

## New plant breeding techniques for Swiss agriculture – significant potential, uncertain future

The genetic makeup of plants can be modified precisely by means of new breeding techniques. Typically, these changes could also occur naturally and, in the process, no genetic material that is unrelated to the species remains in the plant. The techniques significantly broaden the possibilities for plant breeding, thereby potentially helping to make Swiss agriculture more environmentally friendly, economically viable and ultimately more sustainable.<sup>1</sup> As some varieties developed by using the new breeding techniques already exist and new ones are expected to follow shortly, it is imperative to determine whether or not plants engineered by means of these new techniques are subject to the Gene Technology Act. From a natural scientific point of view, there is no reason for a strict regulation of plants bred in this fashion.

### Challenge: Producing more with less

An astonishing feat has been achieved in the past with conventional plant breeding. It made a significant contribution to the improvement of the global food supply since 1960. And this happened in spite of the fact that the population skyrocketed from 3 to more than 7 billion people, while the global cultivation area only increased by 10% during the same timeframe.<sup>2</sup> In the past, the focus of plant breeding was on maximising the yield. To do so, fertilisers and crop protection products were used, resulting in agricultural intensification with undesirable side effects also in Switzerland.

According to forecasts, the global need for food products is projected to increase by approx. 70% until 2050.<sup>3</sup> This development likewise affects Switzerland, which in 2013 only produced 50% of its calorie requirement on its own.<sup>4</sup> If it is

impossible to enlarge the cultivation area and the reliance on imports should not increase, yields need to continue to rise in Switzerland in the future. At the same time, the change in the framework conditions for the Swiss agriculture with regard to climate, resource availability, socio-economic aspects and societal needs is foreseeable.<sup>5-7</sup> Resource-efficient, robust varieties and better cultivation methods will play a key role in solving this task.<sup>8</sup> In this context, the development of new varieties is of particular significance for the intended reduction of crop protection products, which would otherwise be associated with a major loss in yield.<sup>9</sup>

## Potential: more possibilities, more targeted breeding

Conventional plant breeding has two significant disadvantages that make it difficult to achieve the necessary progress: Firstly, it takes a long time to develop new varieties – 10 to 20 years, depending on the plant species. Secondly, desired characteristics are only present in the available genetic resources to a limited degree. But this is precisely what constitutes the potential of the new plant breeding techniques: They enable the targeted and efficient breeding and the broadening of the genetic resources by applying the know-how obtained with the research of the genomes from many wild plants and cultivars. The new breeding methods are based on the latest findings of genome research and novel approaches in molecular genetics (Table).

The term “Novel plant breeding techniques” covers a wide range of methods. As a common feature, they start on the level of the genetic information, by modifying the DNA sequence or the regulation of the conversion of this information into plant traits in a way that would also be possible under natural conditions – albeit in that case only by chance and considerably less common. As the understanding of the genetic basis of important plant traits is constantly improving, it becomes possible to impart new characteristics directly onto agronomically valuable varieties in a way that would virtually be impossible with conventional breeding (Box 1). For instance, higher yields along with better resource efficiency, resistance to diseases and pests or tolerance to drought and hostile temperatures are interesting propositions for a sustainable agriculture. The removal of allergens and toxic substances or the improvement of the nutrient composition are promising with respect to peoples’ health.<sup>10</sup> The first varieties developed on the basis of the new techniques have already been authorised for use in North America, and corresponding applications are pending in the EU.<sup>11</sup> More varieties are expected to follow.<sup>12</sup> Whether the corresponding breeding techniques will also be used and the resulting varieties cultivated on the fields in Switzerland in the future, will depend largely on their regulatory status.

## Safety and legal basis: no increased risks, but a greater degree of regulation?

Before new techniques are used for commercial plant breeding, they are subject to rigorous research. It is important to keep in mind that no breeding technique is categorically risk-free. A multitude of research organisations and regulatory authorities in Europe have already commented on the safety of the new plant breeding techniques.<sup>13–26</sup> They have unanimously concluded that the new techniques considered to date are as safe as the breeding methods used in the past, while additionally achieving a more accurate effect. Moreover, all newly developed varieties undergo several years of extensive variety assessment, irrespective of the techniques used to breed them, before they are included in the National Catalogue of Varieties.<sup>27</sup> Based on the agreement on agriculture

### Box 1: Disease-resistant, healthier potatoes offering better transportability and a longer shelf-life

Apart from grain and sugar beets, potatoes are the main crop in Switzerland.<sup>36</sup> Traits that would also be attractive for Switzerland have already been imparted onto potatoes by means of new breeding techniques (see Table for details about the techniques):

