

BIOTECHNOLOGY AND BIOLOGICAL SCIENCES RESEARCH COUNCIL

NEW TECHNIQUES FOR GENETIC CROP IMPROVEMENT

Position statement

1. This statement sets out the position of the Biotechnology and Biological Sciences Research Council (BBSRC) on new and emerging genetic techniques that have the potential to contribute to producing crops with improved performance.

Key messages

- There is a pressing need for agriculture to produce more output with less input, and to do so sustainably within social, political, economic and environmental contexts. Genetic crop improvement can contribute towards solving this complex problem.
- Novel genetic techniques have been developed in recent years and are advancing rapidly. They include techniques commonly referred to as 'genome editing' that allow targeted changes to be made to genomes, such as adding, removing or replacing DNA at specified locations.
- All genetic crop improvement methods rely on introducing genetic changes. Genetic changes, however produced, can add beneficial characteristics or remove undesirable ones.
- The risks and benefits of genetic crop improvement are associated with the particular changes that have been introduced, not the method by which they were introduced.
- The new techniques offer the possibility of making genetic changes more precisely than previously possible by targeting them to specific sites in the genome.
- In some cases it will be impossible to tell what method was used to produce a new crop variety, because exactly the same DNA changes could be introduced using a variety of conventional breeding or newer techniques. The boundaries between established genetic modification (GM) and non-GM techniques will become increasingly blurred as techniques develop.
- This raises questions about how new crop varieties should be regulated. A regulatory system based on the characteristics of a novel crop, by whatever method it has been produced, would provide more effective and robust regulation than current EU processes, which consider new crop varieties differently depending on the method used to generate them.

The context

2. Agriculture is facing many challenges, which can be summarised as a pressing need to produce more output with less input.
3. Achieving a sustainable and secure supply of agricultural products is a complex problem requiring a multi-faceted solution, including improvements in crop genetics,

agronomic practices and post-harvest systems. Agriculture takes place in diverse social, political, economic and environmental contexts, which shape the choice of priorities for research, the consideration of alternatives approaches, the potential for specific technologies or products to deliver their anticipated benefits, and their acceptability.

4. In this context, genetic crop improvement is an attractive target because of the potential for rapid dissemination via seed distribution. Furthermore, genetic improvement can contribute to diverse aspects of crop biology. These include improved agricultural properties such as resource use efficiency or disease resistance, and improvements in the harvested product, such as nutritional content, storage performance, or processing properties for both food and non-food uses.
5. Because of the potential contributions of genetic crop improvement to the sustainable and secure supply of agricultural products, there is considerable excitement around the rapid emergence of a new suite of techniques to add to the existing tool box (see below and Table).
6. While it is important to guard against the notion of a simple “technological fix” to the many challenges facing agriculture, it is equally important to reap the benefits of available and emerging technologies where they can contribute effectively. This can only be achieved by considering the social, political and economic factors that will support the equitable delivery of these benefits. This, in turn, points to the need for proactive discussion with interested parties such as farmers, consumers and food-based industries, who are likely to have different viewpoints on what constitutes a benefit. Such discussion should seek to listen to views, support information needs and respond to concerns. It is in this context that new technologies for genetic crop improvement should be evaluated.

The techniques

7. A range of novel genetic techniques, particularly techniques often collectively referred to as ‘genome editing’, have been developed that allow targeted changes to be made to genomes. Changes can include adding or removing DNA at a specified location in the genome or replacing a specified segment of DNA with a different one. It is also possible to make epigenetic changes, where the DNA sequence remains unchanged but gene expression is altered because of chromatin changes that may be heritable. For examples of such techniques and comparison with established methods, see the **Table**; further technical detail is provided in the **Annexes**.
8. Adding a gene at a specified location is similar to established genetic modification (GM) approaches, except that the new gene is introduced at a specific site, potentially reducing problems such as unpredictable expression levels that can be associated with random insertion.
9. Deleting a gene can remove an undesirable trait from a crop more rapidly and easily than conventional breeding or mutational breeding techniques, and more reliably than

established GM approaches such as anti-sense gene expression.

