Genetically modified plants
and
plants derived from pollen

Dr. M.C.M. Iqbal, Project Leader, Biotechnology, IFS

GENETIC ENGINEERING

Introduction

In the 1980's scientists were able to introduce genes from other organisms into the chromosomes of plants. Such gene transfer from one organism to another is popularly called **Genetic Engineering**. Plants or animals with foreign genes in their chromosomes are known as **transgenic** plants or **transgenic** animals.

It is, however, much easier to transform single celled bacteria than multicellular plants or animals. Bacteria have a single large chromosome and many **plasmids,** which are circular loops of DNA (Fig. 1). Genetic engineering is basically introducing a gene into a plasmid, which reenters the bacterial cell and so introduces a new gene to the bacteria. The plasmids multiply within the bacteria and produce the products of the new gene. Since plasmids are passed onto the next generation the new colonies of bacteria would have the new gene.

Fig. 1: Electron micro-graph of a plasmid.

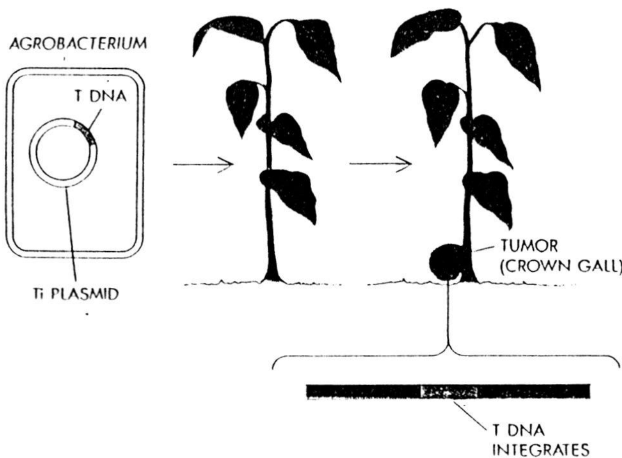


Fig. 2: The Ti plasmid causes 'Crown Gall' in plants.

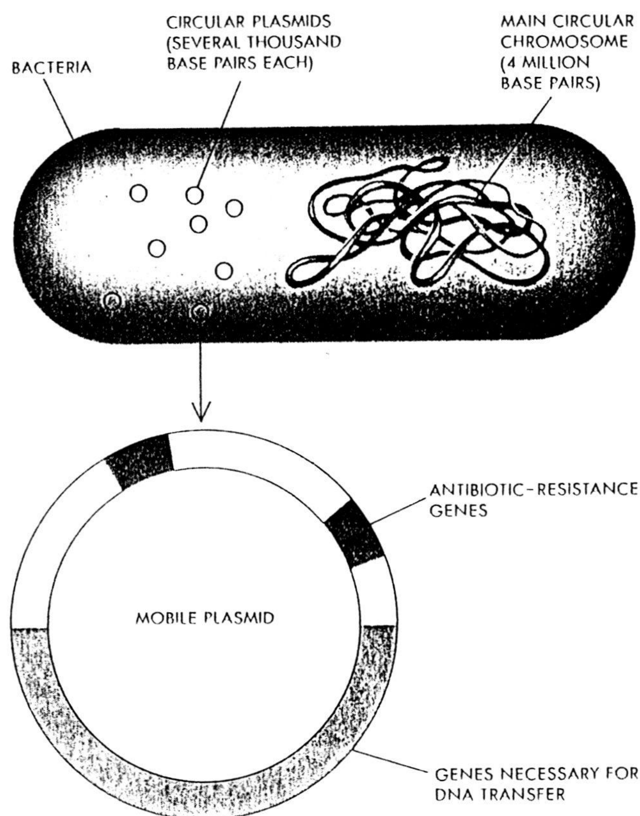
Agrobacterium tumefaciens

Scientists discovered a soil bacterium called *Agrobacterium tumefaciens* that causes a disease in plants. The bacteria cause a lump or **tumor** at the base of the plant called a crown gall. Even if the bacteria is eliminated from the tumor with antibiotics the tumor cells remain tumorous. This showed that the bacteria had introduced a new gene into the cells of the plant. The tumor-inducing agent in the bacteria

was found to be a plasmid and is called a Ti plasmid. The plasmid transfers part of its genes into the chromosomes of the plant (Fig. 2). These genes cause the plant cells to form a mass of unorganized cells called a 'gall'.

Why does the bacterium cause tumors in the plant?

The tumor cells synthesize unusual amino acids called **opines**. These amino acids are not found in plants. The *Agrobacterium* now uses these amino acids as a source of carbon and nitrogen. The plant cells cannot use these opines. Thus the bacterial infection takes over the plants metabolism to make new amino-acids which can only be used by the bacteria as food.



The Ti plasmids are circular DNA molecules (3-5% of the bacteria chromosome) and are independent replicating units in the bacterial cell (Fig. 3). *Agrobacterium* without Ti plasmids do not cause disease or synthesis of opines in infected plants. A part of the DNA in the plasmid causes the tumor in plants and the synthesis of opines by the tumor cells. This part of the DNA is called T-DNA (Fig. 2). Only the T-DNA moves from the Ti plasmid to the host plant cell chromosomes. Thus the Ti plasmid is an ideal **vector** to introduce desired genes into plants cells.

Fig. 3: Bacteria with plasmids.

The Ti plasmid can carry genes into plant cells

To introduce a new desired gene into the plant, a culture of cells or tissues of the plant are prepared by tissue culture. In plant tissue culture, it is possible to culture plant cells or tissues and obtain whole mature plants in the laboratory.

