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Agrobacterium Mediated Gene Transfer: an Overview

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1. Introduction.

Agrobacterium tumefaciens and *Agrobacterium rhizogenes* are common gram-negative soil borne bacteria causing induction of 'crown gall' and 'hairy root' diseases. These bacteria naturally insert their genes into the genome of higher plants. Virulent strains of bacteria introduce a part of their genetic material into the infected cells where it gets integrated randomly with the genetic material of the host cell. The bacterial genes are able to replicate along with the plant genome and uses the machinery of plants to express their genes in terms of the synthesis of a special class of compounds, called opines, which the bacterium uses as nutrients for its growth but are useless to the host cells. *A. tumefaciens* attracted to the wound site via chemotaxis, in response to a phenolic compound. Infected tumorous plant cells were found to contain DNA of bacterial origin integrated in their genome.The transferred DNA (named T-DNA) was originally part of a small molecule of DNA located outside the chromosome of the bacterium.This DNA molecule is called asTi (tumor-inducing) plasmid.

2.1.Ti Plasmid

- Ti plasmid ranges from 180-205kb in size.
- It has **T DNA** -20kb ,**vir** genes , **ori** gene , **tra** genes,and **genes for opine synthesis.**
- Opines are derivatives of amino acids which are of two types; **octopine** and **nopaline**.
- Octopine is formed with two amino acids; Arginine and Alanine. Nopaline is made up of Arginine and Glutamine
- Ti plasmid encode enzymes for catabolism of **opines** such as **permease** and **oxidase**.
- The opines are catabolized and used as the energy source by the bacterium.
- Ti plasmids are classified based on the type of opines they produce in the host cell during infection.
- Octopine Ti plasmids produce Octopine ($C_9H_{18}N_4O_4$).
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- Shi and Roi sequences regulates shoot induction and root induction respectively.
- Nopaline Ti plasmids produces an opine called as nopaline ($C_9H_{16}N_4O_6$).

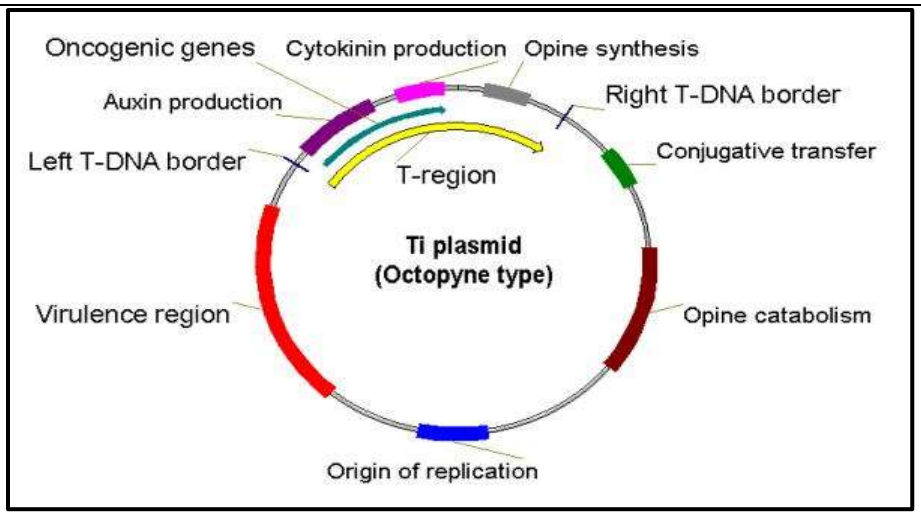

Fig.1: Ti Plasmid

2.2. Ri Plasmid

- The Ri–plasmid contains a distinct segment of DNA which is transferred to plant genome during infection.
- The *A. rhizogenes* have Ri plasmid
- strains of *A. rhizogenes* are known to produce agrocinopine and few opines of the agropine group