- Several genes from wild potatoes have been transferred to an agronomically viable variety by means of **cisgenesis**, thereby making it resistant to late blight (Illustration 1). As a result, a massive reduction of both the use of crop protection products used in large amounts to combat this disease (fungicides or copper-based preparations) as well as the disease-related losses would be possible.<sup>37,38</sup>
- **TALEN** was used for the targeted removal of a few nucleotides from the potato genome, thereby inactivating a gene that is required for the conversion of sucrose to glucose and fructose. As a result, potatoes can be stored longer without the loss of quality.<sup>39</sup>
- A gene that codes the enzyme polyphenol oxidase was inactivated by means of **RNAi**. This enzyme is responsible for the formation of pressure marks (a common occurrence during transport), thereby rendering the potatoes unsuitable for sales.<sup>40</sup>

A potato variety that combines all of these traits has already been authorised in the USA, although it should be noted that all of the traits were attained with RNAi in this case.<sup>41</sup> This could generally also be achieved by way of conventional breeding, as only genes from cross-compatible species were used. Still, it would take a very long time for the introduction of just one of these traits, while a number of favourable characteristics of the original variety would be lost at the same time.

with the EU, varieties of all field and forage crops authorised in Switzerland are automatically authorised for use in the EU and vice versa (except genetically modified varieties).<sup>28</sup> Switzerland is highly dependent on this access to seed; for many crops, there are no breeding programs or seed production in this country, and the degree of self-sufficiency of those seeds produced in Switzerland is very low (with the exception of grain).<sup>27</sup>

A global debate is currently underway as to whether or not varieties generated with the new breeding techniques should

## Overview of select new breeding techniques

Technique	Description	Does the variety contain any foreign DNA? <sup>1</sup>	Does the intermediate product contain any foreign DNA/RNA? <sup>2</sup>
Cisgenesis	Introduction of a gene into the genetic makeup of a plant by means of conventional genetic engineering, with the use of an intrinsically unmodified gene from the same or a cross-compatible species.	No	No
Intragenesis	Analogous to cisgenesis, albeit the DNA is composed of several parts that do not belong together in equal measure in the donor organism, for example the regulating part of a gene and the coding part of another gene. However, all parts originate from the same or a cross-compatible species.	No	Yes
Oligonucleotide-directed mutagenesis (Odm)	Short DNA or RNA sequences are temporarily introduced into plant cells, which usually only differ from the sequence of the target gene in one nucleotide. They adhere to the target sequence, whereupon the cell's own repair mechanism modifies the target sequence according to the introduced template.	No	Yes
Genome engineering with designer endonucleases	Artificial restriction enzymes containing a DNA-binding module and an endonuclease module ("genetic scissors"). The DNA-binding module can be tailored to match the desired target sequences. This allows the separation of the plant DNA at a specifically selected location. Thanks to the cell's own DNA repair mechanism, nucleotides can be removed, modified or additionally inserted at this location. Different techniques with analogous function exist (RNA-mediated endonucleases (e.g. CRISPR), TALEN, meganucleases, zinc finger nucleases). Genome editing is a special case of genome engineering, whereby the generated sequence is precisely predefined with the use of an artificial repair template.	No <sup>3</sup>	Yes
RNA interference (RNAi)	Short pieces of RNA are inserted into the cell, where they serve as mediator for an enzyme that recognises and decomposes messenger RNA (mRNA) with a complementary sequence. mRNA is formed in the cells when the genetic code is read. It conveys the information needed for protein synthesis. Through decomposition of a specific type of mRNA, the corresponding protein (genetic product) is synthesised to a lesser degree or not at all, resulting in targeted downregulation of the target gene without altering the DNA itself.	No <sup>3</sup>	Yes
Grafting with a genetically modified rootstock	A shoot of a conventional plant is placed ("grafted") onto the rootstock of a genetically modified plant. With this process, new traits can be imparted upon the rootstock, without the presence of foreign DNA sequences in the fruits of the plant.	Plant: Yes Fruits: No	Yes
RNA-directed DNA methylation (RdDM)	A gene whose product induces the methylation of the regulatory units of a specific target gene is temporarily inserted into the cell. This enables the reduction or increase of the activity of this target gene. Methylation can be transferred across several generations, albeit not permanently. The gene responsible for methylation is no longer required and can be removed once the modification is complete.	No <sup>3</sup>	Yes
Accelerated breeding	Genetic engineering techniques are used to insert a gene that triggers early flowering. New traits can be crossed into this plant by means of conventional breeding, whereby the generation time is reduced thanks to the early flowering, thereby accelerating the breeding cycle. In the end, the early flowering gene is removed again and is no longer present in the variety used for agricultural purposes.	No	Yes
Reverse breeding	The recombination of chromosomes during meiosis (maturation division with separation of the chromosomes) is suppressed in hybrid plants, such that the germ cells contain only one set of chromosomes. After that, the chromosomes are duplicated, and the cells consequently possess two identical sets of chromosomes. It is subsequently possible to select pure-bred parent plants, which always produce the original hybrid plant when crossed.	No	Yes
Transient agroinfiltration	A suspension with genetically modified agrobacteria containing specific genes is injected into the leaves of a plant. The bacteria transfer the inserted genes to the plant cells, which transform this genetic information into corresponding genetic products. The transgene is only read off temporarily and locally. In this fashion, it can easily be tested whether a genetic construct in a plant is working or high-quality proteins can be produced.	No	Yes

