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Gradual Optimization of Gene Transfer Technology to Get Genetically Modified Organisms

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Abstract:

There is an urgent need for the biotechnology assisted crop improvement, which ultimately aimed to obtain novel and improved plant traits. Numerous approaches are followed for gene transformation, It may be through different vector or with vector less direct transformation. In the field of genetic engineering, the most important tool developed was the plasmid vector. This technology has been continuously expanding and undergoing adaptations. Some other vectors like cosmid, bacteriophage, BAC, PAC etc. Agrobacterium-mediated gene transfer one of the primitive and mostly followed method but gradually direct gene transfer methods developed which makes the transformation easy and efficient. biolistic process probably has more potential than has been realized so far. Biotechnology tools like direct gene transfer methods advance by reducing the time to get improved varieties, Other than conventional methods. This article summarises the types of vector, Ideal vector and recent progress which has been made in the field of gene transfer.

Keywords: Vector, Gene transfer, Genetic engineering, Plasmid, Transgenic

Introduction:

The vector itself is generally a DNA sequence that consists of an insert (transgene) and a larger sequence that serves as the "backbone" of the vector. The purpose of a vector which transfers genetic information to another cell is typically to isolate, multiply, or express the insert in the target cell. All vectors may be used for cloning and are therefore cloning vectors, but there are also vectors designed especially for cloning, While others may be designed specifically for other purposes, such as transcription and protein expression. (Christou P. et al., 1990) Vectors designed specifically for the expression of the transgene in the target cell are called expression vectors, and generally have a promoter sequence that drives expression of the transgene. Simpler vectors called transcription vectors are only capable of being

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transcribed but not translated, they can be replicated in a target cell but not expressed, unlike expression vectors. Transcription vectors are used to amplify their insert (Schell J. et al., 1989).

Features of a vector:

Origin of replication: Necessary for the replication and maintenance of the vector in the host cell. Promoter: Promoters are used to drive the transcription of the vector's transgene as well as the other genes in the vector such as the antibiotic resistance gene. Cloning site: This may be a multiple cloning site or other features that allow for the insertion of foreign DNA into the vector through ligation. (Weber G. *et al.* 1990). Genetic markers: Genetic markers for viral vectors allow for confirmation that the vector has integrated with the host genomic DNA. Antibiotic resistance: Vectors with antibiotic-resistance open reading frames allow for survival of cells that have taken up the vector in growth media containing antibiotics through antibiotic selection. Epitope: Some vectors may contain a sequence for a specific epitope that can be incorporated into the expressed protein. It allows for antibody identification of cells expressing the target protein. Reporter genes: Some vectors may contain a reporter gene that allow for identification of plasmid that contains inserted DNA sequence. Targeting sequence: Expression vectors may include encoding for a targeting sequence in the finished protein that directs the expressed protein to a specific organelle in the cell or specific location such as the periplasmic space of bacteria. (Weising K. *et al.*, 1988)

Criteria of an ideal vector:

Vectors are those DNA molecules that can carry a foreign DNA fragment when inserted into it. A vector must possess certain minimum qualifications to be an efficient agent for the transfer, maintenance and amplification of the passenger DNA. (Draper J. et al., 1988) (1) The vector should be small and easy to isolate. (2) They must have one or more origins of replication so that they will stably maintain themselves within host cell. (3) Vector should have one or more unique restriction sites into which the recombinant DNA can be inserted. (4) They should have a selectable marker (antibiotic resistance gene) which allows recognition of transformants. (5) Vector DNA can be introduced into a cell. (6) The vector should not be toxic to host cell.

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Types of Vector:

(a) Plasmids: Plasmids are the extra-chromosomal, self-replicating, and double stranded closed and circular DNA molecules present in the bacterial cell. A number of properties are specified by plasmids such as antibiotic and heavy metal resistance, nitrogen fixation, pollutant degradation, bacteriocin and toxin production, colicin factors, etc. (Bouvier L.A. *et al.*, 2013) (b) Bacteriophage: The bacteriophage has linear DNA molecule, a single break will generate two fragments, foreign DNA can be inserted to generate chimeric phage particle. But as the capacity of phage head is limited, some segments of phage DNA, not having essential genes, may be removed. This technique has been followed in λ (Lambda) phage vectors to clone large foreign particle. (Palazzolo MJ. *et al.*, 1990) (c) Cosmid: Cosmids are plasmid particles, into which certain specific DNA sequences, namely those for cos sites are inserted which enable the DNA to get packed in X particle. Like plasmids, the cosmids perpetuate in bacteria without lytic development. The cosmids have high efficiency to produce a complete genomic library. (d) Phagemid: These are prepared artificially by combining features of phages with plasmids. One commonly used phagemid is pBluescript IIKs derived from pUC-19. (e) Plant and Animal Viruses: A number of plant and animal viruses have also been used as vectors both for introducing foreign genes into cells and for gene amplification. Cauliflower Mosaic Viruses (CaMV), Tobacco Mosaic Viruses (TMV) and Gemini Virus are three groups of viruses that have been used as vectors for cloning of DNA segments in plant system. SV 40 (Simian Virus 40), human adenoviruses and retroviruses are potential as vectors for gene transfer into animal cells. (f) Artificial Chromosomes: Yeast Artificial Chromosome (YAC) or Bacterial Artificial Chromosome (BAC) vectors allow cloning of several hundred kb pairs which may represent the whole chromosome. It can be cloned in yeast or bacteria by ligating them to vector sequences that allow their propagation as linear artificial chromosome. (Monaco, A *et al.*, 1994) (g) Transposons: Transposable elements like Ac-Ds or Mu-1 of Maize, P-element of Drosophila may also be used for cloning vector and transfer of gene among eukaryotes.