10. Replacing a DNA segment with a different one could allow swapping different versions of the same gene more quickly and precisely than conventional breeding. This is particularly attractive for crops where it is possible to do this directly in existing elite crop varieties.
11. An important limitation of this sort of targeted approach is the requirement to know which genes to target. This requires a good understanding of which genes have relevant functions. Currently, the main application for these new technologies is in basic laboratory-based research to understand gene function. This understanding can be applied to agriculture in many different ways, including any of the available methods for genetic crop improvement, or through changes in agronomic practice.
12. Although there is still much work to be done to understand important plant traits, for some, such as resistance to some diseases, we already know enough about their genetic basis to be able to use the new technologies for crop improvement. It is therefore expected that crop varieties produced using these approaches will be developed world-wide over the coming years.
13. All genetic crop improvement methods rely on genetic changes. The availability of rapid and relatively inexpensive methods for DNA sequencing has taught us that genomes are highly dynamic and constantly changing. Conventional breeding approaches have relied on this dynamism to drive substantial improvements in productivity both historically – during the original domestication of crops – and subsequently within more targeted breeding programmes.
14. The genetic changes underlying the development of conventionally bred modern crop varieties range from small changes in DNA sequences, through more substantial rearrangements caused by transposable DNA elements, to combining entire genomes through species hybridisation.
15. As more methods for introducing genetic changes become available, in an increasing number of cases it will be impossible to tell what method was used to produce a new crop variety, because exactly the same DNA changes could be introduced using a variety of conventional breeding, established GM or newer techniques. If epigenetic approaches are adopted, there will be no DNA sequence changes at all.
16. These developments present a number of important questions about how the potential benefits from these new techniques can be realised. How can the social, political, economic and environmental factors that will influence the realisation of anticipated benefits be identified and evaluated to determine whether, when and how these tools should be used in agriculture? How can the perspectives of different stakeholders be taken into account? What kind of regulation is appropriate for the techniques and products generated using them? Active engagement of a wide range of stakeholders is needed to explore these questions.

Regulation

17. How a new crop variety is handled under the current EU regulatory system is determined by the process used to generate the crop. For new conventionally bred crop varieties, the main requirements are to demonstrate that the variety is distinct, uniform and stable. If the crop is GM it is also subject to an evidence-based risk assessment to determine whether its cultivation would pose significant additional risks when compared to an equivalent conventionally-bred variety.
18. By contrast, Canada operates a trait-based system under which plants with novel traits/characteristics, however introduced, are assessed for their safety and environmental impact. A BBSRC-led workshop in June 2014 showed strong support for this approach.
19. The new techniques place even more emphasis on the advantages of a trait-based system, since the boundaries between GM and non-GM techniques will become increasingly blurred as new techniques develop.
20. These new techniques therefore provide a stimulus to develop a proportionate and evidence-based regulatory framework that will be sufficiently robust to accommodate both existing and new technologies and agronomic practices as they arise.
21. It is recognised that changes to the current EU regulatory processes would be neither quick nor easy. Nonetheless, it is important for policy-makers to recognise that the new techniques are already widely used in research, and commercial applications are likely to follow. Regulatory processes need to be able to accommodate them.
22. Indeed, policy-makers need to be aware of the many costs of *not* introducing appropriate regulatory processes. These include inhibition of the development of potentially important applications that could contribute to addressing some of the key challenges facing agriculture; deployment of novel traits without appropriate consideration of risks when new products fall outside the remit of existing regulatory processes; and additional costs arising from the need to detect particular types of novel products in traded goods.
23. A trait-based regulatory system would allow more meaningful debate about agricultural priorities. Many of the concerns raised about GM crops are not related to the technique used but rather to the traits that have been introduced. These concerns are twofold. There are general concerns about the decision-making processes involved in selecting which traits should come to market and how they are marketed, and there are specific concerns about the impacts of particular traits. A trait-based regulatory system would focus the discussion explicitly on both these factors.
24. Trait-based regulation should be delivered via development of the evidence-based risk assessment and management approaches used currently. The regulatory end-points should remain the effective mitigation of hazards associated with human health and the environment. If the regulatory system is to become fit for purpose, some significant issues will need to be reviewed relating to: proportionality; the type, quality

and extent of the evidence needed to assess applications; and the appropriate basis of comparison of new crops with existing ones.

25. Evidence-based risk management should remain separate and distinct from regulations on traceability and labelling that are intended to support consumer choice once crop varieties have been approved for cultivation.

