

Kristofer Vamling

Plant Science Sverige AB
SE-268 81 Svalöv
Sweden

New Tools in Plant Breeding

Background

Plant breeding is a never-ending challenge to improve our crops. To create a better variety you have to improve an already present trait, or to add a new trait. Typical traits are high yield, resistance against pathogens or special quality characters. In some cases it can also be to develop plants that do not express a trait, e.g. a harmful or unwanted compound.

The unconscious improvement of plants species, to better meet our needs, started already during the Stone Age. The trait specific and science based plant breeding started more than a hundred years ago.

From trait to variety

When an idea for a new trait has been identified, it is the first step in a long process. First the plant breeder has to investigate if the trait is already present in the crop plant species. Perhaps is the trait at hand in the many thousands of seed samples that the plant breeder already has on the shelves, i.e. the plant breeder's gene pool and working material. If not, the search is most often extended to closely related plant species in Gene banks (a place where samples of numerous different plant species are collected and stored). If the search is successful, and a crossable plant species carrying the desired trait is identified, the next step in the process can be started.

Conventional cross-breeding and back-crossing

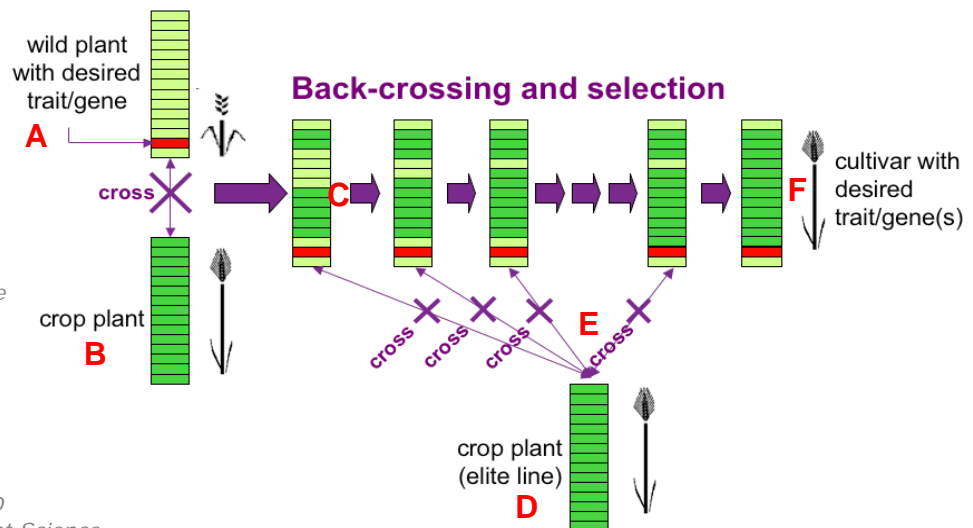


Figure 1. Hybridization is the most frequently used technology to improve our crops. The letters A-F refer to information in the text below)

CORRESPONDENCE TO
Kristofer Vamling, Plant Science
Sverige AB, SE-268 81 Svalöv
Sweden, Email:
Kristofer.Vamling@plantscience.se

Hybridization

In order to be able to transfer the wanted trait into the crop, the plant breeder crosses the plant carrying the wanted trait^A with a crop plant (breeding line) carrying all other wanted traits^B. The cross results in a progeny combining the two parents traits^C. In order to attain a plant^F with all the traits that the breeding line had^B including the new wanted trait^A and as few as possible of the unwanted traits from the donor parent plant (eg. "wild" traits if the donor parent is a wild related plant species) selected plants among the progenies are crossed again with the elite line^D. This is done repeatedly, in a so-called backcross series^E, between the progeny and the breeding line. In every backcross generation there has to be made a selection of the material carrying the new trait in addition to as much as possible from the breeding line. This material will be used in the next backcross with the crop plant. Often 6-8 backcrosses need to be performed before a new variety^F is created.

