

The Canon of Potato Science:

6. Genetic Modification and Cis- and Transgenesis

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What is it?

Genetic modification is the act of inserting one or more agriculturally important genes into the genome of a potato plant by *in vitro* techniques and by using modified *Agrobacterium tumefaciens* as a natural gene transfer tool. The end product is a genetically modified (GM) plant. Important preconditions for transformation are *in vitro* regeneration and transformation ability of individual varieties or genotypes that have to be improved with the gene(s) of interest. Transgenesis is the use of agriculturally important genes which (partly) originate from other organisms (such as bacteria or fungi) or non-crossable plant species. These genes are called transgenes and they can be considered as belonging to a new gene pool for potato breeding. Examples of transgenes are those coding for resistance against viruses, insects, herbicides and fungi or oomycetes. Another important class of transgenes is based on RNAi for silencing existing traits coding for starch composition, processing traits or other quality traits. Because of the new possibility to introduce genes from every organism into a crop plant, additional safety rules have been developed and imposed. In Europe these rules are found in the EU Directive 2001/18/EC and non-governmental organizations have started a discussion on the safety of GM plants and kept the debate on the political agenda. For that reason, very few GM varieties have been approved in Europe. The use of antibiotic- or herbicide-resistance genes as selection markers for transformation is also questionable and meanwhile has resulted in a change of rules.

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At the moment there are two important developments:

1. The generation of marker-free transformants and their use in potato.
2. The routine cloning of genes from the crop species itself or from crossable species.

The cloning of genes is much stimulated by the sequencing of total genomes of important crops like rice and maize. Currently the potato genome is being sequenced in the PGSC (Potato Genome Sequencing Consortium; (<http://www.potatogenome.net>)) in which 14 countries are involved. All these natural genes from the plant species itself or from crossable species are called cisgenes and they are treated in the EU Directive 2001/18/EC in the same way as transgenes. The combination of marker-free transformation and the use of cisgenes only enables us to create cisgenic plants.

The current definition of genetic modification is broader than necessary. It is, for example, including the techniques of inducing mutations and fusing protoplasts. Therefore, in the Annex 1b of the EU Directive 2001/18/EC both techniques have been exempted. We believe that cisgenesis with only one or a few cisgenes can be added to the Annex 1b for exemption. The transformation process creates a GM plant but the genetic content is within the gene pool used by classical plant breeding as is the case with mutation induction and protoplast fusion.

Why is it Important in Potato Science?

Breeding of potato is becoming increasingly complex, as more desired traits are required. The number of seedlings needed for selecting a new variety nowadays is 200,000. Potato is autotetraploid, heterozygous and vegetatively propagated. Improvement of existing varieties, with a long-term safe history, by classical means such as induced mutations is restricted. The main reasons are that most of the mutations of interest are inherited recessively, that changes occur in loss of function and that normally a sexual step is needed for homozygosity. In potato it is impossible to have a sexual step without recombining many other important traits.

Transformation of potato with transgenes or cisgenes is a new possibility to improve existing varieties. As indicated above, with transgenes, it is possible to improve varieties for a range of different resistances and quality traits. The quality traits are nice examples to mimic recessive loss of function mutations in a dominant way by using the GM-based gene silencing technique. It is sure that gene transformation introduces fewer additional side effects than induced mutations will do. However, acceptance of GM plants with transgenes is problematic in Europe and other countries worldwide. Cisgenic GM plants are also very important for potato because of the possibility to introduce genes from wild species without linkage drag problems. We know from resistance breeding how much pre-breeding is needed for the introduction of a new resistance trait. The biggest challenge involved is not the introduction of the resistance genes itself but the frequent linkage with genes coding for traits with a negative impact. Stacking of resistance genes from different sources

(species or accessions) increases this linkage drag problem tremendously. With cisgenes these resistance genes can be stacked and introduced into the plant in one step without these linkage drag problems.

Why is it Important for the Potato Industry?

As shown above, GM plants can bring new possibilities to plant breeding and can enhance its efficacy. This is particularly true in potato breeding. It is a pity that until now transgenic potato varieties – with their new possibilities in resistance and quality breeding – are not accepted. I believe that the possibility of cisgenesis should be exploited in the short term by exemption from the existing GM regulation. It brings new strategies for resistance breeding to late blight (*Phytophthora infestans*), nematodes and warts, thus enabling a considerable reduction of the quantities of crop protecting agents applied. It makes important processing potatoes like cvs Bintje and Russet Burbank more acceptable by the potato industry and the general public. Gene silencing is an efficient tool to manipulate individual traits such as starch composition, processing quality and storage characteristics. Cisgenesis may not only introduce resistances more easily but also introduce the health-related tuber flesh pigmentation.

Scientific Developments

The most important scientific development in the field of genetic modification with transgenesis is the new possibility of cisgenesis and the impact of it. Cisgenesis is possible because of marker-free transformation. In potato the simplest way of marker-free transformation can be achieved using vectors without selection marker. They only contain the gene(s) of agricultural interest. PCR analysis of sufficient regenerated plants shows a relatively high (5%) or even a much higher percentage of cisgenic plants.

The worldwide potato genome sequencing project will make available many more new genes. The next step is to carry out a functional analysis in order to detect the functions of each of these genes and their function within the plant in all kinds of biochemical and/or developmental networks. These functional analyses will bring detailed information about the function of individual genes and their potential use in plant breeding. The dominantly inherited traits could easily be used as cisgenes and silencing of their function by RNAi.

Another important scientific development is the unravelling of the interaction of the plant with their pathogens in more detail. For *Phytophthora infestans*, it is already known that the interaction between *R*-genes (i.e. resistance genes of the host) and *Avr*-genes (i.e. avirulence genes of the pathogen) is crucial. Cloning of *R*-genes with their related *Avr*-genes gives more information about the number of different classes of *R*- and *Avr*-genes and an indication of the durability of individual *R*-genes. Stacking of selected *R*-genes will bring enhanced durable resistance to this pathogen closer.

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