FROM GENETIC MODIFICATION TO GENETIC ENGINEERING

Conventional crop breeding, assisted by biotechnology or not, involves identifying agriculturally advantageous mutations (spontaneous or induced) and moving the modified gene into successful crop line. In Canada, all crop breeding is considered **genetic modification** because it alters the genome of the plant. **Genetic engineering**, on the other hand is more narrowly defined as genetic modification using a laboratory technique that allows the direct addition or removal of a gene. Genetic engineering uses **recombinant DNA** molecules, or DNA sequences that have been put together in the laboratory. Often, recombinant DNA (rDNA) combines genes, parts of genes, or other useful DNA sequences from different species.

With all the modern biotechnology tools available to crop breeders, why bother with genetic engineering (GE)? Conventional crop breeding, while extremely successful, has some limitations, in addition to being laborious, time-consuming and frequently inefficient. Gene introgression through backcrossing results in the transfer of linked genes that can sometimes have a negative effect on the crop plant. In other words, it can be technically difficult to transfer just the desired gene through backcrossing (even with the benefit of a DNA marker), and the best-case scenario is that the linked genes that are co-transferred have no effect. Also, the traits that can be transferred by conventional breeding are limited to those found in the same crop species or, rarely, one that is so closely related that it is sexually compatible. For instance, **triticale** is a grain created by crossing wheat (*Triticum sp*.) with rye (*Secale sp*.), two crops that are related enough for crossbreeding.

Genetic engineering circumvents these limitations by directly transferring one or a few genes into the target crop. Importantly, the genes do **not** have to be from the same species because the genetic code is universal. The genomes of all living things are encoded using DNA, and DNA is molecularly compatible whether it comes from a soybean plant, a tomato plant, or a bacteria. The term **transgenic crop** is sometimes used instead of GE crop. A **transgene** is a gene that has been transferred from one variety, strain, or species to another. This can happen naturally, but is more often the result of genetic engineering.

A Crash Course in Genetic Engineering Procedures

To learn more about the procedure of crop genetic engineering, let's examine the example of herbicide-tolerant soybeans (HT-soybeans), mentioned in the GE food case study. Farmers are constantly battling weeds, which compete with their crops for water and nutrients, harbour pathogens, contaminate their harvest with weed seeds and reduce crop quality. The two main methods for getting rid of weeds are tilling, which churns the topsoil and buries the weeds, and applying herbicides, which are chemicals that kill plants. Broad-spectrum herbicides, which are useful because they kill most weeds, also kill crop plants. Soybeans are an increasingly important crop in Canada, and most soybeans that are commercially cultivated have been genetically engineered to be resistant to a type of broad-spectrum herbicide called glyphosate.

The advantage of HT-soybeans is that herbicides can be applied to the entire field to effectively kill the weeds without damaging the crop plants. How is this possible?

Glyphosate kills plants by blocking the activity of an enzyme that is required for the biosynthesis of certain amino acids. The enzyme is called 5-enolpyruvylshikimate-3-phosphate synthase, thankfully abbreviated as EPSPS, and it is found in all plants, bacteria and fungi. One strain of the soil bacteria called Agrobacterium has a form of the EPSPS enzyme that is not blocked by glyphosate, so the bacteria are resistant to this chemical. This glyphosate-tolerant version of EPSPS was transferred from Agrobacterium to soybean plants to create HT-soybeans. The Agrobacterium-derived herbicide-tolerant

EPSPS is a transgene in soybeans. (For more information about HT-soybeans, see the backgrounder about soybean cultivation in Canada.)

