, not the only answer to meeting future challenges in crop production but they do present breeders with powerful new tools to meet current and future challenges. It is now 20 years since the first GM or 'Biotech' crops were commercialized (http://www.isaaa.org/resources/publications/briefs/52/executivesummary/default.asp, ISAAA 2017), and it is clear that these crops have delivered substantial economic, agronomic and social benefits to farmers and to the environment. The benefits conferred so far by crops developed using NBTs have been quantified at the global level [30]. For the period 1996-2013, these include:

- an increased in crop production and productivity valued at $133 billion,
- an increase in profits of farmers who used GM technology of 68%,
- the enabling of large-scale practice of no-till agriculture, thus helping to reduce soil loss and erosion,
- a reduction in use of chemical pesticides by 37% (583.5 million kg of active ingredients),
- an increase in crop yields by 22%,
- increasing the options available to farmers for agronomic practices,
- a halo effect in which conventional and organic farms adjacent to GM farms benefit from fewer insects,
- a reduction in $C0_2$ emissions, and
- alleviation of many poor farmers from poverty.

The benefits from growing Biotech cotton and canola crops in Australia between 1996 and 2015 have also been quantified [30]. In summary, these include:

- a benefit to Australian cotton and canola farmers of $1.37 billion,
- an increase in farm income from growing GM canola alone of $98.9 million,
- an increase in production of canola of 226,000 tonnes,
- a reduction in insecticide and herbicide use of 23%,
- benefits to the environment through more targeted genetic control of insect pests of cotton, reduced spray drift, fewer tractor passes and less soil compaction,
- a reduction in the exposure of farm workers to chemical pesticides.

(The use of Bt cotton in Australia is discussed later as a 'case study'). The speed with which Biotech crops could be commercialised in the future could enable breeders to meet the challenges of climate change and the arrival of new biosecurity pests and diseases faster than by conventional breeding.

5.3 Food safety

The scientific consensus on the safety of GM foods is strong and consistent. Numerous studies have been undertaken to assess the safety of GM foods. These have included research by national science bodies of the major economies which

all agree that, “The science is quite clear: crop improvement by the modern molecular techniques is safe” (AAAS). The European Commission “A decade of EU-funded GMO research” presents the results of 50 projects, involving 4,400 research groups and concluded that ‘‘biotechnology, and in particular GMOs, are not *per se* more risky than, e.g. conventional plant breeding technologies’’. Similar conclusions have been reached by the Royal Society of Medicine (UK); Food Standards Australia New Zealand, The French Academy of Sciences; and Academies of Sciences from many countries (e.g. Brazil, China, India, Mexico, US, UK, Russia).

GM foods are subjected to more testing than any other food, and the overwhelming body of evidence indicates that they are safe [31].

The experience of the last twenty years and extensive scientific research has demonstrated the value of NBT crops (as a category) to producers, society and the environment. Food produced from NBT crops are consistently demonstrated to be safe and plant varieties produced using NBTs are less likely to have unintended genetic changes than when similar traits are introduced via conventional introgression. In the following sections we will consider the more recent NBTs individually.

5.4 RNA interference

In the normal expression of a gene, coding sequences of DNA are transcribed into messenger RNA (mRNA), which is then translated into functional proteins. RNA interference (RNAi) is a natural biological process which is triggered by the presence of double-stranded RNA (dsRNA) in a cell, and results in the down-regulation or silencing (switching off) of the expression of mRNA with the same sequence as one strand of the dsRNA (Figure 6). This reduces or prevents the synthesis of the functional protein.

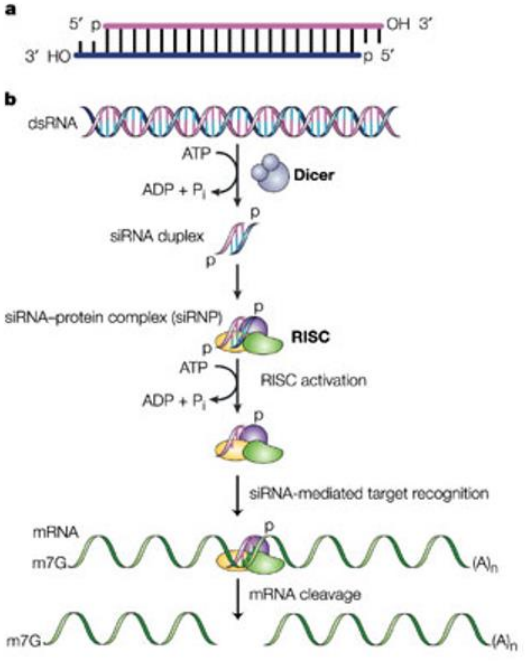

Figure 6. Simplified diagram of RNAi, in which dsRNA is cut into short interfering RNAs (siRNA) by Dicer-like enzymes, one strand of which is bound to an RNAi silencing complex (RISC) which mediates recognition of messenger RNA (mRNA) with a complementary sequence, resulting in cleavage of the mRNA, so preventing its translation into a functional protein (Source: Nature Reviews – Molecular Cell Biology).

When the phenomenon was first studied, it was known variously as *co-suppression*, *post-transcriptional gene silencing* (PTGS), and *quelling*, but since 1998 it has become known as RNA interference (RNAi). It soon became evident

that RNAi could be used to down-regulate or suppress the expression of a specific 'target' gene and that there were many potential applications for this technology, including for crop improvement.

The RNAi pathway is present in many plants, animals, insects and invertebrates and fungi. The process starts with the enzyme Dicer, which cleaves long dsRNA molecules into short double-stranded segments referred to as small interfering RNAs (siRNAs), 20-24 nucleotide in length. The 'guide' strand of the siRNA is incorporated into an RNA-induced silencing complex (RISC), and binds with the complementary sequence in the mRNA molecule. This mRNA is then cleaved by an Argonaute 2 (Ago2) protein to prevent translation of the mRNA. In many organisms, including plants, there is an amplification process to generate more siRNAs, and the siRNA signal can spread systemically in the plant to silence similar mRNA sequences in other cells.

In basic studies, RNAi is often used to knock down (reduce) expression of genes to understand their function, and this is similar to targeted mutagenesis. Practical applications include reducing expression of browning in cut apples [32], bruising in potatoes and reduced production of acrylamide in deep fried produce. Some examples of the application of RNAi are provided in more detail as case studies.

5.5 Host-Induced Gene Silencing (HIGS)

As described above, gene silencing or RNAi is an effective way to silence or down-regulate expression of genes in plants. This technology can be extended so that the plant produces siRNAs that silence genes in pests or pathogens whose expression is vital for some aspect either of plant infection or the establishment and completion of the life cycle of the pest or disease. This process is known as Host-Induced Gene Silencing (HIGS).

HIGS is thus an RNAi-based process in which small RNAs (siRNAs) made in the plant, silence genes of pests or pathogens that attack the plant. The siRNAs are typically made by introducing gene cassettes which produce double-stranded RNA (dsRNA) in transgenic plants. The sequences used are chosen to eliminate any effects on non-target organisms. In effect, the pests or pathogens are tricked into silencing their own vital genes, using their own gene silencing pathway. There is no effect on the host plant since the target genes chosen are not present in the plant; they are only present in the target pest or disease.

So far, HIGS has been commercialised to confer resistance to plant viruses, the most notable example being for papaya ringspot virus resistance, an NBT trait that saved the papaya industry in Hawaii [33]. There is also considerable interest in using the technology to confer plant resistance to nematode and insect pests as well as to fungal and bacterial pathogens. There is now good evidence to show that the three major genera of plant endo-parasitic nematodes (root-knot, cyst and root lesion nematodes) are amenable to control using HIGS (e.g. [8], [34,35]). Similarly, both sucking pests (e.g. aphids) and chewing insects (e.g. lepidopteran larvae) are amenable to control using HIGS. Transgenic maize plants expressing a dsRNA to target the vacuolar H^+ATPase of western corn rootworm were significantly protected, and cotton plants expressing dsRNA of a cytochrome P450 gene enhanced resistance to bollworms. There are many potential gene targets to which the HIGS approach can be applied, including those coding for secreted effector proteins, neuropeptides, and proteins involved in RNA processing.

More recently HIGS has been shown to enhance resistance to fungal diseases and to oomycetes [36]. Applications include improving the resistance of avocado rootstocks to damage by *Phytophthora cinnamomi*, and inhibition of the production of aflatoxins by *Aspergillus* that infects maize cobs [37].

In work to control nematodes that attack roots of banana plants using HIGS, the combination of two genes, one based on HIGS, and a second based on expression of a protein which inhibits gut protease enzymes, has been proposed as conferring more effective and durable resistance to nematodes, by combining two different modes of action [38,39].

Current evidence suggests that movement of small RNAs between fungal pathogens and host plant cells is bi-directional, and this is probably the case for many plant diseases and pests.

5.6 Chloroplast delivery of HIGS

Whereas the HIGS approach has mainly relied on expression of long hairpin dsRNA in the nuclear genome, which is then

processed to siRNAs, an interesting variant is based on the fact that chloroplasts have their own genomes that are of bacterial origin. Bacterial genomes lack the RNAi machinery that are encoded in the nuclear genomes of most higher organisms. Thus, chloroplasts cannot pre-process long dsRNA into siRNAs before ingestion by a pest. In addition, because there are many chloroplasts per cell, there are far more copies of chloroplast genomes than nuclear genomes per cell. When each chloroplast genome in a plant has been selected to have copies of an introduced gene which expresses a long dsRNA, a much higher concentration of long dsRNA is ingested by a pest feeding on the plant cells. The cells are broken down in the insect gut and the long dsRNAs released into the insect, which then processes them into siRNAs by their own RNAi pathway. This can increase the effectiveness of HIGS. Recent work shows that such chloroplast-borne RNAi constructs are highly effective at controlling *Helicoverpa armigera* (budworm) over multiple generations, and hold the promise of providing durable pest resistance in crop plants [40]. Another possible advantage is that because chloroplasts are maternally inherited and so not passed on in pollen, NBTs that target the chloroplast genome address a concern of regulators and others that transgenes may be transmitted to other plants through pollen [41].

5.7 Spray-Induced Gene Silencing (SIGS)

HIGS is categorised as a GM technology since siRNA silencing constructs are integrated into the genome of plants. However, an alternative approach is possible, which is technically not breeding technology. It has been demonstrated that if dsRNA is applied topically it can enter the plant, but being RNA it is not integrated into the host genome. Further, it there is good evidence that it can move systemically in from shoot to root and vice-versa from the point of application. Delivery of dsRNA by spraying it directly onto plants (SIGS), or by otherwise applying it, avoids the need to develop transgenic plants. In effect, the applied dsRNA can be regarded as an agrochemical, and is unlikely to need to comply with all the regulations and expense that commercialisation of GM plants entails. SIG's application could be used to control pests and diseases, or control expression of endogenous plant genes [9].

The practical use of SIGS is still under development. Current research indicates that dsRNA is unstable in the environment, especially when applied under field conditions, and although it is able to enter the plant, the rate of uptake is relatively low when applied to leaves. For SIGS to be developed into an efficient method of gene silencing in the field, the stability and uptake of the dsRNA must be improved and the factors governing the systemic movement of dsRNA within the plant need to be understood. These processes are influenced by factors such as the size of the dsRNA used, the plant cuticle composition, and the mode of entry of the dsRNA into plant tissues.

The main approach being explored to address the issues of environmental stability and plant uptake is by loading the dsRNA onto anionic nanoparticles. The nanoparticles are effective in protecting the RNA from UV degradation. They may also assist in uptake and systemic movement of dsRNA in plants. One of the most promising approaches is the use of clay nanosheets by Professor Neena Mitter and colleagues at the University of Queensland [42], who showed that dsRNA can be loaded onto non-toxic, degradable, layered double hydroxide (LDH) clay nanosheets (known as 'BioClay'). When sprayed onto leaves, this treatment delayed the degradation of the dsRNA and provided sustained release to the plant. The BioClay was still detectable on sprayed leaves 30 days after application although it was ultimately washed from the leaf and degraded. The researchers developed a formulation of dsRNA active against the cucumber mosaic virus (CMV) 2b gene loaded on BioClay nanoparticles. A single foliar spray provided 20 days protection against CMV infection on the sprayed leaves. Interestingly, leaves that emerged from the plant after spraying were also resistant to the virus, indicatingindicative that the dsRNA retained its integrity and was transported through the plant in the vascular system.

These results indicate the great promise of SIGS for practical control of pathogens, insects and pests. Research also suggests the potential to modify plant development and the properties of plant produce, for example, to reduce phenolic browning or delay fruit ripening. Indeed many of the applications which currently employ transgenic RNAi could be assessed for their suitability for implementation via SIGS. The agrochemical company Monsanto, through its 'BIoDirect' technology, has been developing this strategy, for example for virus and insect control, and to control

herbicide-resistant weeds.

5.8 Genome editing

Random mutagenesis occurs naturally in all organisms. It can also be induced to occur at a much higher frequency by treating plants with physical or chemical mutagens, or by somaclonal (tissue culture induced) variation. According to the International Atomic Energy Agency, more than 3,000 plant varieties have been produced by induced mutagenesis (Mutant Variety Database, https://mvd.iaea.org/). As technology has advanced, other forms of mutagenesis have been developed, such as insertional mutagenesis, in which a transposable element is activated or a transgene may insert in and disrupt a functional gene.

However, more recently, targeted mutagenesis has become possible: individual bases at a precise site in a gene within a living cell can now be changed or deleted. Technology in this field is developing rapidly. Genome (gene) editing now provides a powerful new set of technologies which can be used to modify plant genomes, with or without the insertion of new DNA.

5.8.1 Oligonucleotide Directed Mutagenesis (ODM)

The ability to provide plant breeders with defined mutations (or single nucleotide polymorphisms, SNPs) began with the technology of Oligonucleotide Directed Mutagenesis (ODM). Here, a long oligonucleotide (Gene Repair Oligonucleotide, GRON) is introduced into a cell. The sequence is identical to part of the target gene except for a single base mismatch, and the ends are modified to prevent DNA integration. Natural mismatch repair mechanisms result in a directed mutation [43]. The GRON is eventually broken down by the cell. (Figure 7). This mechanism of gene conversion based on ODM is called RTDS^{TM} (Rapid Trait Development System) and the technology has been pioneered by the company Cibus. Canola tolerant of sulfonylurea herbicide was developed by Cibus and has been commercialised in Canada, where it is exempt from biotechnology regulation. Canadian authorities assess crops with traits produced via ODM in the same way as crops resulting from conventional breeding methods.

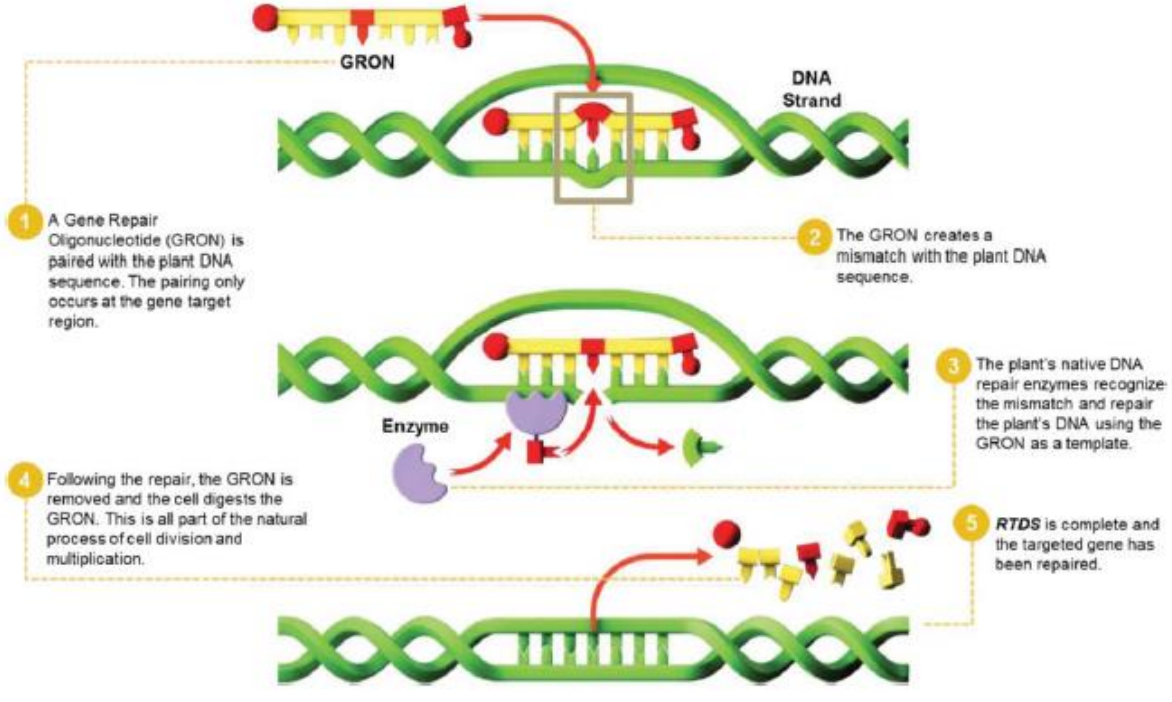

Figure 7. The process of Oligonucleotide Directed Mutagenesis, in which a single base change can be made in a gene using a long Gene Repair Oligonucleotide (GRON) [43].

5.8.2 Genome editing with Site-Directed Nucleases (SDNs)

After the invention of ODM, the quest to develop site-directed methods to modify plant genomes continued with the development of zinc finger nucleases (ZFNs) and TAL effector nucleases (TALENs). These technologies can be used to mutagenise genomes at specific loci in living cells, but each system requires two specifically designed recognition peptides that bind to the DNA flanking the target sequence to be modified. These are linked to a nuclease (Fok I) to make a double-stranded cut in the DNA. Because each target sequence needs two customised recognition peptides, which is not trivial to achieve, these methods have not been adopted widely. Their use has now been superseded by the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) -Cas9 system.

5.8.2. CRISPR-Cas9

The CRISPR-Cas9 system was developed by simplifying a bacterial defence system. In this approach a Cas9 double-stranded DNA nuclease is introduced into a cell at the same time as a synthetic nucleotide guide RNA (sgRNA), 20 bases long. The two form a DNA-RNA duplex within the cell and the Cas9 cuts both strands of the DNA molecule at the site defined by the sgRNA. The break is made beside the protospacer adjacent motif (PAM) preceding the DNA target site (Figure 8). The CRISPR-Cas9 system is a remarkably versatile and powerful tool, with many variations being developed.

