



Plant breeding techniques in a new era

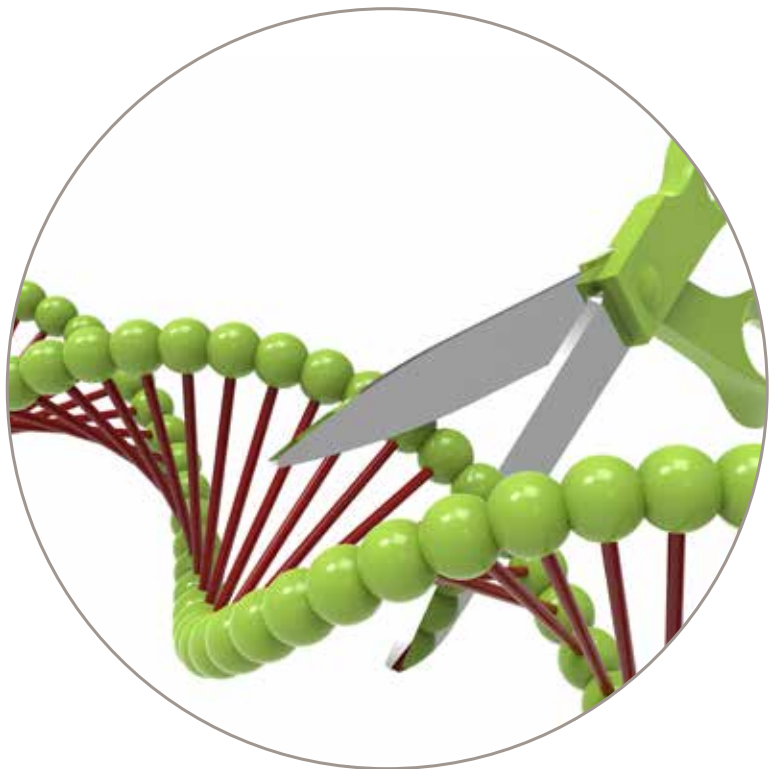


New opportunities for the sustainable production of healthy food

Worldwide, agriculture is facing serious sustainability issues which need to be addressed through an integrated approach that covers the entire production chain. Positioned at the base of the chain, plant breeders will have to develop robust varieties in a shorter period of time to keep up with changes in climatic conditions, soil quality and pathogen & pest pressures, increasing food demand, and the evolving health and taste preferences of consumers.

Fortunately, a range of New Plant Breeding Techniques (NPBTs) have been developed to assist breeders in a more efficient and precise adjustment of the genetic constitution of crops. These include genome editing techniques, which have made tremendous progress since the introduction of CRISPR-Cas in 2012. They will be helpful in improving important traits in crops that have, until now, been difficult to improve via classical breeding alone.

This booklet takes a look at the potential of new scientific developments to assist plant breeders in making the production of healthy and attractive food more sustainable. It describes and explains concrete examples of how three of the NPBTs, namely cisgenesis, induced early flowering, and in particular genome editing, are applied.



New Plant Breeding Techniques: cisgenesis, induced early flowering and genome editing

New Plant Breeding Techniques (NPBTs) are a diverse set of techniques developed to improve the efficiency and precision of plant breeding. They can shorten breeding programmes considerably, depending on the crop and the plant traits targeted, sometimes from decades to years. Genetic modification is frequently used in these techniques but only as a tool during the breeding process. No DNA from non-crossable species is present in the variety produced.

Genome editing, such as with CRISPR-Cas, allows DNA to be changed at precisely specified points in the plant genome, so that plant traits can be adjusted in an accurate and efficient way.

Cisgenesis is a technique that introduces genes from the same species or a crossable species into the plant. The DuRPh programme has shown as proof of principle that by using this technique, potato varieties can be produced with durable resistance to the devastating disease late blight by combining disease resistance genes from wild potato species.¹

In induced early flowering, a transgene induces fruit tree seedlings to flower years before they normally would.



This enables fruit tree breeders to cross plants every year, allowing for the fast introduction and combination of desirable traits, after which the transgene is eliminated. This accelerates introgression of disease resistance genes from wild species into new varieties through crossing.

This cisgenesis demonstration field trial within the DuRPh research programme shows the effect of late blight on the potato variety Désirée.¹ The plants in front contain no resistance against the disease while those at the back contain one or more resistance genes introduced by cisgenesis from a wild potato species. Plots on the left have been sprayed with fungicides. Introduction with classical breeding would have taken decades where cisgenic insertion only took a few years. The resistant potato plants need approximately 75% less fungicide and improve harvest security.