HT-crops make weed control easier for farmers, but there are other advantages too. Glyphosate is considered non-toxic to animals, and therefore humans, because animals don't have the EPSPS target enzyme. Glyphosate also degrades quickly in the environment so it doesn't contaminate groundwater. The commercial success of HT-crops means that glyphosate has replaced herbicides that are much more toxic, with negative environmental effects and possibly negative health effects for farmers. Another benefit of HT-crops is an increase in low-till and no-till agriculture, due to a decrease in the need for tilling as a method of weed control. Tractor tilling disturbs the rich topsoil, increases soil erosion, and contributes to greenhouse gas emissions through fossil fuel burning. Low-till and no-till agriculture leaves a smaller environmental footprint.

Here are the main steps involved in the genetic engineering of crop plants (see **Figure 1** for a pictorial representation):

1. Extract the DNA from an organism that has an agriculturally valuable trait.
2. Isolate the gene responsible for the trait and make more copies of it in a host cell. This process is called **cloning**, and bacteria are commonly used as host cells for gene amplification.
3. Insert the gene into a **plasmid**, which is a piece of DNA, usually circular, that can be replicated in microorganisms without being integrated into a chromosome. A plasmid is a type of vector, which is basically a gene-delivery system. Plasmids can also contain DNA sequences called **regulatory elements** that control when the gene is expressed during the plant's development, and in what part(s) of the plant (leaves, fruit, seeds, etc.).
4. Make more copies of the plasmid by amplifying it in a host cell, again commonly bacteria.
5. Insert the plasmid into plant cells, usually done by **biolistic transformation** or ***Agrobacterium*-mediated transformation**. Transformation is the process through which foreign DNA is taken up, integrated, and expressed in non-animal cells. Biolistic transformation uses a gene gun to shoot plasmid-coated metal particles into plant cells. Agrobacteria can readily transfer some of their DNA to plant cells (it happens naturally), so they are used in the laboratory to transfer plasmids.
6. Identify the plant cells that have successfully integrated the plasmid DNA into their genome.
7. Grow whole plants from the plant cells, select for high quality plants, and backcross the engineered gene into a commercially successful line.

Plant cell transformation, whether biolistic or Agrobacterium-mediated, is an inefficient process. The vast majority of the cells will not integrate the plasmid DNA into their genome. To identify the transformed cells, a selectable marker that is part of the plasmid is used. In the case of a plasmid delivering an herbicide-tolerance gene, the HT-gene functions as the **selectable marker**. When plant cells are exposed to herbicide, the only ones that survive are the ones that have integrated the HT-gene. Another option is to include a gene on the plasmid that confers antibiotic resistance. Exposing the plant cells to antibiotics will select the transformed cells. (For more information about the issues around selectable markers, and their alternatives, see the backgrounder on regulation, labelling and safety of GE foods.)

Here is a quick "compare and contrast" of conventional crop breeding and genetic engineering. They are similar because they both result in changing the genome of crop plants to incorporate desirable traits. Conventional breeding can transfer altered (mutated) genes within the same crop species, or between very closely related species, but genetic engineering can add genes from any species. Conventional breeding transfers many genes in addition to the one encoding the desirable trait, but genetic engineering transfers one or very few.