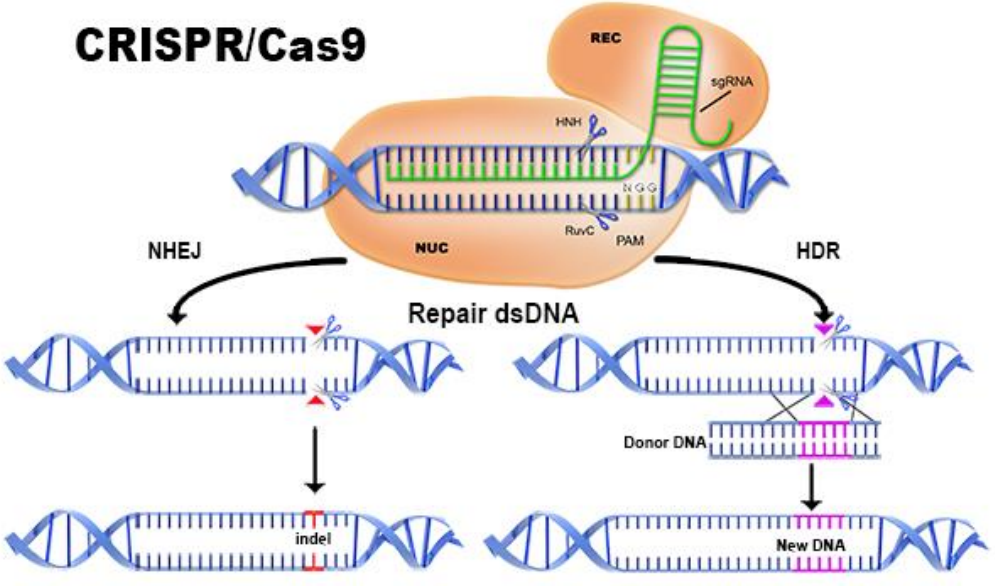

Figure 8. CRISPR-Cas9 targeted double-strand DNA break. Cleavage occurs on both strands, 3 base pairs upstream from the NGG protospacer adjacent motif (PAM) sequence on the 3' end of the target sequence. The target specific guide sg RNA sequence defines where the DNA will be cleaved, and the remainder of the RNA complexes with the Cas9 protein, shown in orange. In Non-Homologous End Joining, NHEJ insertions and deletions can occur; Homologous End Joining', HEJ, or HDR can take place of a donor oligonucleotide is present, to introduce new DNA at that site (Source: http://www.labgene.ch/img/cms/AATI/crispr-dual-editing-method.png).

This system can be applied in a variety of ways. This simplest approach is to make the cut at the target site and allow the natural repair mechanisms in the cell to repair it (a process called Non-Homologous End Joining, NHEJ). In a certain percentage of cases (5-60%), the cells repair system makes errors in joining the cut ends of DNA together, deleting or adding bases, thus generating mutations ('SNPs' or 'indels') at the cleavage site. Alternatively, a 'repair' or 'donor' oligonucleotide can be designed with a novel central sequence flanked by end sequences homologous to each side of the cleavage site. If this is introduced into the cell, a process called 'Homologous End Joining', HEJ (or HDR) takes place to repair the break and the novel central sequence becomes incorporated at the target site. The latter approach

enables the addition of single bases to the site or the insertion or DNA sequences up to the size of whole gene cassettes. The underlying process often make use of expression vectors which are non-integrative: when the DNA double strand breaks are created and the double homologous recombination achieved, there is no detectable trace of the process used to edit the resident genome - only the expected change [44].

A third approach is to introduce two sgRNA guides. This will result in two cuts in the DNA. During repair (NHEJ), the section of DNA between the two target sites may be lost. For improved efficiency, it is also possible to make changes at multiple locations at the same time, directed by different guide sgRNA sequences.

The power of CRISPR/Cas9 to cleave DNA at exact sites in the host provides the capacity to generate site specific mutations in a vastly more efficient way than the random breaks cause by classical mutagenesis. The use of the sgRNA to guide the cleavage rather than the peptide-DNA interactions makes it much more versatile, efficient and flexible than the ZFN or TALENs systems. The power of this system is demonstrated by the fact that It is possible to edit many target genes (up to 14 so far) at the same time using different guide RNA sequences [45].

Variations to this process are described under definitions (Section 3), and include: removal of a genome editing cassette; virus-induced genome editing (VIGE); agroinfiltration and ribonucleoprotein genome editing.

The different outcomes of genome editing are generally sub-classified as:

SDN-1 – non-homologous end joining (NHEJ), in which natural repair mechanisms can result in small nucleotide deletions, additions or substitutions. This is no different to the natural break-and-repair mutations that occur in cells.

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 deliberately included in the repaired sequence.

SDN-3 – is the same as SDN-2, but a longer DNA sequence is inserted, for example up to a full gene expression cassette.

Since genome editing can be achieved by different methodologies, edited plants can be generated which do not contain any integrated DNA from another source. Two examples that achieve this end are (i) introducing externally constituted Cas9 and sgRNA as a ribonucleoprotein complex, in which no DNA is present, into a cell to edit a sequence, after which the ribonucleoprotein is degraded, and (ii) removal of a gene editing cassette by crossing to the untransformed genotype and selecting lines without the cassette. The latter is possible because, unlike RNAi, the edited sequence is at a different chromosomal location from the editing cassette.

These new technologies now pose major issues for the regulators of gene technology, since they must decide whether edited plants without introduced DNA can be de-regulated. Regulation of NBTs is covered in detail later (Sections 9 and 10).

The following two Tables (Tables 3 and 4), provide a comparison of NBTs (established GM technology and Genome Editing) with conventional breeding (Table 3), and a comparison of ease of use/efficiency of NBTs (established GM technology and Genome Editing) with conventional breeding (Table 4).

5.9 Trajectory of technologies.

It is highly likely that genome editing, especially SDN1, will replace many of the applications of RNAi technology, since the latter requires insertion of a gene cassette to generate specific dsRNA sequences to silence target genes, whereas the targeted mutagenesis achieved by SDN1 does not involve any addition of external DNA. It is also highly likely that SDN2 and SDN3 technologies will replace previous transgenic technologies, since the site of any insertion of genetic material will be precise, and the gene editing cassette employed can be removed by crossing and selection.

Table 3. A comparison of NBTs (established GM technology and Genome Editing technologies) with conventional breeding

	ODM	SDN1	SDN2	SDN3 and GM	Cisgenesis	Intragenesis	Reverse breeding
Crossing	The end product of sexual crosses contain a mixture of DNA sequences from the genomes of two different, sexually compatible plants. Changes may include a range of sequence rearrangements and new combinations of genes, or introduction of genes of unknown effects from wild species. These genetic manipulations are excluded from GM regulation.			Not relevant	Not relevant	Not relevant	Not relevant
Cyto-genetic changes	Cytogenetic techniques can be used to translocate large segments of chromosomes between species, for example segments of rye chromosomes in wheat. These genetic manipulations are excluded from GM regulation.			Not relevant	Not relevant	Not relevant	Not relevant
Mutation breeding	Random changes in the genome caused by mutagenic treatment. Many more unintended mutations than desired changes. May include unintended effects on expression of other genes and *pleiotropic* effects (mutation in one gene unintentionally influencing two or more seemingly unrelated traits). Mutation breeding is excluded from GM regulation.			Not relevant			
Established GM	Not relevant			In established GM, a gene or genes, or a partial gene sequence (RNAi) from an unrelated organism or plant is introduced.	GM plants are cisgenic if they contain a cassette with a gene from a compatible species.	GM plants are intragenic if they contain a cassette with gene sequences from the same species.	
Genome editing	The end product either contains a single or a few base changes in a target gene (ODM, SDN1, although for SDN1 the extent of the mutation is variable) or sequences with a history of safe usage (SDN2). Low level or no unintended effects: these are detectable if present. If there is unintended integration of exogenous DNA (ODM or SDN1), this can be removed in one generation by backcrossing. No introduced DNA present in the end product. Products of ODM and SDN1 indistinguishable from mutations or products of conventional breeding.			In SDN3, a gene encoding a desired trait is intentionally inserted at a specific site in the genome. The genome editing cassette can be removed in one generation by backcrossing.	One or a few cisgenes (alleles from a sexually compatible species) are present in the end product.	One or a few intragenes (DNA sequences or alleles from the same or compatible species) present in the end product.	A transgene is only present as an intermediate step to prevent meiotic Recombination. End products are doubled haploids with no exogenous DNA.

Table 4. A comparison of ease of use/efficiency of NBTs (established GM technology and Genome Editing technologies) with conventional breeding

	ODM	SDN1	SDN2	SDN3 and GM	Cisgenesis	Intragenesis	Reverse breeding
Crossing	Conventional plant breeding is time-consuming and expensive, especially so for tree crops. It can be made faster using marker-assisted breeding and whole genome selection as markers spanning genomes are developed, and variants of desired alleles are found. End products have additional alleles of many other genes throughout the genome in addition to those intended to be combined.			Not relevant	Not relevant	Not relevant	Not relevant
Mutation breeding	The technique is simpler than SDN1 and 2, but has a low efficiency. Compared with mutation breeding which requires screening of thousands of mutant lines, genome editing is more efficient because screening simplified (can save 6 to 8 years to obtain a commercial variety, depending on the species, the targeted genomic locus, the new desired trait and on whether a desired trait can be identified readily). Since most genome editing applications require an *in vitro* phase, ease of use depends on how amenable the target plants are for regeneration from *in vitro* culture.			Not relevant			
Established GM	All the genetic changes that can be achieved by ODM, SDN1 and SDN2, and more, can be achieved by established GM technologies, but since they contain DNA from other sources they are regulated under current Gene Technology Regulations. The developmental processes can be quick, but the regulatory hurdles and public acceptance issues continue to limit their use on minor crops.			As for ODM, SDN1 and SDN2, including combining stacked genes and RNAi technology. 10% of the world's crops fall in this category, and this situation will continue. However, many RNAi based end products will now be developed using SDN1 or SDN2 technology, achieving the same target gene silencing, but with end products lacking introduced DNA.	Established GM technologies can be used to generate cisgenic GM plants. Acceptance is improved compared with GM plants containing unrelated genes.	Established GM technologies can be used to generate intragenic GM plants. Acceptance is improved compared with GM plants containing unrelated genes, and marginally so than cisgenic plants.	Although there is a temporary transgenic phase, the final products are not GM. If this technology were filly developed it would be very attractive to breeders.
Genome editing	Plants without exogenous DNA and with only limited targeted changes to a specific gene or genes. This is in contrast to the numerous random mutations in plants obtained by mutation breeding. ODM allows the directed modification of several nucleotides which is impossible through mutation breeding. It can be replaced by SDN1 or SDN2 which are much more precise and efficient. These technologies are the most likely to be applied to vegetable crops. SDN1 has already been used to generate some commercial plants. Of the different genome editing systems, the CRISPR-based systems are the easiest to handle and the most efficient.			SDN3 is likely to generate GM plants with gene(s) introduced at a precise location much faster because of the limited need for backcrossing and targeted insertion of DNA at a precise location and so will be less costly to generate. The genome editing cassette can be crossed out for many crops, but the nature of the introduced DNA means that release of products of SDN3 technology will still be regulated.	Much more efficient than introduction of the same alleles by sexual crossing (if possible) because backcrossing is not needed, and the desired allele is introduced without any linkage drag of other unwanted alleles, as occurs with sexual crossing.	The products obtained by Intragenesis may be achievable by chance after sexual crossing, but not in a specific and targeted manner.	Parental lines cannot be re-constituted from the progeny of a conventional cross. Reverse breeding offers to do just that. However, data on the application of this technique are limited.

5.8.3 Synthetic Gene Drives

A recent discussion paper from the Australian Academy of Science (www.science.org.au/gene-drives, May 2017) describes gene drive mechanisms (or synthetic gene drives) as mechanisms which 'cause a gene to spread throughout a population at a rate higher than would normally occur'. The paper also highlights the potential benefits, hazards and possible applications of synthetic gene drives and how the technology might be managed in Australia's governance arrangements.

In Mendelian genetics, there is a 50% chance of offspring inheriting a modified gene from their parents, but for a gene drive mechanism, modified genes may eventually be inherited by all offspring (Figure 9, www.nature.com).

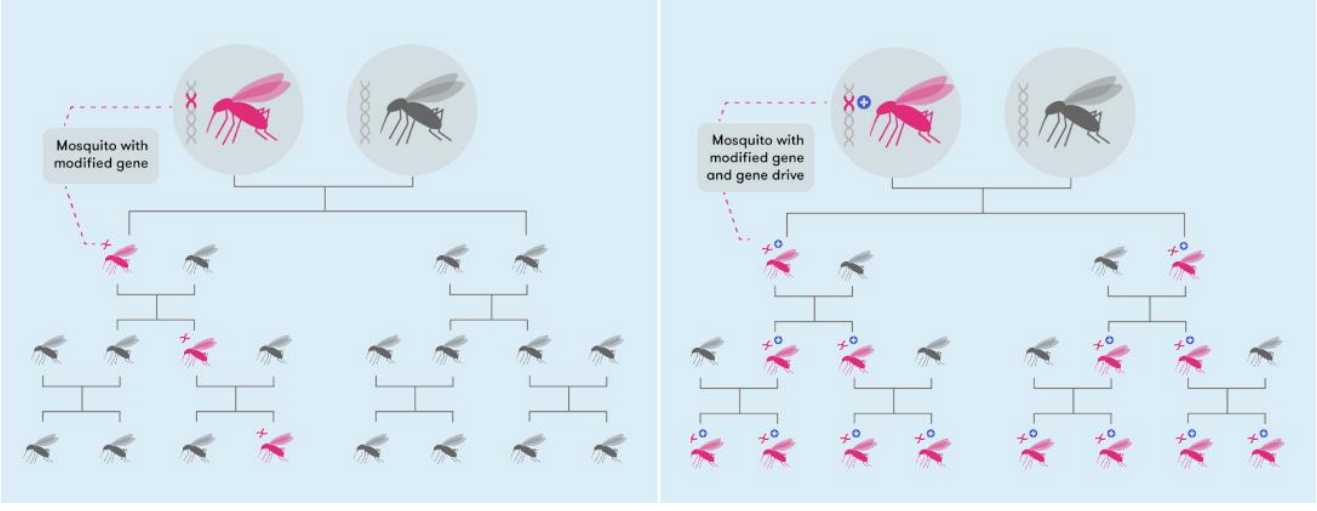

Figure 9. A comparison of the spread of a gene by Mendelian genetics (left) with potential spread of the gene via a synthetic gene drive.

Potential applications in horticulture include insect pest control by releasing pests carrying a potentially lethal gene. Mating with the wild population leads to the spread of the lethal gene through subsequent generations. Insects carrying such gene drives have not yet been released.

5.8.3.1 How a synthetic gene drives work

It is important to note that genome editing and gene drives are significantly different. To generate a synthetic gene drive, both the guide RNA and Cas9 protein are introduced and stably integrated into the DNA of an organism resulting in a GMO. The guide RNA directs the Cas9 to cut both strands of the target DNA, which in turn triggers the cell repair mechanisms to copy the whole inserted construct. The inserted cassette can then be passed on to progeny to edit a target gene in subsequent generations.

This process is illustrated in Figure 10, in which a sg (guide RNA), Cas9 (DNAse) and a 'cargo' gene (an introduced sequence to be inherited in subsequent generations) is inserted in the target (TARGET) gene sequence. The guide RNA then directs the Cas9 to make a double cut in the other chromosome, and the cell repair machinery copies the whole cassette.

One limitation to the application of gene drives is that the process can only work if an organism repairs double-stranded DNA breaks by the mechanism of homologous recombination, and for some plants, such DNA repair occurs principally by non-homologous end joining.

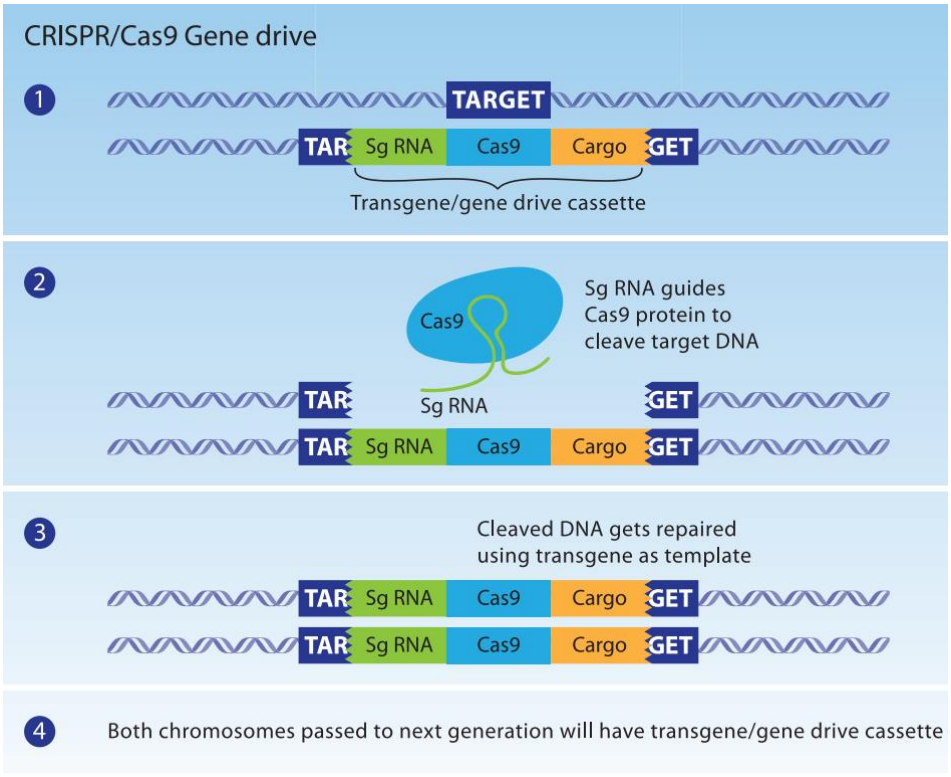

Figure 10. A representation of a mechanism for a synthetic gene drive. From: [46], www.science.org.au/gene-drives

5.8.3.2 Examples of potential applications of synthetic gene drives in agriculture

An exciting potential application for synthetic gene drive is the introduction of mutations that reverse pesticide resistance or herbicide tolerance thus improving the capacity to control pests and weeds using currently available conventional options. Susceptible pests include fruit flies, various moths, mites, thrips and other pest invertebrates which attack vegetables and broad acre crops. Another approach of great potential impact would be gene drives designed to modify insect or nematode vectors to remove or reduce their ability to transmit major plant viruses.

