

# **BT503-ASSIGNMENT 01**

**FALL 2022**

## **SAMPLE SOLUTION**

**Assignment topic :Transgenic Plants**

**Muhammad Kashif 03460319909**

***Note: A student must write the assignment in his own words to secure good marks.***

### **TRANSGENIC PLANTS**

Transgenic plants, or plants which express foreign gene products, can be generated by a variety of procedures, such as *Agrobacterium*-mediated transformation or biolistic delivery. Recent advances in these technologies have resulted in the development of commercially successful disease and herbicide resistant plants which both increase crop yield and reduce costs for the farmer. Safe and inexpensive production of recombinant proteins in large quantities have also been produced in transgenic plants, as well as plants which possess enhanced nutritional traits. In this section, current techniques employed in plant transformation are investigated. Transgenic plants resistant to plant viruses, insects and herbicides are discussed. The manufacturing of proteins in plants, including edible vaccines, immunotherapeutic agents such as antibodies, and biopharmaceuticals are examined. The development of nutraceuticals in transgenic plants is also discussed. Finally, future directions of transgenic plant research and obstacles which remain to be overcome are considered.

### **1. Introduction**

There is virtually no place on earth where the term ‘transgenic plant’, referring to plants that contain foreign genetic material, is unfamiliar. Transgenic plants were first developed and introduced as crops in the early 1980’s. Since transformed plants were found to be fertile and the foreign gene of interest could be continued throughout the progeny, the enormous commercial potential of transgenic plants and their role in crop improvement was fully realized. The first genetically modified crops were soybean and corn, and appeared on the US market in 1996. Since then, transgenic plants have been commercialized in many other countries.

Transgenic plants which exhibit increased pest and disease resistance can prevent global production losses which are currently greater than 35 percent. Transgenic plants also present enormous possibilities to become one of the most cost-effective and safe systems for the large-scale production of proteins for industrial, pharmaceutical, veterinary and agricultural uses. In these cases, the plant derived protein must be biologically identical to its native counterpart and be produced at levels high enough to be purified by relatively simple procedures.

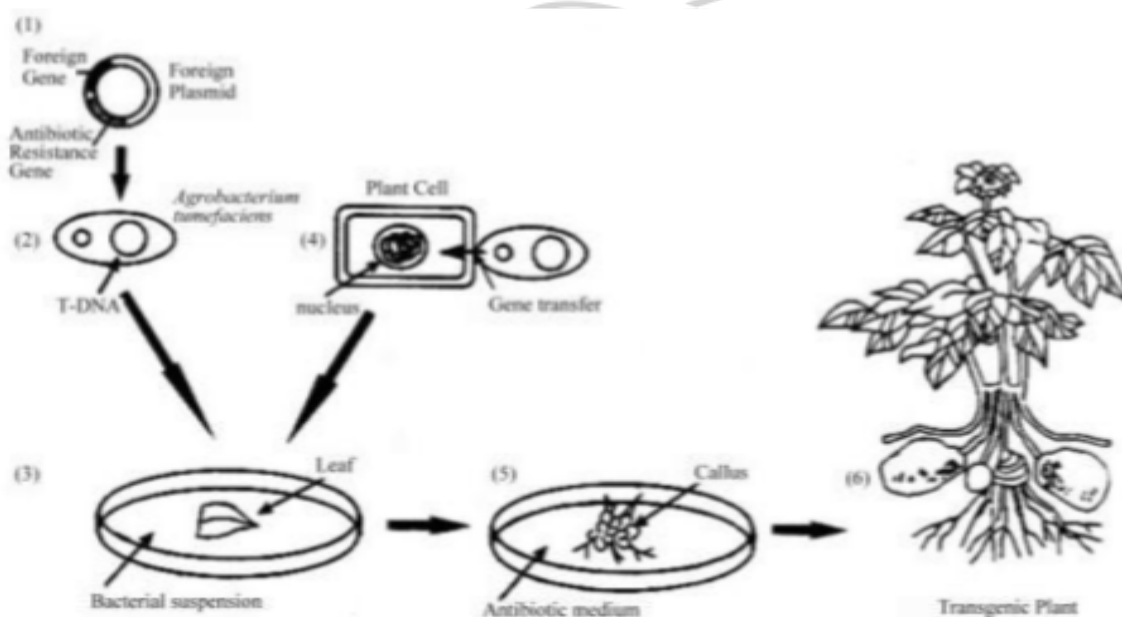
### **Transformation of Plants**

Plant transformation, meaning the stable integration of the gene of interest into the plant genome, was originally conducted using a modified strain of *Agrobacterium tumefaciens*, the bacterial strain responsible for crown-gall disease [see also - *Genetic engineering of plant cells*]. *Agrobacterium tumefaciens* harbours a large tumourinducing (Ti) plasmid and during infection causes a mass of mainly undifferentiated cells to form on a plant's stem at the soil line (crown). The transfer DNA (T-DNA) portion of the Ti plasmid and its delimiting right and left border sequences become integrated into the nuclear genome of a susceptible plant cell that is in contact with the bacterium. The T-DNA encodes enzymes for synthesizing plant hormones that stimulate cell division and the proliferation of undifferentiated cells into the tumour. Vectors used for transformation today lack the genes for hormone-synthesizing enzymes and therefore can introduce foreign DNA into a nuclear chromosome of a plant cell with minimal damage.

Insertion of DNA into a plant by *A. tumefaciens* involves insertion of a foreign gene between the borders of the T-DNA, which in turn is cloned within a small plasmid (Figure 1). The construct is then transformed into a modified version of *A. tumefaciens* which lacks the virulence genes. Upon infection, the T-DNA is transferred into the plant cell, and the gene of interest is incorporated into the host chromosome. The plant cell can then be regenerated from tissue culture into a mature transgenic plant by transferal through a series of culture media with different hormone contents.

