This response was submitted to the Call for Evidence held by the Nuffield Council on Bioethics on *Genome editing* between 27 November 2015 and 1 February 2016. The views expressed are solely those of the respondent(s) and not those of the Council.

Nuffield Council on Bioethics

Consultation on Genome Editing

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Plant Genome Editing in Context

The domestication of crops began over ten thousand years ago. This domestication fixed traits, particularly those related to high yield, but also reduced genetic diversity in breeding populations. Compared to wild species, the breeding populations of many crop plants are less diverse [1]. We therefore need to employ technologies to increase genetic variation in crops in order to identify and fix desirable traits, e.g. to improve yield, to confer disease resistance, to improve the shape or colour of fruits, or to increase nutritive value.

Crop improvement practices include the use of traditional selective breeding to fix mutations that are the product of natural variation in individual plants into the population. Many mutations occur on their own because of exposure to UV light, radiation sources or just though errors in the cell. Since the early twentieth century, we have also used X-rays and mutagenizing chemicals on plant seeds to induce small breaks in DNA. These breaks are sometimes imperfectly repaired, resulting in mutations. Many thousands of crop varieties contain traits conferred by changes in their genomes made using these techniques [2]. Induced mutagenesis is, however, a random process – it is not possible to control where in the genome mutations are made; mutations are induced throughout the whole genome, and then plants are selected that show new or desirable traits. Similarly, genome editing technologies enable us to program proteins called nucleases, proteins that induce breaks in DNA strands, to target specific sequences of DNA. The mutations induced by new genome editing technologies are not random or scattered through the whole plant genome. Instead we are able to target specific genes for which we either already know the function, or would like to learn more about.

Over the past 40 years, plant scientists have been able to identify many of the specific genes associated with useful traits by using a number of different molecular techniques. This has transformed our understanding of the relationship between genome sequence and plant characteristics. We can now use genome editing technologies to learn more about how genes function. We can also use them to make mutations in specific known-function genes to produce desirable traits in crop plants.

What is the current state of the art in the field? What are the current technical limitations and constraints/ bottlenecks?

Genome editing technology has been successful in an ever-increasing number of plant species including model plants useful for laboratory studies and a number of food plants including sorghum, rice, maize, rice, wheat, sweet orange, barley and brassicas [3–16]. In most of these studies the aim was to produce a mutation in the

targeted gene(s) that would disrupt the coding sequence(s) and prevent the gene(s) from being expressed, thus creating a functional 'knock-out'. In most cases, the exact nature of the mutation was not controlled, only the location. Typically the mutations, the result of imperfect DNA-repair, were just one or two base-pairs, although in some studies two breaks were induced in close proximity to excise a portion of DNA perhaps a few hundred bases pairs in length. In the majority of cases, plants with induced mutations were recovered by the delivery of a transgene cassette encoding the elements required to induce the break in the DNA and a suitable selectable marker. This was done using established DNA delivery and plant cell regeneration technologies. Subsequently, plants in which a mutation was detected at the target locus were progressed to the next generation. In most cases the transgene was integrated randomly and therefore not genetically linked to the mutation. It has therefore been possible to segregate the transgenes, demonstrating both the heritability of mutations and the production of transgene-free mutated plants [4, 8–11, 15, 17–19]. In animal cells, efficient genome editing has been achieved by delivering the nuclease proteins directly to the cell - no DNA is delivered and therefore a transgene cannot integrate into the genome [20, 21].

The plant cell wall provides a natural barrier to large molecules such as nucleases, however, very recently, scientists have shown that mutations can be induced in plants following direct delivery of genome-editing proteins to cells in which the cell wall has been removed [22, 23]. One technical limitation is that expertise in recovering plants from such cells is uncommon in academic laboratories and the process is very laborious. Several agribiotech companies have publically spoken of their investment and expertise in these techniques. It is therefore very likely that plants aimed at the commercial market that have never had a transgene integrated but that have small mutations in specific genes to confer desirable traits will be produced.

At present there have been few large-scale studies performed on plants to quantify the frequency of off-target mutations; i.e., mutations induced in genes other than the intended target. Whole genome sequencing and analysis has reduced in cost sufficiently that surveying genomes for mutations is possible for desirable or elite individuals and such mutations can be removed by conventional breeding to a parent line. Nevertheless, there is interest in improving the technologies to be more specific as well as more efficient.