The following Table (Table 5) indicates some possible applications of synthetic gene drive technology to the vegetable industry (modified from www.science.org.au/gene-drives)

Table 5. Potential applications of synthetic gene drive technology in agriculture

Invasive species and the environment

Problem	Examples of current solutions	Potential problems with current solutions	Potential beneficial consequences of gene drive
Invasive Species, biosecurity	Traps and poisons, and other vector control strategies	Invasive or introduced plants, pathogens or insects can cause major crop losses. Chemical or other forms of control are often non-selective, and vector control strategies can be costly	A gene drive to control an economically devastating invasive species pest or biosecurity threat could be used to remove the pest or reduce or prevent a

		to implement.	biosecurity threat.
		Agricultural applications	
Agricultural pests	Spraying of pesticides.	Spraying of chemicals damages biodiversity and decreases beneficial invertebrates because of the non-selective nature of many chemicals. Pesticides become ineffective when resistance evolves.	A gene drive to eliminate a weed or pest could reduce chemical spraying and potentially increase farmer's crop yields.

5.8.3.3 Challenges posed by synthetic gene drives

Gene drives pose some specific issues regarding their development and application, including risk assessment and public acceptance, especially in relation to possibly environmental consequences [47]. However, the technology is advancing rapidly, and there are methods of 'molecular confinement' which can be used to restrict the gene drive to the target species, reduce its ability to spread or confine the drive to a local population. These include 'split' gene drives and 'daisy chain' drives, which would stop the gene drive after a few generations. Synthetic gene drive will be regulated under current GM regulations. The OGTR is presently reviewing its regulations as they apply to gene drive technology.

6. The Australian vegetable industry

Australia has a diverse vegetable production industry. The wide range of climatic zones across the country means a great variety of species can be successfully grown. However, given that the historical development of the industry was around Australia's major cities, the dispersed geographical distribution of these centres combine with the variation in climate and soils, means that common species are grown under a diverse range of different stresses. While this diversity has many advantages, it also presents distinct challenges.

Structure: Australian horticultural production has historically been predominantly based on the family farm. In recent decades there has been substantial consolidation of businesses. Although the majority of businesses are still family owned, there has also been an increase in other business types within the sector. In addition, there has been the development of a range of trading groups such as purchasing or marketing cooperatives. These vary in size, structure and formality.

Coincident with the trend to consolidation, there has been an increasing effort, although still nascent, to develop brand identity, either on an individual basis or through a trading group. In some cases, these reflect provenance.

Current production and markets: The value of Australian vegetable production is some $3.5 billion pa. Processing and packing contributes further to the economy, although processing is not strongly developed. Commodities with the highest annual value of production are tomatoes, potatoes, mushrooms and salad leaf (Table 6).

Table 6. Value of production for the top ten vegetables in Australia for 2014-15.

Commodity*	Value (AUD millions)
Potatoes	445.3
Mushrooms	349.0
Salad leaf vegetables	315.3
Carrots	190.4
Cucumbers	183.5
Capsicums	144.7
Onions	135.5
Lettuce (hearting)	131.2
Broccoli (including baby)	122.5

*Tomatoes, at a value of $548M, are not shown because they are not an HIA commodity

Although vegetable production occurs in all states, Victoria and Queensland are the largest producers. Production tends to be loosely peri-urban but location is also influenced by climate, land availability and access to irrigation. Given Australia's range of climatic zones, the key crop species vary geographically. Thus, potatoes are grown mainly in South Australia, Tasmania and Victoria, and mushrooms in NSW and Victoria.

Some 93% of Australian vegetable production is consumed domestically, with growth in sales largely linked to population changes. As a result, the total value of vegetable production remained steady at between $3.0 and $3.5 billion for the eight years between 2007/08 and 2014/15 (Australian Bureau of Statistics, 2016). Although only around 7% of the total Australian vegetable production is exported, this component is growing much more rapidly than domestic sales. While domestic returns remain steady, export earnings have increased by about 60% in the last decade.

Australia exports vegetables to over sixty countries, with a strong focus on countries in Asia. Currently, the most important destination countries in terms of total value are Japan, New Zealand, Singapore and the UAE (Figure 11).

Destinations vary widely in terms of key commodities: Japan = asparagus; Malaysia, Singapore and UAE= carrots; Indonesia and Thailand = potatoes; Germany = onions. About 60% of the value of export is fresh produce.

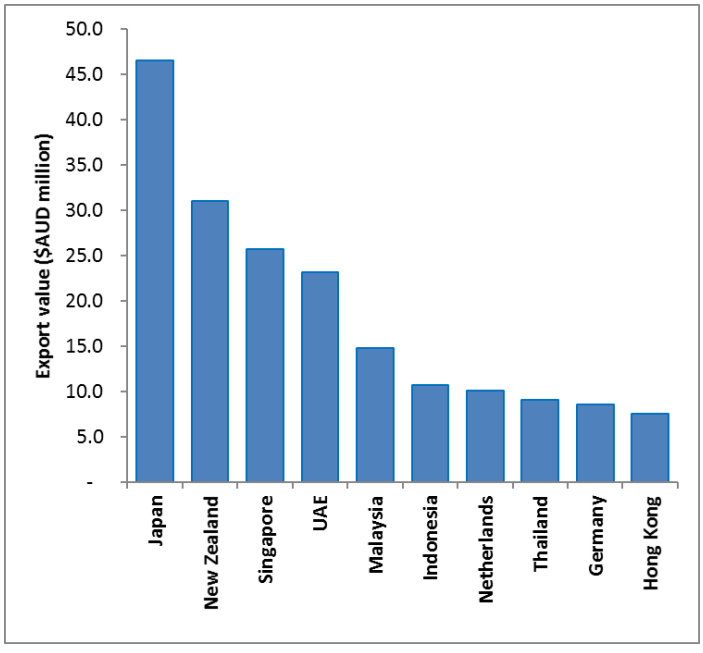

Figure 11. The value of Australian vegetable exports to the top ten destination countries (2013-14).

Export development: With limited potential for growth in the domestic market, increasing vegetable exports will be the primary component of industry growth into the future. With urbanisation, the rising incomes of the middle class and changing consumer preferences, the improved living standards in Asia provide Australia with an unprecedented opportunity to supply the region with high quality, safe and traceable horticultural products. By 2030 it is predicted that two-thirds of the world's middle class will be located in Asia and that the rapidly growing markets, such as Asia, will comprise 63% of global GDP. As incomes rise, consumers in the premium end of the market not only demand greater quality but greater quality assurance and established traceability systems. Health, indulgence, convenience and provenance become more significant factors in shaping consumer preferences. While the key drivers of growth in Asian demand are population, urbanisation and economic development, from the point of view of Australian exports, these are underpinned by the current efforts to liberalise global trade.

Although Australia's international reputation for safe, high quality food from environmentally sensitive production systems gives it good standing in export markets, production and transport costs present a significant impediment. To overcome these constraints and continue to increase export sales, Australia must be able to present a distinctive product with high consumer demand at competitive prices.

NBTs in the Australian vegetable industry? Australia is recognized as having strong plant breeding and genetics skills and has contributed to the development and early implementation of a number of new technologies in these disciplines. Engaging this capacity to generate vegetable varieties with novel traits (e.g. specific health benefits), improved field performance, or superior quality could provide significant advantages for Australian producers. An analysis of the benefits of GM crops showed that growers' profits increased by an average of 68% through a combination of increased yields and reduced agrochemical costs [31]. The change however, was greater in developing than developed countries and would clearly depend on the particular traits in use. The Australian vegetable industry has been under economic pressure for a number of years; the potential economic impact of new technologies on farm

profitability must be seriously evaluated.

While increased yield and decreased input costs will have a positive benefit for producers and consumers regardless of the market, the development of novel traits and superior quality will be of particular benefit in efforts to establish and expand export markets. Not engaging with the opportunity presented by NBTs may result in Australian producers loosing market development opportunities to competitors able to present distinctive products or who are able to provide similar quality at lower cost. The implications for competition between imported lines and Australian products in the domestic market must also be considered.

However, the nature of the Australian vegetable industry presents a number of challenges to successfully pursuing the prospects offered by NBTs. Due to the relatively small size of the Australian industry it has proved difficult for commercial breeding companies. This is not unique to vegetable industries; breeding programs for broad-acre crops are also facing challenges. There are now very few substantial commercial vegetable breeding operations in Australia. The situation is exacerbated by the diversity of environments under which any given crop is produced. Typically, advanced breeding lines or varieties are sourced from large overseas companies and screened for suitability to Australian growing environments and markets. In some cases feed-back channels have been established to allow Australian agents to contribute to the priorities of the overseas programs. For the implementation of NBTs, access to a successful breeding program producing high quality, appropriately adapted genotypes is essential. Thus, partnerships with overseas entities is the most likely path forward. This situation is reinforced by the small number of large enterprises within the Australian vegetable industry that could support research, development or commercialisation.

Conclusion: Australia has a diverse vegetable production industry that is fundamental to supporting its large population centres. However, the domestic market offers little scope for industry growth. NBTs provide the opportunity for a step change in on-farm profitability. Further, the diversity of the Australian production environment and its reputation for safe, high quality produce places us in an excellent position to capitalize on opportunities in the expanding markets of Asia. However, to do so we must be able to present distinctive, high quality produce at competitive prices. The implementation of NBTs provides an exciting opportunity to establish a unique competitive advantage.

7. Consultation with industry stakeholders

7.1 Consultation with researchers

We spoke with leading Australian researchers who have worked on the genetic improvement of vegetables or the application of NBTs to plant improvement. In general, responses were strongly positive with regards the potential of NBTs to contribute to Australian plant industries and vegetable production in particular. The technological capacity for NBTs to contribute to genetic gain was seen as very high.

Perceived industry attitude: Researchers perceived that currently, the industry is receptive to the use of NBTs. It was considered that there was much more concern on whether the resultant variety was likely to be classified as 'genetically modified'. This concern was based on any potential impact on customer acceptance (this aspect is discussed below). It was felt that beyond the question of consumer acceptance, growers want the best possible traits in varieties, and were generally less interested in the method by which the variety was generated.

Researchers also felt that the vegetable industry was very positive about their particular research. This was based on enthusiastic responses from growers regarding their research and the willingness of commercial organisations operating in the industry to fund or partner in research activities. However, the strong support did not preclude opposition and some researchers indicated that there was an entrenched minority in the industry in which the researcher worked that expressed strong opposition to NBT research activity. Some individual researchers who worked closely with their industry considered that growers in this minority were opposed to a range of innovations, not only to NBTs.

Importantly, some commercial enterprises have been willing to support research activities even before a clear path to market has been defined. This included a number of large overseas and multinational organisations. Some of these organisations also have active research programs of their own.

Consumer attitudes: There was believed to be a definite gradation in terms of likely consumer acceptance of different types of products. It was considered that plants developed using NBTs would meet very little resistance if the end product was not for human consumption; for example, ornamental products and essential oils. Where the product undergoes significant processing before being consumed by humans (e.g. extraction of edible oils), while some opposition may occur, this is not expected to be a major issue. It was perceived that there was most likely to be resistance when NBT-generated products are eaten in an un-modified state. This would include many vegetables.

It was also perceived that consumers in Australia are more conservative relative to consumers in many other countries although it was acknowledged that consumer attitudes varied dramatically among other countries.

A strong view was expressed that consumers had limited understanding of the difference between various plant improvement technologies and that a thoughtfully developed education campaign could significantly change public attitudes to the products of NBTs for the benefit of all stakeholders.

Changing attitudes: All researchers believed that attitudes towards novel plant improvement technologies were changing and that this change would continue. When questioned with regard timeframes, most considered that food crops produced using NBTs could be on the market in Australia within 10 years. This was partly seen as resulting from a general trend, but also due to a generational change because younger people are more familiar with the technologies. However, a researcher from Tasmania expressed the view that food crops produced using NBTs were "simply not going to happen" in that state, although there was potential for relaxation of regulations and attitudes around non-food NBT-produced crops.

The widely held view that NBT food crops would be in use in the medium term was held to be true for many other countries, with examples cited of NBT food crops already in the market place in countries in Africa, Asia, the Americas and the Pacific. Europe was seen as a distinctive exception. It was also perceived that if resistance did persist, a challenge to the international food supply, particularly around core commodities, would most likely precipitate industry and market uptake.

Potential applications: Researchers were asked to nominate potential areas in which they could see NBTs making a substantial contribution to the Australian vegetable industry. The most common responses related to improved plant defence against pests and diseases, and product quality improvement. Potential for contribution to the control of intransigent diseases and diseases known to be developing high levels of resistance to the existing suite of chemicals were seen as particularly important.

A significant constraint to the use of NBT crops was seen as the cost of bringing NBT products to market. For this reason, it was considered that initial applications would be for 'world crops' and diseases or abiotic stressors such as tolerance to water deficit or salty soils, which affect multiple crops.

NBTs are seen to have a special role in the improvement of heterozygous, outcrossing crops that are vegetatively propagated. This is particularly the case where cultivars have gained a high level of consumer acceptance. For example, some potatoes cultivars have held significant market share for decades, and maintaining that status is of significant commercial value. NBTs provide the only opportunity to introduce improvements without losing the overall genetic package and the associated high market standing.

Choice of Technologies: In discussions with researchers, two technologies stood out as holding particular promise: CRISPR-Cas9 and whole-genome selection (although the latter does not fall within the accepted definition of NBTs). CRISPR-Cas9 is seen as a particularly powerful method both for gene silencing and for the introduction of novel genetic material; be it trans-, cis- or intragenic. The approach is considered simple and efficient, and has a low risk of unwanted genetic alterations. Whole-genome selection provides scope to radically increase the efficiency of both conventional breeding and the implementation of NBTs through the efficient selection of material for sequential crossing and dramatically reducing the number of generations of subsequent selection before a variety can be released.

7.3 Consultation with plant breeders and seed merchants

Vegetable breeders and seed distribution agencies have a critical role in the introduction and ongoing use of new varieties. In comparison to grain crops, there is much less seed retention by vegetable growers; seed predominantly being purchased from a supplier every season.

The most important potential targets for NBT manipulation as nominated by breeders related to crop protection, especially diseases, and product quality traits including shelf life and reducing wastage.

GM versus non-GM: The vegetable seed merchants and vegetable breeders who were interviewed held a conservative but open stance toward NBTs. As with other stakeholders, the concerns of breeders centred on the question of whether or not a product would be classified as GM, the major consideration being any potential influence on market access. If NBTs were not regulated as GM and did not require specific labelling, the expectation is that growers, retailers and consumers would see no difficulty and any improved crop or product performance would be well received. Breeders believed that growers were much more focused on the performance of a variety in the field than the technology used to develop the variety.

As the peak industry body, the position of the Australian Seed Federation (ASF) is (i) to support the choice of members on whether or not they engage with crop biotechnology and (ii) that members should respect the right of others to exercise their choice. ASF members include organisations that have chosen to engage fully with advances in crop biotechnology, those who have chosen not to be involved, and those who have adopted various 'wait and see' positions.

All the breeders have explored, or continue to explore, the potential for NBTs to contribute to their breeding programs, with the specific intention of producing non-GM varieties. The two key questions are the current regulatory uncertainty and the cost of commercial implementation.

Cost of implementation: The total cost of bringing a regulated product to market includes regulatory cost, additional promotional cost, industry stewardship programs and the capacity to cover risk. Breeders considered it unlikely that a small breeding company will have the financial or technical capacity to bring products to market without the support of

a larger entity. It is therefore seen as imperative that before embarking on the implementation of NBT technology, a clear path to market is planned that includes a large organisation that has the financial capacity and skills required. Critical to this process will be formulating a clear intellectual property position and commercial access agreements prior to commencing development.

Some breeders (and other industry operators) also considered that the involvement of large international breeding companies is needed to provide access to elite genotype packages into which specific traits could be incorporated using NBTs. In contrast, some companies want to be able to access publicly-funded NBT research, under licensing agreements, to incorporate new traits into their own varieties. In either case, it was considered essential that excellent conventional breeding programs must be maintained as a basis to underpin any NBT activities.

Some breeders also expressed the view that there was significant scope remaining to explore the existing gene-pool within vegetable species and their wild relatives; a number of companies investing independently in the collection of wild germplasm. However, no specific data were presented or cited to support the view that genetic diversity within the primary gene pool of vegetable species is more poorly utilised than that of other crops. This may be worth further assessment. Associated with this idea is the question of whether the most cost effective approach to developing new traits is *via* conventional breeding or NBTs.

Regulatory uncertainty: Current regulatory uncertainty was seen as a major difficulty to the development of genotypes using NBT and to a lesser extent to the initiation of significant research and development activities in the field. Breeders saw the final classification of a product as having a substantial influence on the feasibility of bringing a product to market because of both the direct financial cost of regulatory approval and the influence on consumer acceptance of the products of NBTs.

To provide regulatory clarity and to minimise the possible impediment to innovation, the ASF propose that:

> *"the genetic variation in a final plant product should be excluded from regulation under the Gene Technology Act 2000 where:*
>
> *a) there is no novel combination of genetic material (i.e. there is no stable insertion into the plant genome of one or more genes that are part of a designed genetic construct) or*
>
> *b) the final plant product solely contains the stable insertion of inherited genetic material from sexually compatible plant species or*
>
> *c) the genetic variation is the result of spontaneous or induced mutagenesis. "*

Overall then, the representatives interviewed were positive about acceptance of genotypes developed using NBTs so long as they were not classified as GM varieties. Given the costs of bringing a product to market, the involvement of some large entities (commercial or otherwise) early in the development process is seen as imperative.

7.3. Consultations with Growers

In general, leading growers were less positive about the practical potential for new breeding technologies to contribute to the Australian vegetable industry. The most frequently and most strongly expressed concern was the problem of consumer acceptance. The second most commonly expressed concern was the cost involved in developing new technologies for the wide diversity of crops grown in small amounts. This was seen as a particular challenge where the number of medium and large producers in Australia may be very small.

GM versus Non-GM: No interviewees expressed concern with the products of NBT *per se*, and most growers recognised the very great technical scope for NBTs to deliver varieties with improved traits. However, there was very strong opposition to the introduction of GM varieties if there was not a positive change in consumer attitudes to GM fresh

food. Therefore, if a particular NBT (or specific product of an NBT process) was classified as GM, if there was a need to label, or if a particular NBT was perceived as being associated with GM, there was scepticism about the potential to successfully deliver the product into the Australian fresh market. Conversely, if NBTs were not classified as GM, growers would be very happy to access the potential benefits.

