Genetic Engineering: A Biotechnological Tool for Crop Improvement

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Abstract

Genetic engineering or recombinant DNA (rDNA) technology involves artificial transfer of genes or gene fragments from one organism to another to produce novel traits in the recipient living organism. Thus, it is the process of manipulation of the genetic material with useful gene. New genes are added or existing genes are changed so that they are made at different times and/or different quantities. The transfer process involves cutting the desired gene out of a chromosome of a particular plant, animal or bacteria and putting that gene into a cell; the genetically modified cell is then regenerated to produce a 'transgenic' or genetically modified organism or GMO. The modified organism passes the new gene onto its progeny.

Introduction to Genetic Engineering

In the late 1960s, Werner Arber identified the restriction enzymes in bacteria that were designed to cleave DNA and in 1970, Temin and Baltimore independently identified the viral enzyme reverse transcriptase, which resulted in the birth of recombinant DNA technology-the first recombinant DNA was produced in Boyer Laboratory in 1972 and in 1976, the first biotechnology company Genentech was born. The big biotech boom was seen in the 1980s, especially after the invention of the polymerase chain reaction (PCR) by Kary Mullis in 1983. Genentech's recombinant interferon gamma and Eli Lilly's recombinant human insulin appeared in the market in 1982. The Human Genome Initiative, later to be renamed the Human Genome Project, was launched in 1986 and its completion was announced nearly two decades later. Another biotech company, GenPharm International, Inc., created the first transgenic dairy cow to produce human milk proteins for infant formula in the 1990s, and in the same period the first authorized gene therapy began on a four-year-old girl with an immune disorder known as ADA, or adenosine de-aminase deficiency.

Essential steps of rDNA Technology

1. Generating DNA fragments (by using PCR)
2. Cutting and joining the DNA fragments to vector DNA molecules (by using REs and Ligases)
3. Introducing the vectors carrying the foreign DNA into host cells where they can replicate (For high copy no.)
4. Selecting the clone(s) of recipient cells that have acquired the recombinant DNA molecules (By antiobiotics or under any selection pressure)
5. Multiplication/Expression

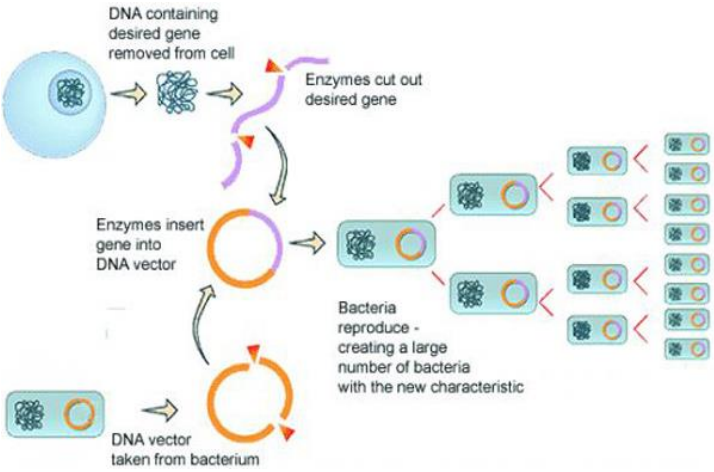

Fig. 1. Steps and mechanism of genetic engineering

Tools of Genetic Engineering

Restriction Endonucleases

Restriction endonucleases are very useful and effective in genetic engineering as they recognize and cut specific target sequences. There are four known different subtypes of restriction endonucleases (Type I, Type II, Type III and Type IV) that are grouped together based on the level of enzyme complexity, cofactor requirements, recognition sequence properties and many other parameters. Type I were characterized first of all and they consist of one enzyme with different subunits for recognition, cleavage, and methylation (all-in-one). Their mechanism of cleavage relies on the translocation of DNA until a mechanistic collision occurs (usually quite some distance away, up to 1000 bp), producing fairly random fragments. Thus, their lack of a specific cleavage point makes them unsuitable for specific

gene cloning purposes. Type II are the most common types of restriction enzymes used in general cloning experiments as they are very specific and have fairly constant cut positions. They can be tetra, hexa or octa cutters and cleave up to 20 bp away on one side of the recognition sequence and are palindromic in nature. Type III consists of one complex of two subunits encoded by the mod (modification) and res (restriction) genes. The recognition sequence is a set of two copies of nonpalin-dromic sites in inverse orientation. The enzyme then cleaves at a specific distance (24 to 26 bp) away from one of the copies. Since the exact cut site is not predetermined, these enzymes are not suitable for cloning purposes. Type IV enzymes can recognize modified (even methylated) DNA. They are rather large proteins with two catalytic subunits, and they cleave outside their recognition sites.

