

Can DNA from Transgenic Plants be Transferred to Soil Micro-organisms?

Editor's Note: In 2006, 252 million acres of transgenic crops were planted in 22 countries by 10.3 million farmers. The majority of these crops are herbicide – and or insect-resistant soybeans, corn, canola, and alfalfa. Other transgenic crops such as sugar beet are gaining acceptance. The question of GMO's and safety is paramount in discussing transgenics, but well conducted studies directed toward answering questions are not ubiquitous. A well designed study reported several years ago, provides some evidence using transgenic sugar beet. This study conducted by scientists at the Institute for Plant Virology, Microbiology and Biosafety; Braunschweig asked the question, can horizontal gene transfer of transgenic plant DNA to soil bacteria be detected under laboratory or field conditions?

The possibility of horizontal gene transfer was investigated both in the natural environment in the soil and in the laboratory under specific conditions conducive to gene transfer.

Transgenic beet remains were ploughed into the soil after the harvest. In the spring and autumn of the following years, samples were taken and analyzed. In addition, the persistence of free DNA in the soil was investigated in the laboratory: soil samples were mixed with transgenic sugar beet DNA and observed over a period of six months using PCR.

As evidence of horizontal gene transfer in the field the researchers looked for bacteria in the soil samples that had taken up parts of the genetic construct introduced into the sugar beet. The detection methods involved PCR and hybridization

Since only a small proportion (0.1-1%) of soil bacteria can be cultured under laboratory conditions (culture-dependent methods), the project also investigated DNA extracted directly from the soil samples (= culture-independent), which contains fungal, plant and free DNA as well as bacterial DNA. This DNA was analyzed for the presence of transgenic DNA using PCR and primers specific to the genetically engineered construct. These primers facilitate the specific and sensitive detection of the transgenic DNA.

Two experimental approaches were chosen for horizontal gene transfer under optimized laboratory conditions. The bacterium *Acinetobacter* was used as a model organism.

In a first step, the uptake ability of bacterial cells was investigated using different types of DNA (from bacteria, plasmid DNA, transgenic plant DNA).

Horizontal gene transfer is a very rare event, which is therefore difficult to detect. For this reason, a special

Acinetobacter strain was developed with ideal conditions for gene transfer: an incomplete nptII gene (kanamycin resistance) was introduced into the bacteria. The complete gene is present in the sugar beet as a marker gene. Using special mechanisms (homologous recombination), the special *Acinetobacter* strain can complete its nptII gene comparatively easily by taking up transgenic plant DNA.

The number of transformants and the transformation frequency were measured for both approaches.

Results of the study

Persistence ability of transgenic DNA in the soil

- The transgenic sugar beet DNA was detected in the total DNA extracted from the soil samples (culture-independent analysis) over two years. It was, however, not possible to say for sure whether the transgenic DNA detected is present as free DNA or 'packed' in rotting plant remains.
- In the laboratory experiment (culture-dependent analysis) the transgenic DNA was still detected after one, three or six months, depending on the PCR method used.

Horizontal gene transfer

- (1) Outdoor conditions–The number of bacteria increased temporarily following the addition of the shredded beet material as a result of the addition of nutrient in two rotting fields. Around 4500 bacteria that showed resistance to kanamycin were examined more thoroughly. No instances of transgenic DNA sequences were found.
- (2) Laboratory conditions–No transformants that had taken up transgenic plant DNA were found under laboratory conditions either. This meant that there was no indication of horizontal gene transfer.
- (3) Under optimized laboratory conditions, the 'prepared' *Acinetobacter* strain with an incomplete nptII gene was found to have taken up transgenic sugar beet DNA in isolated cases. Transformations with non-transgenic sugar beet DNA were not found. It was possible to attribute the gene transfer of the transgenic DNA to a homology (similarity of DNA sequences) between the DNA of the *Acinetobacter* strain and the transgenic DNA.
- (4) Gene transfer from transgenic plants to *Acinetobacter* sp. BD413 was found only with homologous sequences and only under optimized conditions.



Garry Smith can be reached at garrypatsmith@msn.com