Consumer Focus: One grower likened the problem of introducing GM or NBT varieties to the choices presented between conventional genotypes of differing characteristics. He explained that he currently grew a variety that was agronomically more challenging and had a somewhat lower yield than the main alternative because the more challenging variety had much higher consumer acceptance: "It is all about the consumer". Growers who had close relationships with large retailers also felt that the primary supermarket operators would take a conservative view, reflecting the perceived attitude of the consumer.

In light of consumer concerns, one grower suggested that the traits which should lead the way in the introduction NBTs should be traits that directly provided consumer benefits, such as significantly improved shelf-life, additional health benefits, product appearance or flavour. It was felt that traits that provided a primary benefit to the grower, and therefore only indirectly benefitted the consumer, would have little chance of acceptance. However, traits aimed at the consumer, such as better taste, nutrition, shelf life, etc, could also provide benefit to growers via a competitive advantage in the market place.

The growers interviewed also had a more negative attitude regarding the rate of change in the attitudes of consumers towards the implementation of GM. Most considered that, without the advent of a significant food shortage, a considerable education campaign would be necessary to gain worthwhile change. With regards consumer acceptance of non-GM products developed using NBT technologies, growers generally felt that such products would not be influenced by consumer attitudes. However, discussions with social scientists suggest caution in assuming that there will not be a reaction. This is discussed in Section 7.4.

Cost of implementation: Consistent with attitudes expressed by plant breeders and some researchers, a level of concern was also expressed regarding the cost of implementing NBTs for Australian vegetable production. There are few vegetable species for which particularly large volumes are produced and in some cases the number of producers is small. In addition, the suite of primary challenges for crop production varies geographically across the country. As suggested by breeders and researchers, close collaboration with international breeding companies will be very important.

Target traits: In terms of target traits, growers saw consumer traits as important in their own right as well as for encouraging consumer acceptance. In terms of production traits, opinions were diverse and reflected local issues. Desired new traits included yield and vigour, resistance to abiotic and biotic stresses, traits targeted to increase or improve the consistency of the marketable proportion of the yield (pack out) and traits to improve the efficiency of resource use.

7.4. Australian consumer attitudes

This section has been developed after discussions with social scientists working in the area and issues raised by the other stakeholders interviewed. It also incorporates consideration of recent relevant literature.

Across all groups consulted, there was consistent recognition that the critical hurdle for the implementation of NBTs in the Australian vegetable industry will be public/consumer acceptance. All stakeholders held the view that very few people would currently understand what is encompassed within the definition of NBTs and that fewer still would have any basis on which to form an opinion of food safety. Stakeholder concerns regarding their likely acceptance relate to the current negative attitude of the public towards GM food and whether non-GM varieties developed using NBTs will be differentiated from GM varieties. The question of differentiation will have two components: (i) whether the non-integrative NBTs as a class of technologies will be perceived by society simply as an extension of existing GM technology and (ii) whether products of particular NBTs will be explicitly classified as GM by regulatory bodies. Differentiation from

'traditional' GM varieties was the most frequently expressed concern of researchers, plant breeders, growers and other industry operators.

Consumer attitudes to GM: Given the concern expressed about differentiation of NBTs from currently adopted GM technologies (referred to as GM forthwith), it is pertinent to consider the current status of public acceptance of GM food in Australia.

There is very limited reliable data available on the attitudes of Australian society toward GM food and virtually no information available on attitudes towards food from other NBTs.

Many of the researchers we interviewed expressed the opinion that the public were becoming more accepting of GM foods. In contrast, leading producers felt there had been little positive movement and expected little change in the future. FSANZ, OGTR and state governments now receive fewer public responses or comments regarding the safety of GM crops or foods than in the past. However, this change cannot necessarily be taken to reflect increased acceptance; it may equally reflect a shift in focus to other issues or an increasing sense of disenfranchisement.

Survey results show that Australians have significant concerns about GM food [48,49]. The Swinburne National Science and Technology Monitor used a scale of 0 (not at all comfortable) to 10 (very comfortable) to gauge consumer feeling toward GM. The results show that consumers remain somewhat uncomfortable (score ≈4) about the use of GM plants for food and this improved only slightly over the nine-year period from 2004 to 2012 [49].

World-wide, the best information available on consumer acceptance is from the Eurobarometer survey comprising data for eight time points at irregular intervals between 1978 and 2010. The data set shows a dramatic decline in support for biotechnology in general between 1993 and 1999 [50]. Although attitudes toward biotechnology in general now appear substantially higher, support for GM food is still low and is showing little sign of increasing: in 2005, 27% of respondents felt that GM foods should be supported and in 2010, support was only 23% [51]. Extending this by considering anecdotal information about recent developments, Lucht concluded there was

> *"a hardening of the negative environment for agricultural biotechnology in Europe, a growing discussion—including calls for labelling of GM food—in the USA, and a careful development in China towards a possible authorisation of GM rice that takes the societal discussions into account"* [51].

Importantly, although survey results show a low level of acceptance of GM foods in Europe, there is reason to believe that this data gives an unduly negative assessment. Experimental purchasing scenarios show a greater willingness to purchase GM food than reflected by the surveys [52]. Further, actual purchasing data show that purchases do not reflect survey results: little avoidance of GM foods took place, even where there was obligatory labelling [53]. No work has been found that measures the actual purchasing choices made in Australia in response to the presence of GM foods in the market place. It will be necessary to allow for the exaggerated negativity of survey data when interpreting the results as a guide for industry decision making.

The concern in perspective: The level of concern over the use of GM in foods needs to be put into a wider context. Problems of an environmental nature figure much more strongly in the minds of the Australian public than the use of GM in food [54]. When asked to select the most important issue for Australia as a whole, five times as many people selected water shortage or using-up natural resources as selected GM food. Climate change was selected three times more often (Figure 8). Further, when Australians were asked to rate their level of concern about various food integrity issues on a scale of 1 to 7 (1 = not concerned, 7 = extremely concerned), the use of genetic engineering was rated similarly to all the other issue (Figure 12; [55]).

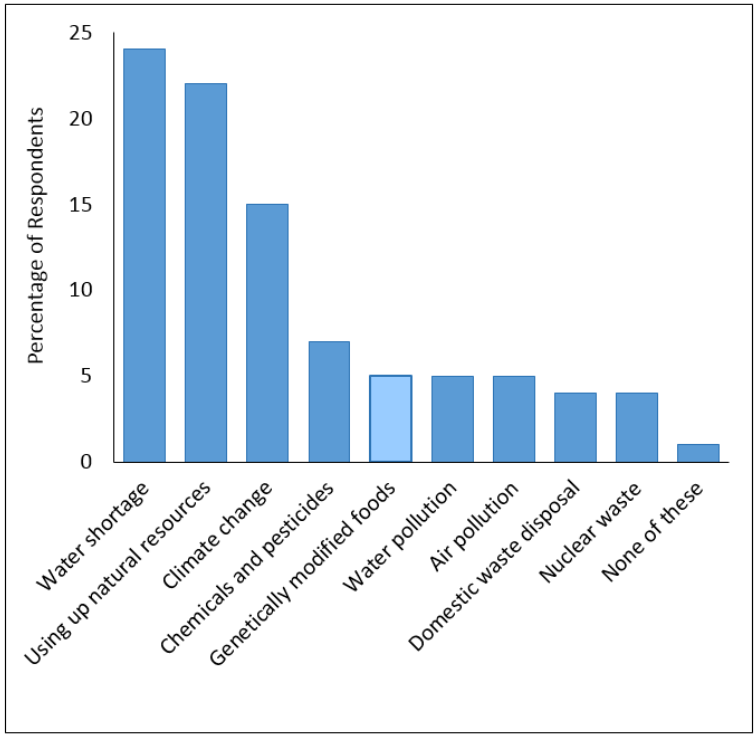

Figure 12. Perceptions of the most important issues for Australia. Percentage of respondents who perceived different issues as the most important for Australia as a whole. Data for 2010 [54].

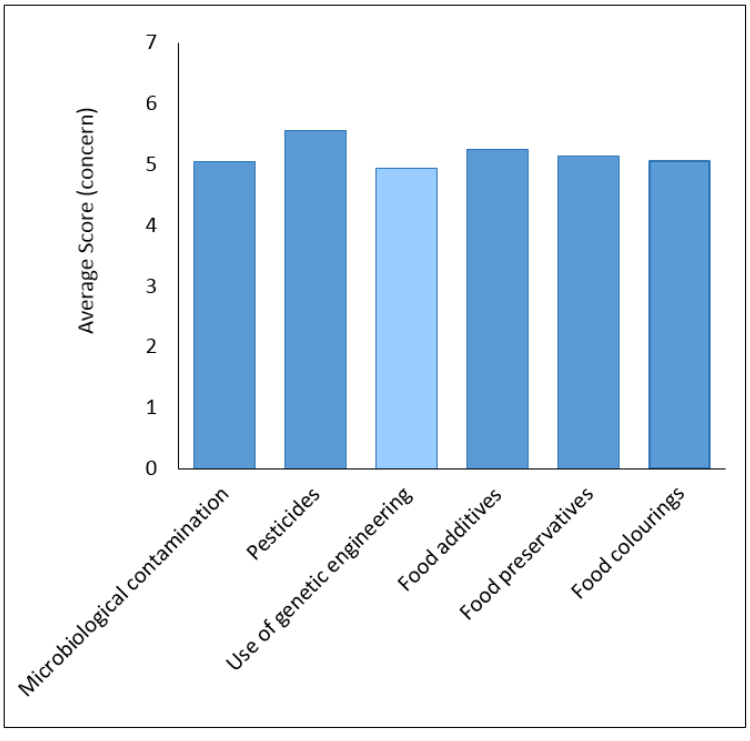

Figure 13. The level of concern among Australian consumers regarding six food integrity issues (1 = not concerned, 7 = extremely concerned). Data from [55].

Not surprisingly, the findings of these two surveys align with the Eurobarometer data on the attitudes of consumers to different potential applications of GM in foods. Support was gauged in terms of whether a consumer would purchase GM food. Willingness to purchase GM foods in general was 37.3% and respondents indicated that this would not be altered by lower prices (in contrast to the results of purchasing studies). However, willingness to purchase increased to 48.8% if the GM food was more environmentally friendly, 50.7% if there was less risk of pesticide residues and 54.8% if it was healthier [56].

Untangling the complexity of public attitudes towards agricultural biotechnology

From many years of surveying public attitudes towards agricultural biotechnology, Cormick and Romananch [48] concluded that simple figures alone do not do justice to the complexity of ways that the public view GM technologies in agriculture. Attitudes vary with gender, age, level of education, country; the intended end use, e.g. non-food products vs food products (processed or non- processed); personal risk and benefits analysis; trust of scientific institutions and regulators; receptiveness towards science and technology, and how media present technology. They summarised public attitudes under four distinct consumer segments. 1. The concerned and disengaged, 2. The risk averse, 3. The cautiously keen and 4. The science fans. The responses of these consumer segments to a series to questions is summarised in Figure 14.

Results of the survey show the values that most predict acceptance or rejection of biotechnology applications, also provide some insight into the best way to engage best with different segments, by aligning messages with their values of concern.

For example, the 'Concerned and Disengaged' would be more receptive to messages along the lines that: 'biotechnology allows nature to adapt to our changing global climate'.

The Risk Adverse would be more receptive to messages of balancing the benefits of any biotechnology applications with strict regulation and safeguards, and long-term testing.

Cormick and Romananch (2014) consider that with awareness of such segments it is possible to determine if interactions are predominantly 'preaching to the converted' – who tend to self-select for most science engagement activities – but allows understanding of the values and attitudes of people with whom one might not normally communicate, and also to make our messages more receptive to differing segments by aligning them with their values.

In summary, there is some evidence from survey data and personal discussions that there is an easing in public opposition to GM/biotech foods at least in Australia, but not in Europe. However, the level of concern as reflected by survey data is more negative than indicated by actual patterns of purchase. Further, the willingness to purchase a GM product is expected to vary depending on the function of the specific trait and price differentials with competing products.

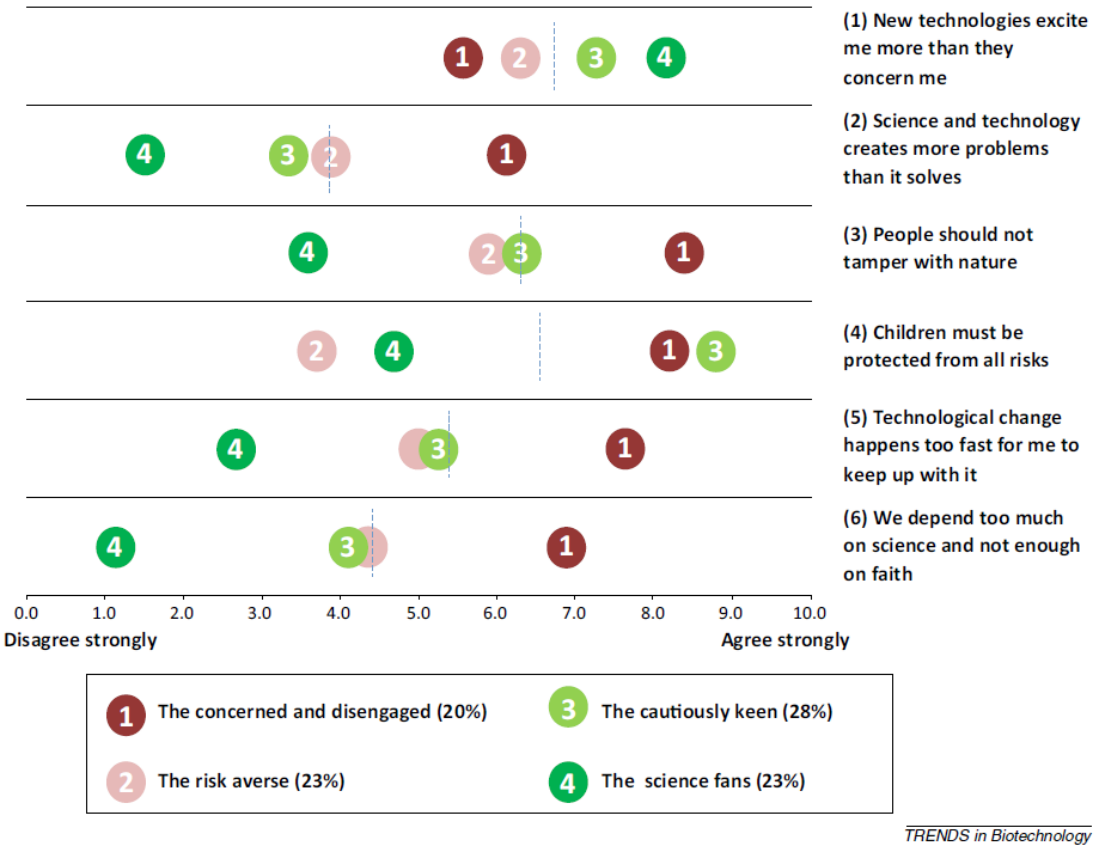

Figure 14. Mapping the spread of segments across value statements indicates that different values segments align with different values statements in different ways. Of note, the segment order of 1 through to 4 does not always occur in that order, and also Segments 1 and 4 tend to be at extreme lengths from the average point of the population, indicating the gap that exists between people in those values segments trying to understand the values of the other [48].

The need for a positive dialogue: The view was presented by a number of researchers and plant breeders that if NBTs avoided regulatory classification as GM, then a 'business as usual' strategy could be adopted and that producers, retailers and consumers would be likely to express little interest in the methodology by which a variety was developed. While this may be true, the approach involves a certain level of risk. Some science communicators hold that the course of the debate around GM crops and food has shown that a low-key or poorly coordinated approach is more likely to allow the proliferation of poorly based claims and at worst, misinformation. At present there appears to be very limited understanding of NBTs in the Australian community at large and very little discussion. This provides an opportunity for science and industry to be proactive in presenting a clear and coherent message on the benefits and risks of the application of NBTs with a view to securing a more favourable outcome for food produced by new NBTs than food produced using traditional GM.

Comments from both NBT researchers and social scientist interviewed emphasise the importance of a constructive community dialogue in ensuring a social licence to operate, a view supported in the literature [56-59]. Integral to effective community engagement will be increased transparency on behalf of decision makers and the proponents of technology [58] which will contribute to re-establishing trust in the institutions and processes involved. European studies have shown this to be a major driver in public response to GM foods [56,60].

As mentioned earlier, consumers are more willing to accept GM foods if they perceive value in the new trait [61]. Therefore, promoting the value that a trait has delivered can be beneficial [62,63]. In the case of NBTs, this should also

be considered in the decision of which traits to implement first. European consumers responded most positively to GM foods if they delivered health benefits or reduce the likelihood of pesticide residues.

Successful commercialisation of vegetable varieties developed using NBTs will thus depend as much on a considered approach to constructive community engagement and demonstration of consumer benefit as on effective technical implementation.

8. Learnings from other industries: Case Studies

8.1 Market premiums for novel traits: Florigene Moon Carnations

Flower colour is one of the most important traits of ornamental plants, especially for cut flowers. True (royal) blue flowers do not occur naturally in the most valuable cut flower species: carnations, roses and chrysanthemums. There have been several attempts to use NBTs (by combining transgenic and RNAi technologies) to manipulate these species to generate royal blue-coloured cultivars, all without success. Instead, a range of mauve-purple cultivars of all three species has been generated by genetic manipulation, the most famous of which is the Moonshade™ range of mauve carnations with extended vase life. Varieties in this range (Figure 15) were developed in Australia by Florigene, which was later bought by the Japanese brewing company Suntory.

Bluish carnations and roses were modified by expression of delphinidin (Dp)-type anthocyanins in their petals *via* the heterologous expression of a *flavonoid 3',5'-hydroxylase* gene. Anthocyanins with multiple aromatic acyl groups (often referred to as polyacylated anthocyanins) in the 3'- or 7-position tend to display a more stable blue colour than non-acylated anthocyanins.

Genes were transferred to into white carnation carnations using *Agrobacterium tumefaciens*. The genes incorporated encoded the biosynthetic production of delphinidin pigments originated from *Antirrhinum majus* (snapdragon) and *Petunia hybrida* (petunia). A gene from a *Viola* species (black pansy) has been included in some plants to provide the deep purple colour.

In addition to the anthocyanin genes responsible for altered flower colour, an extra copy of the carnation ACC synthase gene has been included to down-regulate the production of ethylene and extend vase life. They also carry a mutant ethylene receptor gene that reduces sensitivity to external ethylene. Expression of the ethylene transgenes is strictly confined to the floral organs because ethylene is an important endogenous hormone involved in disease sensitivity and regulating vegetative propagation.