If the desired trait cannot be found in the crop plant species, or within any crossable related plant species, one option is to try to create the wanted trait in the crop. A trait depends on the genetic information that the organism is carrying. It can be the result of a single gene or due to the interaction between hundreds of genes in the organism. Even if the trait is linked to a single gene, it is important to be aware of that there is always a need for a certain genetic and metabolic background in the organism to enable the trait to be expressed.

To establish a new trait it is therefore necessary to modify already present genetic information via *mutation* or *genetic modification* and have the trait expressed in a suitable genetic and metabolic environment.

Mutation breeding and back-crossing

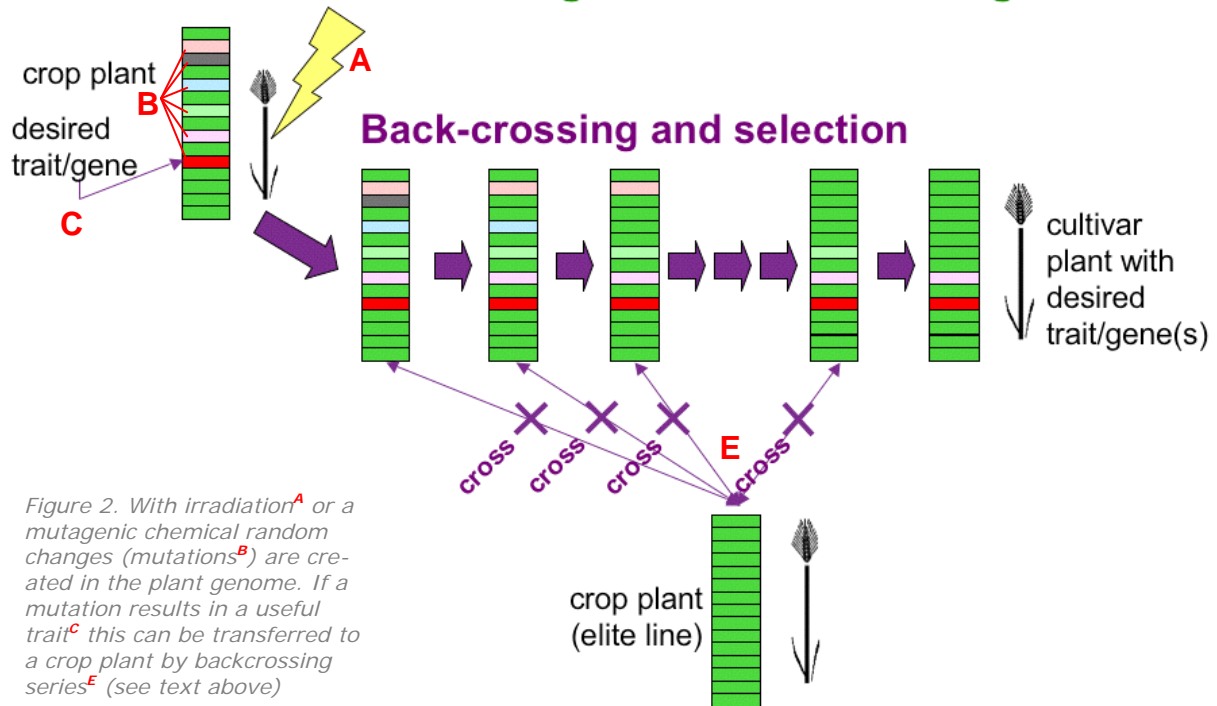


Figure 2. With irradiation^A or a mutagenic chemical random changes (mutations^B) are created in the plant genome. If a mutation results in a useful trait^C this can be transferred to a crop plant by backcrossing series^E (see text above)

Mutation

One of the fundamental aspects of evolution and the variety of life on earth is the spontaneous changes in the genetic information that constantly takes place in the genome of an organism. This process is called mutation. More than fifty years ago scientists found out that the frequency of mutations can be increased with some specific chemicals or radioactivity. Plant breeders can use this technology to create a population of plants with alterations in their genetic information. Some of these changes also result in changes in the properties of the plants. More than 99% of these changes are however worthless for the plant breeder. However in some cases valuable a "change"/trait can be identified. This "change"/trait is then transferred to a breeding line by the hybridization technology describe above. Plant breeding based on mutation technology is rarely used today, but with the development of new molecular techniques, the use of mutations will probably increase in importance in the future.

