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Plant breeder's toolbox through history

Selection breeding

More than ten thousand years ago people began to cultivate the land and grow different plants. Thus they did not need to wander around to hunt and collect their food. That was the time when plant breeding history began. Wild plants were adapted to become crops when humans began to pick and sow the seeds from the plants with the best properties. They chose plants with for example many seeds that remained long on the plant and thus gave them a lot of food. Through thousands of years, it was in this way that plants bred in agriculture have provided the foundation on which today's plant breeding is based. This method can be called selection breeding, since the selection of properties had not occurred naturally without human intervention. Since then, the plant breeding has evolved to include more advanced tools to modify and combine plant genomes to achieve improved properties of crops for adaptation to human agriculture and quality needs.

Cross breeding

By cross breeding the genes from two plants chosen as parents are “mixed” and you will get a combination of traits from both plants, both the good and bad ones. Technically a crossing is done by ensuring that pollen from one (the father) plant fertilizes the egg in the other (mother) plant. The seeds that form will then get properties from both parents. Crossing may for example be made between an existing crop and a wild relative of this crop to bring in new properties.

Mutation breeding

In mutation breeding plant cells are exposed to radiation or chemicals which induce random changes anywhere in the plant genome; *e.g.* so that a gene loses a base or gets an extra base in its DNA sequence producing a 'reading error' when the gene is to be used by the plant. The result can be that the protein for which the gene codes either stops functioning or gets a different function. By growing up thousands of plants with different such changes you can select among these the ones where the change happened to be favourable, and use these plants further in a breeding program. Mutation

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breeding can be compared to a lottery where you with luck and plenty of time can win something good and useful.

Gene technology breeding

In recent decades a new method has been added to the plant breeder's toolbox that can be called gene technology breeding. With this method you choose in advance a certain gene that codes for a particular protein whose function is already known. This gene is put into a crop for example via a soil bacterium (*Agrobacterium tumefaciens*) that has naturally developed a transport system for entering its own DNA into the plant genome. Thereby, the bacteria can fool the plant to produce certain nutrients for its own use. With the help of bacteria, we can introduce a certain property into a plant which is then called *transgenic*. In order to know which of the plants that successfully received the gene, a marker gene (such as a gene encoding a particular antibiotic resistance) is simultaneously introduced into the plant. The plants that manage to grow despite exposure to the antibiotic will also contain your gene of interest.

Choice of breeding tools

Depending on which properties are targets of a breeding process you can choose different tools in the breeder's toolbox. A very complex trait may be the result of interplay between many different genes, and then crossing of this trait from a wild relative into a crop can be the most effective approach. Perenniality is such an example of a complex trait in plants. It can provide great benefits for the environment. It may also save time and money since you do not have to spend as much time and energy into preparing the soil every year, and furthermore you get a reduced nutrient leakage from the fields. For another trait where only one or a few known genes are needed (and are known), some method of gene technology can be a very effective and time-saving tool. Sometimes such a method may be essential to introduce the trait into a crop. Below are two examples from our own research where we, by using gene technology, have introduced such novel properties into crops. The idea is to change the oil quality and quantity with the purpose to replace fossil oil in the chemical industry in the future.

'Conductor gene' makes potatoes produce oil using genetic engineering

Vegetable oils are chemically very similar to fossil oil (long hydrocarbon chains with different number of double bonds) and can therefore be used as replacements in many applications. The amount of fossil oil that we use today, however, is far too large to be replaced by plant oil since the world's arable land would not be sufficient for this purpose if we still want something to eat. However, a small but significant part of the fossil oil used by the chemical industry for specialty products today (*e.g.* lubricants, plastics and paints) can be replaced with plant oil in the future if the global plant oil production increased significantly. The oil crops we grow today are few and have little potential for further production increases; oil palm, soybean, sunflower and rapeseed. The crop today that by far provides the largest amounts of plant oil is oil palm grown in rainforest areas. An increase of this crop would be at the expense of rainforest devastation, something that is obviously not desirable. In order to increase global oil production from plants, we therefore need to develop new high-yielding oil crops. For this reason, we are interested to find genes controlling why various plants store energy (carbon) in different forms; for example sugar in sugar beet, oil in rapeseed, and starch in potato tubers.

The building blocks (carbon in the form of sugar) for energy storage come from photosynthesis in all plants, but are by certain genes directed into the different synthesis pathways for oil or starch. These genes are examples of so-called transcription factors that simplified might be called 'conductor genes'. The function of such genes can be compared to what a conductor does in an orchestra: *i.e.* determines which instruments (genes) that should play simultaneously for a given piece of music (such as an oil or starch piece). Turning on or off a conductor gene in a plant may have a large effect as they turn on or off many other genes simultaneously, compared to if you turn on or off the individual genes in a particular pathway alone. One such interesting conductor gene with great importance for oil accumulation in plant storage tissues is called the 'wrinkled gene'. It was discovered in a mutant of Thale cress (where the gene was turned off) that had seeds nearly lacking oil and therefore making them wrinkled. The gene could more fairly be called the ‘oil conductor gene’, since it is a gene that regulates the use of many other genes, both in glycolysis (which provide the building blocks for fatty acids) and in the fatty acid synthesis which further gives oil. We have in our research found that this gene is essential for oil formation in various plants, including underground tubers from a plant called nutsedge or tigernut (*Cyperus esculentus*) that has an unusual high amount of oil in their tubers. By transferring this ‘wrinkled gene' to potato by using gene technology, we have made potato tubers, which usually only stores energy in the form of starch, to also produce oil! The level of oil is still far from sufficient to provide a significant amount of oil, but it is an example of how genetic engineering and conductor genes can drastically alter the metabolism of a plant that could be of benefit to the environment (Figure 1).