Figure 15. Varieties of 'Moonshade' carnations developed by Florigene.

The Moonshade™ carnation series are GM products but their method of development is not a component of their

marketing. Because they are not food items, they demonstrate the value of NBTs for the development of novel market traits in the absence of the complication of negative messages regarding GM food. Moonshade™ carnations are grown in Colombia, Ecuador and Australia and marketed in North America, Japan and Europe. The GM products attract a price premium because of their novel colours, even in arenas with a negative attitude to GMOs such as Japan and Europe.

8.2 Bt Cotton in Australia: benefits to industry, community and the environment

The cotton industry in Australia effectively began in the mid-1960s. In fifty years it has grown to be a major component of the Australian agricultural sector, generating $1.5 billion p.a. in export earnings. Up to the 1990s, cotton production in Australia relied heavily on insecticide application for the control of *Helicoverpa armigera* and *H. punctigera*. In addition, there was chemical application for other insect pests (such as *Tetranychus urticae*, two spotted mite), diseases and weed control. By the mid-1990s, insecticide use had selected for resistance in *H. armigera* and *T. urticae* rendering control more difficult and more costly. In addition, there was concern that the heavy and increasing reliance on chemicals could undermine the cotton industry's social 'licence to operate'.

Bt cotton was introduced in 1996. Bt varieties contain one or more genes from the soil dwelling bacterium *Bacillus thuringiensis* that encode the production of insecticidal proteins active again *H. armigera* and *H. punctigera*. The original Bt cotton releases contained one transgene (Cry 1 Ac) but were followed in 2003 with varieties containing two complimentary genes (Cry 1 Ac and Cry 2 Ab). In 2015 *Bollgard 3* was introduced, with the *Cry1Ac* and *Cry2Ab* genes plus a gene coding for a third protein, *Vip3A*. Adding genes which express proteins with different modes of action reduces the rate of development of resistance in the target species, since each protein kills larvae in a different way, thus increasing the longevity of the technology (Figure 16).

Figure 16. Genetically modified insect protected cotton on the left, next to a closely related conventional cotton variety on the right showing damage from heavy insect feeding pressure. Greg Kauter, Courtesy of Australian Cotton Growers Research Association Inc, Narrabri, NSW.

Benefits: The introduction of Bt cotton was a dramatic success. It was rapidly and extensively adopted by the Australian industry with over 98% of the cotton area now using this technology. This has led to a striking reduction in the use of insecticides, by over 85% (Figure 17).

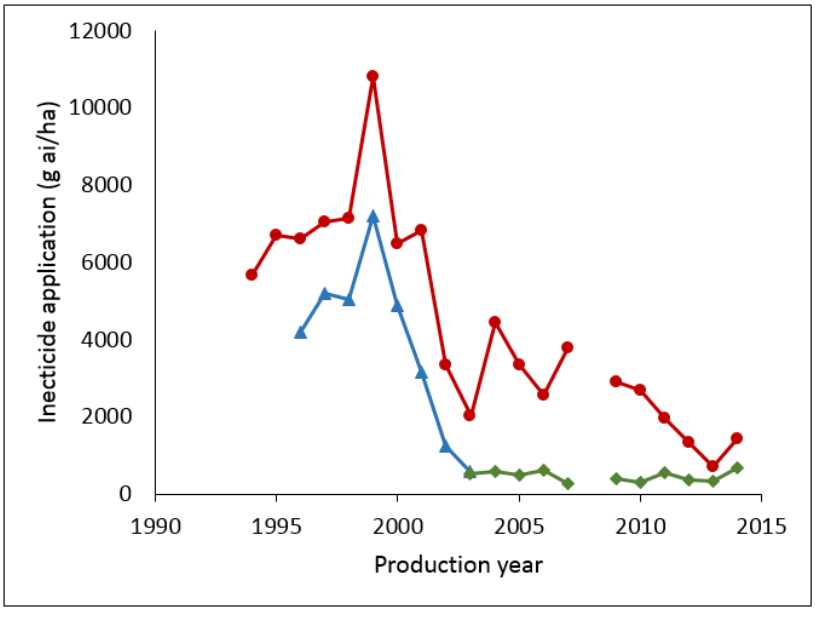

Figure 17. Trend over time in total insecticide application (g ai/ha) to Australian cotton crops after the commercial introduction of Bt cotton varieties. Red line = Non-Bt varieties; Blue line = INGARD varieties (single Bt construct); Green line = Bollgard II (double Bt construct). Data from Crop Consultants Australia Annual Market Audit and Cotton Research and Development Corporation.

The major change in insecticide use has had both direct and indirect benefits for the grower, the industry, and the environment. For example, it has:

- Contributed significantly to a substantial reduction in pesticide levels in river systems in cotton production areas,
- Reduced the risk of exposure to aerially applied agrochemicals for growers and nearby residents,
- Reduced urgency of insect control interventions and the intensity of management required for insect pests,
- Seen a decline in the resistance of *H. armigera* to a range of insecticides,
- Improved survival of beneficial species within cotton crops,
- Provided growers and managers with more opportunity to focus on improved agronomy,
- Improved agronomic water use efficiency (yield per unit of water used), and
- Provided more flexibility in sowing dates, which in turn has
- Allowed early sowing to be avoided, thus reducing the risk of seedling diseases favoured by lower temperatures.

Emerging challenges: Although the introduction of Bt technology has had many substantial benefits, it has not been without negative repercussions. The insecticides used to control *Helicoverpa* species before the release of Bt, had also been suppressing other species. With the reduced input of these compounds, a number of previously minor pests increased in significance. In particular, new research and extension activities for the appropriate control of green mirids

(*Creontiades dilutes*) were required. There have also been occasional damaging outbreaks of jassids, thrips, whitefly and cotton strainers in various localities. Without sound knowledge of the significance of these pests and the development of appropriate intervention strategies, the benefits of the Bt technology could have been reduced by the misuse of broad-spectrum insecticides at inappropriate times.

Social analyses have raised a number of concerns relating to the adjustment of local communities during the period of initial implementation that may warrant further assessment. For example, the dramatic decline in demand for agrochemicals challenged the viability of some local agricultural supply businesses and caused a level of disruption in supply chains. For some consultants and resellers, there was also a need to develop new skill-sets or to alter business focus. While these challenges can be overcome, the impact on the individual and local support facilities should not be overlooked.

There are a number of lessons can be learned from the successful introduction of Bt cotton to the Australian industry.

Addressing a key production constraint: The control of *Helicoverpa* species had become problematic due to increasing levels of resistance, requiring significant cost in crop monitoring and control, and raising concerns about the public perception of the cotton industry due to the high levels of insecticide use. Bt cotton provided a novel solution at a critical time for the industry. As a result, the development of the Bt technology was strongly supported by grower organisations. Once released, it was rapidly and extensively adopted.

Benefits beyond producers: While the introduction of Bt cotton yielded clear benefits for growers, it also generated benefits for the local community and society at large. The reduction in insecticide usage, particularly by aerial application, reduced the risk (real or perceived) of possible exposure for nearby residents and contributed to reduced contaminant levels in waterways, providing major positive outcomes for the natural environment and hence the community at large. These wider benefits are important for gaining acceptance and addressing the question of the industry's social 'licence to operate'.

Local validation is essential: The Bt technology was initially developed in the USA and was effective against the key lepidopteran pests in cotton production systems in that country. However, while controlled environment tests showed that it was also able to control the key lepidopteran pests in the Australian industry, field studies showed that under certain environmental stresses, the production of insecticidal protein fell to levels which were no longer efficacious against the Australian target species.

Research needs go beyond the question of efficacy: Considerable research was required before release, not only to test the efficacy of the technology in the local production systems, but also to explore the response of the system to the altered management regime. In addition, questions of pollen movement, cross compatibility between transgenic lines and native species and the risk of escapes establishing themselves in the environment all need to be considered. As this was a pest control application, considerable research was also conducted into the frequency of resistance alleles in the pest populations and the likely rate of development of commercially significant levels of resistance. A comprehensive resistance management plan was developed and became part of the grower licence for use of Bt cotton. Research also continued after the implementation of the technology to assist in the management of emergent challenges.

8.3 Roundup® ready canola

Roundup Ready® is the commercial name for GM crops which are tolerant to the herbicide Roundup (active ingredient: glyphosate). The trait was developed by the agricultural company Monsanto. In Australia, the only commmercialised food crop using this technology is Roundup® Ready canola (*Brassica napus*). Glyphosate inhibits a key enzyme in the shikimate pathway, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is required for synthesis of aromatic amino acids. Plants treated with glyphosate cannot make aromatic amino acids and so they die (Figure 13). Roundup

Ready canola now contains two introduced genes, the first is the *cp4 epsps* gene from the soil bacterium *Agrobacterium tumefaciens* strain CP4. Expression of this gene results in synthesis of the CP4 EPSPS enzyme which is not inhibited by glyphosate, allowing plants treated with Roundup to grow normally. The *gox* gene from *Ochrobactrum anthropi* codes for production of the enzyme glyphosate oxidase (GOX) which catalyses the breakdown of glyphosate into glyoxylic acid and aminomethylphosphonic acid (AMPA). When both of these enzymes are expressed in canola plants, they provide tolerance to glyphosate (Figure 18). With 20 years commercial deployment of glyphosate tolerant crops, the expression of these genes is consistent, with stable inheritance over many generations.

It is worth noting that farmers have the option to use two other herbicide tolerance traits available in canola: tolerance of triazine and imidazolinone herbicides. Both of these traits are derived from mutagenesis, and so are not classified as GM. Nevertheless, farmers have embraced Roundup technology since its introduction.

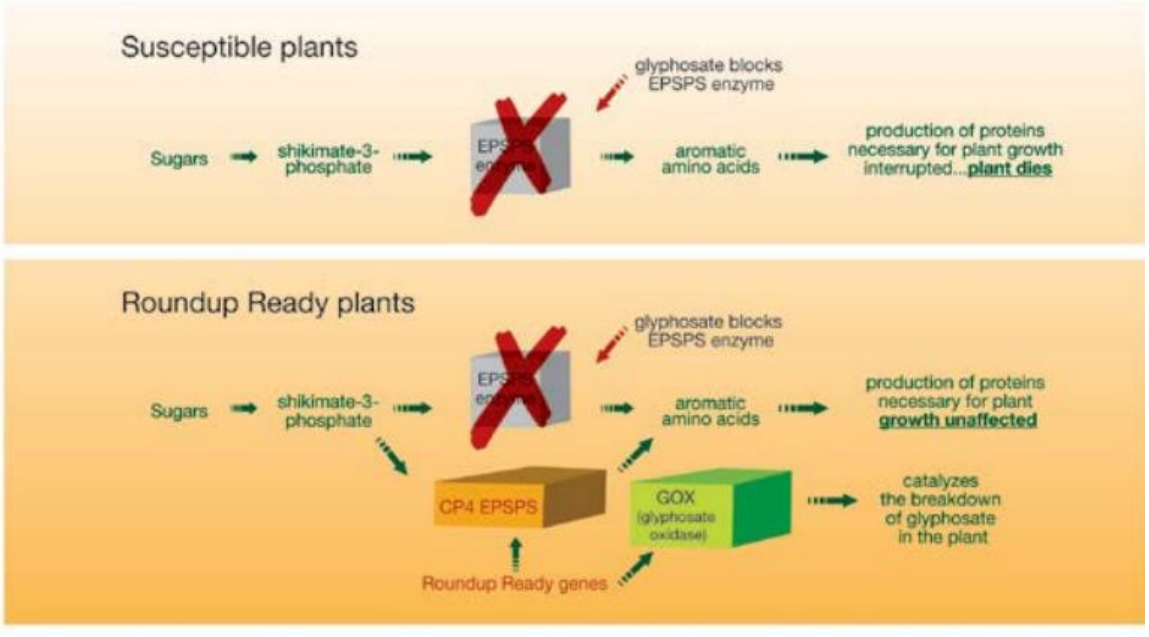

Figure18. The herbicide-insensitive CP4-EPSPS allows Roundup Ready canola to tolerate applications of Roundup Ready Herbicide with PLANTSHIELD, whilst susceptible plants cannot produce essential amino acids and therefore senesce (Source: Monsanto).

8.4 Purple Tomatoes

Anthocyanin pigments are anti-oxidants that can confer benefits to human health when consumed. Independent studies show that antioxidants and anthocyanins can reduce the incidence of cancer, improve vascular function and improve health and well-being.

To achieve expression of high levels of anthocyanins in tomatoes, genes from two plants were used. The expression of these genes, known as transcription factors (Delila, *Del,* and Rosea1, *Ros1*), result in the production anthocyanins in snapdragon (Antirrhinum) flowers, and the MYB12 gene from Arabidopsis, which is a flavonol-specific activator of flavonoid biosynthesis was also used. Fruit expression was achieved using the fruit-specific E8 promoter.

These modifications resulted in plants in which tomatoes the containing Del/Ros1 genes are orange, those only containing the MYB12 gene are purple, and tomatoes with all three genes are dark indigo.

The introduction of one gene from a snapdragon plant into tomato plants results in the synthesis of high levels of anthocyanins, and the production of purple tomatoes (Figures 19 -21). The purple tomatoes contain the same

compounds that are present in blueberries and cranberries, offering the same health benefits to consumers, but provided in a food that is widely available and reasonably priced. These tomatoes were made at the John Innes Centre in Norwich, U.K. with further development at Norfolk Plant Sciences, but commercial production is underway in greenhouses in Canada, with the production of purple tomato juice. Although there are purple-skinned heritage tomatoes available, they do not accumulate useful levels of anthocyanins.

Figure 19. Left, a comparison of purple and wild type tomatoes, right, stages in purple tomato development (Norfolk Plant Sciences).

Figure 20. Wild type tomato (top left), DEL/ROS orange tomato (top right), MYB12 purple tomato (bottom left) and indigo tomato containing all three genes (bottom right).

Figure 21. Left to right - juice from orange (DEL/ROS), purple (MYB12) and indigo tomatoes, which contain all three genes, Right, sections through indigo tomatoes. (All images from Norfolk Plant Sciences).

Similar high anthocyanin-containing purple or red fleshed Mexican lime fruits have also been developed which express the *VvmybA1* anthocyanin biosynthesis regulatory gene (from red grape), and the *Ruby* anthocyanin biosynthesis regulatory gene from blood orange [64]. This type of change intentionally generated by transgenic technology is known as 'metabolic engineering': another well-known example is 'golden rice' in which insertion of two genes enables rice grains to make pro-vitamin A at levels which would alleviate vitamin A deficiency for people whose diet is mainly polished rice.

8.5 Innate potato: reducing wastage in a key global food crop

Potato is the fourth most important food crop in the world. It is an established part of the western diet and production and consumption are rapidly increasing in Asia. Unlike the other major crops, wheat, rice and maize, harvested potato has a high moisture content and respiration rate. As a result, wastage is a major concern.

The J.R. Simplot Company in the USA released their first 'Innate' Russet Burbank potato variety in 2015. The variety used RNAi technology to reduce the expression of (a) genes in the biochemical pathway that leads to black spot bruising and browning after peeling, as well as (b) genes in the pathway that can lead to the production of acrylamide on cooking. The aim was to reduce wastage in the supply chain and to provide a possible health benefit to consumers. This is now referred to as Generation 1 Innate and is sold as White Russet in the USA fresh market (Figure 22).

The second generation of Innate potato (Innate Generation 2) also includes resistance to 'cold sweetening', another cause of postharvest wastage that can occur in cool storage or during transport, as well as a cisgene from a wild potato relative that provides significant tolerance to late blight caused by *Phytophthora infestans* (Figure 23).

Figure 22. Innate Russet Burbank potatoes have a 44% reduction in bruising compared to conventional potatoes. Sources: http://www.innatepotatoes.com

Figure 23. Innate Generation 2 potatoes resist Phytopthora late blight http://www.innatepotatoes.com

Benefits: Innate potatoes provide benefits to the grower, along the supply chain, to the consumer and to the environment.

- Reduced postharvest losses from black spot bruising, browning after peeling, and cold induced sweetening provide benefits to growers, processors, wholesalers, retailers and consumers
- High tolerance to *Phytophthora*, may provide (i) significant benefits to growers by reducing disease control costs and yield losses and (ii) potential benefits to the environment by reducing agrochemical application and so reducing risks of off-site or off-target impacts.
- Reducing losses due to disease and postharvest wastage increases effective resource use efficiency, most importantly water use efficiency and fertilizer use efficiency, providing benefits to the grower as well as to the environment and hence to the wider community.

- While these benefits may be substantial for commercial growers in advanced productions systems, they are likely to be of far greater significance if the genotypes can be deployed in less developed regions where potato is a major component of food security.

Challenges in the path to market: Generation 1 Innate potatoes are approved in both the USA and Canada. They are targetted at the fresh market in supermarkets and restaurants where they have been accepted and are selling strongly. For Generation 2 Innate potatoes with benefits to the processing sector, there is still a level of consumer resistance. Simplot has a long standing relationship with the McDonalds Company. In response to concerns over consumer acceptance, McDonalds has issued statements to emphasise that it does not use GM potatoes. However, Generation 2 Innate potatoes are already being processed as potato crisps (chips) by more than a dozen regional processors.

An additional challenge has been gaining international approvals for both generations to protect foreign exports of conventional frozen fries and dehydrated products. In the interests of good stewardship, this has meant significant extra effort and cost for Simplot in obtaining food safety approvals for a number of non-target destination countries. This is on top of the considerable cost in gaining approval for production and sale for the intended markets.

There are a number of lessons that can be drawn from the development and release of the Innate potato genotypes.

Research and development processes are not trivial: Simplot has been successful in developing genotypes with the desired characteristics and using the technologies that can be consider to exploit genetic material 'innate' to potato and its relatives. However, this was not facile; taking scientists and breeders 15 years to accomplish. While new methodologies may increase the speed of delivery, planning must account for a realistic delivery timeframe.

Realising returns on investment: Securing return on investment can be challenging for companies working with new methods of plant breeding. It is important to understand the trait value and target those segments that provide obvious benefit without the stigma of GMO. For Simplot, this has meant focusing on fresh potatoes for its first releases and potato chips for its second generation before broadening to larger markets with big consumer brands. This raises the challenge of securing clear market arrangements. While these agreements might best be finalised early in the R&D process prior to major investment, at that stage the success of new technology and the time frame for delivery may still be uncertain.