A number of problems exist with this mode of transformation. Primarily, the restricted host range of *Agrobacterium* renders infection of monocots difficult. For this reason, other transformation procedures have been developed. Maize, for example, is commonly transformed by particle bombardment, a procedure in which high velocity microprojectiles carrying DNA can be 'shot' with compressed gas using a 'gene gun' into plant tissue.

In addition to this, foreign gene expression in nuclear transformed plants can vary markedly from one transgenic plant to another. Chromosomal position effects are partially responsible for this problem, since the insertion of the transgene into the plant genome is uncontrolled. Other difficulties include the ability of nuclear transformed plants to express more than one transgene. Since many agronomic traits are in fact multigenic and stem from the action of several genes, the production of transformants expressing multiple genes is a painstakingly long process.



**Figure 1: Stages involved in generation of transgenic plants by *Agrobacterium* mediated transformation.**

1. Gene of interest is cloned into foreign plasmid which contains an antibiotic resistance gene
2. Plasmid is transformed into *Agrobacterium Tumefaciens*
3. Cut leaf is exposed to a suspension of *Agrobacteria* containing the gene of interest
4. The gene of interest is integrated into the genomic DNA of individual leaf cells.
5. The leaf is exposed to an antibiotic to kill non-transformed cells. The surviving cells form a callus which then sprouts roots and shoots.
6. The plantlets produced from the callus are transferred to soil. Mature transgenic plants generated now contain the foreign gene of interest.

More recently, genes have been introduced directly into the plastid genome. This was first accomplished for *Chlamydomonas reinhardtii* by biolistic transformation. Plastid transformation is unique from nuclear transformation as the transgene is incorporated directly into the plastid genome by homologous recombination and can be predictably directed to a specific site within the plastid chromosome. Recently, two new procedures involving polyethylene glycol and direct *in situ* injection have also been developed for plastid transformation.

## **2. Herbicide and Disease-Resistant Crops**

### **2.1. Plant Virus-Resistant Crops**

#### **2.1.1. Coat protein-mediated resistance**

Transgenic plants which carry nucleotide sequences derived from plant viruses have been constructed and are capable of protecting against viral diseases (Table 1). The presence of a viral sequence or gene product in a plant can interfere with infection, resulting in cross-protection against the challenger virus. This process is thought to act in a similar manner to that of classical cross-protection, in which infection of plants with a virulent strain of the virus is suppressed by the prior inoculation with a mild strain of the same virus. However, in the case of classical cross-protection, the use of mild strains of the virus may be of disadvantage in agriculture, since viral strains which are seemingly harmless to one crop type may cause severe damage to another, or may act synergistically in conjunction with another virus to create a more severe disease condition.

#### **Resistance conferred by other gene products.**

Since the initial studies with TMV, numerous examples of cross-protection with viral coding sequences other than those encoding the coat protein have been established in other virus-host systems (Table 1). Expression of the replicase of Brome Mosaic Virus (BMV), pea early browning virus (PEBV) as well as Potato Virus X (PVX) has all demonstrated to confer resistance against infection. However, it is uncertain which portion of the replicase protein is responsible for protection. More recently, tobacco plants transformed with the sequence containing an additional open reading frame encoding a 54 K protein located within the replicase gene of TMV were confirmed to be resistant to infection.

Transformed plants expressing a defective movement protein of TMV or protease of soybean mosaic virus (SMV) were also demonstrated to be resistant to viral infection. Transgenic potato plants expressing mutant PLRV movement protein exhibit a broad range protection against virus infection. These plants were found to be resistant to PLRV and unrelated PVY and PVX.

Transgenic plants have also been generated which express ribozymes directed against specific viral sequences in an attempt to inhibit virus infection and spread. Transgenic plants which express mammalian antibodies directed against plant viral proteins have been shown to reduce the incidence of infection with some degree of success. Finally, plants expressing sequences coding for the defective-interfering particles (DI particles) of cymbidium ringspot virus (CyRSV) demonstrated a high level of resistance against infection by the corresponding viruses. It appears, then, that the expression of a variety of viral gene products can disrupt one or several stages of the viral life cycle during infection.

## **2.2. Resistance against Other Pathogens**

The expression of foreign genes in plants has been used to employ a number of active defence mechanisms which can protect plants against infection by viruses as well as a variety of other pathogens [*see also - Crop protection through pest resistance genes*]. For example, expression of ribosome-inactivating proteins (RIP's) such as the pokeweed antiviral protein (PAP), a 30 kD protein isolated from *Phytolacca americana* in transgenic plants, results in the inhibition of protein synthesis in cells which are infected. Pokeweed antiviral protein inhibits translation by catalytically removing a specific adenine residue from the large rRNA of the 60S subunit of eukaryotic ribosomes. Transgenic plants expressing this protein possess a high level of resistance against a wide spectrum of viruses and other pathogens including TMV, PVX and the fungal pathogen *Rhizoctonia solani*. Increases in pathogenesis-related (PR) proteins, but no increase in salicylic acid levels was observed, suggesting that PAP may elicit a signal transduction pathway that is independent of salicylic acid (SA).

Salicylic acid was overproduced in transgenic plants by transforming tobacco with two bacterial genes coding for enzymes that convert chorismate into SA. This resulted in constitutive expression of the PR proteins, and the plants were resistant to viral and fungal infection in a manner that resembled systemic acquired resistance (SAR).