The 2021 Banks Memorial Lecture: Before and after genetic engineering: Perspectives from a plant biologist

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Before the advent of the new breeding technologies, which include genetic engineering and gene editing, plant breeders used many techniques to modify plants genetically. Genetic engineering – generally defined as the transfer of a gene (transgenics) from one species to another species with which it would never naturally breed – has raised the spectre of 'tinkering with nature', and includes issues such as unexpected changes, the contamination of organic produce, escape into the wild, farmers being unable to save seed, and multinational companies gaining control of the seed supply. As will be discussed below, these issues could equally apply to several of the techniques used by plant breeders over many decades and yet, in New Zealand, genetic engineering is highly regulated. The second new breeding technology, gene editing, has been placed under the same strict regulations as genetic engineering, a situation that is currently being challenged in New Zealand. Because of the precision with which gene editing can be carried out, the question is whether gene editing should be treated differently to genetic engineering.

With all plant breeding techniques, the basis for comparison should be traditional plant breeding, which relies on successful pollination, fertilisation, and seed development. This typically occurs when plants of the same species are used, and the parental chromosomes can pair. Seed can often be saved and used by the farmer the next season.

However, even if traditional breeding techniques are used seed cannot always be saved. The first example of farmers not being able to save seed occurred following the development of hybrid maize by George Shull in 1909. This followed the discovery of hybrid vigour, a phenomenon exhibited when different inbred lines of maize are crossed. The hybrids exhibit enhanced yield. Much of the world's maize production is hybrid maize. The Maize Hybrid Seed Production Manual produced by CIMMYT (The International Maize and Wheat Improvement Center) details hybrid maize production (MacRobert et al., 2014).

However, seed from the hybrid maize plants that the farmers grow cannot be used the next season as the yield in the offspring will be highly variable. In other words, the seed cannot be saved to good effect. For the home gardener, seed for F1 hybrid plants is commonly available (e.g., selections of broccoli, carrot, eggplant, pak choi, radish, etc.), but saved seed will not return uniformly high yielding plants the next season.

'Hybridisation' in plant breeding generally refers to wide crosses between different species or genera. If the parents have different chromosome numbers, the offspring are usually sterile because the chromosomes of the hybrid cannot pair, preventing further breeding. However, it was discovered in 1937 that if the alkaloid colchicine is used, chromosome doubling can occur. If colchicine is absorbed by a dividing cell during mitosis, the chromosomes split but do not migrate to opposite sides, because the colchicine inhibits the microtubules, and division of the cell is prevented. The cell ends up with twice as many chromosomes with each one doubled up. The doubling means that each chromosome now has a pair and the hybrid gains fertility. The review by Eng and Ho (2019), "Polyploidization using colchicine in horticultural plants", lists a substantial number of horticultural plants developed using colchicine, and provides details of the processes used over the last 80 years.

With some wide crosses (i.e., those beyond the species barrier), fertilisation may occur, but the embryo does not develop because the endosperm (the food that feeds the growing embryo) does not function properly and the embryo does not develop. However, techniques were developed so that the embryo could be 'rescued' and grown either on a medium containing endosperm (such as banana or coconut milk) or on an artificial medium (Picó et al., 2002; Ohnishi et al., 2011; Pathirana et al., 2015). Embryo rescue has been used in the development of intergeneric crosses in orchids, such as crosses between *Vanda* orchids and *Phalaenopsis* moth orchids leading to the development of orange moth orchids (× *Vandaenopsis*; Fig. 1).

Fig. 1 × *Vandaenopsis* Kdares Orange Girl, an intergeneric orchid. Photo: '阿橋 HQ' (CC BY-SA 2.0).

A combination of embryo rescue plus colchicine treatment was used in the development of the new cereal triticale (× *Triticosecale*; Fig. 2), which is the result of wide crosses between wheat (*Triticum*) and rye (*Secale*) – a crop currently grown in New Zealand and elsewhere. Such 'tinkering with nature' has no legislation governing its use.

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Fig. 2 Triticale (× *Triticosecale*). Photo: 'God Emperor' (CC BY-SA 3.0).