In addition, the cost of stewardship, including seeking approvals in international destinations where the product is not intended to be sold, can be considerable and can further erode potential returns.

8.6 Arctic® non-browning apples: a test case for NBTs in fresh food

Wastage in apple has been estimated to be as high as 40%. One contributor is the discolouration of apples caused by bruising or by oxidative browning after cutting. As for potatoes, when the cells of an apple are damaged, an enzymatic reaction is initiated that results in the apple's flesh turning brown. While there is more than one type of apple browning, the primary form is caused by a group of enzymes called polyphenol oxidases (PPOs). PPOs catalyse the oxidation of polyphenols to quinones, causing oxidative browning. Although the damage is superficial it can affect the taste and texture of the apple, and give it an un-appetising appearance.

The Arctic® brand was developed by Okanagan Speciality Fruits. Arctic® brand apples were derived from the popular varieties Granny Smith, Fuji and Golden Delicious. These apples were genetically modified using RNAi technology, by incorporating a transgene that produces siRNAs specific complementary to the four apple PPO genes (the apple genome has been sequenced and the PPO genes were identified from it). Fresh apples are physiologically active and the expression of the siRNAs in the fruit induces the RNA interference (RNAi) pathway, which prevents expression of polyphenol oxidases, thus preventing browning (Figure 24).

The browning of apple imposes certain constraints during processing. Work plans must be organised so that peeling, coring, slicing or dicing take place as soon as possible before subsequent steps in the process. Oxidative browning is a

particular difficulty for pre-packaging of fresh cut apples. Pre-packaged fresh fruit and vegetables is growingly as a snack food industry. Previously, food service companies that cut and packaged apple segments added browning inhibitors such as the antioxidant calcium ascorbate. However, this additive alters the flavour of the fruit. Further, the cost of treating conventional sliced apple segments with antioxidants accounts for 35% of the costs of production. Thus, it is expected that Arctic® apple segments will be cheaper than the equivalent conventional product as well as having better flavour.

Figure 24 Non-browning Arctic apple and juice from it compared to conventional Golden apple. (Source: http://www.okspecialtyfruits.com/arctic-apples/)

Arctic apples were approved for release by the USDA in 2015. Okanagan has also applied for regulatory approval the Arctic® Apple in Canada. About 85,000 Arctic® trees were planted in Washington State in 2015 and 2016, and another 200,000 are budded for 2017. All Arctic® apple orchards are being managed by Okanagan.

The first sales of 500, 20 kg boxes, of Arctic apples were sent to retailers in the American Midwest in February 2017. The fruit is not required to be labelled as GM. The next harvest in the northern autumn of 2017 is expected to yield 6,000 boxes (ca 120 tonnes).

Non-browning apples provide benefits to producers, processors and consumers:

- Reduced bruising reduces losses during harvesting, storage, packing and processing.
- The inhibition of oxidative browning removes the time constraint between cutting or peeling and subsequent processing steps, allowing more flexibility in work planning and reduced losses for processors.
- New markets can be created for loose, fresh apple pieces, including from vending machines, without the need for adding antioxidants.
- Consumers, especially children, are expected to be more amenable to eating pre-cut apple pieces that have not turned browned and have not required additives.

Arctic apples will pioneer NBT fruit to the consumer, and responses to these apples will provide a litmus test for consumer acceptance and marketing of other NBT-based products that will be approved for general release in coming years.

8.7 PRSV resistant papayas: NBT saving an industry

Papaya ringspot virus (PRSV) decimated papaya production in Hawaii in the early 1990s, causing production losses of US$17 million per year. PRSV-resistant GM papaya, an RNAi-based trait, was developed and commercialised in 1997 in Hawaii, and in the US in 1998. By 2001, papaya production recovered in Hawaii to the extent that exporting of GM papayas to Canada began in 2002 (Figure 25). Since 2012, Hawaii has exported GM papaya to Japan [65].

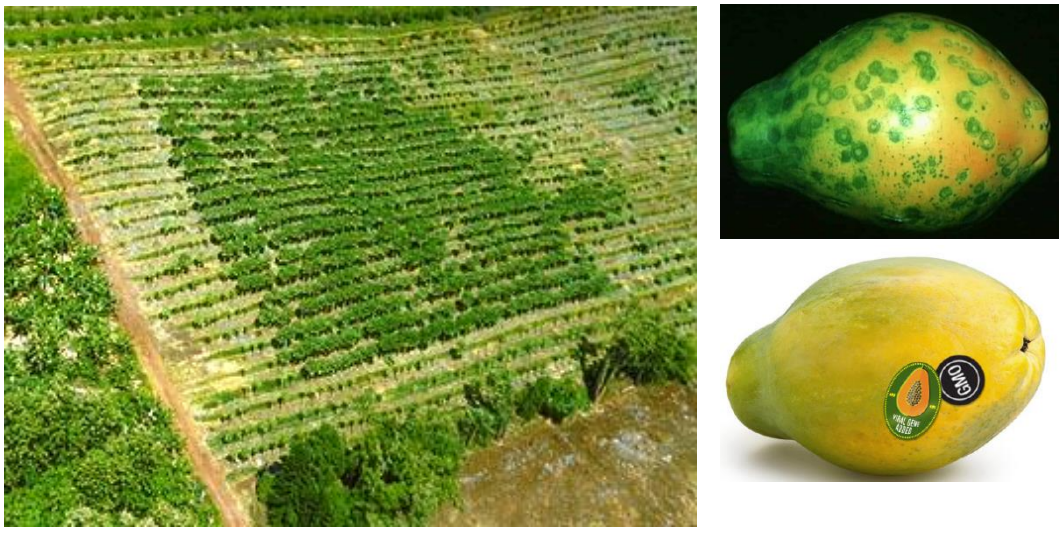

Figure 25. Left: PRSV-resistant plants in the centre of the square, surrounded by wild type papaya plants decimated by PRSV. Right: PRSV infected papaya and GM PRSV-resistant papaya.

9. Food Standards Australia New Zealand

Food Standards Australia New Zealand (FSANZ) is responsible for the pre-market approval of food products. While OGTR focuses on the production of a crop and assesses safety with respect to the environment and the community in general, FSANZ focuses on the safety of food for human consumption.

Definitions: In relation to the application of gene technology, the definitions used by FSANZ are set out in Standard 1.5.2 of the *Australia New Zealand Food Standards Code* which deals with "Food produced using gene technology". The Standard states:

> "*Food produced using gene technology* means a food which has been derived or developed from an organism which has been modified by gene technology.
>
> Note: This definition does not include food derived from an animal or other organism that has been fed food produced using gene technology, unless the animal or other organism is itself a product of gene technology.
>
> *Gene technology* means recombinant DNA techniques that alter the heritable genetic material of living cells or organisms."

From this it can be seen that, consistent with the Gene Technology Act 2000, the definitions used by FSANZ are process based rather than output based. The application of the Standard is based on the fact that gene technology was used in developing the food rather than the character of the modification. Standard 1.5.2 (or Standard A18 as it was known before 2001) was adopted in 1998 and came into effect in 1999 and therefore slightly pre-dates the Gene Technology Act 2000 under which the OGTR operates. As a result, the definitions used by FSANZ differ somewhat from those used by the OGTR. Both agencies recognise the importance of harmonising definitions, but this is difficult in the present fluid regulatory environment and against a background of rapidly evolving technology.

As is the case for the definition of OGTR, definitions of GM food used by FSANZ were designed to deal with the technologies being used in 2003 when the Standard was put in place. They clearly capture transgenics and also capture cis- and intragenics, but the technology has advanced far beyond that today. Recently developed NBTs are more problematic. When NBTs are used to stably insert a gene into an organism, they are clearly captured within the existing definition. However, when a technology such as genome editing to induce male sterility in a parent line, for example, has been used in a breeding program, and the resultant plant contains no foreign genetic material, the applicability of Standard 1.5.2 is less clear.

FSANZ hosted a technical workshop on NBTs, in 2012 that included an invited panel of experts (workshop report available at http://www.foodstandards.gov.au/consumer/gmfood/Pages/New-plant-breeding-techniques-in-the-spotlight.aspx). While FSANZ has not yet made any determination on any of the NBTs, it has indicated that it will have regard to the workshop conclusions in considering any application that might be made for a food produced using any of the NBTs. At the same meeting, the expert panel concluded that food produced from a plant that had a GM rootstock and non-GM scion would still be regarded as GM food and hence be captured by Standard 1.5.2.

Assessment: For NBT-generated food legislated under Standard 1.5.2, the FSANZ safety assessment is undertaken in accordance with internationally established scientific principles and guidelines developed through the work of the Organization for Economic Cooperation and Development (OECD), the Food and Agriculture Organization (FAO) of the United Nations, the World Health Organization (WHO) and the *Codex Alimentarius* Commission. In particular there are two Codex Guidelines:

- CAC/GL 44-2003: Principles for the risk analysis of foods derived from modern biotechnology, and
- CAC/GL 45-2003: Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants.

All assessments are evidence based.

A food approval, if given, is for food from a particular line (genotype) rather than a construct. Under the definition of a 'line' in Standard 1.5.2, food derived from the progeny of that line when crossed with a non-GM line or another approved GM line is similarly approved. An approval covers all food products coming from a line. For example, approval of a corn line covers corn meal, flour, oil and starch as well as the whole grain of that line.

Implementation: FSANZ has had a number of enquiries regarding their likely stance on developing NBTs, but no applications for approval (other than conventional GM) have yet been submitted. As NBTs encompass a wide range of technologies and potential modes of application, no single stance is possible. FSANZ recognise the need for industry, regulators and compliance agencies to have clarity in this area and are actively working to address this issue.

There are currently no plans to move toward a product-based definition of what falls under Standard 1.5.2. While this may be considered desirable by some in the sector, it would require a fundamental change in approach.

10. Regulation of Gene Technology in Australia

The State, Territory and Australian governments and interested parties developed the Gene Technology Act 2000. This Act was passed by the Australian Government in December 2000 and took effect on 21 June 2001. The legislation is the Australian Government's component of a national scheme for the regulation of genetically modified organisms (GMOs), which includes legislation in every Australian State and Territory.

The aim of the Act is to protect the health of people and the environment by identifying any risks posed by, or resulting from, gene technology and by managing possible risks. The Act establishes three key advisory groups to provide advice, on request, to the Gene Technology Regulator and the Gene Technology Ministerial Council. These are:

- the Gene Technology Technical Advisory Committee (GTTAC), which provides advice on scientific and technical matters,
- the Gene Technology Ethics Committee (GTEC), which provides advice on ethical matters and,
- the Gene Technology Community Consultative Committee (GTCCC), which provides advice on community issues regarding gene technology.

The Gene Technology Regulations Act 2001 determines how the Gene Technology Act 2000 is implemented. Note that the Gene Technology Act 2000 relates to living organisms, and that Food Standards Australia New Zealand (FSANZ) is responsible for the safety, content and labelling of food, including pre-market safety assessment for food produced using gene technology. The Therapeutics Goods Administration (TGA) deals with GM medicines and therapeutics, and the Australian Pesticides and Veterinary Medicines Authority (APVMA) regulates agricultural chemicals, the Department of Agriculture, Forestry and Water Resources (DAFWR) (Formerly the Australian Quarantine and Inspection Service, AQIS) regulates importation of GMOs and GM materials under the Gene Technology Act 2000.

10.1. The Office of the Gene Technology Regulator

The Office of the Gene Technology Regulator (OGTR) is located in Canberra. Dr Raj Bhula is the current Gene Technology Regulator, appointed for a period of five years in 2016. The Regulator is an independent statutory office holder responsible for administering the Gene Technology Act 2000 and corresponding state and territory laws, and OGTR staff are part of the Department of Health.

In administering the gene technology regulatory system the Regulator has specific responsibility 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.

Section 27 of the Gene Technology Act sets out the functions of the Regulator to perform functions in relation to GMO licences as set out in the Act (Part 5), which outlines the licensing system under which a person can apply to the Regulator for a licence authorising dealings with GMOs. It also functions to develop draft policy principles and policy guidelines, codes of practice, issue technical and procedural guidelines in relation to GMOs and provides information and advice to other regulatory agencies and to the public about GMOs and GM products, and about the regulation of GMOs. It includes the Legislative Governance Forum on Gene Technology to cover operations of the Regulator and the Gene Technology Technical Advisory Committee, the effectiveness of the legislative framework for the regulation of GMOs, including in relation to possible amendments of relevant legislation.

The Regulator can also undertake or commission research in relation to risk assessment and the biosafety of GMOs, promote the harmonisation by regulatory agencies of risk assessments relating to GMOs and GM products, monitor international practice in relation to the regulation of GMOs, maintain links with international organisations that deal with the regulation of gene technology and with agencies that regulate GMOs in countries outside Australia, and conduct other functions conferred by the Act, the Regulations or any other law, such as monitoring and enforcing the legislation and reporting quarterly to the Minister and Federal Parliament.

The OGTR provides a series of definitions for genetically modified organisms, and for dealings associated with GMOs. In brief, a GMO is:

a. An organism that has been modified by gene technology; or
b. An organism that has inherited traits from an organism, where the traits occurred in the initial organism because of gene technology.

'Dealing' in relation to a GMO is defined in the Act as meaning:

- conduct experiments with the GMO
- make, develop, produce or manufacture the GMO
- breed the GMO
- propagate the GMO
- use the GMO in the course of manufacture of a thing that is not the GMO
- grow, raise or culture the GMO
- import the GMO
- transport the GMO
- dispose of the GMO or
- possess, supply or use the GMO for the purposes of, or in the course of, any of the above.

From a regulatory point of view, there are four main categories of dealings in relation to a GMO:

1. ***An exempt dealing***

Exempt dealings are dealings with GMOs that have been assessed over time as posing a very low risk (i.e. contained research involving very well understood organisms and processes for creating and studying GMOs).

2. ***A Notifiable Low Risk Dealing (NLRD)***
   Activities with GMOs undertaken in containment (i.e. not released into the environment) that have been assessed as posing low risk to the health and safety of people and the environment provided certain risk management conditions are met.

3. ***Dealing Not involving an Intentional Release (DNIR)***
   Dealings with GMOs in contained facilities which do not meet the criteria for classification as either an exempt dealing or NLRD

4. ***Dealing involving an Intentional Release (DIR)***
   Dealings with GMOs which take place outside containment facilities.

All licences issued for DNIRs and DIRs are listed on the publically available GMO register (http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/reg001-1)

10.2 Review of the Gene Technology Regulations

It has become increasingly clear that gene technology regulations in Australia, now approaching 20 years since their formulation, have not kept up with advances in science and with the history of safe usage of GM crops. As a result, in October 2016 the Gene Technology Regulator initiated a Technical Review of the Gene Technology Regulations 2001, on Options for Regulating New Technologies.

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

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

Views were sought on the following Options:

Option 1: no amendment to the Gene Technology Regulations.
Option 2: regulate certain new technologies (ODM, SDNs1, 2 and 3).
Option 3: regulate some new technologies based on the process used (ODM, SDN2 and SDN3).
Option 4: exclude certain new technologies from regulation on the basis of the outcomes they produce (i.e. regulate only SDN3)

These Options are presented in Figure 26, which relates the process used to make changes to the type of breeding technology employed, indicating mutagenic processes (but not other plant breeding processes which result in genetic manipulation) not regulated as gene technology (green), those regulated as gene technology (red), and those NBTs for which the regulatory status is not clear (yellow).

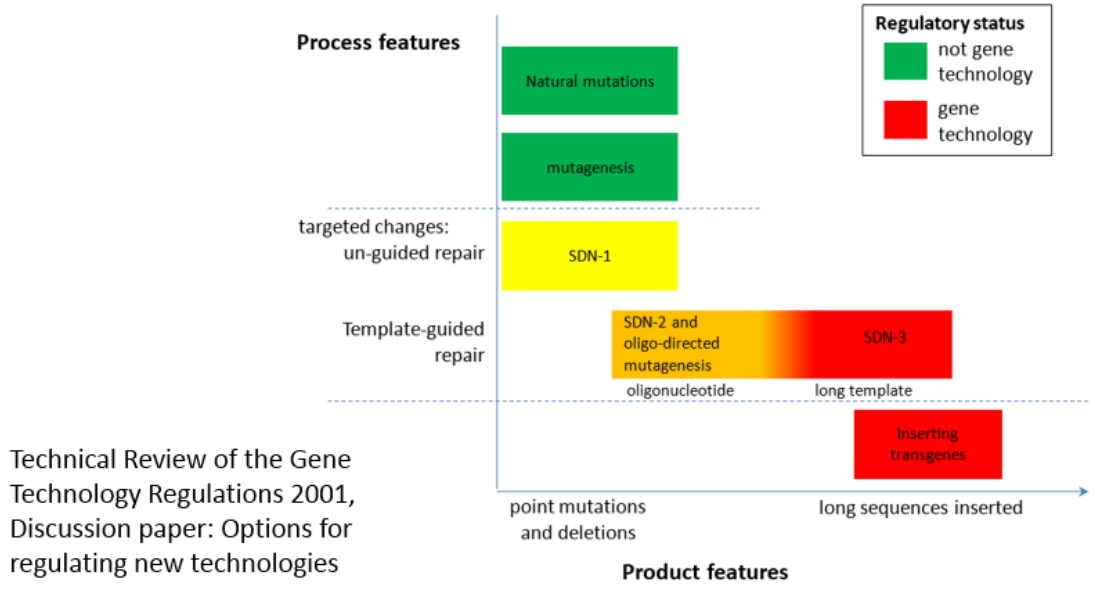

Figure 26. Overview of options for discussion by the OGTR for the Technical Review of the Gen Technology Regulations Act 2001.

A summary of the responses received is provided in Appendix 2, with the majority of evidence–based responses opting for Options 3 or 4. This clearly indicates that definitions in the GT Act 2000 need to be adjusted to take into account (i) a history of 20 years of safe usage of major GM technologies and (ii) new technologies, particularly those based on genome editing, which are classified as SDN1 and SDN2 (see above), and especially in products in which there is no introduced DNA.

In particular, 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. The latter include chemical and radiation mutagenesis methods, natural mutations, the natural variations present in nucleic acid sequences between genotypes, or established breeding activities 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.

The view expressed by the plant crop industry overall was that 'Plant varieties developed through innovative breeding methods should not be differentially regulated if they are similar to or indistinguishable from varieties that could have been produced by established breeding methods'

However, the Gene Technology Act 2000 covers microbial and medical manipulations as well as plants, and it may be difficult for a one-size-fits-all solution to be found. Although there will still need to be a trigger to determine whether a dealing falls under the GT2000 Act, it is suggested that there should then be a product based filter to exclude those products which have a history of safe usage of are classified as SDN1 or SDN2.