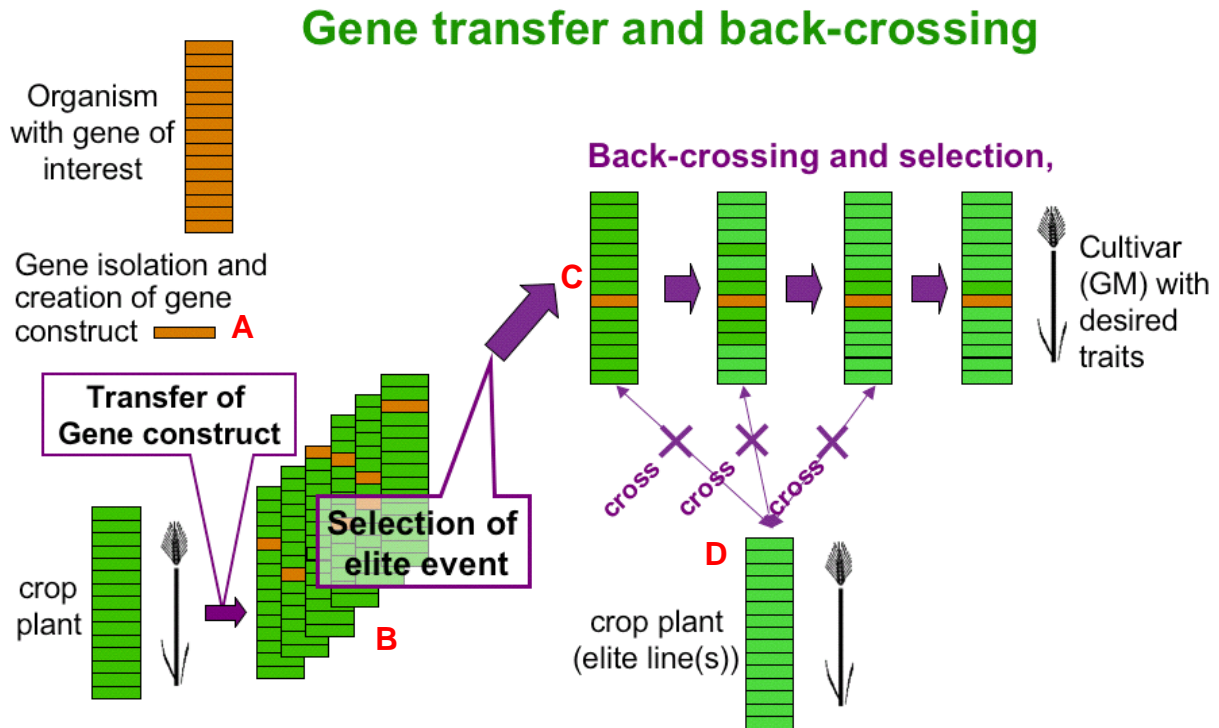


Figure 3. Genetic modification is a technique where a specific piece of genetic information is isolated from the genome of one organism and transferred to the genome of another organism. Since it is still not possible to direct the insertion of the new genetic information to a specific site, a number of transgenic plants has to be created^B. Out of these transformation events one is selected (the elite event^C) for final safety assessments and further plant breeding efforts^D.

Genetic modification

Genetic modification (GM) is a relatively new technology that provides a method for the plant breeder to introduce a new or improved trait in a crop plant. In order to create the new trait in the plant, a specific isolated section of genetic information (a gene construct)^A has to be transferred to a single plant cell. Such a "section of genetic information" can be isolated from any organism.

From the single plant cell a complete new plant is generated. If the new genetic information is "read" (transcribed) in a proper way the regenerated plant will exhibit a new trait.