Mutation breeding, which is the purposeful application of mutations, has been used in plant breeding for more than 80 years. There is little control over the magnitude or kind of genetic change that occurs. The changes, induced by mutagenic chemicals (e.g., ethyl methane sulfonate, EMS) or irradiation (often ^{60}Co delivering gamma rays), are mostly point mutations, but these are random, multiple, and unspecific. Such mutations in the DNA may lead to a change in one or more proteins. As these changes are random, unexpected changes are the norm and most of the variants will be discarded from the test tube or later during field testing.

The International Atomic Energy Agency (IAEA), whose role is in the promotion of the safe, secure, and peaceful use of nuclear technology, has a register of some 3,364 mutant selections of more than 210 different plant species from over 70 countries, including numerous crops, ornamentals, and trees, which have officially been released for commercial use (www.iaea.org/topics/mutation-breeding). Approximately 25% are ornamentals or decorative plants. More than 500 ornamentals are registered – mostly developed in the Netherlands. Popular in the FAO/IAEA Mutant Variety Database are named selections of alstroemerias, chrysanthemums, gladioli, orchids, roses, and *Streptocarpus*.

However, the other 75% of the plants developed using mutagenesis are crop plants, including barley, grapefruit, pears, peas, rice, and wheat. The latest registered varieties on the FAO/IAEA database include two rice selections, registered from India and the Philippines in 2019. Useful mutations that have been selected for include those resulting in increased yield, enhanced quality attributes, disease resistance, herbicide tolerance, tolerance of acid and saline soils, and drought tolerance (Pathirana, 2011).

Even though there is little control over the changes induced using mutagenic agents, there is no legislation governing mutation breeding in New Zealand. Food companies offering organic products have, possibly inadvertently, used fruit or seeds from cultivars derived through mutation breeding. Classic examples on the web show organic beer derived from 'Golden Promise' barley. This cultivar was induced with gamma rays and was widely grown for several decades. 'Golden Promise' barley was selected for its semi-dwarf stature, salt tolerance and high malting quality. It is still used in fine whisky and craft beers (Schreiber et al., 2019). A second common example is that of 'Rio Red' grapefruit (Fig. 3), derived by treating bud sticks with thermal neutrons. Selected attributes included improved fruit and juice colour (deeper red) and tolerance to a wide range of growing conditions. 'Rio Red' fruit juice is sold as 100% organic.

Fig. 3 *Citrus* × *paradisi* 'Rio Red' grapefruit. Photo: 'Babij' (CC BY-SA 2.0).

In New Zealand, herbicide tolerant brassicas have been developed by PGG Wrightson in partnership with the Crown Research Institute Plant and Food Research using a combination of mutagenesis and traditional breeding. The mutagenesis was initiated in 1992, by soaking *Brassica napus* seeds in EMS and selecting seedlings tolerant to the broad-spectrum sulfonyl urea herbicide DuPont® Telar®. Plants from more than 30,609 seeds were screened in the second generation and traditional breeding was subsequently used to introgress the herbicide tolerance into other brassicas. Dumbleton et al. (2012) describe the development of the DuPont® Telar®-resistant swedes, forage rape, bulb and leafy turnip, currently marketed as the Cleancrop™ Brassica System. The seed and herbicide are sold as a package (much the same as Monsanto sold Roundup® alongside its Roundup Ready soybean), along with a best practice and stewardship plan.

When examined in detail, it becomes apparent that many of the arguments against genetic engineering could equally be aimed at several accepted plant breeding techniques. But genetic engineering has caught the public eye. It is a more powerful technique as genetic material can be transferred across kingdoms, such as the transfer of a firefly gene into tobacco which, when supplied with the appropriate substrate, luciferin, will glow in the dark (Fig. 4) (Ow et al., 1986).

Fig. 4 Transgenic tobacco plant incorporating the luciferase gene from the firefly. The luciferase gene is used by researchers to 'report' where, in a genetically engineered plant, a gene is being expressed. This is achieved by taking the promoter of the gene under investigation and linking it to the luciferase 'reporter' gene and supplying the plant with luciferin. Image courtesy of the National Science Foundation.