The induction of the break in DNA at a specific genetic locus also allows DNA to be integrated at this site. The sequence of inserted DNA might be designed or it might come from another species or it might be largely identical to the target and serve to 're-code' the existing gene or introduce a specific change in the DNA sequence. There have, however, been relatively few reports of such targeted integration or gene conversion by replacing a section of recoded DNA in plants. Targeted insertion requires the simultaneous delivery of a sufficient amount of the repair template along with the programmable nuclease. Initial reports of targeted integration demonstrated the insertion of transgenes at genome locations in which a non-functional selectable marker or reporter gene had previously been randomly integrated, thus allowing targeted events to be positively selected by repairing this gene [24]. An alternative strategy was the recoding of the acetolactate synthase gene (*ALS*). Several herbicides are known to inhibit this enzyme, killing the plant. Specific mutations that remove the ability of these herbicides to inhibit the gene product and therefore allow the plant to survive its presence, were introduced by the simultaneous delivery of genome editing tools and a repair template with the desired mutations [25]. However, the frequency of success of targeted integration is low enough that positive selection for targeted integration is necessary. The general recoding of, or integration of foreign DNA as specific genes that cannot be selected for during plant regeneration

has not yet been widely reported. However, techniques that enable delivery of larger quantities of DNA to plant cells to increase the amount of DNA template delivered to the cell have been shown to increase the frequency of targeted integration [22, 26, 27].

Genome editing technologies have been shown to work in plant cells in many species. The primary bottlenecks are those traditional to plant biotechnology: the time and effort required for delivery of DNA to plant cells and the regeneration of plants with the programmed changes. These techniques are labour-intensive, slow and require significant investment in technical expertise and training. Technical developments in plant cell culture have for some time been considered supporting technologies and do not typically result in high-profile publications. For this reason most academic institutes cannot invest in the area.

Although, as shown by the number of publications, many laboratories are able to use genome editing for induced mutagenesis, at present only a small number of academic laboratories have sufficient technical, human and infrastructural resources to attempt experiments in targeted integration. Technical improvements may change this in the future however, to date, companies in the private sector (Dow AgroSciences, DuPont Pioneer, Cellectis) have been the primary contributors in this area.

What are the main directions of travel? What are the envisaged endpoints/ applications?

The possible uses of genome editing technologies are varied. In academic research they will be used to further the understanding of plant gene function. As it is possible to target multiple genes at once, which has been very difficult or laborious with other technologies, they will enable studies that were previously very difficult or impossible. There is also a large amount of existing information on the characterisation of gene function from knock-outs made using mutagens in model species that might quite rapidly be applied for the improvement of crops.

Genome editing tools have been used to knock out the gene encoding inositol-1,3,4,5,6-pentakisphosphate 2-kinase (*IPK1*) from the maize genome in order to reduce the quantity of phytate in the seed [16]. Phytate is an anti-nutrient that binds to minerals including iron, zinc, manganese. Its consumption slows their absorption in the human intestine [16]. Other targets of interest have included the removal of sequences that enable infection by pathogens. The bacterial pathogen that causes rice blight secretes proteins that bind to the rice sucrose-efflux transporter gene. Gene-editing tools were used to induce mutations in the specific part of this gene to which these pathogen proteins usually bind, reducing the virulence of the pathogen [11]. Also, the disruption of particular genes in the genome of bread wheat has conferred resistance to powdery mildew [13] and in brassicas, induced mutations produced plants that were smaller in stature and with altered seed-pod properties [15].

Plants produce many unwanted products including allergens and toxins that could be removed through genome editing. It may also be possible to induce mutations in some of the genes that confer susceptibility to plant diseases.

When the efficiency at which DNA can be integrated is improved, it will be possible to not only knock genes out, but also to recode them to desirable sequences, and to integrate transgenes at known locations to achieve predictable levels of transgene expression. Transgene expression is generally acknowledged to be affected strongly by the position of integration in the genome. Furthermore, if multiple transgenes can be inserted at the same site they can be more easily transmitted in breeding crosses.

Known integration loci in which transgenes can be integrated without disrupting an endogenous gene are also sometimes described as ‘safe-harbours’. The identification and characterisation of such sites is also a current area of investigation.

What is the rate of travel? What are the expected timescales for realising the envisaged endpoints?

The speed of progress is largely defined by the time to regenerate an edited plant and to test it. It is desirable to show heritability of mutations across generations and therefore, depending on the species, it can take several years for a single experiment to go to term. However, many experiments have been underway for some time and the technology is being rapidly adopted by more scientists. It is likely that in the next handful of years a large number of plants with mutations induced with genome editing tools will have been produced. Outside of the private sector, however, the production of plants with targeted integrations and recoded genes will probably remain less common that induced mutagenesis for a few more years due to the constraints as outlined above.