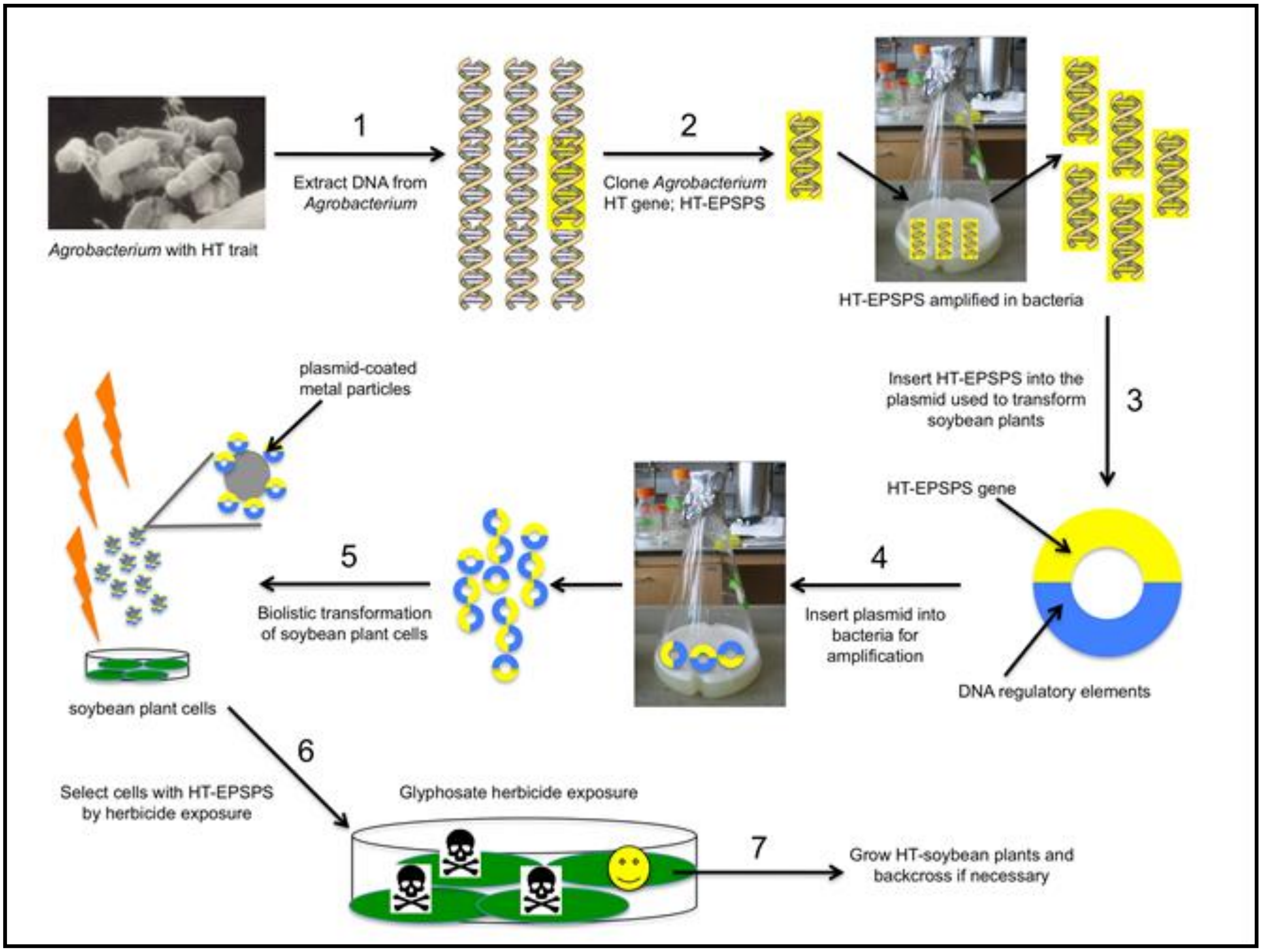

Figure 1: Major Steps to Genetically Engineer HT-Soybeans.
Refer to text for description of each step. Yellow highlighted DNA represents gene for glyphosate-tolerant enzyme, HT-EPSPS. DNA regulatory elements present in plasmid include sequences to control plasmid amplification in bacteria and sequences to control timing and location of HT-EPSPS expression in soybean plants. Orange lightning bolts represent biolistic transformation using a "gene gun" apparatus. Soybean plant cells that do not integrate the HT-EPSPS gene are killed by glyphosate exposure (☠). Successfully transformed cells survive and can form entire plants (☺).

We have used herbicide tolerance as one example of GE food crops, but there are many more traits that have been engineered such as insect resistance, virus resistance, drought tolerance and altered and/or improved nutrient content. Table 1 gives an overview of several different GE crops, many that are widely grown and some that are "in the pipeline". Also the backgrounder about soybean cultivation in Canada has more information about the different kinds of GE-soybeans that are grown here.