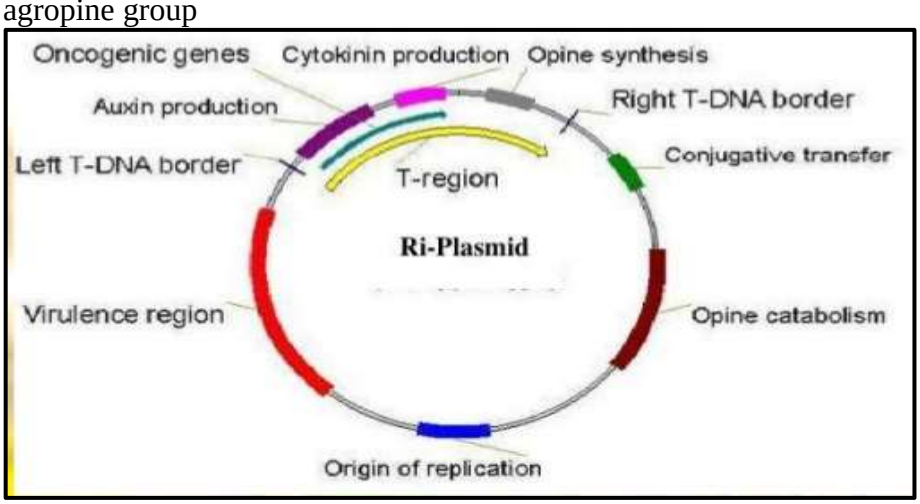

Fig.2: Ri plasmid

3.Organization of T-DNA

The transfer DNA (T-DNA) is the transferred DNA of the tumour inducing plasmid (pTi) of some *Agrobacterium* species of bacteria. T-DNA has both its side 24 kb direct repeat border sequence and contains the gene for tumor / hairy root induction and also for opines biosynthesis (Figure). pTi has three genes, two of these genes (*iaaM* and *iaaH*) encode enzymes which together convert tryptophane in to IAA (Indol-3-acetic acid) a type of auxin. If these two genes are deleted then shooty crown gall will produce. Therefore, the locus was earlier called ‘shooty locus' and the genes were designated as *tms* 1 (tumour with shoots) and *tms* 2. The third gene, *ipt*, encodes an enzyme which produces Zeatin-type cytokinin isopentenyl adenine.

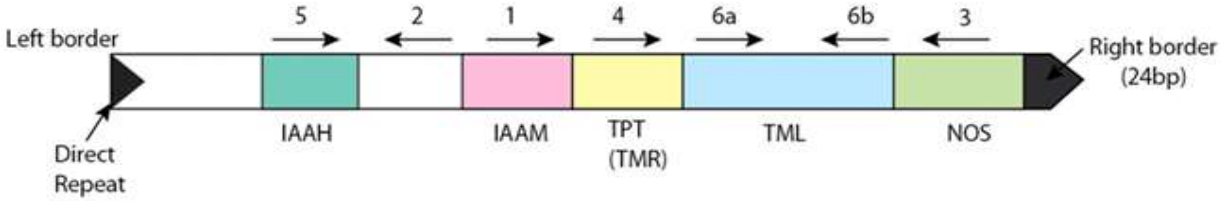

Fig.3: Nopaline type Ti plasmid T-DNA (Arrows indicating the direction of transcription and number indicates the transcriptional unit)

The deletion of *ipt*, causes rooty crown galls and the region was earlier designated as 'rooty locus' and denoted by *tmr* (tumour having roots). In addition to these, another locus called *tml* and the deletion of which results in large tumours. Besides, T-DNA also contains genes involved in opine biosysnthesis which are located near the right border of T-DNA.