Apple seedlings do not flower until they are 5-6 years old, but an inserted early flowering gene can induce seedlings such as this one to flower a few months after germination, allowing the next cross to be made immediately. Proof of principle was the combination of several disease resistance genes from wild apple species in a cultivated apple over three generations, which only took three years instead of the decades normally required with conventional breeding.² Accelerating the availability of apple varieties resistant to fungal diseases such as apple scab will considerably decrease the use of crop protection agents.



¹ Haverkort AJ et al. (2016), *Potato Research* 59: 35-66

² Flachowsky H et al. (2011), *New Phytologist* 192: 364-377



Three examples of desirable traits produced by genome editing

Genome editing has already been used to create a number of valuable traits, generating improvements that range from more sustainable cultivation to food that is adapted to the needs of specific consumer groups.

³ Li T et al. (2012), *Nature Biotechnology* 30: 390-392

⁴ Schouten HJ & Bai Y (2017), *Prophyta Annual* 2017:44-45

⁵ Andersson M, Turesson H, Nicolai A, Fält AS, Samuelsson M, Hofvander P (2016) Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR-Cas9 expression in protoplasts. *Plant Cell Reports* 36: 117-128. <http://rdcu.be/EUpR>

⁶ Sánchez-León S et al. (2017), *Plant Biotechnology Journal*

Example

1

Resistance to diseases is crucial for more sustainable agriculture. It makes it possible to use fewer crop protection chemicals during cultivation, reducing environmental impact. This represents an improvement for a range of stakeholders, from farmers to consumers. CRISPR-Cas broadens the range of genes amendable for disease resistance to include susceptibility genes – the plant genes that get 'hijacked' by a pathogen during the infection process. Genome editing has enabled American researchers to produce rice resistant to bacterial blight³, in a way that would have been practically impossible via classical breeding techniques. Research on susceptibility genes is now flourishing and several relevant genes have been identified in a range of crops including maize, potato, tomato, pepper and citrus which provide resistance to a diverse array of pathogens, including fungi, oomycetes, bacteria and viruses.⁴ This approach is especially interesting for many pathogens for which no natural disease resistance exists in the germplasm of the crop.



Example

2

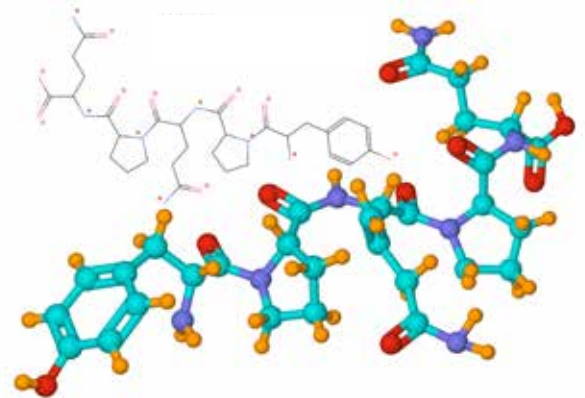
Genome editing also offers opportunities to improve sustainability in the processing industry. A potato variety which contains only amylopectin in its starch (as opposed to the mix of amylopectin and amylose that potatoes normally have) is appreciated, as amylopectin has high-value applications, especially in non-food product chains. Separating amylopectin from amylose is costly in terms of both energy and chemicals, so a potato that contains only amylopectin would reduce costs both to the environment and the economy. The biosynthesis pathway of amylose and amylopectin shows that a single knockout mutation of one crucial gene would make this possible. Such a mutation would be most useful in existing modern starch potato varieties, as they already have high yields and resistances to diseases and pests, but is very difficult to obtain with random mutagenesis as potatoes are tetraploid, i.e. they have four sets of chromosomes and four copies of every gene. Proof of principle was recently reported of efficient targeted mutagenesis of all four copies of this gene in the same plant thanks to the use of genome editing.⁵



Example

3

Intolerance to gluten in coeliac patients affects one to two per cent of the human population. The only remedy is to avoid gluten altogether, which is difficult because wheat gluten is an additive used to give structure to a wide range of consumer products, including snacks, soups, sauces and sausages. In addition, gluten-free products usually have lower nutritional value and a shorter shelf life. Coeliac symptoms could be prevented if most gluten in wheat could be removed or modified while retaining baking quality. As gluten proteins are encoded by five large gene families, classical breeding cannot achieve this. Recently, the first study was published that demonstrated that the modification or knockout of part of these genes led to a reduction in immuno-reactivity.⁶