The plant material that is used in the gene transfer process is most often not a top performing plant variety. It works well for the gene transfer but is not competitive enough in the agricultural field. In order to introduce the new GM-trait into a competitive plant variety the "GM-plant" is crossed with a modern and competitive breeding line. This is done in the same way as described above under "Hybridization".

Genetic modification – Step by Step

The properties or traits of a plant in the field are the response by the plant genome to the environment. Sometimes only one gene is responsible for a certain trait, e.g. resistance to a fungus. In other cases many genes work together to give a trait, e.g. high yield. In cases where many genes work together there are most often some major dominating genes responsible for the "major part" of the trait.

In order to be able to use genetic modification, the gene(s) responsible for the desired trait have to be

identified. Most often it is a time consuming task that involves several steps and technologies. Depending on the type of trait it may take from months to several years before the genetic information (gene(s)) behind a trait is determined. Next step is thereafter to investigate if the gene(s) provides the desired trait in the crop.

In order to do this, the gene(s) has to be transferred to the crop plant.

The vast majority of all genes in a plant are located in the nucleus of the plant cell (genes are also present in chloroplasts and mitochondria), where they are organized in chromosomes. In one chromosome there are thousands of genes linked together in a long row.

A basic genetic function is the regulation of the expression of the genes. In a cell there are genes that are always active. Other genes are just active during germination, others only at flowering. The gene-expression pattern in a plant is a very complex process with a lot of interactions inside the plant as well as reactions from outside stimuli.

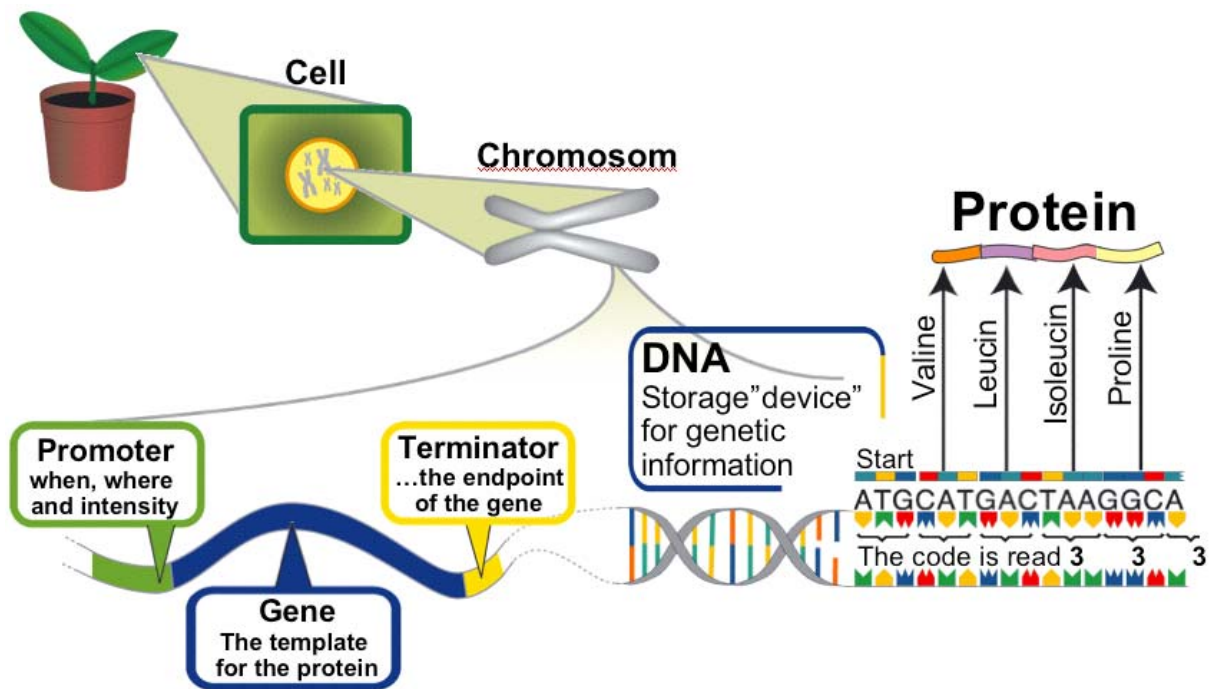


Figure 4. All genetic information of an organism is harbored in the cell. Most of the information is located in the nucleus of the cell. In the nucleus the genetic information is positioned in chromosomes

In order to be able to use the tool, genetic engineering, a gene with its genetic information has to be isolated from the genome of an organism. The isolated gene is placed in a gene construct (see Figure 6) and transferred into the genome of a recipient plant cell.

