

# Bombardment: using the particle gun to transform plants.

By Amanda Hudswell

**At ACPFG we use a variety of techniques to help improve the tolerance of cereal crops to environmental stresses such as drought and salinity. These techniques are both GM and non-GM. Genetically Modifying (or transforming) a plant involves inserting a gene into a single cell of the plant and then growing that cell into a new plant which is then referred to as a transgenic plant.**

In our last issue we detailed the role of *Agrobacterium* in transformation. This method takes advantage of a natural process called pathogenesis – the infection of an organism by a bacterium such as *Agrobacterium* (see Vector issue 12).

In this issue we look at using the particle gun (also known as biolistic transformation) to transform plants.

## History: The particle gun

Biolistic transformation was first invented in 1987 by American Geneticist, Associate Professor John C. Sanford from Cornell University where he and his colleagues Theodore Klein, Edward Wolf and Ray Wu showed that small micro projectiles could be delivered into a cell without killing it. The first transformation gun was developed by John Sanford and Edward Wolf, and was a converted Crossman air pistol which was modified to fire dense tungsten particles coated with the DNA containing the gene of interest.

‘Tungsten was first used in the transformation process but was replaced by gold particles because tungsten could be oxidised easily and was potentially harmful to the cell,’ said Dr Serik Eliby, Plant Transformation Group Leader at ACPFG.

‘There have been a number of different improvements on the early models of gene guns resulting in the more sophisticated apparatus used today,’ he said. ‘The key principle behind the gun is to accelerate particles by explosion.’



## How the particle gun works

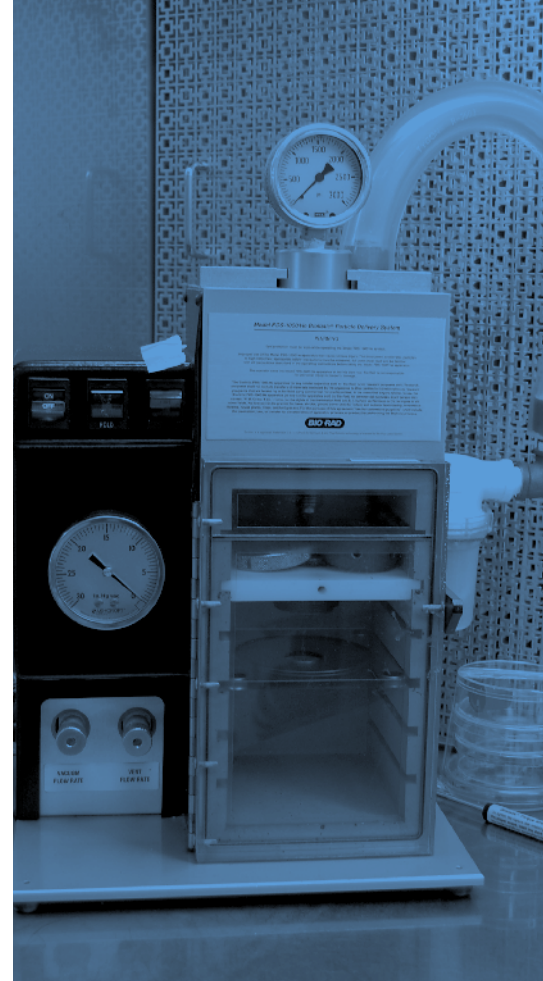
‘Transformation using biolistics is a physical process rather than a biological one as used in *Agrobacterium* dipping,’ said Dr Eliby ‘It’s a process of mechanically inserting the gene of interest into the cell.’

The process involves placing gold particles into a suspension and adding the DNA containing the gene of interest along with calcium chloride (CaCl<sub>2</sub>) and Spermidine which help to ‘pellet’ the DNA onto the gold particles. The DNA is not actually attached, but rather it gathers around the gold particle. This solution is then spun down and washed with ethanol to ensure no free DNA is floating around in suspension. Researchers from ACPFG have improved this process by using polyethylene glycol and magnesium (together referred to as PM) instead of CaCl<sub>2</sub> and Spermidine as PM is very stable and cannot be oxidised.

The gold suspension is then pipetted onto a disc called a ‘macrocarrier’. Gas flow is blocked initially by a ‘rupture disc’ which ruptures at a certain pressure. The pressure build up that ruptures the disc forces the gold particles to fly through a stopping screen which scatters the gold particles over immature embryos sitting on a plate underneath. The explosion forces the gold particles into the cell some of which penetrate the nucleus.



ACPFPG's Ainur Ismagul operates the particle gun during the transformation process.



'Ideally the gold particles will fly through the cell and land in the nucleus,' said Dr Eliby, 'and by a natural repair mechanism within the nucleus called 'recombination' the enzymes will collect the new DNA and integrate it into the DNA of the cell.'

The embryos are then subjected to a selection pressure to identify which have been transformed. These embryos are grown into new transgenic plants.

'The process is a statistical one,' said Dr Eliby, 'thousands of gold particles are shot onto the embryos, of which some are bound to enter the nucleus.'

'This is the benefit of the particle gun,' explained Dr Andrew Jacobs, Enabling Technologies Focus Group Leader at ACPFG. 'Some plant species are not susceptible to Agrobacterium infection, but few organisms can withstand the particle gun due to the physical nature of this process.'

However, there are some drawbacks to the particle gun process.

'The main problem with this method is that you might not get full integration of the DNA. You can get fragmentation of the transformation construct\* due to the nature of the process – shooting DNA in is a random thing. It's a bit hit and miss as to whether the entire construct of interest will be incorporated into the repairing DNA,' Dr Jacobs said.

Despite the drawbacks, the biolistic method has been successfully used to produce improved commercial crops.

'Both Agrobacterium dipping and the particle gun have their advantages and disadvantages, so it's a matter of assessing which method works best for each plant we transform. Generally speaking, barley transformation works well using Agrobacterium and wheat, which is not readily transformed by Agrobacterium, can be transformed with the particle gun.'

### Where is transformation going?

'Improvements in transformation processes are always on our radar. We're looking at other plant pathogenic bacteria, but efficiencies aren't as high as with Agrobacterium' said Dr Jacobs.

'As far as the particle gun goes, improvements could be made in the chemical solutions chosen in the process, as well as looking at more advanced particle accelerating devices, like the ones using laser induced shock waves' said Dr Eliby.

\*The transformation construct is the entire transfer (T-DNA) sequence that researchers want to integrate into the host genome. It commonly includes two open reading frames; the first, a promoter controlling expression of the gene of interest with a terminator code, and the second another promoter driving the expression of a selectable marker gene with a terminator.