Traceability of plants generated through NPBTs such as genome editing

Traceability depends on the technique used. Information that is reported by a breeder may be helpful in this respect. Varieties made with induced early flowering are identical to plants made using conventional breeding. Varieties made using genome editing contain changes that in many cases also may arise spontaneously or already exist in other varieties or in wild relative species. In

practice, it will not be possible to deduce with certainty which breeding method or combination of breeding methods was used. In most cases, it will be possible to trace and identify a specific change in the DNA corresponding with a new trait, should this, in exceptional cases, be desirable for specific reasons.

Economy and consumer perception

Just as for conventional breeding, the net benefits of the use of NPBTs depend on the specific plant trait or traits on which the breeding is focused, the crop that is improved, and the manner and place of cultivation. NPBTs make breeding faster, bringing the benefits forward in time. While the largest gain in time will be realised in vegetatively propagated crops, the largest economic impact may be seen in vegetables and arable seed-propagated crops.

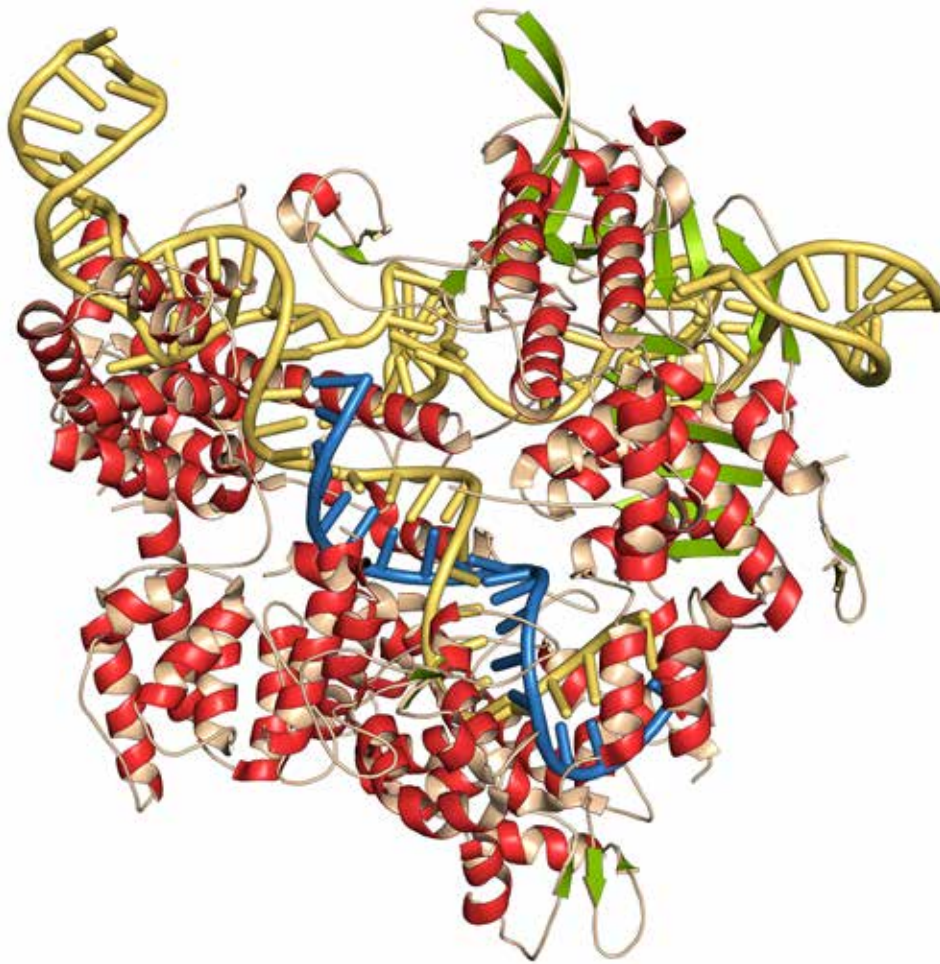
The first results from economic research suggest that NPBTs will have a positive effect on farm income growth⁷ and may result in lower pressure on the environment and more attractive food. Moreover, NPBTs will enable breeders to create new varieties that correspond more closely to market demand.