Once such approach is presented here in Figure 27.

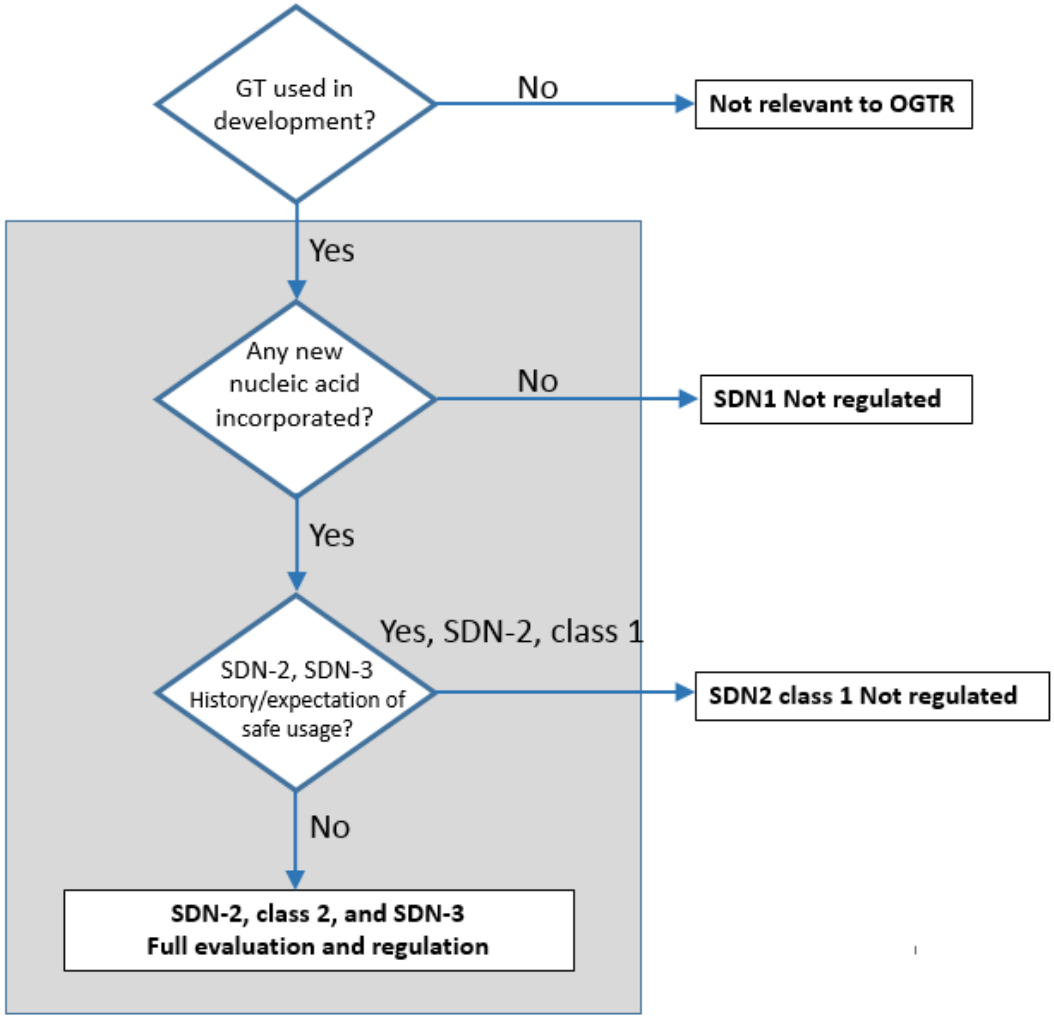

Figure 27. Suggested changes to the Australian Gene Technology Regulations (Jones, Wylie and Milroy).

This scheme retains the same trigger for consideration by OGTR: it includes genotypes that have had gene technology used in their development, but contain no new RNA or DNA in the product, it avoids excessive regulation and the stigma of GM for SDN-1 and most SDN-2 dealings. It also takes into account a history of safe usage, and the source of any introduced DNA (e.g .cisgenic, intragenic) of an NBT change in determining the level of intervention required for SDN-2. Two classes are suggested for SDN-2: class 1 if equivalent to non-regulated forms of manipulation or if there is a history of safe usage, and class 2 if there is any reason for concern. It needs clarity and transparency around determination of 'history of safe usage'. SDN-2 class 2 and SDN-3 treated products are still regulated as GM plants.

The aim for industry is to encourage revised Gene Technology regulations, to push the vertical arrow (Figure xxx below) as far to the right as possible, since this will enable the vegetable industry to use SDN-1 and SDN-2 technologies without the stigma or unnecessary costs triggered by Gene Technology regulation.

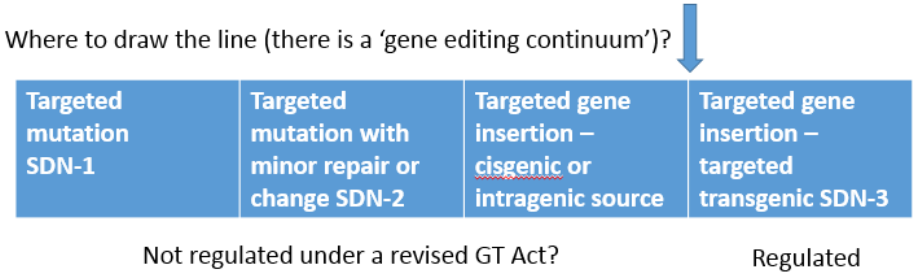

Figure 28.. The genome editing continuum. To the left of the vertical line products should be exempt from GT regulation: its exact location will be determined by a review of the GT regulations.

This scheme and Figures 27and 28 are provided because clarity in the Regulations is vital for the future implementation of NBTs by the vegetable industry in Australia.

11. Regulatory and market status of NBTs in major international markets

11.1 The Cartagena Protocol

The major international agreement on Living Modified Organisms (LMOs), more commonly referred to as GMOs, is the Cartagena Protocol on Biosafety (the Protocol) to the Convention on Biological Diversity. The Protocol was adopted in January 2000, and became effective on September 11, 2003. It is designed to protect both biological diversity and human life from any adverse effects of organisms modified by modern biotechnology. There 166 parties to the Protocol, a notable exception is the USA. Australia is a party to the Protocol, and so this must be taken into account when considering changing the definition of LMOs/GMOs, and international trade. pro

Biosafety was one of the key issues addressed by the Convention on Biological Diversity, which stressed the need to protect human health and the environment from the possibility of negative outcomes of modern biotechnology, while at the same time seeing the potential for good results of innovation in such areas as improving food supplies through agriculture.

The Protocol itself states that its objective is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.

The Protocol defines "living modified organism" as "any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology." "Modern biotechnology" is defined as the application of:

> i) *In vitro* nucleic acid techniques, including recombinant DNA and direct injection of nucleic acid into cells or organelles, or
>
> ii) Fusion of cells beyond the taxonomic family,

that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection.

11.2 Attitudes to GM commodities in Australia's major export markets

A summary of attitudes to GM food and the importation of GM produce by each of Australia's major export markets for horticultural produce is given in Table 5, and below.

Table 5. Summary of GM status in major trading partners

Country	Signatory Cartagena Protocol?	GM crops grown commercially in country	Imported GM produce used for food or feed	Public attitudes to GM food
Indonesia	Y	None	Soy, cotton, corn	Low level of awareness of GM issues. Little opposition
China	Y	Tomato, cotton, petunia, sweet pepper and chili pepper, papaya, rice, and corn.	Cotton, soybean, corn, and canola	Low public awareness of the safety of GM crops and food. Much misinformation.
Japan	Y	None	Soy, corn, potato, canola, cotton seed oil, alfalfa, sugar beet, papaya	Low public awareness of the safety of GM crops and food
South Korea	Y	None	Soy, maize, cotton, canola, potato, alfalfa, sugar beet, and a microorganism	Low public awareness of the safety of GM crops and food
Singapore	Y	None	Soy, corn	Medium level of public mistrust of GM food
Egypt	N	Cotton, corn	Soy, corn, potato, canola, cotton seed oil	Low level of awareness of GM issues
Israel	N	None (extensive research on GM crops)	Soy, corn	Some concern about safety and kosher nature of GM food
Kuwait, Oman, Qatar, UAE	N	None	Soy, corn	Low level of awareness of GM issues
Europe	Y	Most EU countries prohibit GM crops. GM maize grown widely in Spain	55 GM crops are approved for import as feed and food into Europe, but used only for feed	High level of public mistrust of GM food
USA	N	Soy, corn, potato, canola, cotton, alfalfa, sugar beet, papaya	Soy, corn	High level of public acceptance of GM food
New Zealand	Y	None	Soy, corn	High level of controversy regarding safety of GM crops and food

11.2.1 Indonesia

The government of Indonesia's policy on agricultural biotechnology is to "accept with a precautionary

approach" with respect to environmental safety, food safety, and/or feed safety based on scientific approaches as well as taking into consideration religion, ethical, socio-cultural, and aesthetic norms. Public awareness of the issues surrounding GMOs is low.

Imports of GM food and feed. Indonesia imports $1.5 billion of GM products annually including Bt cotton, herbicide tolerant soybeans and meal, Bt corn and a variety of food products. Indonesian soybean consumption is growing in correspondence with population and economic growth. Total soybean consumption is about 2.7 million tonnes, which is mostly fulfilled by imports: U.S. GM soybeans contribute 92% of the market share in Indonesia.

Cultivation of GM products. Indonesia is preparing to grow GM crops. A GM corn event has been approved for planting. In addition, two GM soybean varieties and two further GM corn varieties are in the pipeline for food safety approval. A food safety assessment application for GM late blight resistant potato has been submitted to the National Agency of Drug and Food Control (BPOM).

11.2.2 China

China is generally supportive of the growth and consumption of a wide range of GM crops. The testing, production, and marketing of GMOs in China are subject to government approval. Foreign companies that export GMOs to China, including GMOs as raw materials, must apply to the Ministry of Agriculture and obtain GMO Safety Certificates. There is public concern in some quarters over the safety of GM food.

Imports of GM food and feed.
Licenses have been granted for the import four GM plant products: cotton, soybean, corn, and canola. Among them, only GM cotton can be grown in China - the other crops can only be used as raw materials. Imported GM soybeans constitute 65% of the soybeans consumed domestically.

Cultivation of GM products.
*I*n 2012 China grew 4.0 million hectares of GM crops, including cotton, papaya, poplar, tomato, and sweet pepper constituting the largest biotech crop area among developing countries, and the sixth largest worldwide. There are rumours that GM rice is also grown widely, but this is not officially recognised.

11.2.3 Japan

Although it is legal to plant GM crops in Japan if certain procedures are followed, no commercial planting of GM crops (aside from the 'blue' rose and carnations developed by Suntory and Florigene) has not occurred in Japan to date, mainly because of political pressure by a general public sceptical about the safety of GM crops.

Imports of GM food and feed. Despite some public opposition to GM products being grown, Japan is one of largest importers of GM foods, including *soy, corn, potato, canola, cotton seed, alfalfa, beet, and papaya*. Fresh papaya ringspot virus resistant GM papaya fruit grown in Hawaii has been sold in Costco supermarkets in Japan since 2012.

Cultivation of GM products. None to date.

11.2.4 South Korea

The government has enacted a range of legislation governing the importation and cultivation of GM crops in South Korea. Public awareness of GMOs is high in South Korea. Public sentiment trends against GMOs. Generally, people are more tolerant of the pharmaceutical or medical use of GMOs, but less so when GMOs are used in food or feed for livestock.

Imports of GM food and feed. GMOs so far approved and imported for food and food additives, include soybean, maize, cotton, canola, potato, alfalfa, sugar beet, and a microorganism.

Cultivation of GM products. None to date.

11.2.5 Singapore

At present, Singapore does not have a dedicated umbrella legislation specific for the regulation of GM technology and its products. However, The Genetic Modification Advisory Committee (GMAC) of Singapore has released two sets of guidelines covering the commercial release of agriculture-related GMOs (for growing and/or commercial sale) and for research on GMOs. There is little public controversy over consumption of GM products.

Imports of GM food and feed. Twenty-one imported GM products are approved for release as food in Singapore. These are events of cotton, maize, soybean, and sugar beet.

Cultivation of GM products. None to date.

11.2.6 Egypt

Egypt takes a permissive approach to GMOs, and its public policy does not oppose growing, importing, and exporting genetically modified crops. In 2008, Egypt became the first North African country to grow GM crops, and it is now one of the five countries worldwide to export biotech crops to other countries. Egypt ranks third in Africa in planting and importing GM crops. There is little public controversy over consumption of GM products.

Imports of GM food and feed. Egypt imports GM maize and soybean.

Cultivation of GM products. Since December 2010, GM crops have been planted without restriction in ten different Egyptian provinces, primarily GM maize and cotton. Egypt not only engages in growing and trading GM crops, but also provides training to other countries to develop their capacity to produce such crops, one example being Tanzania.

11.2.7 Israel

*I*sraeli law permits the development and growth of GMOs for research purposes in accordance with requirements established by subsidiary legislation. Although commercial growth of GMO crops is not permitted, GM products may be imported, sold, and used in production of food and pharmaceuticals. Israel's religious kashrut authority has determined that the use of GM ingredients in food does not affect its kosher status because GMOs are used in small proportions.

Imports of GM food and feed. GM soy and maize are imported for feed.

Cultivation of GM products. No commercial cultivation to date.

11.2.8 Kuwait, Oman, Qatar and the United Arab Emirates (GCC-4)

There regulations governing the growth and importation of GM plant are poorly defined. Import of GM agricultural products to the GCC-4 are all regulated by the Office of Agricultural Affairs in Dubai. There is a low level of public awareness of the international debate surrounding GM food.

Imports of GM food and feed. Severe climatic conditions and limited water resources limit commercial agricultural production in the GCC-4. As a result, the GCC-4 relies heavily on the importation of raw, semi, and fully processed foods to satisfy consumer demand. GM maize and soy is sourced from the USA, Argentina and Brazil.

Cultivation of GM products. None to date.

11.2.9 Europe

The European Union (EU) has a comprehensive and strict legal regime in place for GMOs, food and feed made from GMOs, and food/feed consisting of or containing GMOs. The EU's legislation and policy on GMOs, based on the precautionary principle, is designed to prevent any adverse effects on the environment and the health and safety of humans and animals. It reflects concerns expressed by consumers, farmers, and environmentalists. In many EU countries, public opinion is generally against growth and consumption of GM food.

Imports of GM food and feed. The EU imports about 30 million tonnes of GM products annually. Forty-nine GMO events, consisting of eight GM cottons, 28 GM maizes, three GM canolas, seven GM soybeans, one GM sugar beet are approved for import.

Cultivation of GM products. Spain is the largest grower of GM crops in the EU (about 140,000 ha). Smaller acreages of GM crops are, or have been, grown in the Czech Republic, Slovakia, Portugal, Romania and Poland.

11.2.10 USA

The USA is the world's leading producer of genetically modified (GM) crops. Compared to other countries, regulation of GMOs in the US is highly favourable to their development. GMOs play a significant role in the US economy. Public opinion is generally supportive of GM technology, with some exceptions.

Imports of GM food and feed. The USA is a net exporter of GM food and feed. The products of GM crops are widely used domestically as food and feed.

Cultivation of GM products. About 90% of the soybean, cotton, and corn grown in the US are GM for herbicide tolerance and/or insect resistance. Other crops approved and/or grown in the USA include cotton, tomato, rapeseed, canola, potato, squash, beet, sugar beet, papaya, rice, alfalfa, flax, tobacco, plum, rose, and apple.

11.2.11 New Zealand

Research into GM crops has taken place in New Zealand over 20 years. Hazardous Substances and New Organisms Act 1996 (HSNO Act) regulates GM plant research and governs release of GM plants and products. GM products must get the approval of the Environmental Risk Management Authority (ERMA). There is a medium level of public opposition to GM food.

Imports of GM food and feed. Some processed foods containing genetically modified ingredients (eg, soy or corn flour) are imported. No whole viable grain from GM varieties is imported.

Cultivation of GM products. No commercial cultivation to date.

12. Conclusions

New Breeding Technologies present remarkable new potential to contribute to the productivity, quality and profitability of the Australian vegetable industry. NBTs have the potential to benefit growers through increased yields, reduced input costs, increased flexibility in crop management and improved product quality. Importantly, these benefits, when combined with the potential for novel product characteristics present the opportunity to develop a competitive advantage in both domestic and export markets over produce from origins where production costs are lower. Benefits to consumers will accrue through improved quality, improved shelf life and enhanced health benefits, as well as other novel, produce characteristics. Reviewing the history of GM use in broad-acre crops demonstrates that major benefits can also accrue to the environment, benefiting society as a whole.

The technical potential of NBTs recognised by researchers, breeders, growers and other industry stakeholders in the Australian vegetable industry. However, three key concerns were expressed by the various stakeholder groups: (i) the risk of poor consumer acceptance, (ii) the cost of development and commercialization relative to the small size of many vegetable industries, and (iii) current regulatory uncertainty.

For the successful commercialisation of produce developed through NBTs, it will be necessary for industry to engage in a constructive dialogue with regulators, politicians, consumers and the community at large. Currently, there is very limited knowledge or understanding of NBTS or their potential benefits. Grower and community education, public outreach and strategies for consumer involvement will be critical components of a cooperative approach that leads to broad community recognition of the range of benefits for society that can result from a carefully considered use of NBTs.

The cost of developing and commercialising NBTs is significant, although the technical costs are decreasing at a remarkable rate. Regulatory and stewardship costs remain a major consideration. The size of this barrier will vary dramatically depending on the regulatory status of any given NBT or type of product. Indeed NBTs have the potential reduce the cost of breeding programs where products do not fall within the regulatory framework.

Given the current cost of development and commercialisation, the small size and great diversity of vegetable production industries in Australia mean that initial efforts to develop NBT vegetables needs to be targeted carefully. To maximise returns on investment, traits will need to have wide applicability and be incorporated into one of the major crops. Choosing a trait with clear consumer benefit will assist in re-forming perceptions of how society as a whole can benefit from the implementation of NBTs.

To achieve the full potential offered by NBTs there also needs to be revision of the Gene Technology Act 2000 and Gene Technology Regulations 2001 to accommodate more appropriately the diversity of NBTs and their potential applications. A system needs to be developed that allows for de-regulation of (i) those products in which the genetic changes are similar to or indistinguishable from the products of conventional breeding, and (ii) those technologies for which there is clear scientific evidence of a history of safe usage.

