***Agrobacterium* mediated gene transfer in plants**

*Agrobacterium tumefaciens* is a soil-borne, Gram-negative bacterium. It is rod shaped and motile, and belongs to the bacterial family of Rhizobiaceae. *A. tumefaciens* is a phytopathogen, and is treated as the nature’s most effective plant genetic engineer.

*Agrobacterium*-mediated genetic transformation is the dominant technology used for the production of genetically modified transgenic plants.

Agrobacterium tumefaciens, is commonly used as a vector for the introduction of foreign genes into plants and consequent regeneration of transgenic plants.

*A. tumefaciens* naturally infects the wound sites in dicotyledonous plants and induces diseases known as crown gall. The bacterium has a large plasmid that induces tumor induction, and for this reason, it was named tumor-inducing (Ti) plasmid. The expression of T-DNA genes of Ti-plasmid in plant cells causes the formation of tumors at the infection site. The molecular basis of *Agrobacterium*-mediated transformation is the stable integration of a DNA sequence (T-DNA) from Ti (tumor-inducing) plasmid of *A. tumefaciens* into the plant genome.

*A. tumefaciens*-mediated transformation has some advantages compared with direct gene transfer methods such as integration of low copy number of T-DNA into plant genome, stable gene expression, and transformation of large size DNA segments.

Vectors are made by making certain changes to the Ti-plasmid. The foreign DNA is inserted between the right border and left border of the Ti-plasmid and then integrated into the plant genome without causing tumors. **(refer to the topic Ti-plasmid based vectors)**

**The process of T-DNA transfer and its integration into the host plant genome are as follows: -**

1. Signal induction to *Agrobacterium*: - The wounded plant cells release certain chemicals-phenolic compounds (example- acetosyringone) and sugars which are recognized as signals by *Agrobacterium*. The signals induced result in a sequence of biochemical events in *Agrobacterium* that ultimately helps in the transfer of T-DNA of T-plasmid.
2. Attachment of *Agrobacterium* to plant cells: - The *Agrobacterium* attaches to plant cells through polysaccharides, particularly cellulose fibres produced by the Bacterium.
3. Production of virulence proteins: - As the signal induction occurs in the *Agrobacterium* cells attach to plant cell, a series of events take place that result in the production of virulence proteins. To start with, signal induction by phenolics stimulates vir A which in turn activates (by phosphorylation) vir G. This induces expression of virulence gene of Ti-plasmid to produce the corresponding virulence proteins (D1, D2, E2, B etc.).
4. Production of T-DNA strand: - The right and left borders of T-DNA are recognized by vir D1/vir D2 proteins. These proteins are involved in the production single-stranded T-DNA (ss DNA), its protection and export to plant cells. The ss T-DNA gets attached to vir D2.
5. Transfer of T-DNA out of *Agrobacterium*: - The ss T-DNA –vir D2 complex in association with vir G is exported from the bacterial cell. Vir B products form the transport apparatus.
6. Transfer of T-DNA into plant cells and integration: - The T-DNA –vir D2 complex crosses the plant plasma membrane. In the plant cells, T-DNA gets covered with vir E2. This covering protects the T-DNA from degradation by nucleases. Vir D2 and vir E2 interact with a variety of plant proteins which influences T-DNA transport and integration. The T-DNA – vir D2, vir E2- plant protein complex enters the nucleus through nuclear pore complex. Within the nucleus, the T-DNA gets integrated into the plant chromosome.