The techniques are presented in more detail at [www.naturalsciences.ch/plant\\_breeding](http://www.naturalsciences.ch/plant_breeding)

- 1 Does the variety contain any DNA of a variety that is not cross-compatible and would it therefore not be producible by way of conventional crossing?
- 2 Does the breeding result in an "intermediate organism", i.e., is any foreign DNA or RNA inserted temporarily?
- 3 Special cases of these techniques exist, in which genes are permanently incorporated into the plant genome.

be deemed genetically modified organisms (GMOs). The need for clarification also exists in Switzerland.<sup>29</sup> The key question is whether the new breeding techniques result in plants whose genetic material was modified in a way that is not happening under natural conditions with cross-breeding and natural recombination. Only if the answer to this question is yes should plants be qualified as GMOs within the meaning of the Gene Technology Act (GTA).<sup>30</sup> To the extent that the new breeding techniques are based on mechanisms that also occur in nature and identical plants could theoretically also be produced with conventional breeding techniques, this does not result in GMOs in the legal sense. However, nucleic acid molecules are in some cases introduced into the target plant temporarily or even permanently (Table), thereby rendering the new techniques into genetic engineering methods as defined in the Release Ordinance (RO)<sup>31</sup> and the resulting plants potentially into GMOs. But again, this only applies to modifications that cannot occur under natural conditions, and are still present in the plants to be evaluated in inheritable form. In the EU, which is by far the most important trading partner for seeds and agricultural goods, the legal situation is virtually identical with the one in Switzerland.<sup>32</sup> Based on the global nature of the market, the decisions of our trading partners, in particular the EU, regarding the handling of the new techniques, will also be of major significance for Switzerland. While the EU has postponed a decision on this issue on several occasions, individual member states have already issued a position statement and concluded that at least some of the new methods or certain applications of these methods are not resulting in GMOs.<sup>33</sup> Other countries (for example Canada, the USA or Argentina) have already come to a decision: If a variety's genetic makeup could also be produced identically by means of conventional methods, the plants should not be deemed GMOs.<sup>34</sup> There is no identification requirement for such varieties in these countries and it is impossible to distinguish them from conventionally produced varieties; in connection with different regulatory rules, this would pose a problem for the traffic of goods. Research and development in the field of novel plant breeding techniques is relatively unattractive due to the legal uncertainties. This is true especially for small- and medium-sized plant breeders, for whom investments are linked to a higher existential risk.



**Illustration 1:** The healthy potato plants (left) carry late blight resistance genes deriving from wild potatoes that were transferred using cisgenesis (Box 1, Table). In contrast to the unmodified plants (right), they are durably immune to this plant infection.

## Possible courses of action: Clear legal basis to promote plant breeding

Various scenarios exist for the future handling of the new breeding methods. Two options are basically available on the level of the Gene Technology Act:

- *The valid definition of GMOs is interpreted unilaterally with a focus on processes and the varieties created by means of the new techniques are classified as GMOs.* A moratorium for the cultivation of GMOs is currently in place in Switzerland until 2017, and an extension until 2021 is to be expected. The options for the time thereafter include the renewed extension of the moratorium, a blanket ban for the cultivation of GMOs or a co-existence of cultivation systems with and without GMOs. In the latter case, virtually no varieties developed with the use of these new breeding techniques would likely be authorised and cultivated in Switzerland as a result of the strict regulation of genetic engineering in agriculture and the low acceptance of GMOs among the population. In the other cases, the new techniques would not be feasible for the creation of new varieties for Switzerland. This scenario would mean that both the cumulative focus on processes and production as well as the protective purpose of the applicable legislation would be neglected. After all, it is only the product but not the process of its generation that is relevant with respect to the existing risk.<sup>25</sup>
- *The legal definition of GMOs is interpreted in a narrow sense and the new varieties are not deemed GMOs.* A number of passages in the text of the relevant laws leave room for interpretation that can be construed in favour of the novel techniques. On the one hand, all techniques are based on natural mechanisms, and on the other hand, the resulting changes can also occur under natural conditions. If we adhere closely to the wording of the legal definition, breeds developed by means of these techniques would therefore not represent GMOs. When interpreting legal formulations, special attention should be paid to the purpose of the law, which is the prevention of specific harm to human beings and the environment. Because plants that can also be produced naturally are not associated with a special inherent risk, they must not be covered by the GTA's scope of application.