Vectors

Vectors are DNA molecules which can replicate autonomously and thus can be used to carry the insert DNA into organisms and amplify this DNA in vivo. There are many types and many functions of vectors. The most commonly used vectors are (a) plasmids (b) phage vectors (c) cosmids (d) bacterial artificial chromosomes and (e) yeast artificial chromosomes. All of these vectors change in the size of the insert they can carry and the purpose for which they can be used. Plasmids can carry inserts of up to 10 kb, while phage vectors go up to 20 kb inserts, BAC Bacterial artifical chomosomes (BACs) are based on the Fertility (F) plasmid that is designed to carry large DNA sequences of usually 150 to 350 kb while YAC vectors can carry 100 to 1000 kb inserts. One can also choose vectors based not on the size of the insert, but on the application purpose: cloning, sequencing, preparing RNA or DNA probes, or expressing proteins. Plasmids are perhaps the most commonly used vectors and are extra chromosomal DNA molecules that are present in prokaryotes. They are usually small, circular double-stranded DNA molecules that have the capacity to replicate autonomously. If we want to synthesize specific proteins for further analysis we need to use expression vectors.

Modifying Enzymes

DNA polymerases are DNA-dependent DNA polymerases, which use a DNA strand as a template and synthesize a complementary DNA molecule in a 5′-to-3′ direction, also called the 5′-3′-polymerase activity. DNA ligases catalyze the covalent bond formation between a 5′-phosphate group and a 3′-hydroxyl group. Alkaline phosphatases (AP) modify nucleic acids by removing the 5′ phosphate groups. Site-specific recombination can also be employed for cloning using recombinases, which in the long term is an effective way to subclone DNA sequences from one vector to many others.

Methods of Gene Transfer

During gene transfer, the desired DNA is extracted by physical or chemical means (a whole collection of methods like blotting, washing, transfer, chemical methods are used to obtain intact DNA). The desired gene is then inserted into the target organism. For this, usually, a microorganism (*Agrobacterium* species) is used as a carrier or vector to smuggle the gene of interest into the target organism. Biolistic is another method. A ‘gene gun’ allows the DNA containing the gene, coated on microscopic gold or tungsten particles, to be blasted into plant cells. Once inside the cell nucleus, natural processes are expected to allow incorporation of the foreign DNA into the plant’s own DNA in its chromosomes. There are different methods used in genetic engineering or recombinant DNA (rDNA) technology to transfer a gene of interest. These can be categorized as vector-mediated or indirect gene transfer and vector less or direct gene transfer methods. Vector-mediated or indirect gene transfer involves Ti plasmid of *Agrobacterium tumefaciens* to transfer a gene. This bacterium is known as “natural genetic engineer” of plants because these bacteria have natural ability to transfer T-DNA of their plasmids into plant genome upon infection of cells at the wound site and causes an unorganized growth of a cell mass known as crown gall. In the direct gene transfer methods, the foreign gene of interest is delivered into the host plant cell without the help of a vector. The gene transfer system using genetically engineered vectors do not work out well particularly in monocot species. Vector less or direct gene transfer can be done by different chemicals (PEG, Calcium phosphate etc.) or physical methods like microinjection and macro-injection, electroporation method, microprojectiles or biolistics or particle gun, liposome mediated gene transfer or lipofection, gene transformation using pollen or pollen tube etc.

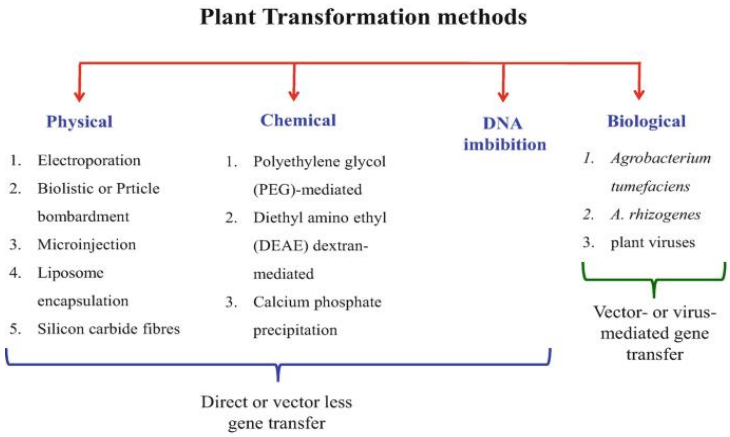

Fig. 2. Different methods of gene transfer

Screening for Recombinants

There are many different ways to screen for recombinants, depending on which vector one uses for cloning. The most common method used for the screening of transformants is antibiotic screening lthough other methods like blue-white screening and colony PCR are also available. However, if cloning is done into a phage vector, then a different method like plaque screening would be needed.

Advantages of rDNA Technology

1. Fractionation of individual DNA components of complex genomes.
2. Amplification of cloned genes.
3. Opportunity to study the expression of individual genes thus cloned.
4. Potential to create new genetic combination.

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