Genome as a Tool of Genetic Engineering: Application in Plant and Plant Derived Medicine

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Abstract

The study was conducted from different modern research data to review the innovative latest technology in the genomics and its application in Agriculture, biomedicinae and plant derived medicine. Application of genome in genetic engineering and molecular biotechnology have been exhibited well. Genetically Modified Organism (GMO), Agrobacterium mediated recombination (T-DNA) and genetic engineering using molecular Biotechnology in plant, medicine and biomedicine have been highlighted from technology based different research data. Moreover, molecular biotechnology in biopharmaceuticals, pharmacogenomics, new medical therapies, genetic testing, transgenic fruit, vegetable and flower production, using Agrobacterium mediated gene, DNA cloning have been presented well showing innovative data.

Keywords - *genomics, agriculture, biomedicinae, medicine, genetic engineering*

I. INTRODUCTION

A genome (DNA or RNA) is an organism's complete set of DNA, including all of its genes. Each genome contains all of the information needed to build and maintain that organism [1, 2]. In human, a copy of the entire genome more than 3 billion DNA base pairs is contained in all cells that have a nucleus. New DNA may be inserted in the host genome by first isolating and copying the genetic material of interest using molecular cloning methods to generate a DNA sequence, or by synthesizing the DNA, and then inserting this construct into the host organism [3]. Genes may be removed, or knocked out, using a nuclease. Gene targeting is a different technique that uses homologous recombination to change an endogenous gene, and can be used to delete a gene, remove exons, add a gene, or introduce point mutations. Genetically modified organism (GMO) is considered as an organism that is generated through genetic engineering. The first GMOs were bacteria in 1973, GM mice were generated in 1974 [4]. Insulin-producing bacteria were commercialized in 1982 and genetically modified food has been sold since 1994. Glofish, the first GMO designed as a pet, was first sold in the United States December in 2003 [4]. Genetic engineering biotechnology has been applied in numerous fields including agriculture, industrial biotechnology, and medicine. Enzymes used in laundry detergent and medicines such as insulin and human growth hormone are now manufactured in GM cells, experimental GM cell lines and GM animals such as mice or zebra fish are being used for research purposes, and genetically modified crops have been commercialized [4]. The objective of the study was to review the various superlative techniques of genome application in plant, fruit and vegetable, biomedicine and medicine from different research data.

II. APPLICATION OF GENOME IN GENETIC ENGINEERING AND MOLECULAR BIOTECHNOLOGY

A. Genetically Modified Organism (GMO)

Plants, animals or micro organisms that have changed through genetic engineering are termed genetically modified organisms or GMOs [5]. Bacteria were the first organisms to be genetically modified. Plasmid DNA containing new genes can be inserted into the bacterial cell and the bacteria would then express those genes. These genes can code for medicines or enzymes that process food and other substrates. Plants have been modified for insect protection, herbicide resistance, virus resistance, enhanced nutrition, tolerance to environmental pressures and the production of edible vaccines [6]. Most commercialized GMO's are insect resistant and/or herbicide tolerant crop plants. Genetically modified animals have been used for research, model animals and the production of agricultural or pharmaceutical products. They include animals with genes knocked out, increased

susceptibility to disease, hormones for extra growth and the ability to express proteins in their milk.

B. Agrobacterium-mediated recombination

1. Agrobacterium-mediated recombination in animal cell

1% of bacteria are naturally able to take up foreign DNA but it can also be induced in other bacteria. For example, stressing the bacteria with a heat shock or an electric shock, can make the cell membrane permeable to DNA that may then incorporate into their genome or exist as extra chromosomal DNA. DNA is generally inserted into animal cells using microinjection, where it can be injected through the cells nuclear envelope directly into the nucleus or through the use of viral vectors. In plants the DNA is generally inserted using *Agrobacterium*-mediated recombination or biolistics [7].

2. In Agrobacterium-mediated recombination the plasmid construct contains T-DNA

DNA which is responsible for insertion of the DNA into the host plants genome. This plasmid is transformed into *Agrobacterium* that contains no plasmids and then plant cells are infected. The *Agrobacterium* will then naturally insert the genetic material into the plant cells. In biolistics transformation particles of gold or tungsten are coated with DNA and then shot into young plant cells or plant embryos. Some genetic material will enter the cells and transform them. This method can be used on plants that are not susceptible to *Agrobacterium* infection and also allows transformation of plant plastids. Another transformation method for plant and animal cells is electroporation [8].