This single cell will thereafter be "forced" to develop into a whole plant. Since all cells in the plant are copies of the first recipient cell, all cells will contain the transferred gene in their genome. If everything works as planned the transferred gene will also introduce a new

trait to the plant. The method where a gene construct is transferred to a recipient cell and a new plant is re-generated is called transformation.

The process from the first genetically modified plant to a variety with a new trait introduced with genetic engineering is very long. Normally a large number of independent (originating from different transformed single cells) transgenic plants have to be produced in the transformation process in order to find one plant (one transformation event) that expresses the new trait in an optimal way and at the same time does not exhibit any other unintended changes. This selected plant is the "elite event". The elite event, originating from one single cell, has to go through a series of tests and trail cultivations to verify the stability and expression of the trait. The extensive safety assessment that all new traits have to go through before they are allowed to be commercialized is also based on the "elite event". However before the new trait can be introduced to the farmer there is more work to be done by the plant breeder. In most crops the elite event has to be used in a conventional backcross program (Figure 3^{C, D}) to introduce the "elite event" trait into lines representing the best and most competitive material.

The final step for all new plant varieties, transgenic as well as non-transgenic, is the official plant variety testing. This is a test where the variety has to prove "its" ***Distinctness*** (possible to distinguish from other varieties on the market), ***Uniformity*** (all plants have to look the same and have the same quality and quantity characters) and ***Superiority*** (it has to be better or offer a new quality compared to already existing varieties). After passing the ***DUS***-test and the official testing it is released and sold as at new plant variety on the market.

FROM A TRAIT TO GENES (step 1)

The following are just a few simple examples of methods to identify the genetic information behind a trait. There are several more techniques already available and more will come since this research area is expanding rapidly.

The traits an organism exhibit can be described as the response of the genes to the environment. Some trait, e.g. flower colour, is most often completely independent from the surrounding environment. Other trait, e.g. the grain yield of a crop, is highly influenced by the environmental conditions in the field.

In plant breeding, with or without the use of genetic modification, it is very important to know whether a trait is a result of a single gene or several genes. Is the trait resulting from a single gene, the breeding work is much easier. If the trait is the result of a combination of several genes located at different places in the plant genome, the plant breeding work will be much more complex and most often more time consuming.

Today we can only handle a few genes in the same gene transfer process. The hybridization technique, on the other hand, includes one copy of the whole genome (thousands of genes) at the same time. This offers possibilities to breed for traits dependent on many genes. The limitation is that all genes of the plant are involved, not only the genes responsible for the trait. The implication of this is that more traits than wanted are transferred in the cross. In order to remove these unwanted traits a repeated backcross program is necessary (See above, fig 1. ^{C-E}).

When a desired trait has been identified the first step is to investigate if this trait is possible to achieve with conventional cross-hybridization. If this is impossible, or it is anticipated that it will take too long time (depending on e.g. genetic complexity), the search for a suitable source for the new genes (genetic information) starts. The source for the new genes is most often a plant or even a bacterium. The genetic information in a gene can be "understood" in the plant also when the transferred gene(s) comes from another organism as e.g. a bacterium. The challenge is to find out what genetic information (gene(s)) that is necessary to create the new trait in the crop plant. Below you can find three very simplified examples that will give an idea of how this work can be done.