The first genetically engineered plant was reported in 1983. Genetic engineering involves inserting DNA into a plant via *Agrobacterium tumefaciens* or via a 'gene gun'. The insertion event is essentially random, and traces of the insertion mechanism remain. Internationally, the most common events include the production of herbicide tolerant plants

(e.g., Roundup Ready® soybean and canola) and insect tolerant plants (e.g., Bt maize, cotton and eggplant/brinjal), or plants carrying both events. Commercially grown transgenic virus-tolerant plants are not as common, although papaya (pawpaw) grown in Hawaii is most likely transgenic. Some transgenic virus tolerant papaya is also grown in China. Other virus-tolerant crops are in development, including potatoes. The International Service for the Acquisition of Agri-biotech Applications (ISAAA) website (www.isaaa.org) provides a link to a 'GM Approvals Database', by crop and by country, as well as links to scientific papers, including those on gene editing. The ISAAA lists 26 transgenic crops. In terms of flowers, only petunia, rose and carnation are listed. Transgenic carnations with novel mauve/purple blooms were first developed by Florigene Ltd in Australia and commercialised in 1996, following attempts to obtain blue flowers (Okitsu et al., 2018). Eleven selections of the Moonseries of carnations (e.g., Moonvista™; Fig. 5A–B) are now grown commercially and/or sold in several countries (Fact Sheet. Genetically modified (GM) chrysanthemums in Australia[2]). Florigene/Suntory Holdings Ltd also developed the transgenic rose 'Applause'. A Biosafety Clearing-House link (https://bch.cbd.int/database/record.shtml?documentid=115379) describes the transgenic events leading to the (not quite) 'blue' rose (Fig. 6A–B). Unfortunately, the quest to produce genuinely blue carnations or roses has not yet been successful.

While appropriate crop husbandry is necessary to prevent the escape out of cultivation of any transgenic plant (Gilbert, 2010), it has been confirmed that bright orange transgenic petunias have 'escaped into horticulture'. The transgenic event involved the transfer of a maize gene (A1) into a pale pink petunia. The resulting petunia had novel, bright orange flowers (Meyer et al., 1987). While these petunias were not commercialised, they were spotted growing in Helsinki in 2015.

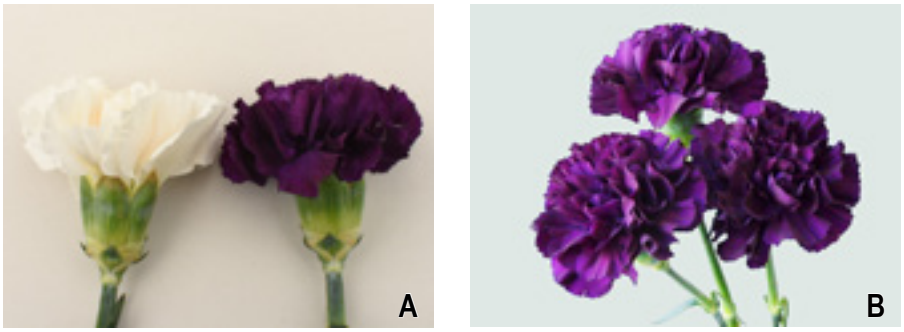

Fig. 5 Moonvista™ carnation. **A**, white flowered parental control (left) alongside transgenic Moonvista™ (right) expressing genes incorporated from pansy and petunia and producing the pigment delphinidin. **B**, close-up of flowers. Photos courtesy of Dr Steve Chandler, Suntory Flowers Ltd, Japan.

Fig. 6 Attempts to produce a blue rose led to the commercial production of the mauve *Rosa* 'Applause'. **A**, bunch of flowers. **B**, close-up of flower. Photos courtesy of Dr Steve Chandler, Suntory Flowers Ltd, Japan.

Fig. 7 Orange-flowered *Petunia* 'African Sunset', which received an AAS bedding plant award in 2014, before its fall from grace following the realisation that it was transgenic. The decision to ban petunias containing the A1 gene from maize appears to have been recently reversed (2021) in the US. Photo courtesy of All-American Selections.

[2] www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/9AA09BB4515EBAA2CA257D6B00155C53/$File/11%20-%20Genetically%20modified%20(GM)%20crops%20in%20Australia.pdf.

They were identified as transgenic and numerous selections across Europe and the US were destroyed (Fig. 7). Refer to the article by Servick (2017) "*How the transgenic petunia carnage of 2017 began*" for more details. A more recent publication suggests that there were two different lines of transgenic petunias that had 'escaped into horticulture' (Voorhuizen et al., 2020).