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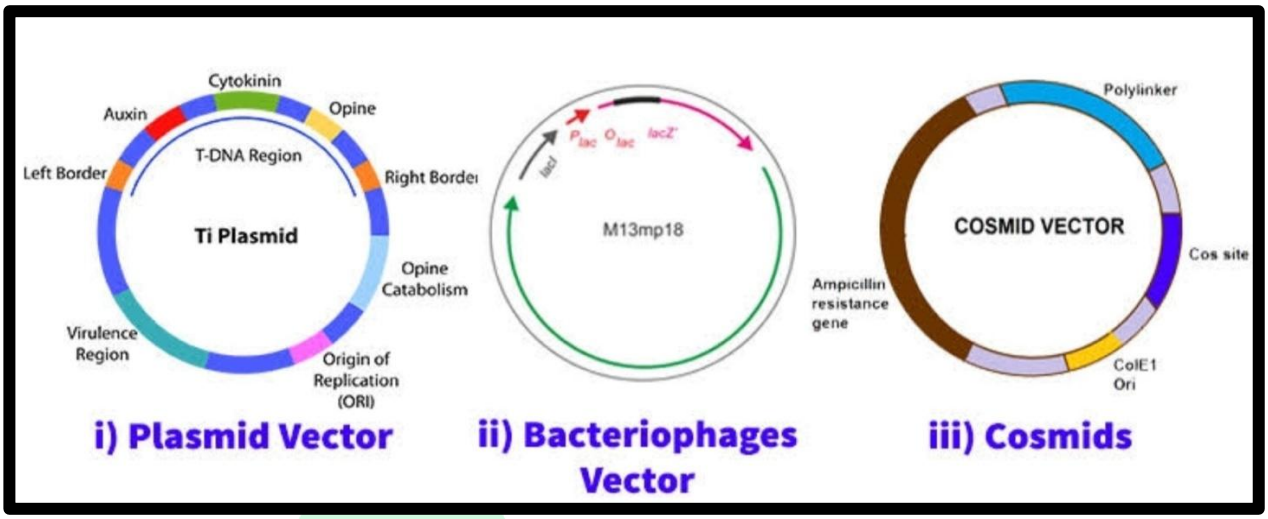

Fig 1.1: Some example of vector.

Recombination Procedure:

Insertion of a vector into the target cell is usually called transformation for bacterial cells, transfection for eukaryotic cells,although insertion of a viral vector is often called transduction. Procedure involves, Isolation of the DNA of interest (or target DNA), Ligation, Transfection (or transformation), and A screening/selection procedure.

Gene Transfer Technique:

Gene transfer technique is used very widely both in basic research and applied biology. The delivery of DNA into animal cells is a fundamental and established procedure. It has become an indispensable tool for gene cloning, the study of gene function and regulation and the production of small amounts of recombinant proteins for analysis and verification.

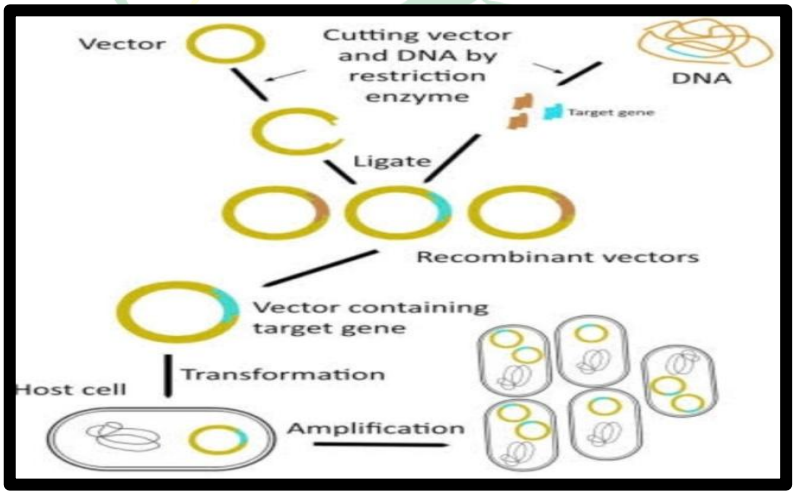

Fig 1.2: Process of recombination and amplification

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Gene transfer experiment helps to express the introduced genetic construct (or transgene) in the recipient cells or to disrupt or inactivate particular endogenous genes (resulting in a loss of function. There are many applications of gene transfer like large-scale commercial production of recombinant antibodies and vaccines and gene medicine or gene therapy. They range from the use of mammalian and insect cell cultures to transfer of DNA into human patients for the correction or prevention of disease

Gene transfer methods:

Various gene transfer techniques are grouped in two main categories like Vector mediated gene transfer and Vectorless or direct DNA transfer .

