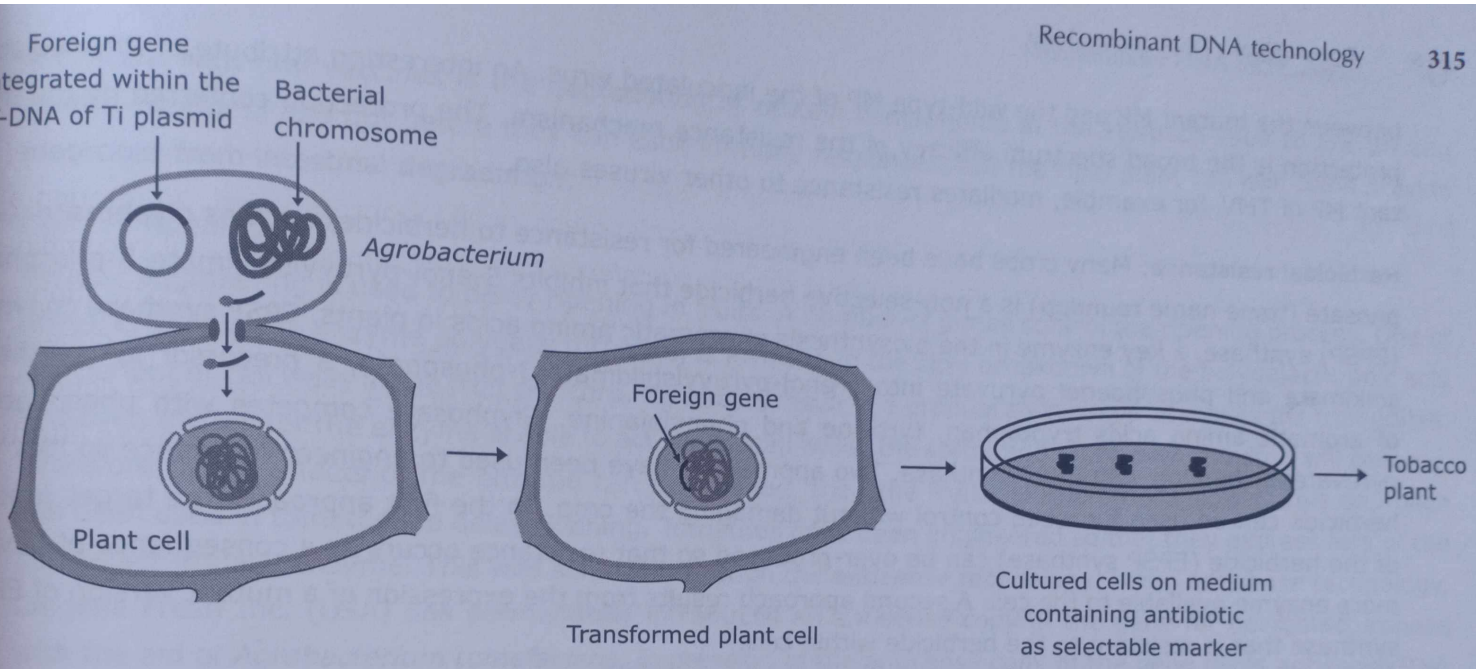

Figure 2.36 A procedure used to make a transgenic plant. Plant tissue in culture is incubated with *Agrobacterium* cells that carry a recombinant plasmid that contains both a selectable marker gene and a desired transgene. When the *Agrobacterium* recognizes a plant cell, it efficiently passes a T-DNA strand that carries transgene into the plant cell. Only those plant cells that take up the T-DNA and express the selectable marker genes survive to proliferate and form a callus. The manipulation of growth regulators and nutrients supplied to the callus induces it to form shoots, which subsequently root and grow into adult plants carrying the transgene.

2.15 Transgenic plants

Transgenic plants are those plants, which carry additional, stably integrated and expressed, foreign gene(s) from trans-species. The whole process of transgenic plants' development involving introduction, integration and expression of a foreign gene(s) in the host, is called *genetic transformation*. The combined use of recombinant DNA technology, gene transfer methods and tissue culture techniques has led to the efficient transformation and production of transgenics in a wide variety of crop plants. Unlike conventional breeding, only the cloned gene(s) of agronomic importance is being introduced without the cotransfer of other undesirable genes from the donor. The recipient genotype is least disturbed and there is no need for repeated back crosses. This will serve as an effective means of removing certain specific defects of otherwise well adopted cultivars.

2.15.1 General procedure used to make a transgenic plant

Agrobacterium-mediated transformation

The first step in the process of development of a transgenic plant involves the formation of the recombinant plasmid and its transfer to plant cells. For the recombinant formation, T-DNA needs to be *disarmed*. To do this, the genes encoding the proteins for the production of auxin and cytokinin are simply removed from the T-DNA fragment. New DNA can, then, be inserted between the left and right border repeats.

Recombinant T-DNA plasmid vectors contain a number of functional units in addition to the right border and left border elements. These include:

1. A broad host range origin of replication,
2. An antibiotic-resistance gene for plasmid selection in bacteria,
3. A multiple cloning site located between right and left border elements for insertion of the target gene,
4. A dominant selectable/screenable marker gene for selection of transformed plant cells.

   Most such genes used in plants are dominant selectable markers that confer resistance to antibiotics or herbicides. The commonly used selectable marker genes include those conferring resistance to the antibiotics kanamycin and hygromycin; and herbicides glyphosate, phosphinothricin, etc.

Table 2.7 Examples of some commonly used selectable markers

Selective agent	*Gene*
Kanamycin (antibiotic)	NPT (encoding *neomycin phosphotransferase*)
Streptomycin (antibiotic)	SPT (codes *streptomycin phosphotransferase*)
Hygromycin (antibiotic)	HPT (encoding *hygromycin phosphotransferase*)
Bromoxynil (herbicide)	BXN (codes *bromoxynil nitrilase*)
Phosphinothricin or Glufosinate (herbicide)	BAR (codes *phosphinothricin acetyltransferase*)
Glyphosate (herbicide)	EPSPS (codes *EPSP synthase*)
Chlorsulfuron (herbicide)	ALS (codes *acetolactate synthase*)

Agrobacteria that are carrying a recombinant plasmid with both a selectable marker and a desired transgene are incubated in culture with plant cells. The wounded plant cells at the edge release substances that attract the *Agrobacteria* and cause them to inject DNA into these cells. Only those plant cells that take up the appropriate DNA and express the selectable marker gene survive to proliferate and form a callus. The growth factors supplied to the callus induce it to form shoots and roots, and grow into adult plants carrying the transgene.