Consumer studies show that technologies seen as more natural and newer are perceived as less risky and more beneficial (and evaluated more positively) by consumers.⁸ It stands to reason that plants derived from NPBTs fit well in such a positive consumers' perception: the techniques are innovative and increase the efficiency and effectiveness of breeding, while the plants they produce contain no foreign DNA, just as those created by conventional breeding.



⁷ Wesseler J et al. (2016) Conference "Transforming the Bioeconomy: Behavior, innovation and science", Ravello (Italy): June 26 - 29, 2016

⁸ Ronteltap A et al. (2016), *Journal of Agricultural and Environmental Ethics* 29: 643-672. <http://rdcu.be/EVt2>



Further developments

The opportunities of cisgenesis and induced early flowering to improve the sustainability of agriculture are most prominent for vegetatively propagated crops, as they enable stacking of e.g. disease resistance genes in potato, apple and pear, banana, tulips and lily, and many other crops.

The opportunities for CRISPR-Cas to improve the sustainability of healthy food production are not limited to gene knockouts to create useful traits.⁹ Targeting gene promoters results in subtle changes in gene expression, which is likely to become a fruitful genome editing approach as well. A recent study showed that branching and flowering patterns in tomato could be modified through a series of promoter mutations that introduce

new genetic variation in three regulatory genes.¹⁰ This would enable achieving an optimal balance between leaves and fruits, improving the yield of high-quality fruits.

Genes involved in many aspects of fruit maturation and quality, such as sugar content, aromatic compounds (volatiles), firmness and colour, are already being targeted using CRISPR-Cas as part of basic research into the genetic pathways involved.¹¹ Likewise, genes involved in plant resilience, such as drought tolerance, are being discovered and tested.¹² Knowledge gained in this fundamental research will make the traits amenable to improvement by breeding through the use of conventional approaches combined with new plant breeding techniques.

⁹ Schaart JG et al. (2016). *Trends in Plant Science* 21: 438-449

¹⁰ Rodríguez-Leal D et al. (2017), *Cell* 171: 470-480.e8

¹¹ <https://www.wur.nl/en/Persons/dr.-RA-Ruud-de-Maagd.htm>

¹² Shi J et al. (2017), *Plant Biotechnology Journal* 15: 207-216

Outlook

21st century agriculture faces the huge challenge of producing more food in a more sustainable way. Plant breeding must bring forward crop varieties that are disease-resistant and resilient, and require fewer inputs. NPBTs are increasingly being applied as tools to assist breeders in a more efficient and precise adjustment of the genetic constitution of crops. Rapid developments in these techniques, particularly in genome editing, are leading to many promising new applications.

This brochure describes examples of the use of New Plant Breeding Techniques (NPBTs) for:

- combining resistance genes into varieties with durable disease resistance
- adapting susceptibility genes to create plant resistance against pathogens
- producing a potato with only amylopectin starch for high-quality applications in food and non-food processing
- removing gluten from wheat for the benefit of coeliac patients.



There are many more opportunities, including the improvement of oil composition in oil seeds, better keeping quality in potatoes or mushrooms, lower acrylamide content in potatoes after frying, and fewer seeds in fruits. Low-phytate grains used for feed will also increase nutrient use efficiency in animal husbandry. This will reduce phosphorus levels in manure and improve the environmental sustainability of animal keeping.

Further developments in NPBTs indicate that traits such as resilience against drought and salt stress, and consumer quality factors such as fruit taste, can also be effectively addressed using genome editing. The successful change of susceptibility genes in rice is an early demonstration of an achievement that would have been virtually impossible by conventional breeding alone.



Legal status and market introduction

In the European Union, the approval of GMO plants has been harmonised since 2001, and is subject to long and expensive procedures. Most genome editing techniques and other new plant breeding techniques use a genetic modification step during the breeding process, but the final variety does not contain foreign DNA. The status of such plants is unclear in relation to the present GMO regulation, which is based on the state of science prevailing in the 1990s. As long as this remains unclear, breeding companies or institutes are unlikely to develop and market plant varieties with these new techniques in Europe.

In the US, bacterial blight-resistant rice, amylopectin-rich – so-called waxy – maize and non-browning mushrooms, all developed using genome editing, have been deemed outside of USDA-APHIS' regulatory purview. Performance tests aimed at introduction to the market are ongoing, and many more varieties are expected to follow.

In view of developments elsewhere in the world, the EU will shortly need to find a balance between assuring appropriate safety assessment and stimulating innovations based on NPBTs.



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