While there are no commercially grown genetically engineered crops in New Zealand, medicines derived through the use of transgenic microbes are widely used, the earliest being insulin introduced in 1983, and the most recent being the Pfizer-BioNTech COVID-19 vaccine first administered in New Zealand in 2021. Foods derived from 10 transgenic crop plants are currently on supermarket shelves, e.g., products from canola, maize, potato, and soybean. Food Standards Australia New Zealand provides a list of such foods under Standard 1.5.2 – Food produced using Gene Technology (www.foodstandards.govt.nz/consumer/gmfood/applications). The ISAAA 'GM Approvals Database' referred to previously includes New Zealand, but these approvals are for food use only.

Contained laboratory experiments using recombinant DNA technology are permitted in New Zealand under tight regulations (approved by the EPA) and inspections (conducted by MPI). Field trials are permitted with controls set by EPA. "Conditional release" (farm scale with controls) has been permitted since 29 October 2003, but no crop plants have been granted either conditional release or "release" (farm scale, with no controls). The issue of cost (and destruction of trials) is such that some field trials have been conducted overseas and/or the intellectual property rights (IP) sold. One could infer that our restrictive legislation has simply fed into multinational companies gaining control of New Zealand-developed IP.

As reported by Matveeva and Otten (2019), naturally occurring transgenic plants may not be all that uncommon. Indeed, Kyndt et al. (2015) present convincing evidence that the 291 tested accessions of cultivated sweet potato, which include kumara, contain specific portions of DNA from an *Agrobacterium* spp. As this DNA was not present in closely related wild relatives, Kyndt et al. (2015) suggest that the *Agrobacterium* DNA provided a trait or traits that were selected for during domestication of the sweet potato. In other words, this was a case of natural genetic engineering.

With respect to genetically engineered plants, New Zealand is still where we were at the time of the Royal Commission at the turn of the century. Interestingly, South Australia has just lifted a 16-year moratorium on the growing of genetically engineered plants. Elsewhere in Australia, Bt-cotton and GE canola have been grown commercially for some years (refer www.ogtr.gov.au).

The most recent new breeding technology utilises gene editing, a technique that has taken the biological world by storm. The 2020 Nobel Prize in Chemistry was awarded to Emmanuelle Charpentier and Jennifer Doudna for their development of the CRISPR/Cas9 editing system, the basis for the current surge in gene editing of crop plants. Gene editing refers to molecular techniques that make changes in the DNA sequence at specific sites within the genome (Fig. 8).

The Royal Society Te Apārangi has several resources explaining gene editing (www.royalsociety.org.nz/major-issues-and-projects/gene-editing), including a video. A recent article by Hudson et al. (2019) canvassed Māori perspectives of gene editing in Aotearoa New Zealand.

While a genetic engineering step is required to transfer the CRISPR/Cas9 editing system into a plant, subsequent crossing means that no trace of the insertion event remains. The gene to be edited (mutated or replaced) must be known. Applications of gene editing are numerous, including, for example:

- increasing resilience to climate change
- enhancing disease resistance
- reducing use of fertiliser
- increasing yield by increasing the seed number and/or seed size in various crops, or by decreasing seed shedding in perennial ryegrass seed crops
- increasing herbage digestibility
- decreasing the impact of neurotoxins by editing genes in endophytes
- increasing herbicide tolerance
- speed breeding in tree crops
- changing flower and fruit colour

