New breeding technologies
for the Australian vegetable industry

Methods for improving crop varieties are advancing very rapidly. In particular, New Breeding Technologies (NBTs) promise a dramatic revolution in plant breeding. The expression New Breeding Technologies describes a suite of biotechnology-based methods designed to rapidly and precisely improve plant traits. Depending on how the techniques are applied, the new varieties generated may or may not be considered to be genetically modified organisms (GMOs). Regulators are currently assessing how to classify varieties produced using the various NBTs. Where the demarcation line falls will have profound implications for the Australian vegetable industry; it will dictate the types of new traits available, and the time and cost needed to commercialise each variety. The technological capacity for NBTs to benefit the Australian vegetable industry is very high. The challenge is to develop a constructive dialogue with regulators, consumers and the wider community, to ensure the benefits of new varieties derived from NBTs are widely valued.

Industry setting

The Australian vegetable industry makes a critical contribution to the health and well-being of the population by ensuring the ready availability of a diversity of quality fresh produce. However, it has been under economic pressure for some decades. To ensure its success into the future it must improve on-farm profitability and increase both domestic and export market share. The domestic market accounts for 93% of Australian vegetable production but expansion of this market is largely constrained to the rate of population growth. In contrast, the export market is only 7% of production but has grown by 60% in the last decade. While Australia has a good standing for safe, high quality produce, to expand market share it will be increasingly important to meet the shifting demands of the market place. New breeding technologies (NBTs) provide major new opportunities that will benefit the vegetable industry by improving farm profitability through reduced input costs and improved yields, and enhancing sales by providing a range of new traits designed for consumer benefit.

New breeding technologies

NBTs are a broad and varied suite of biotechnologies that have developed from our increasing knowledge of how genes work, and the mechanism by which genetic information is translated into traits in plants.

Descriptions of the most important NBTs are given on the last page. While NBTs include the older methods of genetic modification (GM) already in use, they also include a range of new and less well known techniques that cannot easily be classified as GM. Products from NBTs range from clearly GM to clearly not GM. The NBTs which most clearly do not fit under the conventional definition of GM are (i) those which induce small targeted changes in existing plant genes which could have readily happened by natural means, and (ii) those which modify the activity of genes without changing the genetic code itself, a process known as epigenetics.

Targeted mutations: The DNA in all cells is frequently breaking and being repaired. Sometimes, during repair, an alteration is made in the sequence of bases that make up a gene. Genetic changes (mutations) that occur naturally are known as sports and are the source of many traditional horticultural varieties. The well-known apple variety Royal Gala arose from a natural mutation that occurred in variety Gala. NBTs can be used to generate beneficial changes in a far more predictable, precise and safe manner than the process of mutation that occurs naturally in all organisms.

Epigenetics: Every cell within an organism contains an identical copy of the genome (the total compliment of genes of the organism), but the activity of each gene is differentially controlled. For example, although root cells contain flowering genes, they remain inactive.

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Murdoch UNIVERSITY

Some NBTs provide the capacity to alter gene activity without changing the underlying DNA code. For example, reducing the activity of enzymes that soften cell walls during ripening can extend the shelf life of fruit and vegetables without negatively affecting taste.

Challenges for implementation

The technological capacity for NBTs to contribute to the future success of the Australian vegetable industry is very high. However, there are three challenges to their implementation: regulation, cost, and market acceptance. The regulatory environment will have a major impact on the other two.

The regulation of the various NBTs is currently being considered by regulators. In Australia, the two key bodies for the regulation of new products created through NBTs are the Office of the Gene Technology Regulator (OGTR) and Food Standards Australia New Zealand (FSANZ). Both organisations recognise that there will be challenges in applying the current legislative and regulatory arrangements to some NBT products in a manner that reflects the original intent of the Gene Technology Act 2000. They also recognise that clarity and certainty are required for investment in both research and commercialisation. The demarcation between GM and non-GM for products developed using NBT will have important implications. If a particular product falls within the definition of GM, the cost of commercialisation will be high and its GM status may negatively influence market acceptance. Other products of NBT will not be defined as GM; they are likely to be less costly to commercialise and more readily accepted in the market.

The cost of development and commercialisation using NBTs is currently relatively high but is falling rapidly as technology develops. For the Australian vegetable industry, the economic challenge is exacerbated by the relatively small size of each industry within Australia and by the regional variation in key production issues. Initial efforts will need to address industry-wide challenges to maximize economic return.