Example: Improved fungal resistance in a crop plant

A wild plant species resistant to a fungal disease can react on a fungal attack by producing a specific protein that inhibits the growth of the fungus. To find out what protein it is, proteins from plants cultivated with and without the fungus are isolated. The protein composition from the two plant groups is analysed. If the theory is correct, an additional new protein is found among the proteins from plants grown with the fungus. Through a chemical and physical analysis of this additional protein, it is possible to formulate the genetic code (the genetic information) for this specific protein. There are now two possibilities; (1) either synthesis a new gene based on the code, or (2) isolate the gene responsible for the protein from the genome of the resistant plant.

Example: New insect resistance in a crop plant

For many years it has been well known that different groups of proteins that are produced in a bacterium, *Bacillus thuringiensis*, are toxic to specific insect groups. By isolating the gene, from the bacterial genome that is responsible for the desired toxic protein, it is possible to improve or introduce resistance to a specific insect group among crop plants.

Example: Improved drought tolerance in a crop plant

Some moss species are known to be very tolerant to drought conditions. To find out what genes are responsible for this drought tolerance it is necessary to compare genes active in the moss under normal humid conditions with the genes active under drought conditions. This can be done with modern molecular techniques. However, the technique does not give the answer which genes are the most important for the drought tolerance. Neither does it give any information if the genes and which genes can give the trait drought tolerance in a crop plant. This information has to be found through extended research including gene transfer to well known model plants that easily can be tested in greenhouses and special climate chambers.

Genes that give positive results in a model plant are later tested in the crop plant.

FROM GENES TO GENE CONSTRUCTS (step 2)

When the gene(s) has been identified and isolated (or synthesized) the next step is to build the gene construct. The gene construct is necessary to enable the transfer of the gene to a plant cell and its proper function in the plant.

A functional gene construct is made up from three genetic elements: a gene, a promoter and a terminator. The main function of the terminator is to specify the endpoint of the gene. The promoter has a more critical role since it is regulating the intensity of the gene expression as well as it also regulates in what cell types and in what tissues the gene shall be expressed. It is for example the promoter that "turns on" the gene for red pigment in the petals in a plant with red flowers.

This regulating function of the promoter makes it very important when designing a new trait. If the desired trait is a resistance against an insect that harms the crop plant by eating the leaves of the plant, it is important to select a promoter that provides a gene expression in the leaves. When altering the oil quality in the seeds of oilseed rape, the promoter has to be "seed specific" in its way to regulate the inserted gene(s).

Sometimes it is very important to have an altered metabolism in one part of the plant, but no changes can be tolerated in other parts of the plant. In these cases the specificity of the promoter is very important. When changing the starch composition in potato, a tuber specific promoter regulates the activity of the inserted genes in a way that the starch composition in the potato tuber is changed but nowhere else in the plant.

A promoter suitable to regulate a gene that helps the plant to withstand drought most often has a constitu-

tive expression pattern, i.e. the expression of the gene is active all the time in all tissues and cells.



Figure 5. The genetic elements that are needed for a trait are: (1) a promoter with an expression pattern suitable for (2) the gene that is the template for the protein that initiates, or is responsible for, the trait, (3) a terminator that indicates the end of the gene.

In most cases there is also a need for additional genetic elements in the gene construct to enable the gene transfer process to the crop plant to be handled efficiently. The additional genetic information needed in the gene construct is a selection gene. The function of this gene is in some way to promote the plant cells that receive the gene construct in the transformation process and make it possible to identify and select the cells in the gene transfer process. The selection gene often renders the cell that have received the gene construct a resistance to a compound to which other plant cells are sensitive. To get a proper functionality of the selection gene it also has to have a suitable promoter (that activates the expression of the selection gene under the gene transfer process) and a terminator.