BBSRC, September 2014

Table: Some genetic approaches for crop improvement

Method / technique	Benefits	Limitations	When the method is most useful
Conventional breeding	Well established. Accelerated by recent advances including use of genomic information, for example in marker assisted selection.	Slow. Limited to the genetic diversity in current varieties and their relatives. Widespread unintended side-effects from additional genetic changes introduced along with those sought.	For complex multi-gene traits where the genetic basis is poorly understood.
Mutational breeding	Can generate new diversity not readily available in current varieties and their wild relatives.	Untargeted. Widespread unintended side-effects since many mutations are induced at random and these are time consuming to remove.	For single gene traits where there is insufficient diversity available in current germplasm. For traits for which the genetic basis is poorly understood.
GM (transgenic)	Enables introduction of wider range of traits not available through conventional or mutational breeding. Can increasingly make changes directly in elite lines.	Unintended side-effects due to insertion of the transgene into the genome. Many desirable traits are complex and/or there is insufficient knowledge of their genetic basis to introduce them in this way.	For traits that depend on a small number of known genes.
<i>New genetic improvement technologies:</i>			
Site-directed / targeted genome editing	More targeted gene insertion/deletion is possible. Provides a more precise way to increase variation in specific genes. Can increasingly make changes directly in elite lines, allowing rapid and precise swapping of gene variants.	Many desirable traits are complex and/or there is insufficient knowledge of their genetic basis to introduce them in this way. Frequency of unintended side-effects not yet determined.	For traits that depend on a small number of known genes.
Epigenetic modifications	Epigenetic modifications resulting in gene silencing may be targeted to specific sites in the genome without affecting the order of A,C,G and T in the DNA. The gene silencing effect may persist for the lifetime of the plant and even across generations. The epigenetic mechanism can be targeted by RNA that is delivered by a virus or by grafting – plant regeneration from tissue culture may not be required.	More research is needed to identify genetic loci that are susceptible to epigenetic modification and the factors that influence the stability of the epigenetic change either within a plant or between generations.	For traits that can be improved by loss of gene function.

Annex 1: Genome editing

Note: this annex was first produced as a fact sheet for the Science Media Centre and is available at www.sciencemediacentre.org/genome-editing

Genome editing comprises a range of molecular techniques that allow targeted changes to be made to the genomes of organisms.

Also referred to as genome engineering or site-directed mutagenesis, genome editing can:

- modify genetic information within genomes to create new traits
- remove specific regions from genomes
- add transgenes (genes from other organisms) to specific locations in genomes

Genome editing is more precise than conventional crop breeding methods, as well as many standard genetic engineering (transgenic) methods, for introducing desirable traits in crops.

Established genetic engineering techniques include using the naturally occurring plant pathogen *Agrobacterium* to insert genes in the genome of an organism, typically at a random position.

It is unclear how existing regulations for genetically modified organisms apply to plants modified by genome editing methods.

Genome editing precisely modifies nucleotides (A, T, G, C) in the genetic code, and is performed by:

1. using specifically engineered 'molecular scissors' to create precise breaks in the genome
2. repairing and editing the genome by harnessing the DNA repair mechanisms of cells

1. Techniques for creating breaks in the genome: sequence-specific nucleases

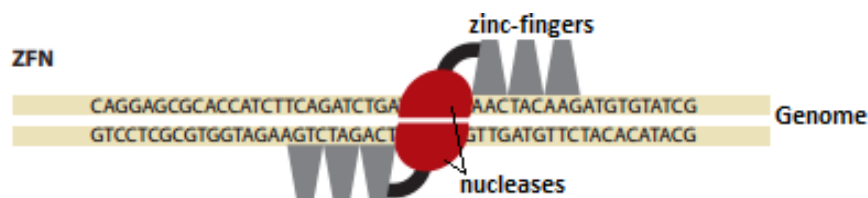
Nucleases are enzymes that sever nucleic acids (DNA, RNA), hence 'molecular scissors'. They can be engineered to target specific sites within genes and create breaks in the genome.

Four kinds of **sequence-specific nucleases (SSNs)** are currently used in genome editing:

Zinc-Finger Nucleases (ZFNs)

Zinc-fingers, protein structures containing zinc molecules, can be designed to recognise and bind to unique sequences in a genome.