4. T-DNA transfer and integration

The steps involved in T-DNA transfer and integration in to the plant genome are explained in Figure

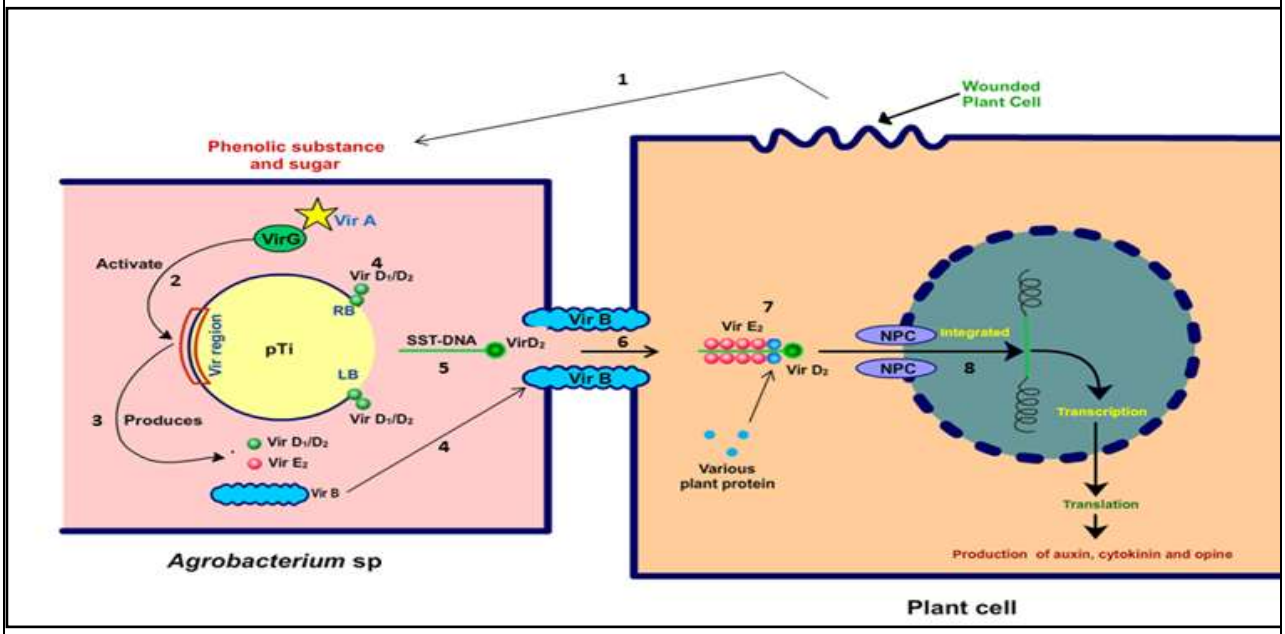

Fig.4: T-DNA transfer and integration

Wounded plant cell releases phenolics substances and sugars (1)which are sensed by *vir* A, *vir* A activates *vir* G, *vir* G induces expression of *vir* gene of Ti-plasmid (2); *vir* gene produce all the

vir -protein (3); *vir* D_1 and *vir* D_2 are involve in ssT-DNA production from Ti-plasmid and its export (4) and (5); the ssT-DNA (with associated *vir* D_1 and *vir* D_2) with *vir* E_2 are exported through transfer apparatus *vir* B (6); in plant cell, T-DNA coated with *vir* E_2 (7); various plant proteins influence the transfer of T-DNA + *vir* D_1 + *vir* D_2 + *vir* E_2 complex and integration of T-DNA to plant nuclear DNA(8). (LB= left border; RB= Right border; pTi = Ti plasmid, NPC = nuclear pore complex)

4.1.Signal recognition by *Agrobacterium* spp.

The wounded plant cells release certain chemicals, such as phenolics and sugars. These chemicals are recognized by *Agrobacterium* as signals. This in turn results in a sequence of biochemical events in *Agrobacterium* that helps in transfer of T-DNA of Ti plasmid.

4.2. Attachment to plant cell

Attachment of this bacterium to plant cells is a two step process. It involves an initial attachment via a polysaccharides (the product of *att* R locus). Subsequently, a mesh of cellulose fibres is produced by *Agrobacterium*. Several chromosomal virulence genes (*chv* genes) are involved in attachment of bacterial cells to the plant cells.