Are gene drives an area of particular interest or concern and, if so, why?

Gene drives are primarily of interest for allowing the rapid spread of a mutation in a ‘wild’ or ‘natural’ population. Crop plants are typically raised in confined populations from seeds that have come from breeding programs where gene drives would not confer enormous benefits. For them to be used in crop plants, transgenic plants bearing the gene drive constructed in the laboratory would need be released into the breeding population to mate with wild-type individuals to spread the drive through the wild population. As stated in Esvelt et al [28], the total time required to spread to all members depends on the number of drive-carrying individuals that are released, the generation time of the organism, the efficiency of homing, the impact of the drive on individual fitness, and the dynamics of mating and gene flow in the population. In general, it would take several generations.

However, gene drives could potentially prevent the spread of crop diseases through the release of edited pathogens. Furthermore, they could be used on wild insect or weed-plant populations (provided that they reproduce sexually) to, for example, confer or reverse pesticide and herbicide resistance. We are not aware of any laboratories undertaking research in this area at present.

What are the main ‘drivers’ and ‘obstacles’ for plant genome editing in relation to envisaged endpoints?

Academic, discovery-driven scientists are interested in the use of the technology to enhance understanding of plant gene function. For this group speed, efficiency and cost of producing edited plants are the primary obstacles.

For translation-focused academics, spin-outs, start-ups and small enterprises, the drivers are to apply current or emerging knowledge to the production of better plants for agriculture and biotechnology. This group also faces technical obstacles but additionally face issues in the complicated intellectual property (IP) landscape surrounding plant biotechnology and genome editing - the requirement to navigate the intellectual property landscape is noted as a disincentive for pursuing commercialization [29, 30]. Patent claims in plant science are not limited to engineered traits and plants and also include basic molecular tools, plasmid vectors and many enabling technologies [31]. Regulatory challenges and finding a market (public acceptance) are also of concern.

Large agritech companies have the resources to overcome the technical problems by increasing the human, laboratory and glasshouse resources to overcome problems in efficiency and by investing in plant tissue culture technologies that, although critical, are not as heavily rewarded as the discovery of gene-function in academic plant sciences. These companies are also less affected by IP as they have the resources to navigate the complex landscape and negotiate licencing [31, 32].

Uncertainty of regulation is, however, an issue.

Programmable nucleases have enabled the production of transgene-free plants with specific genetic changes. These new crops currently sit in a grey area between those that have been mutagenized using chemical agents or radiation and which are not regulated, and those that have DNA inserted into their genomes using recombinant DNA technologies and are regulated as products of Genetic Modification (GM). The induced mutations may involve just a few nucleotide bases and the plants may not contain any foreign genetic elements. Mutations created with programmable nucleases, therefore, may be indistinguishable from the allelic variation that occurs naturally or from that induced using chemicals and radiation. In addition, targeted mutagenesis has been achieved without incorporating foreign DNA into the genome [26].

The USDA is the regulator of biotech and GM in the United States. It has responded to DOW Agrosciences that maize lines in which genome editing was used to reduce levels of phytate by mutation of the *IPK1 gene* falls outside its regulatory authority [16]. Similar responses have been received by researchers at Iowa State University, who reduced susceptibility to disease by disruption of the *OsSWEET* gene [11] and by Cellectis and CIBUS for induced mutations in potato and *Brassica napus,* respectively [33]. Other countries, including Australia and Brazil, have adopted similar attitudes and, although broadly using process-based regulatory frameworks, have ruled similarly to the US and Canada on plants with targeted mutations created using programmable nucleases [34, 35]. An extended period of uncertainty in Europe will certainly mean that companies will invest in other regions by preference – a loss of opportunity for the European plant biotechnology industry.

Because of the different applications and outcomes of applying genome-editing tools to plants, it is unlikely that all plants using such technologies will be (or should be) subject to the same regulatory process. Many scientists have conveyed the opinion that genome edited crops, particularly those that only contain small mutations, should not be regulated in the same way as transgenic crops [35–41]. At present it seems likely that the regulatory approach will differ between global territories, complicating the trade in the products of such plants. In cases where the induced change is identical to those found in either natural or chemically-mutagenised populations, it will be extremely difficult if not impossible to apply simple tests for contamination such as those currently used for screening product batches for contamination by transgenic seed. The burden of proof will therefore depend on the integrity of the ownership chain.

What direct or indirect influence does historical public discussion surrounding genetic modification of plants have? What is (and what should be) the current level and focus of public debate?