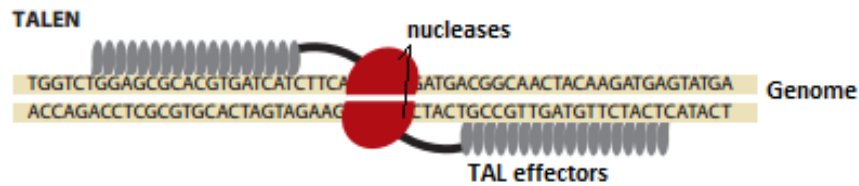
Two combinations of zinc-fingers are then fused to nucleases that create a break when they meet.



Transcription Activator-Like Effector Nucleases (TALENs)

Transcription activator-like (TAL) effectors are proteins produced by *Xanthomonas* bacteria, a family of plant pathogens, which bind to specific sections of a plant's genome.

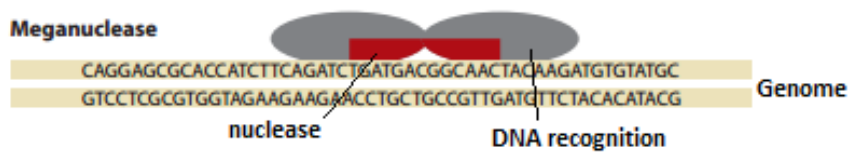
TAL effectors can be engineered to target desired DNA sequences and when fused to nucleases can be used to create breaks in a similar way to ZFNs.



Meganucleases

Unlike ZFNs and TALENs which have separate DNA recognition and nuclease components, meganucleases (also called homing endonucleases) have DNA recognition built in.

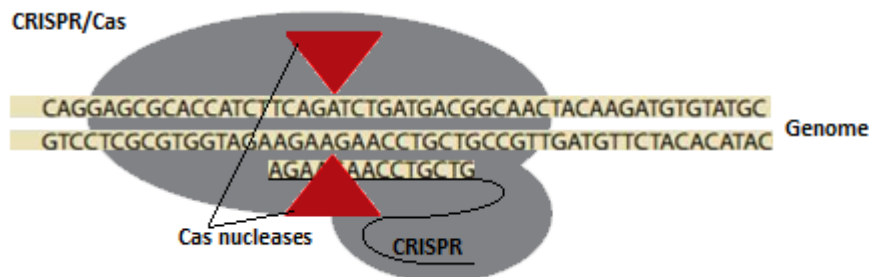
Meganucleases occur naturally and can be engineered, to some extent, to target specific sequences.



Clustered Regularly Interspersed Short Palindromic Repeats (CRISPRs)

CRISPRs are an immune defence system found in bacteria to protect against viruses; the system exploits short stretches of viral DNA that, when matched to the DNA sequence of an invading virus, trigger CRISPR associated (Cas) nucleases to make a break.

CRISPRs can be easily engineered to specify where a break should be made on the genome: the target DNA sequence is provided by a short RNA making it easier to implement than the three systems above.



2. Techniques for repairing and adjusting the genome

Once a break is made in the genome at the desired position the DNA repair mechanisms of a cell are triggered which can be harnessed to make the desired changes via two mechanisms:

Homologous Recombination involves introducing a DNA fragment as a template for repair which contains the desired genetic sequence and can be used to replace or insert nucleotides or full genes.

Non-Homologous End Joining (NHEJ) doesn't require a template and simply repairs the break, in general precisely but occasionally with small deletions or insertions.

Slight changes made to the genome will often stop a gene from functioning, creating a 'knock out', but NHEJ can also be purposefully used to make insertions and deletions of full genes.

Sources / further information

[Precision Genome Engineering and Agriculture: Opportunities and Regulatory Challenges](#)

(PLOS Biology)

[Plant Genome Engineering with Sequence-Specific Nucleases](#) (Annual Review of Plant Biology)

[Genome engineering using the CRISPR-Cas9 system](#) (Nature Protocols)

This is a fact sheet issued by the Science Media Centre to provide background information on science topics relevant to breaking news stories. This is not intended as the 'last word' on a subject, but rather a summary of the basics and a pointer towards sources of more detailed information. These can be read as supplements to our roundups and/or briefings.

For more information about our fact sheets, please contact Robin Bisson at the Science Media Centre on 020 7611 8345 or email robin@sciencemediacentre.org.

Annex 2: Schematic outlining one possible process for genome editing using *Agrobacterium* and CRISPR