Addressing the challenges of regulation and community engagement will ensure a social licence to use NBTs to benefit the Australian vegetable industry, consumers and the environment, and contribute substantially to horticultural crop improvement in the future.

13. Recommendations

13.1 Research and Development

1. HIA and other RDCs should take a 5-10 year perspective to plan progress to the commercial implementation of NBTs.
2. HIA should take a targeted approach to the development and implementation of the first NBT traits in fresh vegetables. Initial traits selected for development using NBTs should:
    a. reflect clear consumer benefit, taking into account the results of research on types of benefits most valued by consumers in GM food, and
    b. target major crops to maximise return on investment, given the relatively small size of each crop in Australia and the diversity of production environments.
3. There is likely to be a need to collaborate with overseas companies for access to genotypes, capital and stewardship programs. In selecting collaborators, HIA should take into account the influence of an organisation's positive consumer profile to the acceptance of GM products.
4. Considering the rapid rate of development of NBTs and the relatively small size of the Australian research community, HIA should establish a dialogue with other agricultural RDCs to seriously consider supporting the development of a highly collaborative national centre for NBT applications or some targeted aspect thereof to help maintain Australia's strong research position.

13.2 Industry and Consumer Communication

5. HIA should assist industry to understand the value of NBTs to the development of the Australian vegetable industry, the environment and the community.
6. HIA should explore opportunities to develop a constructive dialogue with consumers and other stakeholders, and support community education and involvement in understanding the benefits of NBTs
7. HIA should consider strategies to support export market development for the early NBT vegetable varieties which can present distinct consumer benefits, as a means of strengthening Australia's export profile for quality fresh produce.

13.3 Regulatory Environment

8. HIA should contribute to the coming review of the Gene Technology Act 2000 to promote the development of an approach that allows for the deregulation of NBT products in classes SDN1 and SDN2, and the de-regulation of other classes of NBT applications based on a history of safe usage.
9. HIA should encourage OGTR to promote, where possible, harmonisation of regulations for NBTs with overseas countries importing Australian produce.

14. Appendices

Appendix 1. Glossary

Amino acid: The building blocks of proteins (*q.v.*). Organic compounds containing amine (-NH2) and carboxyl (-COOH) functional groups, along with a side chain (R group) that is specific to each amino acid.

Base pair (bp): A pair of purine and pyrimidine bases, in complementary strands of a double stranded nucleic acid. The base A pairs with T in DNA (with U in RNA); while G pairs with C in both DNA and RNA. The size of a double-stranded nucleic acid molecule is often given in terms of the number of base pairs it contains. tTe sequence of the bases along the DNA backbone encodes genetic information.

Biosecurity: A set of preventive measures designed to reduce the risk of transmission of infectious diseases in crops and livestock, as well as to avoid the spread of quarantined pests, invasive alien species, and living modified organisms.

Cassette: (gene cassette): a gene (or set of genes) together with regulatory elements that control its expression.

Chromosome: Each of the DNA molecules containing the genetic material of an organism. DNA is tightly coiled many times around histones (*q.v.*) that support its structure.

Cisgenics: The introduction of a gene or genes into cells *via* artificial gene transfer from a related species that could otherwise have been transferred by conventionally breeding (*see also* Intragenics).

Cloning: The process of producing similar populations of genetically identical individuals.

Construct (DNA construct): A segment of DNA that has been generated *in vitro* that may be transferred into target tissue.

Copy number: The number of copies of a particular gene in a genome.

crRNA: A 20 nucleotide sequence of RNA used in the CRISPR system to guide the Cas9 nuclease (*q.v.*) to the place in the DNA to be cut.

Cytogenetics: A branch of genetics concerned with how the chromosomes relate to cell behaviour, particularly during mitosis and meiosis.

DNA: Abbreviation for deoxyribonucleic acid. DNA is a biological polymer that constitutes the genetic material of all known organisms except some RNA-based viruses.

DNA construct: *see* Construct.

DNA sequencing: Procedures for determining the nucleotide (*q.v.*) sequence of a DNA fragment in their precise order.

dsDNase: An enzyme that specifically degrades double-stranded DNA molecules.

dsRNA: Double-stranded RNA.

Effector proteins: A small protein molecule that selectively binds to another molecule and regulates its biological activity.

Endogenous: Produced within; originating from, or due to, internal causes. Opposite to exogenous.

Epigenetics: Changes in a chromosome that do not affect its sequence, including chemical modification of DNA or histone molecules that affect gene activity and expression.

Eukaryotic cell (Eukaryotic organism): One of the major evolutionary clades (domains), characterized by having the nucleus enclosed by a membrane, and possessing chromosomes that undergo mitosis and meiosis. Eukaryotic organisms include animals, plants, fungi, algae, plant pests and some diseases.

Event (transgenic event): the insertion of a particular transgene into a specific location on a chromosome. The same

transgene inserted into another chromosomal location is another event.

Exogenous: Produced outside of; originating from, or due to, external causes. Opposite to endogenous.

Expression: *see* Gene Expression.

Gene cassette: *see* Cassette.

Gene editing: *see* Genome editing.

Gene expression (expression): The process by which information from a gene is used in the synthesis of a functional product, usually a protein.

Gene stacking: Combining two or more transgenes in a single transgenic organism.

Genetic diversity: The total number of possible genetic characteristics in the genetic makeup of a species; reflects all the possible variation of all traits.

Genetic linkage (linkage): Genetic elements which are located closely together on a chromosome tend to be inherited together.

Genome: an organism's complete set of DNA (or in the case of some viruses, RNA), including all of its genes.

Genome editing: (gene editing): a type of genetic modification in which DNA is inserted, deleted or replaced at a specific site in the genome of a living organism directed by a guide RNA using dsDNA nucleases. The CRISPR-Cas9 system is a widely used genome editing system.

HEJ *see Homologous end joining*

Histone*:* A highly alkaline protein found in eukaryotic (*q.v.*) cell nuclei. Histones are the main protein components of the chromosome. They play a major role in regulating gene expression.

Homologous: similar in position, structure, and evolutionary origin, but not necessarily in function.

Homologous end joining (HEJ; homology directed repair): pathway that repairs double-strand breaks in DNA which requires an oligonucleotide with ends complementary to the DNA sequence on each side of the ds DNA break to guide the insert (repair).

Intragenics: The introduction of a genetic elements or genes from the same species into cells (*see also* Cisgenics).

Linkage: *see* Genetic linkage.

Linkage drag: When crossing two organisms, the transfer of undesirable genes together with desired genes.

Mutation: A permanent change to the nucleotide sequence of the genome of an organism, a virus, or another genetic element.

NGG: In genome editing - the sequence (where N is any nucleotide) of the 'protospacer adjacent motif' (PAM) DNA sequence immediately following the DNA sequence cut by the Cas9 nuclease in the CRISPR genome editing system.

NHEJ *see* Non-homologous end joining

Non-homologous end joining: A pathway that repairs double-stranded breaks in DNA, which are directly ligated without the need for a complementary (homologous) template.

Nucleic acid: A large molecule consisting of a chain of nucleotides (*q.v.*). In living organisms two types are commonly found, DNA and RNA.

Nucleotide: building blocks of nucleic acids (DNA and RNA). They are composed of three subunit molecules: a nitrogenous base, a five-carbon sugar - ribose (in RNA) or deoxyribose (in DNA), and at least one phosphate group. The sequence of nucleotides determines the genetic information present in the nucleic acid.

Nucleotide polymorphism: DNA sequence variation.

Nucleotide Sequence: *see* Sequence.

Oligonucleotide: A short sequence of bases of nucleic acid (*q.v.*) used in research, transgenic organisms, genetic testing, forensics. A short chain of nucleotides (*q.v.*).

PAM *see Protospacer Adjacent Motif.*

Particle bombardment: A method for delivering exogenous DNA/RNA (e.g. transgenes) into cells using a micron size heavy metal particles (gold or tungsten) coated with DNA. Originally designed for plant transformation to transfer DNA across cell walls.

Polymerase chain reaction (PCR): A widely used molecular biology procedure that allows the production of multiple copies (amplification) of a specific DNA sequence, provided that the base pair sequence of each end of the target is known. It allows extremely small amount of the specific DNA sequence to be detected and quantified.

Protein: A large biological molecule. Proteins consist of one or more long chains of amino acids, the sequence of which is dictated by the nucleotide (*q.v.*) sequence of the genes by which they are encoded. Many proteins are enzymes which catalyse metabolic reactions in cells.

Protoplast: the protoplasm (cell contents) of a living plant within its cell membrane, whose cell wall has been removed.

Protospacer Adjacent Motif (PAM): is a 2-6 base pair DNA sequence immediately following the DNA sequence targeted by the Cas9 nuclease in the CRISPR genome editing system.

Ribonucleoprotein: an association between an RNA molecule and an RNA-binding protein.

RNA: Abbreviation for ribonucleic acid. RNA is an essential biological polymer involved in various biological roles in coding, decoding, regulation, and expression of genetic information.

Sequence: (Base sequence; Nucleotide sequence): The linear order of nucleotides along a DNA or RNA molecule (*See also*: DNA sequencing, genome).

Sequencing: *see* DNA sequencing.

Selectable marker: Often an antibiotic or herbicide resistance gene that confers a new trait used for the artificial selection of transgenic cells or organisms. If genetically linked (q.v.) to a desired genetic alteration, it allows selection of cells or organisms containing that trait by exposing a collection of cells or organisms to the antibiotic or herbicide: those without the selectable marker will die.

sgRNA: single-guide RNA. Combines the tracrRNA (trans-activating crRNA) and, crRNA (CRISPR targeting RNA) into a single RNA construct, simplifying the components needed to use CRISPR/Cas9 for genome editing.

Silencing (gene silencing): Loss of gene expression either through an epigenetic alteration in the DNA sequence of a structural gene, or its regulatory region, or because of interactions between its transcript and other RNAs present in the cell.

Stacking *see gene stacking*

tracrRNA: Trans-activating CRISPR-targeting RNAs are small trans-encoded RNAs that are part of a defence system in bacteria against invading viruses and plasmids. TracrRNA acts as a guide for the enzyme Cas9, which cleaves the nucleic acid. The system has been modified for use in eukaryotic organisms for the process of gene editing.

Transcription (transcribed): the process by which RNA (especially mRNA) is generated from the encoding DNA by the enzyme RNA polymerase. The transcript may subsequently be translated into one or more proteins.

Transformation: The uptake of introduced DNA into a cell's native DNA with the aim of changing the phenotype of the recipient organism in a predictable manner.

Transgene: An exogenous gene used to transform an organism, particularly when the gene originates from another species (transgenic), but can originate from a related species (cisgenic) or the same species (intragenic).

Transposable element (transposon): A DNA sequence that can change its position within a genome, sometimes creating or reversing mutations and altering expression of genes, changing an organism's genetic identity, and changing its genome size.

Virus: An infectious agent composed of a protein capsule and a nucleic acid core (DNA or RNA), which is dependent on a host organism for replication.

Appendix 2. Stakeholder consultation

Stakeholders who were interviewed to gauge perceptions towards the potential for new breeding technologies to contribute to the Australian vegetable industry are listed below.

Organisation	Contact	Role/Position
Growers		
The Mitolo Group	Gary O'Neil	Agronomist
Mulgowie Fresh	Andrew Sipple	Agronomist
Patane Produce	Penny Patane	Co-Director
Rugby Farm	Surachat	Agronomist
Vegetables WA	John Shannon	CEO
Zerella Fresh	Matt Bennett	Agronomist
Breeders and Seedsmen		
Abundant Produce	Graham Brown	Executive Director
Australian Seeds Federation	Michael Leader (Monsanto)	Chair of ASF Biotechnology Committee
Fairbanks	Anthony Ladds	General Manager
Nexgen Plants	Brian Ruddle	Interim CEO
Rijk Zwaan Australia	Arie Baelde	Managing Director (Aust)
Biological Researchers		
Dept Economic Development, Jobs, Transport and Resources	Tony Slater	Research Leader, Molecular Plant Biology
Griffith University	Rebecca Ford	Associate Professor
Queensland Univ of Technology	James Dale	Distinguished Professor
Queensland Univ of Technology	Peter Waterhouse	Professor of Molecular Biology
University of Queensland	Jimmy Botella	Professor of Plant Biotechnology
University of Queensland	Neena Mitter	Professorial Research Fellow
University of Tasmania	Calum Wilson	Associate Professor in Plant Pathology
Social Science Researchers		
Think Outside The…	Craig Cormick	Creative Director
University of Adelaide	Heather Bray	Senior Research Associate (Humanities)
Other Organisations		
Crop Life Australia	Osman Mewett	Director – Crop Biotechnology Policy
Food Safety Australia New Zealand (FSANZ)	Janet Gorst	Senior Scientist, Risk Assessment – Biological Sciences
Food Safety Australia New Zealand	Barbara Butow	Section Manager, Risk Assessment –

(FSANZ)		Biological Sciences
Grains Research and Development Corporation (GRDC)	Juan Juttner	General Manager – Genetic technologies
Office of the Gene Technology Regulator (OGTR)	Heidi Mitchell	Director, Plant Evaluation
OGTR	Louise Mathew	Assistant Director, Regulatory Practice and Secretariat Section

Appendix 3. Outcomes from the OGTR 2016-2017 Technical Review of the Gene Technology Regulations Act 2001

As part of the review of the Gene Technology Regulations 2001, the OGTR sought submissions on how site-directed nuclease techniques and oligo-directed mutagenesis (both falling within the definition of NBTs) should be regulated. We examined the responses to provide a broad overview of positions held by different groups.

In an attempt to structure responses, the OGTR prepared a discussion paper in which the advantages and disadvantages of four options were outlined. Respondents were requested to indicate their preference and present a reasoned argument for the position held. The four options were:

Option 1:	**No amendments to the Gene Technology regulations.**
Option 2:	**Regulate certain new technologies.** Amend the Gene Technology Regulations so that dealings with all organisms developed using oligo-directed mutagenesis and all site-directed nuclease techniques are regulated under the Gene Technology Act.
Option 3:	**Regulate some new technologies based on the process used.** The use or absence of a nucleic acid template to guide DNA repair determines whether techniques are regulated under the Gene Technology Act. That is, techniques where nucleic acid template is applied to guide DNA repair (i.e. oligo-directed mutagenesis and the site-directed nuclease techniques known as SDN-2, SDN-3) would result in GMOs, whereas some specific techniques which do not involve the application of nucleic acid template (i.e. the site-directed nuclease technique known as SDN-1) would not result in GMOs
Option 4:	**Exclude certain new technologies from regulation on the basis of the outcomes they produce.** Exclude organisms from regulation as GMOs if the genetic changes they carry are similar to or indistinguishable from the products of conventional breeding (e.g. chemical and radiation mutagenesis methods and natural mutations). This would have the effect that dealings with organisms produced by oligo-directed mutagenesis and SDN-1 and SDN-2 would be excluded from regulation.

Overall, the submissions in response to the discussion paper reflected a general desire to move toward regulation based on the outcomes of any intervention. However, there was a degree of reservation as to whether it would be feasible to implement this at the present time. There was also a definite difference in the responses depending on the background of the respondent.

For the purposes of this report, the responses given were tabulated according to the function of the organisation and whether or not an individual indicated that they had involvement with, or connection to, industry or research (Table A2.1). In the small number of cases when a respondent nominated more than one of the potential scenarios, both responses where included in the table to provide a fuller picture of the range of responses. A coordinated group of 600 *pro forma* responses not addressing the issues presented could not be incorporated in the analysis.

Table A2.1: Preferred options indicated in submissions to the 2016-2017 technical review of the Gene Technology Regulations 2001 categorized by the organization type or the involvement of individuals.

Choice[1]	Industry[2]	Government[3]	Research[4]	Individual With No involvement[5]	Individual With involvement
Option 1	1	0	2	0	1
Option 2	0	1	6	7	0
Option 3	2	3	10	0	1
Option 4	20	3	15	0	4

Notes:

1: In the small number of cases in which a respondent nominated more than one option, both were collated. Most of these cases reflected a view that Option 4 would be preferable in the long term but other options may be expedient at present.

2. Industry organisations and commercial entities.

3. State governments and state and federal government departments

4. Universities, other research organizations and learned academies

5. There were about 40 responses from individuals who did not indicate special knowledge of, or involvement, in the use of biotechnology or NBT's.

Preferences differed between the groups. State governments or their departments and federal government departments supported Options 3 or 4. One Department indicated that while Option 4 was an eventual target, various unresolved issues around implementation made Option 3 preferable at the present time. One respondent considered that dealings with animals should be treated more conservatively than plants and indicated separate options to reflect this stance.

Industry organisations and commercial entities overwhelmingly favoured Option 4. One organisation dealing solely with human therapies indicated that Option 1 covered all requirements. Two organisations indicated Options 3 or 4; in one case the preference being for Option 4 unless consumer sentiment necessitated Option 3.

The most divergence was seen in responses from research organisations. While Option 4 was the most common response, significant numbers of respondents selected Option 2 and 3. Of those who selected Option 2 or Option 3, the two most common reasons expressed against Option 4 were (a) that it was not consistent with the current legal framework under which the regulations are implemented and (b) anticipated difficulties in implementation caused by lack of clarity in the definition and a high degree of subjectivity, resulting in considerable administrative overburden in decision making. For this reason, a few organisations indicated that while Option 4 may be a long term goal, under current condition Option 2 or Option 3 were preferred.

There were about 40 responses from individuals who did not indicate special knowledge of, or involvement, in the use of biotechnology or NBT's. Most of these did not indicate a preference for any of the four options. While a minority indicated blanket opposition to the use of technologies of this type, the more common responses expressed where (i) a need for continued careful regulation and (ii) a desire for appropriate labelling of products that contain components derived from genetically modified organisms. The seven respondents who did nominate an option all indicated Option 2.

In a few cases, individuals indicated involvement with, or connection to, industry or research. These were grouped separately from those not indicating involvement. Respondents in this category tended to support Option 4.

Overall, most categories of respondents tended to support the implementation of Option 4 or a movement toward that end point. That is, there was strong support for movement toward a system based on the outcome rather than the technology used to develop it. In contrast, general individual respondents expressed concern over the need for continued regulation and appropriate product labelling. In the context of this report, this would suggest a significant level of consumer concern around the adoption of NBTs. However, the respondents to the OGTR review are very unlikely to be a representative subset of the wider community**.** An analysis based on actual purchasing data where GM and non-GM products are available and based on the wider Australian consumer community would provide valuable information for decision making.

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16. Acknowledgements

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