The Pew Research Center recently published data showing that the gap between scientific and the public opinion on genetically modified organisms is greater than for any other scientific controversy: 88 percent of scientists from the American Association for the Advancement of Science agreed that genetically modified foods are safe to consume but only 37 percent of the public did [42]. At present, particularly

in the EU, there is uncertainty as to how crops made using programmable nucleases will be regulated (see above) and this is likely to significantly impact public opinion.

It is against this background that the historical discussion of GM is often considered. Scientists are not confident that they can escape the negative associations of GM. However, there is increasing evidence that scientists are misinformed about public opinion. A 2012 study by the Global Food Security program, a multi-agency programme bringing together the interests of the Research Councils, Executive Agencies and Government Departments studied public attitudes and found that seven in ten agreed that 'we need to make greater use of science and technology to increase the world's food supply in the future' (71%).

However, the debate on genetic modification in plants has been heavily influenced by technology ownership as well as by disillusionment in the engineered traits that have reached market. Some of the negative reaction to genetic engineering in plants stems from concern over the ownership of technologies that underpin food production [43]. A relatively small number of 'agritech' multinationals dominate the seed market and control the majority of innovations in crop development [31]. Others doubt that bioengineering can lead to widespread societal benefits rather than improved profit margins for a small number of multinational companies. Clarity of the regulatory status might stimulate growth in plant biotechnology. In particular, if the burden of cost were affordable to smaller, home-grown, European endeavours, this would be positive for the industry, the technology and, potentially, for the public dialogue.

Public debate should focus on issues around food insecurity and rising prices that an increasing population and climate change may bring in the coming decades. The introduced trait should be the focus of debate and conversation rather than the route by which it was achieved. Scientists should continue to seek the opinions of the public on how these challenges are tackled and provide data on the scale of the problems in food production that we will face. We should also continue to engage in scientific communication to increase the level of public knowledge and understanding of science that seeks to address issues surrounding food production and the production of medicines in plants. In is important that scientists are seen as individuals not as a white-coated 'other'. We should represent ourselves as members of the community and our motivations and desire to achieve positive social outcomes should be communicated often and clearly. We should seek to describe the technologies that we employ in terms that are open and transparent, and should be clear about the relative similarity between plants with mutations induced by genome-editing technologies, those produced using older technologies and those that have acquired mutations without human intervention. Scientists should be sensitive to the role of food in human culture and religion and respect the beliefs of those that differ from our own while also speaking to the ethical need to produce sufficient nutritious food for our growing population.

A recent John Innes Centre public dialogue project highlighted that the public was keen that scientists should consider the wider context of a problem, such as economic, societal and political factors which could be affecting food security, and take part in wider discussions on these lines.

What are the main anticipated benefits and costs (including safety and other risks) of genome-edited plants? In what ways, if any, are they significantly different from alternative GM technologies?

The Food and Agriculture organisation estimates that we need to increase food production by as much as 70% in the next 35 years but notes that agriculture already uses 40% of earth's landmass, 70% of fresh water and employs 30% of the human

population. Agriculture and forestry are responsible for over 30% of our carbon emissions. The potential for improving plants using genome-editing technologies is considerable. However, the length of time to achieve success and our current levels of understanding and achievement should not be over-estimated. It is likely that genome-editing will be just one of a very large number of technologies required to address such challenges.

Plants are also a source of medicine, fibers, construction materials and fuels, and provide ingredients for a range of consumer products such as paper, adhesives, dyes and resins as well as food.

Genetically modified plants are currently grown on hundreds of millions of hectares of land and their safety and benefits are increasingly widely reported and recognised within and beyond the scientific community [44, 45]. Even non-food biotech crops such as pest-resistant biotech cotton have indirectly contributed to food security by raising household income levels and improving access to more nutritious food [46]. Gene-editing technologies have the potential to add further benefits in the form of new traits unachievable with GM technologies.

Some genome edited plants, those that contain no transgenes and only a minute change in the sequence of the DNA in a specific gene or genes, are different from GM plants. They are more similar to plants produced by mutagenesis technologies, which are not regulated as GM. Plants in which genome-editing technologies have been used to insert new DNA at a specific genetic location are similar to plants currently regulated as GM.

As noted above, it will be extremely difficult to apply simple tests for genome-edited plants with no integrated DNA. The burden of proof will therefore depend on the integrity of the ownership chain, and breaks or errors in that chain would be impossible to trace. This poses a problem for people who are opposed to the use of biotechnology for the modification of plant genomes, as it would be difficult to provide a reliable assurance which would allow them to maintain this choice. This will be particularly true if edited crops are grown without any restrictions in other world regions with which we trade. At present a large number of tests are performed on GM crops that are not performed on mutagenized plants. Since edited plants may be genetically identical to mutagenized plants it follows that they should be subject to similar tests and not saddled with the financial burden of the GM regulatory process. Evidence-based discussions and regulatory processes are required on the potential risks versus benefits of biotech plants taking into account that both edited and GM plants may have less changes to their genomes than those produced by conventional breeding.