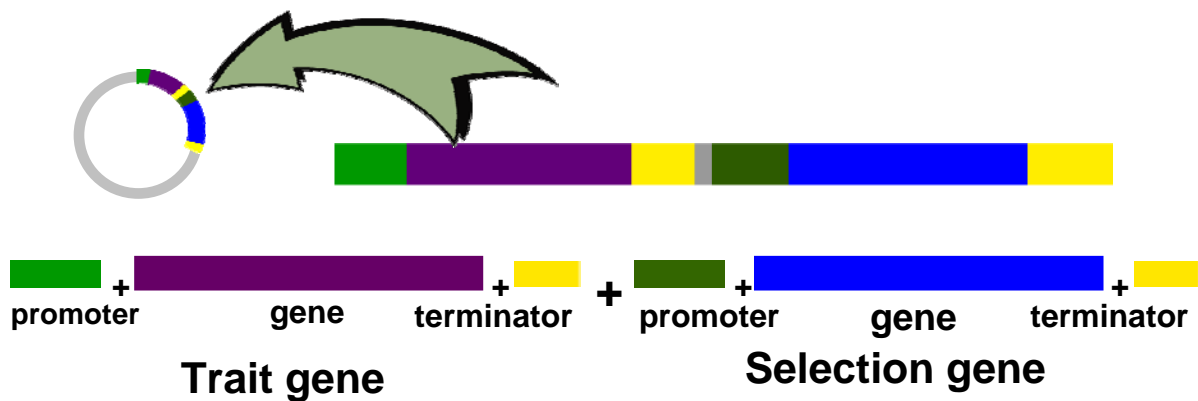
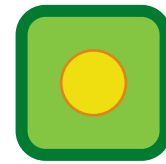
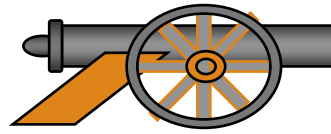


Figure 6. The complete gene construct, where a trait gene and a selection gene, is inserted into a ring-shaped DNA molecule called a plasmid. The plasmid itself is also built of the same molecular building blocks as the gene construct.

FROM GENE CONSTRUCT TO THE PLANT CELL (step 3)

A difficult step in the process is the transfer of the gene construct to the genome of the plant. The step is very much species dependent – easy in plant species such as potatoes and tomatoes but more difficult in cereal species such as wheat and barley. There are several different techniques to transfer the gene construct into the plant cell nucleus. The two most common are the biolistic technique and the method using a specific bacterium, *Agrobacterium tumefaciens*.

Device for particle acceleration



a plant cell

Biolistic technology:

In the biolistic technology the gene constructs are attached to very small particles of gold or tungsten. These particles are thereafter accelerated* to such a high speed that they are able to penetrate the thick wall of the plant cell. Some particles will eventually also end up in the cell nucleus. The gene constructs will separate from the particle and be integrated in the cell genome. The result is a transgenic cell, i.e. a cell with new genetic information integrated.

* The first "gene particle acceleration" was originally made with a rebuilt rifle. This technology is therefore often called the gene-gun method.

Agrobacterium technology:

Agrobacterium tumefaciens



a plant cell

This technology is based on a gene transfer system that has evolved in nature. *Agrobacterium tumefaciens* has evolved a capability to transfer a specific part of its Ti-plasmid (Ti= Tumor inducible) to a plant cell genome with genes containing genetic information that directs the cell to provide "nutrition and a place to live".

When using the *Agrobacterium* for gene transfer in plant biotechnology, the genetic information for "nutrition and a place to live" is replaced with the gene construct with its genetic information. Since the *Agrobacterium* always transfers the same specific part of the Ti-plasmid, the "man made" gene construct is therefore transferred to a plant cell genome. Also here the result is a transgenic cell, i.e. a cell with new genetic information integrated.

Different plant tissues are utilized as starting material depending on what plant species the gene transfer will be performed in. In most cases the gene transfer process is performed under aseptic conditions where the tissue grows on a culture substrate in plastic vessels. Irrespective of what gene transfer technique and plant tissues used, the number of transgenic cells is

very low in comparison to the unaffected non-transgenic cells.