Presently there is very little information available on acceptance by Australian consumers of foods produced by NBTs. Industry leaders and researchers expect that new vegetable varieties produced by NBTs that are not classified as GM are likely to meet little or no market resistance from growers, retailers or consumers. On the other hand, if certain NBTs are ruled to be GM, it is likely that they will be rejected by those consumers who reject the GM products on the market today.

Surveys of attitudes to GM foods show that the market is still largely cautious. However, surveys give a poor indication of actual purchasing behaviour, with much less discrimination occurring in actual purchases than indicated by surveys results.

There is considerable variation between individuals in acceptance of GM foods and in their response to information about the benefits of GM technologies. Further, acceptance is altered by the perceived value of the new trait. Thus, people are generally more likely to purchase a GM product if it provides additional health benefits or reduces any risk of pesticide residues. Current GM varieties have traits designed mainly to benefit producers (herbicide and insect tolerances) rather than consumers. Vegetable varieties with novel traits attractive to consumers have not yet been well tested in the marketplace.

Attitudes are also strongly influenced by the perceived social responsibility of organisations involved in the development and commercialisation of the product.

‘Innate’ potato: lower inputs and less waste

Conventional (left rows in each pair) and Innate (right rows in each pair) exposed to late blight

The J.R. Simplot company in the USA released their first innate Innate potato variety in 2015. A second version is now in the approvals process. It employs RNAi technology to turn off key enzymes in the biochemical pathways that lead to bruising, cold induced sweetening, and acrylamide production. It is also cisgenic in that in includes a gene introduced from a wild relative of potato to confer resistance to *Phytophthora infestans* (late blight). The outcomes are less chemical inputs in production, less waste during storage, processing and preparation, and less risk of any possible effects that may arise from acrylamide. Benefits accrue to all stakeholders from the grower to the consumer.

Export potential

Australia exports vegetables to over 60 countries with a strong focus on Asia. With rising incomes and changing consumer preferences, improved living standards in Asia provide Australia with an excellent

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opportunity to supply the region with high quality, safe and traceable produce. As standards of living rise, consumers demand better quality, greater quality assurance, and assured provenance.

As for Australia, the response of Asian customers to vegetable varieties produced using NBTs is expected to depend on which products are ruled to be GM and non-GM and on the perceived benefits of the new traits. Acceptance of GM varieties by consumers differs between countries and the situation is complicated by the lack of standard definitions of what constitutes a GMO. Table 1 provides a summary of the current status of GM food imports in key countries that import Australian vegetables.

Key points for implementation

It will be important for the industry and research funding bodies to take a medium term view (5-10 years) for the successful implementation of NBTs. This is not only to accommodate development and commercialisation but also to allow for appropriate community interaction to ensure wide support. This will require an open dialogue around the benefits of the approach to the consumer, the environment and the economy and will need to involve government regulators, the vegetable industry and consumers.

To maximise acceptance, the first traits considered for commercialisation should show clear consumer benefit, ideally personal benefit in terms of health or product quality. The choice of industry partners should also consider community perceptions of the social responsibility of the possible partners.

Industry stake-holders and research funding bodies can contribute to the likely success of NBT vegetables by advocating for, and contributing to, the development of appropriate regulatory frameworks, and lobbying for the harmonisation of frameworks within the region.

NBT Papaya saves industry in Hawaii

Papaya fruit infected with PRSV (L) and fruit from the PRSV resistant variety (R) developed using RNAi.

Hawaiian papaya production is concentrated in the Puna district. PRSV was found in the production region in 1992 and by 1995 the industry was in crisis. The virus was causing death of seedlings and decline in the vigour and yield of trees. Many plantations were being abandoned. Production in the region fell by 50%.

Because the threat had been foreseen and pre-emptive research initiated in 1985, RNAi-base PRSV-resistant varieties could be released as early as 1997. The new varieties were taken up rapidly. In just five years, production recovered to the extent that GM papaya was being exported to Canada.

Table 1 Current status of GM food imports for key destinations for vegetables from Australia.

Export destination	Import regulations	Labelling required	Consumer acceptance	Current GM Imports
Japan	Yes [a]	Yes	Low but substantial consumption	High volume. Alfalfa, beet, canola, corn, cotton seed, papaya, potato, soy and 33 processed foods
New Zealand	Yes	Yes	Low	Products from canola, corn, cotton, soy
Singapore	Guidelines	Guidelines	OK	21 approvals including cotton, maize, soy, sugar beet
United Arab Emirates	Yes	Yes	Unknown	Unknown
Malaysia	Yes [b]	Yes	Unknown	Include maize, soy and processed foods
Indonesia	Yes [a]	Yes	Low awareness	High volume. Corn, soybean, sugarcane
China	Yes [a]	Yes	Currently strong debate	High volume. Canola, corn, cotton, soy

a. Local administrative rules may also apply
b. Based on the Australian system

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Important new breeding technologies

NBTs provide a range of exciting and highly controllable ways to generate new plant varieties. Some provide new possibilities not previously available. A number of key ones are explained here.