The gene (sequence of DNA bases) to be introduced into the plant may be isolated from any organism.

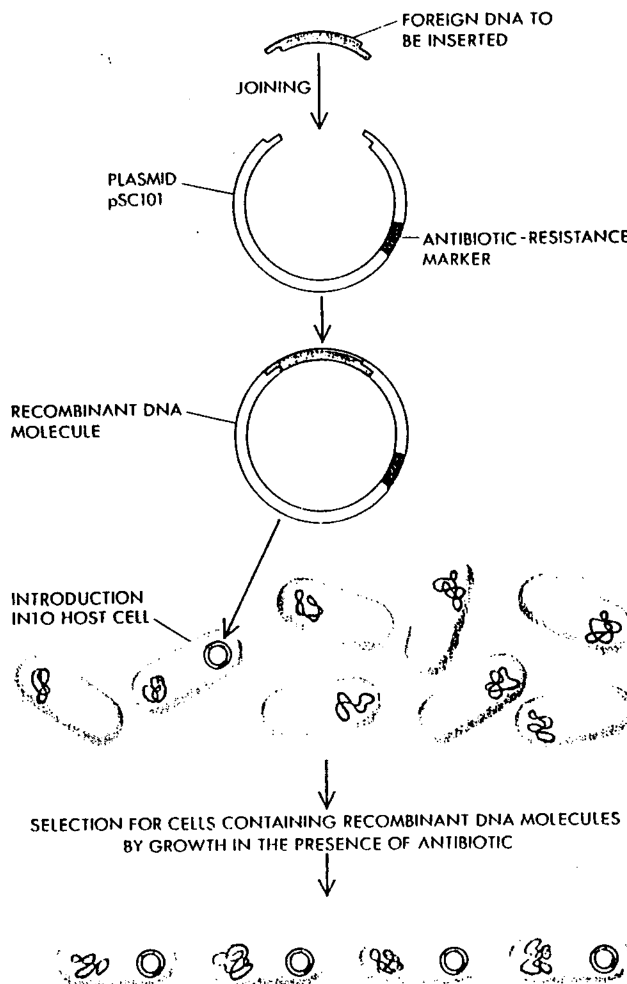


Fig. 4: The cloning of DNA in a plasmid

This particular gene is inserted into the T-DNA using **restriction enzymes**. The new Ti plasmid now has the desired foreign gene in the T-region of its DNA (Fig. 4).

The last step is to infect the plant tissues or cells in culture with the transformed *tume-faciens bacteria*. The plant tissues or cells are cultured with the *Agrobacterium*. The bacteria enter the protoplasts and the T-DNA is transferred to the plant chromosomes. The final step is to regenerate a plant from the protoplasts, which have been transformed. Instead of protoplasts other tissues such as leaf segments, callus, hypocotyl are also used for transforming plants.

The plant cells or callus is finally regenerated to plants.

INDUCTION OF HAPLOIDS

Introduction

The discovery of inducing microspores to develop into embryos was accidental. In 1964, Guha and Maheswarie were studying meiosis *in vitro* in the plant *Datura* innoxia. They found that the microspores developed into embryos. Three years later Bourgin and Nitsch were able to produce entire plants from haploid embryos. Now, nearly 30 years later, microspore-derived plants have been reported in more than 200 species in over 30 families. Most of these species are however in 3 families: *Solanaceae, Cruciferae* and *Graminae.*

Another contains pollen, and anther culture involves the culture of these structures *in vitro*. The immature pollen or microspores within the anther either gives rise directly to embryos or to callus tissue, which in turn is induced to regenerate plants in a culture medium. Pollen is haploid and the cells produced from pollen or microspores during culture are haploid. When plants are regenerated from haploid cells, a haploid plant is produced. Haploid plants are sterile and cannot produce seed. However, a spontaneous duplication of chromosomes often occurs to produce fertile double haploid plants.

Haploid - Sex or genetic cells with half the number of chromosomes. egg. pollen, ovules (in plants); sperm, egg-cell (in animals)

Methodology

The most common form of culture to induce haploids is by anther culture on agar solidified medium. The culture medium contains, macro and micronutrients and various combinations of phytohormones, organic supplements and a source of sugar-usually sucrose. Different media have been optimised for different species.

Anthers are also cultured in liquid medium (*i.e.* without agar). The floating anthers may either produce embryoids or callus on the anthers, or they may shed their microspores in the liquid medium. The microspores then undergo development.

The anther wall is of diploid tissue and often undergoes somatic embryogenesis, which is undesired.

In certain species (e.g. *Nicotinana* and *Brassica*) it is possible to culture isolated microspores which are isolated by:

- crushing the buds/anthers to release the microspores.
- Sieving to filter the debris and microspore.
- Centrifuging to isolate and pellet the microspores and removes the small particles. The microspores are cultured in liquid medium. The single microspores can be followed through their stages of cell division to form embryos.

Normal Pollen development

The most important factor in the induction of embryogenesis is the stage of pollen development. The critical stage in more species is the late uni-nucleate or early binucleate stage.

In the anther, pollen develop from pollen mother cells (2n) which undergo mitosis and meiosis to give a tetrad of haploid microspores. The tetrads are released from the pollen mother cells. Initially the microspores are thin walled and vacuolate with a central nucleus. As the microspores increase in volume, a vacuole develops and the nucleus is pushed to the periphery by this vacuole. At this stage, the first pollen mitosis occurs to produce a large vegetative nucleus and a small generative nucleus. This is an asymmetrical division. The vacuole disappears and a second pollen mitosis of the generative cell produces two sperm cells one of which ultimately fertilizes an ovule.