4.3. Induction of virulence gene

vir A (a membrane-linked sensor kinase) senses phenolics (such as acetosyringone) and autophosphorylates, subsequently phosphorylating and, thereby, activating *vir* G. This activated *vir* G induces expression of virulence gene of Ti plasmid to produce the corresponding virulence proteins (D, D2, E2, B). It has been also identified that certain sugars (e.g. glucose, galactose, xylose etc.) also induce virulence gene.

Table No.1: *Agrobacterium* virulence protein function

Virulence protein	Function in *Agrobacterium* spp.	Function in plant
*vir*A	• Phenolic sensor • Part of two component system with VirG; phosphorylation and activates VirG	-
*vir*G	• Transcriptional factor • Responsible for *vir* gene expression	-
*vir*B1-B11	Components of membrane structure for T-DNA transfer	-
*vir*D1	• In T-DNA processing • Modulate *vir*D2 activity	-
*vir*D2	• Nick the T-DNA • Directs the T-DNA through *vir*B transfer apparatus	-
*vir*E2		• Single stranded DNA-binding protein • Prevents T-DNA degradation by nucleases • Involved in nuclear targeting and helps in passage through nuclear pore complex (NPC).

4.4. Production of T-DNA strand

The right and left border sequence of T-DNA are identified by *vir* D1/ *vir* D2 protein complex and *vir* D2 produces single stranded DNA (ss-T-DNA). After nicking, *vir* D2 becomes covalently attached to the 5'end of ss-T- DNA strand and protect and export the ss-T-DNA to plant cells.

4.5. Transfer of T-DNA out the bacterial cell

The ss-T-DNA – *vir* D2 complex in association with *vir* E2 is exported from bacterial cell by a 'T-pilus' (a membrane channel secretary system).

4.6. Transfer T-DNA into plant cell and integration

The single stranded T-DNA–*vir* D2 complex and other *vir* proteins cross the plant plasma membrane. In the plant cells, T-DNA gets covered with *vir* E2. This covering of *Vir* E2 helps in protection of ss-T-DNA from degradation by nucleases. *vir* D2 and *vir* E2 interact with variety of plant proteins which influence the T-DNA transport and integration. The T-DNA – *Vir* D2 – *Vir* E2 – plant proteins complex enters the nucleus through nuclear pore complex (NPC). In the nucleus, T-DNA gets integrated into the plant genome by a process referred to as 'illegitimate recombination'. This process is unlike homologous recombination as it does not depend on extensive region of sequence similarity.

5. Modifications of Ti plasmid

1. Co-integrated Vectors

Co-integrated vectors or **hybrid Ti plasmids**, these vectors were among the first types of modified and engineered Ti plasmids devised for *Agrobacterium* -mediated transformation**.** These vectors are constructed by homologous recombination of a bacterial plasmid with the T-DNA region of an endogenous Ti plasmid in *Agrobacterium*. Integration of the two plasmids requires a region of homology present in both.

Three vectors are necessary in this system:

Disarmed *Agrobacterium* Ti plasmids

In these Ti plasmids, the oncogenes located in the T-DNA region have been replaced by exogenous DNA.

Examples of these vectors include:

. SEV series: the right border of the T-DNA together with the phytohormone genes coding for cytokinin and auxin are removed and replaced by a bacterial kanamycin resistance gene while the left border and a small part of the left segment (T_L) of the original T-DNA (referred to as Left Inside Homology (LIH)) are left intact.

. pGV series: the phytohormone genes are excised and substituted by part of pBR322 vector sequence. The left and right border sequences as well as the nopaline synthase gene of the Ti plasmid are conserved.

- **Intermediate vectors**

These are small pBR322-based plasmids *(E. coli* vectors) containing a T-DNA region. They are used to overcome the problems derived from the large size of disarmed Ti plasmids and their lack of unique restriction sites. Intermediate vectors are replicated in *E.coli* and are transferred

into *Agrobacterium* by conjugation. They cannot replicate in *A. tumefaciens* and therefore, carry DNA segments homologous to the disarmed T-DNA to permit recombination to form a co-integrated T-DNA structure.

