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Agrobacterium- A Natures Plant Genetic Engineer

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Abstract

Plant transformation has become most commonly adopted as a method of gene transfer. This method depends on the stable introduction of transgenes into the genome of the plant. Various methods have been developed to achieve this and many plant species have been transformed successfully. One of the most important method of gene transfer is *Agrobacterium* mediated indirect gene transfer which is based on utilizing *Agrobacterium,* a pathogen that transfers genes into the plant genome. The present paper discusses about the biology of *Agrobacterium* and how it is used in plant transformation.

Key words: *Agrobacterium tumefaciens*; transformation,

Introduction

Transgenic technology had a profound impact on the rapid development of the plant biology in the past two decades. The development of technologies that allow the introduction and functional expression of genes in plant cells has extended to the production of transgenic plants with improved insect and disease resistance, crops with enhanced nutritional qualities and plants resistance to abiotic stresses. Vaccines against various diseases and other important products have also been developed using transgenic plants (Teli and Timko, 2004). The emergence of new functional genomic strategies for the identification and characterization of genes promises to provide a wealth of information with an enormous potential to enhance traditional plant breeding and to genetically engineer plants for specific purposes. Plant biotechnology is at the threshold of an exciting new era in which the emphasis is on the trans kingdom gene transfer and production of elite genotypes of different crop species.

The production of transgenic plants involves the combination of two distinct basic technologies. The first is the introduction of new genetic material into the plant cells (transformation) and second is the regeneration of the transgenic cell into a plant, via tissue culture methods. For the introduction of a transgene into a cell, several methods have been developed. One of the the most important method of gene transfer is *Agrobacterium* and the present paper discusses about the biology and mechanism of A*grobacterium* gene transfer.

Agrobacterium mediated gene transfer

Agrobacterium-mediated transformation is the most widely used method for the production of transgenic plants. In nature *Agrobacterium tumifaciens* is the causative agent of crown gall disease and was discovered at the turn of the last century. The ability of *Agrobacterium* to genetically transform a wide variety of plant species, contributed much to the basic plant research and modern biotechnology. Single stranded copy of T-DNA molecule produced by the bacterial virulence machinery is transferred into the host cells followed by the integration into the host genome (Chilton *et al*., 1977).

Ti plasmid and DNA

Agrobacterium maintain a plasmid which carries the tumor inducing nature in it and is called Ti plasmid. *Agrobacterium* has the capacity to transfer a portion of plasmid DNA into the host cell and due to this property it is called as natural genetic engineer. The portion transferred to the host cell is called transfer DNA or T DNA. *Agrobacterium* recognizes the two border sequences flanking on either side of the T DNA and any sequences inside the border sequence is get transferred. The desired gene can be inserted in between the borders and can be transferred to the

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plant cells (De Frammond *et al*., 1983; Hoekema *et al*., 1983).