Apart from the GTA, there are several options as to how clarification could be achieved with adaptations in the Release Ordinance (RO). The RO lists the methods that result in the qualification as GMOs.

- *The use of novel techniques for targeted point mutation could be recognised as mutagenesis.* Targeted modifications of the genetic makeup generated with novel techniques – e.g. genome engineering using designer endonucleases or OdM (Table), which demonstrably only represent a point mutation or lead to sequences that are documented in an identical or a cross-compatible variety, should basically be considered generated by mutagenesis, and the resulting plants should be deemed produced without the use of genetic engineering (if need be with additional documentation requirements as proof).<sup>12, 26</sup>

## Box 2: Product- vs. process-based regulation

Whether a new plant variety is deemed GMO in Switzerland and is therefore governed by the Gene Technology Act is dependent on the breeding techniques used. When the GTA was formulated, it was assumed that the plants pose a higher risk for human beings, animals and the environment because they were produced by means of genetic engineering. This is therefore known as a partly process-based authorisation procedure. The alternative is a purely product-based authorisation procedure, where the focus is on the plant's new trait. However, both approaches essentially take into account aspects of the other. For instance, Canada pursues a product-based regulation. In that country, a strict authorisation procedure is used if a plant possesses an entirely new trait (a so called "novel trait"). In contrast, a simplified authorisation procedure is applied if other plants with the same trait have already been authorised. Extensive experience gathered through research and use of genetic engineering techniques in plant breeding has shown that there are no specific genetic engineering-related risks.<sup>13, 22, 25, 42-49</sup> From a natural scientific point of view, the new trait is relevant for the safety, and for this reason, the purely product-based approach is preferable to the process-based one. Analogous to the situation in the EU, the reinterpretation of the valid regulatory conditions might suffice for this purpose;<sup>18, 25</sup> but an extensive amendment of the law may possibly be required.

- *The list in the RO could be supplemented with methods that are not considered genetic engineering techniques.* This list includes techniques such as e.g. mutagenesis, cell and protoplast fusion, which would be considered genetic engineering techniques by definition, but are excluded from the ordinance. These exceptions are due to the long history of their safe use. This list could be supplemented with new techniques, for which a degree of safety to be specified can be assumed. As the RO is an ordinance, it would be easier to implement these additions than to amend the law.
- *The RO could be adapted to the extent that plants without or with only minimal amounts of modified nucleic acids are not considered GMOs.* Two changes would suffice for this purpose:<sup>15</sup> Firstly, the definition of a minimum number of nucleotides that need to be added to the genetic makeup in order for a legally relevant change to be present (the proposal is 20 nucleotides, because all smaller changes with any possible nucleotide sequence can with a realistic probability also be generated by spontaneous mutations and would hence not be distinguishable from engineered,

identical modifications).<sup>24</sup> Secondly, the introduction of the term "intermediate organism" for cases in which intermediate products in the breeding process contain recombinant DNA sequences, but not the variety as end product. The intermediate products would have to be deemed GMOs, and the corresponding development steps would be subject to stricter requirements. In contrast, the varieties used for agricultural purposes would not be deemed GMOs as long as they are free of recombinant DNA (Table).

Another option would be a paradigm shift from partly process- to entirely product-based regulation. The risk of a new variety would be determined based on its traits rather than by taking into account the used breeding techniques like it was done in the past. In this way, technical progress would not be hampered by legislation, and there would still be no need to worry about an increased risk to the environment or the health. A reinterpretation of the existing regulatory conditions might suffice for this purpose, but an extensive amendment of the law may possibly be required (Box 2).

## Conclusion

It is expected that the new breeding techniques will be used to a greater extent. They offer the potential for developing varieties that can contribute to a sustainable agricultural production in Switzerland. The failure to use them will decrease the attractiveness of research based on these technologies. As a result, the clarification of the legal situation with regard to the regulation of these new technologies is urgently required. In addition, the opportunities and risks associated with the techniques, the difference compared to conventional genetic engineering and the consequences of the failure to use them must be communicated to the public.

The GTA was originally introduced because it was assumed that genetic engineering techniques are associated with special risks. Nowadays we know that this is generally not the case. It would therefore be advisable to adapt the legal provisions to the current state of knowledge, and/or to make appropriate use of the available room for interpretation. A transition from partly process- to entirely product-based regulation of plant breeding is indicated (Box 2) from a scientific point of view. This allows the establishment of regulatory conditions that are capable of keeping pace with the rapid developments in plant breeding and enable the exploitation of the benefits associated with the novel technologies without reducing the protection of human beings, animals and the environment.

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