Agrobacterium mediated transformation:

Agrobacterium system was historically the first successful plant transformation system, marking the breakthrough in plant genetic engineering in 1983. The breakthrough in gene manipulation in plants came by characterizing and exploiting plasmids carried by the bacterial plant pathogens Agrobacterium tumefaciens and A. rhizogenes. These provide natural gene transfer, gene expression and selection systems. (Gardner *et al.,* 1986) In recent times, A. tumefaciens has been treated as nature's most effective plant genetic engineer. Agrobacterium are gram-negative rods that belong to the bacterial family of rhizobiaceae. These are often found near soil level at the junction of plant stem and root. It induces crown gall disease. It induces hairy root disease. It is an avirulent strain.

Chemical transfection:

Chemical transfection methods have to overcome a number of boundaries to deliver active DNA into the nucleus. They have to persuade the cell to interact with and process exogenous DNA, and eventually deliver at least some intact DNA molecules to the nucleus. The first boundary to gene transfer is the cell membrane, which is hydrophobic and negatively charged whereas DNA, is hydrophilic and negatively charged. (Potrykus, 1990) DNA can only interact with the cell membrane by a synthetic complex, in combination with DNA that carries a net positive charge or if it is either enclosed in a fusogenic capsule.

Calcium phosphate transfection:

It was the first chemical transfection method to be used with animal cells. Calcium phosphate is probably the most widely used transfection method. This is a simple, reliable method applicable to many cultured cell lines, and the reagents are inexpensive. It can be used both

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for transient and stable transformation.

Transfection with DEAE-dextran:

DEAE-dextran was the first transfection reagent to be developed and was very widely used until the advent of lipofection reagents in the 1990s. (Walbot V. *et al.* 1987) It is a soluble polycationic carbohydrate that forms aggregates with DNA through electrostatic interactions.

Lipofection:

Gene transfer mediated by liposomes was first described by Fengler in 1980. Liposomes are used to form a fusogenic particle with DNA. They are hydrophobic, unilaminar phospholipid vesicles. Such vesicles when mixed with cells in culture, fuse with the cell membrane and deliver DNA directly into the cytoplasm. (Caboche M., 1990)

Physical Transfection:

In physical methods the DNA is delivered directly into either the cytoplasm or the nucleus using some kind of physical force. There is no requirement for interaction with the plasma membrane. (Sanford J.C, 1990)

Electroporation:

This method was first used with animal cells by Neumann and colleagues in 1982. Electroporation is the transfection of cells following their exposure to a pulsed electric field. (Fromm M. *et al.*,1987)

Macroinjection:

It is the injection of DNA solution (5–10 µl) by micropipettes into the developing floral side shoots (tillers) of plants. Within the floral tillers are archesporial cells that give rise to pollen in the developing sac by two meiotic cell divisions. (De la Pena *et al.*, 1987)

Microinjection:

The direct microinjection of DNA into the cytoplasm or nuclei of cultured cells is sometimes used as a transfection method. It is highly efficient at the level of individual cells. The most significant use of this technique is introduction of DNA into the oocytes, embryos of animals. (Neuhaus G. *et al.*, 1987)

Transfection by particle bombardment:

Particle bombardment (also known as biolistics or microprojectile transfection) procedure involves coating micrometer-sized gold or tungsten particles with DNA and then accelerating the particles into cells or tissues. (Klein *et al.*, 1988)

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Transfection by ultrasound:

It involves the exposure of cells to a rapidly oscillating probe, such as the tip of a sonicator. The application of ultrasound waves to a dish of cells or a particular tissue results in the formation and collapse of bubbles in the liquid, including the cell membrane, a process known as cavitation. (Weber G. *et al.*, 1988; Schocher R. *et al.*, 1986)

Virus-mediated transduction:

Viruses have evolved to deliver nucleic acids safely into animal cells. The enveloped and non- enveloped viruses follow different way to interacting with the membrane.

Conclusion:

Gene transfer or DNA uptake refers to the process that moves a specific piece of DNA (usually a foreign gene ligated to a bacterial plasmid) into cells. In plant breeding, techniques involving gene transfer through sexual and vegetative propagation are well established, the aim being to introduce genetic diversity into plant populations, to select superior plants carrying genes for desired traits and to maintain the range of plant varieties. The application of these conventional or classical techniques has produced significant achievements in the yield improvement of major food crops. However, this takes a long time. For example, it usually takes 6–8 years to produce a new variety of wheat or rice by sexual propagation. With the rapid development of genetic engineering techniques based on the knowledge of gene structure and function, plant breeding has been dramatically broadened.

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