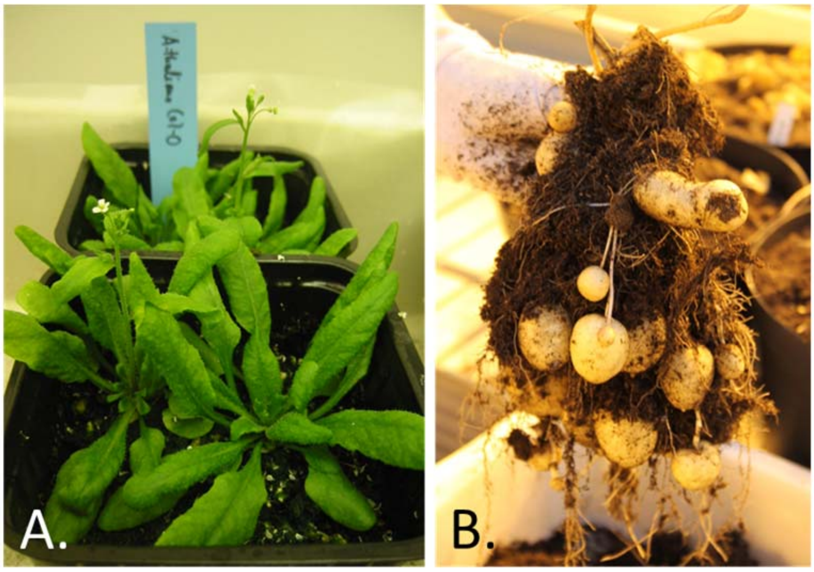

Figure 1. A 'conductor gene ' (a transcription factor) from thale cress (Arabidopsis thaliana, A) was transferred by gene technology into potato (Solanum tuberosum) which thereby started to accumulate oil in its tubers (B). (Photo: Åsa Grimberg, Mariette Andersson).

Genes from a desert shrub give high quality lubricants in an oil crop using gene technology

The gear box of older cars usually had lubricant oil containing wax esters. Wax esters are not as common seed oil (three fatty acids linked to a glycerol molecule with ester bonds), but instead consists of a fatty acid attached to a fatty alcohol with an ester bond. The wax ester oil had excellent properties as a lubricant with high stability. It rarely had to be replaced for the entire lifetime of the car. However, the oil came from the head of Sperm whales. Because of this desired product, these wales were hunted until they were almost extinct. In the 1970s, the sperm whale became protected, and since then lubricants have been based only on fossil oil even if it does not have as good properties. Interestingly, there is a desert plant called Jojoba (*Simmondsia chinensis*), that in its seeds has the same type of wax esters as in the sperm whale. The production of wax esters in Jojoba seeds occurs by two enzymes, a fatty acid reductase and a wax synthase. By using gene technology, the genes coding for these two enzymes were cloned (amplified) from Jojoba seeds and through soil bacteria transferred into a plant called Crambe (*Crambe abyssinica*) that then started to produce wax esters! The oil pressed from these transgene seeds thus get entirely new properties (suitable for a good lubricant), compared to the type of oil these seeds usually produce. This result would not have been possible without using gene technology, because Jojoba is a plant species that cannot interbreed with Crambe, and mutation breeding had not worked since the genes in Crambe had not been able to achieve this. This wax ester Crambe has been grown in field trials and the new product, wax ester oil, has been extracted and its properties as a lubricant will be characterized (Figure 2).

Figure 2. Genes from the desert shrub Jojoba (Simmondsia chinensis, (A.) were transferred by gene technology into the oil crop Crambe (Crambe abyssinica) to make it produce wax esters that can be used as a lubricant instead of fossil oil derived chemicals. Plants of Crambe setting seeds (B.), mature seeds of Crambe before harvest (C.), and seeds of Crambe inside the pods (D.). (Photo: Åsa Grimberg).

Gene technology debate in Europe

The European debate about the use of gene technology in plant breeding has resulted in an exhaustive process for approval of a new transgenic variety that is both very expensive and takes very long time (see the article *GMO or not GMO?* Bioscience Explained Vol 8, no 1). A variety that has been developed by using gene technology must undergo a larger number of controls, compared to a new variety that has been developed with more traditional breeding methods. The control system could instead evaluate all varieties in the same way and focus on the risks of the new traits, no matter what technology they were introduced with. Then also Europe might take part of a greater diversity of new varieties and crops that can be of great benefit for the people and the environment. The majority of new properties introduced in crops by using gene technology that are approved for cultivation in Europe today represent a fairly short list; herbicide tolerance and insect resistance. Companies behind these products have seen the economic benefits of being able to sell their own herbicides and seed as one kit, which has made people doubtful about using these crops. The academic research community does, however, not have as a major goal to make money but instead to serve the society. They can therefore use the toolbox in plant breeding with more imagination to develop new crops with traits that can be of great benefit for society. For example, the use of insect or fungal resistant crops leads to decreased use of chemical pest control in agriculture, new qualities of oil can replace fossil oil in the chemical industry, crops that can resist drought or cold temperatures can be grown in areas previously not suitable for agriculture, and crops with changed nutrient composition can give us more healthy food.

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Read more

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