- **Helper vectors**

These are small plasmids maintained in *E. coli* that contain transfer (*tra*) and mobilization (*mob*) genes, which allow the transfer of the conjugation-deficient intermediate vectors into *Agrobacterium*.

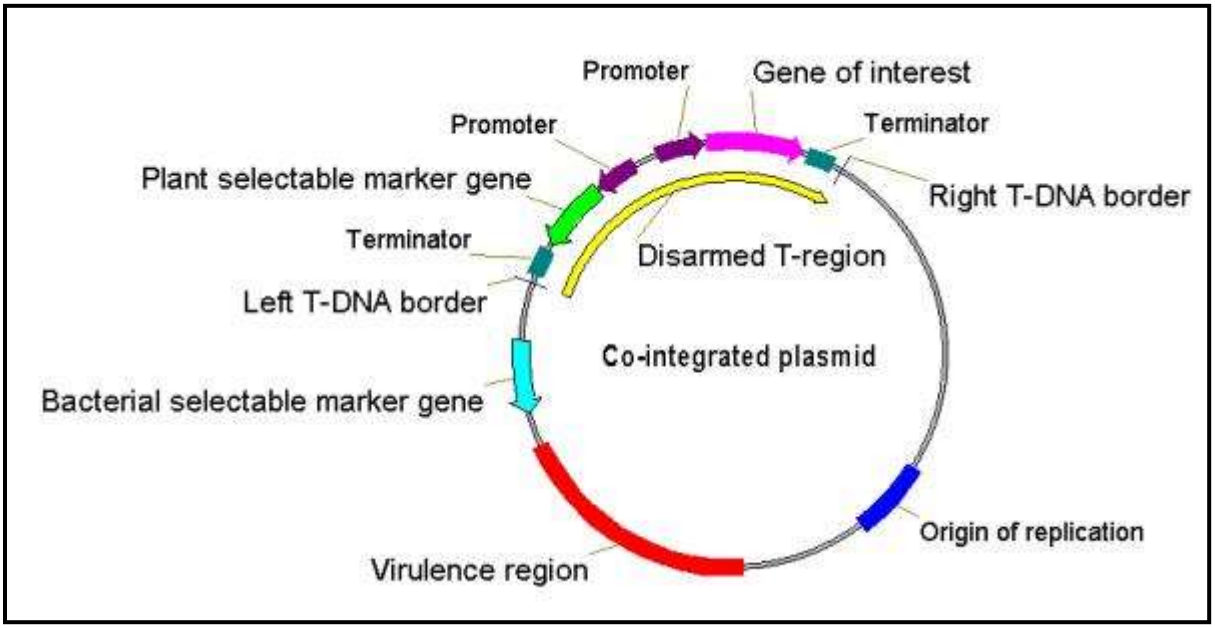

Fig.5:Co integrated Vector

A resulting **co-integrated plasmid** assembled by *in vitro* manipulation normally contains:

1. the *vir* genes,
2. the left and right T-DNA borders,
3. an exogenous DNA sequence between the two T-DNA borders, and
4. plant and bacterial selectable markers.

Advantages of *Agrobacterium* mediated gene Transfer

- Simple and comparatively less expensive
- High transformation efficiency
- Transgenic crops obtained have better fertility percentage
- Protocols for both dicotyledons and monocotyledon are available

Relatively large length DNA segment can be transferred

6. References

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- Reza Mohammad Hassan *et al.*,(2014) Agrobacterium-based vectors: a review *Intl. J. Farm & Alli Sci*. 3(9): 1002-1008
- T. Kondo T., Hasegawa H., Suzuki M . 2000. Transformation and regeneration of garlic (allium sativum l.) By Agrobacterium-mediated gene transfer. *Plant cell reports.* 19(10)pp.989-993

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