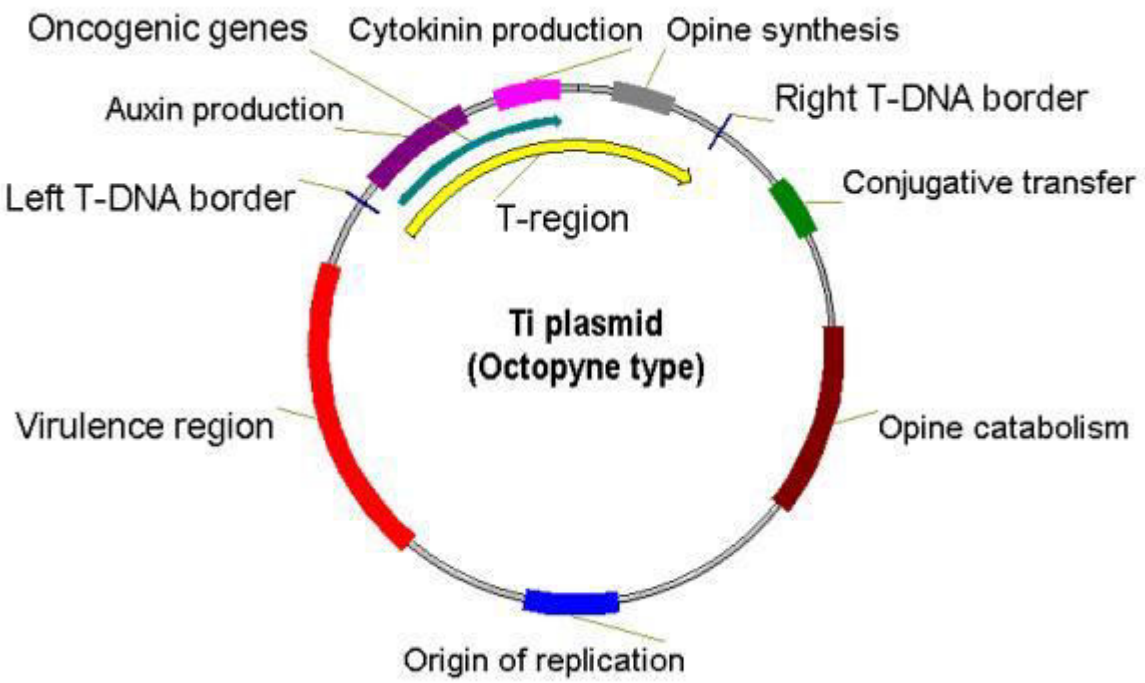

Fig.1. Schematic representation of typical octopyne type Ti plasmid, Adapted from Roa- Rodriguez and Nottenburg (2003)

Features of Ti plasmid

1. They contain one or more T-DNA regions
2. They contain a vir region
3. They contain an origin of replication
4. They contain a region enabling conjugative transfer
5. They contain genes for the catabolism of opines (a class of amino acids)

Mechanism of gene transfer

Agrobacterium is attracted by the phenolic compounds released from the wounded plant tissues and bind to them by a polar attachment mechanism. Then a co coordinated expression from a suit of genetic operons critical to the gene transfer also begins. These operons vir B, C, D, E and G are called 'vir regulon' and are coordinately regulated by a virA/virG two component system. The wound phenolics and monosaccharide cause the autophosphorylation of virA receptor kinase, which in turn activates the soluble cytoplasmic transcriptional factor virG through another phosphorylation event. Activated virG subsequently stimulates the transcription of the individual '*vir* operons' by binding to the upstream 'vir box enhancer element'. Gene products that are generated from transcription of the '*vir* operons' perform functions that are critical to the transfer of T- DNA from the tumor inducing plasmid into plant cells. Once inside the nucleus, the T- DNA is integrated into the plant genome via non homologous recombination mediated by plant encoded proteins that are likely part of recombination and repair process in plants (Lorence and Verpoorte, 2004; Gelvin, 2003; Tzfira *et al.,* 2004).

Table. 1. Agrobacterium virulence protein functions

Virulence protein	Function in Agrobacterium spp.	Function in plants	Plant protein that interact
Vir A	Phenolic sensor kinase ; phosphorylates and activates Vir G		
Vir G	Transcription factor Responsible for induction of *vir* gene expression		
Vir B1-B11	Components of membrane structure for transfer of T-DNA		
VirC1	Overdrive binding protein enhances efficiency of T-DNA transfer		
VirD1	Required for T-DNA processing; modulates VirD2 activity		
VirD2	Nicks the T-DNA and directs T-DNA through the VirB/VirD4 transfer	Nuclear targetting of T-DNA	Karyopherin-ά
VirD4	Components of transfer apparatus		
VirE1	VirE2 chaperone		
VirE2		Single stranded DNA binding protein	VIP1[d]
VirF		Directs protein coating T-complex	
VirJ	T-DNA export		

Conclusion

Among all the different techniques the most widely used and successful transformation methods are the *Agrobacterium* mediated indirect gene transfer. *Agrobacterium* mediated transformations have come up with the considerable successes that plant biotechnology has already achieved and continues to give well into the future.

References

1. **Chilton, M.D., M.H. Drummond, D.J. Merio, D. Sciaky and A.L. Montoya *et al*.,** 1977. Stable incorporation of plasmid DNA into higher plant cells: The molecular basis of crown gall tumorigenesis. *Cell,* 11: 263-271.

2. **De Framond, A.J., K.A. Barton and M.D. Chilton,** 1983. Mini-Ti: A new vector strategy for plant genetic engineering. *Biotechnol*., 5: 262-269.

3. **Gelvin, S.B.** (2003) Agrobacterium-mediated plant transformation: the biology behind the "gene-jockeying" tool. *Microbiol. Mol. Biol. Rev*. 67, 16–37

4. **Hoekema, A., P. Hirsch, P.J.J. Hooykas and R.A. Schilperoort**, 1983. A binary vector strategy based on separation of vir and T-region of the *Agrobacterium tumefaciens* Ti plasmid. *Nature*, 303: 179-180.

5. **Lorence, A. and Verpoorte, R**. 2004. Gene transfer and expression in plants. *Methods Mol. Biol*. 267, 329–350

6. **Roa-Rodriguez, C. and Nottenburg, C.** 2003. Agrobacterium mediated transformation of plants. CAMBIA (http://www.bios.net/ Agrobacterium)

7. **Teli, N.P. and M.P. Timko,** 2004. Recent developments in the use of transgenic plants for the production of human therapeutics and biopharmaceuticals. *Plant Cell Tissue Org. Cult*., 79: 125-145.

8. **Tzfira, T. et al.** 2004. Agrobacterium T-DNA integration: molecules and models. *Trends Genet.* 20, 375–383