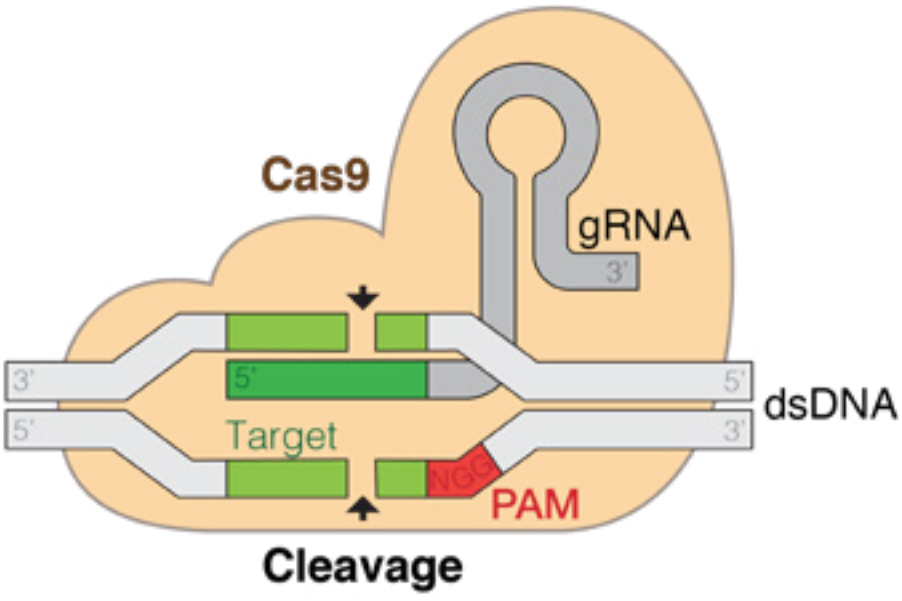

Fig. 8 Model of the action of CRISPR/Cas9 in the cell. The CRISPR guide RNA (gRNA) is designed to match the DNA sequence that is to be mutated. The gRNA and the Cas9 are co-transformed into the plant and form a complex. The gRNA aligns with the target gene and the Cas9 protein acts as a pair of scissors and cuts the DNA. Once this has occurred the gRNA and Cas9 leave the scene. The cut DNA will re-join but the gene sequence may be disrupted, i.e., mutated. Image: 'Mariuswalter' (CC BY-SA 4.0).

- reducing fertility in conifer species to reduce wilding potential etc.

Clearly, there are multiple applications directed to known genes that could be, and in some cases are being, targeted in New Zealand. Internationally, the crops now being genome-edited are mainly cereals, followed by oilseed crops, vegetables, fruits and nuts, and pulses (Lassoued et al., 2021).

Currently, the legislation applied to gene editing in New Zealand is identical to that of genetic engineering – highly restrictive (Fritsche et al., 2018). It is important to be aware that we cannot distinguish between a natural mutation, a chemically or irradiation induced mutation, or a CRISPR/Cas9 induced mutation. Even though CRISPR/Cas9 induced mutations are precisely targeted, whereas mutations induced in mutation breeding are multiple and random, there is no legislation covering the random multiple mutations induced during mutation breeding, but there is legislation covering the more precise gene editing. Interestingly, the US National Academy of Sciences states that there is no justification for regulating genetically engineered crops while not doing the same for mutation breeding crops.

While genetic engineering lacks precision in terms of where the new DNA is inserted into the genome, and traces of the insertion mechanism remain, the same legislation is currently applied to gene editing even though no trace of the CRISPR/Cas9 mechanism remains, and the induced mutation is at a precise location and is indistinguishable from a mutation that may have occurred naturally. It seems that as plant breeding has become more and more precise, the legislation has become more and more restrictive. The perceived risk is simply not proportional to the amount of DNA changed or to the specificity of the change. Moreover, the legislation relevant to genetic engineering and gene editing predates next generation sequencing technology which can be used to monitor changes to the genome.

The Global Gene Editing Regulation Tracker (https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/) summarises gene editing regulations in agriculture and medicine country-by-country, and shows many countries, including the US, Canada, many South American countries, Australia, and Japan, now have no or little regulation governing gene edited crops provided the crop has no new DNA inserted. However, currently, due to the July 2018 ruling by the European Court of Justice, gene editing is prohibited in the UK and in most of the EU, leading Smyth and Lassoued (2019) to suggest that "*Europe can now be known as the death place of agricultural breeding innovations*".

However, this year (2021) the European Commission says the EU should look to the 'major advances' CRISPR gene editing provides to boost yields and reduce the environmental footprint of agriculture, and the UK Government's view in 2021 is that organisms produced by gene editing should not be regulated as GMOs if they could have been produced, in theory, by traditional breeding methods.

The question now is will New Zealand continue with its outdated legislation, limiting our scientists, plant breeders and the public from taking advantage of this Nobel Prize winning technology and join Europe, or potentially be left behind Europe, to become the new "death place of agricultural breeding innovations"? The Lassoued et al. (2021) survey concludes that experts consider genome editing to be a powerful tool for future food security – in a world with a human population approaching 10 billion – that should be enabled rather than delayed. What is needed in New Zealand is the development of appropriate science-based regulations so we can take advantage of this new technology, while respecting cultural concerns and those of the public.

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