Transgenic plants: These are GMOs that contain one or more beneficial genes from unrelated organisms, referred to as transgenes, transferred using recombinant DNA technology. An example is insect resistant crops that contain genes from the bacterium *Bacillus thuringiensis* (Bt) and so produce insecticidal proteins. Multiple transgenes can be put into the same plant; this is referred to as 'gene stacking'. Some GM maize varieties contain up to eight transgenes for various traits.

Cisgenic plants: These are GMOs that contain one or more beneficial genes from a closely related organism, referred to as cisgenes, transferred using recombinant DNA technology. The introduction could alternatively have been achieved *via* conventional plant breeding processes. The approach can be used to rapidly and cleanly introduce a novel trait (such as disease resistance) from a wild relative.

Intragenic plants: These are GMOs that contain one or more beneficial genes originating from the same species as the recipient plant but transferred using recombinant DNA technology. The same result could have been achieved by conventional breeding or over evolutionary time. This approach can provide dramatic savings in time and money when an elite variety lacks a single necessary trait.

Transgenic rootstocks: Non-transgenic scions have been grafted on to transgenic root stocks. The aim is to provide the benefits of certain GM traits (such as resistance to soil borne pests or diseases) whilst maintaining the recognised characteristics of the scion. Australian regulators currently consider the plant as a whole and therefore classify the produce as GM.

RNA interference: RNAi (also known as gene silencing) can be used to reduce or switch off the expression of specific genes. The cell is induced to manufacture small pieces of RNA that bind to the messenger RNA produced by the target gene. This interferes with the gene performing its natural function. RNAi has been used to generate non-browning apples, and to reduce bruising and cold-sweetening in potatoes.

- **Host-Induced Gene Silencing:** In HIGS the interfering RNA that is incorporated into the plant is designed to silence a vital gene in a pest or pathogen. When the pest or pathogen attacks the plant, the interfering RNA triggers silencing of the gene. Thus the host plant has enhanced resistance.
- **Spray-Induced Gene Silencing:** In SIGS the interfering RNA is again designed to silence a vital gene in a pest or pathogen but in this case it is sprayed onto the plant using conventional spraying equipment. As the RNA is not incorporated into the host's genetic structure, SIGS is not actually a NBT.

Reverse breeding: When a superior heterozygous plant is identified in a segregating population from a cross, gene silencing can be used to prevent the mixing of genes during the formation of the spores (held in the pollen and ovule). This means that the parent genotypes can be identified. This allows the unique cross to be reproduced.

Genome editing: includes a set of powerful new and emerging technologies based on recognising specific DNA sequences to direct where an enzyme will cut or modify a gene.

- **Oligonucleotide-directed mutagenesis** introduces few new bases into a gene to generate a specific, targeted mutation.
- **TALENS** (transcriptional activator-like effector nucleases) and **ZNF** (Zinc Finger Nucleases) use specially design proteins to identify the DNA sequence for the enzyme to cut. **CRISPR** uses a short RNA guide sequence to identify the target.
- **Ribonucleoprotein genome editing** is a new method to generate genome-edited plants without introducing foreign DNA at any stage (DNA is usually introduced at one stage and subsequently removed). In this approach, the CRISPR guide RNA and the enzyme to cut the DNA are first mixed together and then introduced into plant cells.
- **Site-directed nucleases:** TALENS, ZNF and CRISPR are used to generate changes termed either SDN1, SDN2 or SDN3. In SDN-1, the DNA in the target gene is cut and repairs naturally. A certain proportion of the repaired cells will incorporate an error; that is, a mutation occurs. In SDN-2, one or a few additional DNA bases are specifically added at the site of the break. In SDN-3 the introduced DNA is longer, up to the size of a complete gene.

Targeted alteration of gene expression through epigenetic control: Epigenetics is the study of changes in organisms caused by the modification of gene activity, rather than alteration of the genetic code itself. Using an RNA guide to locate a gene (as is done for CRISPR) chemical groups can be attached to the DNA to alter a genes activity.

Authors:
Dr Steve Milroy
Dr Steve Wylie
Prof Mike Jones
School of Veterinary and Life Sciences, Murdoch University

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