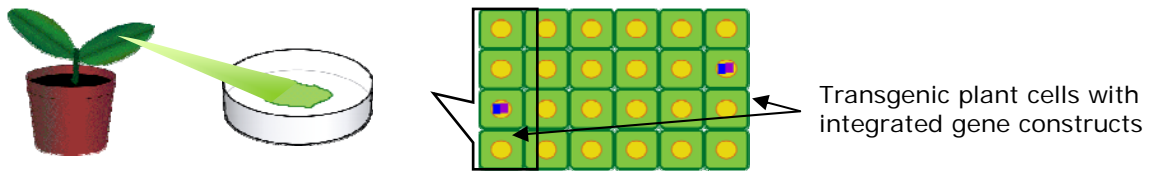


Figure 7. A small piece of a leaf is placed on a culture substrate in a plastic culture vessel. Irrespective of what method has been used for the gene transfer, the number of cells where a successful gene transfer has been accomplished is very low.

FROM A PLANT CELL TO A WHOLE PLANT (step 4)

Depending on the relatively low number of cells where the gene transfer has been successful, it is necessary to have a system to select and favour the growth of these transgenic cells.

It is in this step the selection gene has a critical role to play. By adding a compound to the culture substrate that the cells that have received the gene construct can withstand (due to the function of the selection gene), and all other cells are sensitive to, is it possible to select and “pick out” the cells or group of cells harbouring the gene construct. These transgenic cells will continue to divide, and after some months it is possible to influence them with growth hormones to regenerate a new plant, where all the cells carry the inserted gene construct.

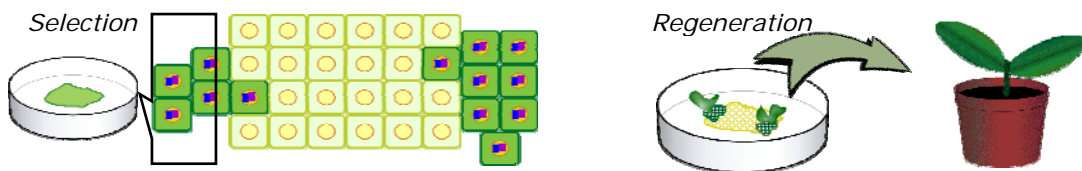


Figure 8. **Selection**, Only cells carrying the selection gene will divide and grow on a culture substrate containing the selection compound. **Regeneration**, By adding defined growth hormones groups of dividing cells will develop into shoots that can be transferred to pots with soil.

FROM A PLANT TO A VARIETY (step 5)

The function of the integrated gene(s) will depend on where in the plant genome the gene construct has been integrated. The insertion place of the gene construct is not totally random but it is still not possible to direct the integration of the gene construct to a specific place in the genome. Therefore the functionality of the inserted genes will vary between different transgenic cells. Plants regenerated from these different transgenic cells will each of them represent one unique transformation event. The potential variation in gene functionality between different events is one of the reasons why the number of individual transgenic plants, that has to be regenerated to be able to achieve one “elite” event plant/line, has to be high. Important is that the gene construct has an optimal function and no other unintentional changes have occurred. In a situation where the aim is just to get a rough judgment of the gene function, twenty plants (transgenic events)

can be enough. To develop an elite event (new commercial trait), with one trait gene, the number of plants (events) regenerated and tested often is 50 times higher. Furthermore, the more genes in the construct that is needed for a proper trait function the more transgenic plants (events) need to be developed.

It often takes several years to identify the transgenic event that best fulfills all commercial criteria and also meets all demands for environmental and health safety. The event goes through a comprehensive testing and legal process before a commercial release on the market is allowed. This event is called the elite event. But in most crops the material is not yet ready for the farmer's field and the commercial market with its end consumers. The elite event from the "biotech track" has to be combined with the best material from the "conventional plant breeding track" (see Figure 3, ^{C-D}). This work is done by conventional plant hybridization. It takes a number of generations before the new variety is ready. The new material also has to go through a variety testing and registration before it can be multiplied and the seeds can be sold to a farmer for further cultivation in the field.

In total it takes approximately the same time, from the first cross in a conventional plant breeding program to the launch of the new variety, as it takes from the gene transfer to the plant cell to the launch of the new variety with a new trait added with the help of gene transfer.