Are there particular issues raised by genome editing in relation to ecological stability, biological diversity, technology transfer between countries, and equitable sharing of the benefits of research?

The issues here are similar to those raised by agricultural technologies and biotechnologies in general. Agriculture and forestry are a threat to ecological stability and biological diversity as they consume so many of our natural resources and produce pollutants. The application of genome technologies to improve agriculture so that it is less resource-intensive has the potential for positive environmental impact.

Considering risks and unintended consequences, the application of genome-editing technologies to plants (except gene drives) does not pose any additional risks

beyond those posed by technologies already in use: edited plants with mutations are similar (and may even be genetically identical) to the products of other mutational technologies that have been used for many decades, while those with inserted DNA are similar to GM. While we can test for the safety and nutrient values of food plants, we do not possess the capacity for extensive testing of the behaviour of every genetic variant in a natural ecosystem, regardless of how they are produced or even if they arise naturally.

While some larger multinational corporations and philanthropic funders have established investment and CSR programmes in developing communities, resource-poor communities are not natural sources of large profits. As is the case for infectious diseases that afflict mainly developing countries, there is little financial incentive for investment in the crops of poor countries that may be most in need of an increase in food production. Bright examples of achievement in biotechnology can already be found in Kenya and Ghana and, particularly with an increase in support for technology transfer and international collaborations, there is potential that locally-led solutions can be found for problems that affect developing countries and poor world regions. Joint working and collaboration between the John Innes Centre and Biosciences for east and central Africa (BecA) is already starting to increase scientific capacity in sub-Saharan Africa.

The landscape of intellectual property around genome editing technologies has yet to settle. For plants, enabling technologies, DNA sequences, molecular tools and techniques are often the subject of IP claims [31]. However, scientists in many developing countries can find greater freedom to operate because of limited patent claims in their regions, which may aid the uptake of technologies in these regions.

To what extent, and in what way, does and should the distribution of anticipated benefits and costs of using genome editing in plants influence research and innovation?

Differences in efficiency, ease of use, access to supporting tools and enabling technologies have all impacted on the number of users of genome editing. The relative technical ease and low cost has greatly aided the uptake of the newest tools that enable plant genome editing (e.g. RNA guided Cas9, the protein found at the CRISPR locus in bacteria and Archea). Earlier tools were more technically challenging and had higher associated costs, and therefore were less-widely used. In the last two years, we have seen a significant uptake in the use of genome editing technologies in plant science laboratories. This has also been aided by parallel advances in DNA-assembly technologies in the same time-period, a key enabling factor for all molecular biology [47]. It can therefore be concluded that the effort, time and financial investments required to apply new technologies is of the greatest influence in academia.

In the private sector, particularly in large and well-resourced companies, there has been significant investment in applying genome-editing technologies for as many as eight years – one of the first papers using a programmable nuclease to edit the genome of a crop plant was published in 2009 from work conducted at DowAgroSciences [16]. Where resources can be channelled at the potential benefits, for the far longer periods of time required to get new technologies to work in plants, and where this is without the need for intermediate achievements and publications, it is likely that effort, time and financial investments are less of a restriction.

To what extent are public and commercial interests in genome editing in plants complementary? In what circumstances might they come into conflict?

The public have a number of different interests depending on where they reside and their cultural associations with food production. Food security, however, has an important role - food shortages and price spikes, even in a single country, can have significant domestic and international consequences. It is in everyone's interest that the costs of foods are affordable, that food is safe and nutritious and that there is sufficient and varied high-quality foods for everyone. For this to be the case the amount of food that we are able to produce (yield) must increase in line with population especially in world regions in which population growth is out-stripping food supply.

From a commercial point of view, increased yield and better nutritional content are desirable marketable traits. To this extent the public and commercial interests are in line. However, the costs of developing new crops require a sustained and significant investment which must be recovered. Resource-poor communities are therefore not natural markets for purely commercial ventures since the cost of food there is necessarily low. Just as government incentives are required for investment in neglected diseases that afflict developing countries, incentives may be needed to stimulate interest in the crops grown in these regions and in the growth of home-grown agri-tech ventures that can use genome editing technologies for the development of their own crops.

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