3. Electroporation

Electroporation involves subjecting the plant or animal cell to an electric shock, which can make the cell membrane permeable to plasmid DNA. In some cases the electroporated cells would incorporate the DNA into their genome. Due to the damage caused to the cells and DNA the transformation efficiency of biolistics and electroporation is lower than agrobacterial mediated transformation and microinjection. As often only a single cell is transformed with genetic material the organism must be regenerated from that single cell. As bacteria consist of a single cell and reproduce clonally regeneration is not necessary. In plants this is accomplished through the use of tissue culture [8]. Each plant species has different requirements for successful regeneration through tissue culture. If successful an adult plant is produced that contains the transgene in every cell. In animals it is necessary to ensure that the inserted DNA is present in the embryonic stem cells. Selectable markers are used to easily differentiate transformed from untransformed cells. These markers are usually present in the transgenic organism, although a number of strategies have been developed that can remove the selectable marker from the mature transgenic plant. When the offspring is produced they can be screened for the presence of the gene. All offspring from the first generation will be heterozygous for the inserted gene and must be mated together to produce a homozygous animal. As for example, Transgenic plant (Fig.1).

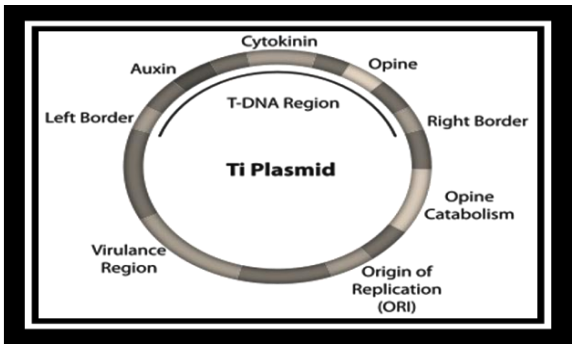

Fig.1. Mechanism of Ti plasmid with T- DNA region.

III. GENETIC ENGINEERING USING MOLECULAR BIOTECHNOLOGY IN MEDICINE AND BIOMEDICINE

In medicine, modern biotechnology finds promising applications in such areas as i. drug production, ii. Pharmacogenomics, iii. Gene therapy, iv.Genetic testing (or genetic screening): techniques in molecular biotechnology detect genetic diseases.

IV. PHARMACOGENOMICS

Fig. 2. Genomic testing

Genetic testing is shown in Fig. 2. Pharmacogenomics is the study of how the genetic inheritance of an individual affects his/her body's response to drugs. It is a portmanteau derived from the words pharmacology and genomics. It is hence the study of the relationship between pharmaceuticals and genetics. The vision of pharmacogenomics is to be able to design and

produce drugs that are adapted to each person's genetic makeup [3].

IV. MOLECULAR BIOTECHNOLOGY IN PHARMACEUTICAL PRODUCTS

Computer-generated image of insulin hexamers highlighting the threefold symmetry, the zinc ions holding it together, and the histidine residues involved in zinc binding (Fig. 3).

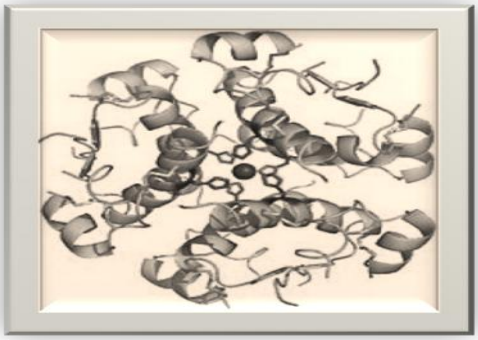

Fig. 3. Computer-generated image of insulin hexamers

VI. BIOPHARMACEUTICALS

Biopharmaceuticals are large biological molecules such as proteins (Fig. 3) that are developed to address targets that cannot easily be addressed by small molecules. Some examples of biopharmaceutical drugs include Infliximab, a monoclonal antibody used in the treatment of autoimmune diseases, a chimeric monoclonal antibody used in the treatment of cancer. Due to their larger size, and corresponding difficulty with surviving the stomach, colon and liver, biopharmaceuticals are typically injected [7].

Fig. 4. *E. coli* or yeast for the production of substances like synthetic insulin or antibiotics.

Modern biotechnology is often associated with the use of genetically altered microorganisms such as *E. coli* or yeast (Fig. 4) for the production of substances like synthetic insulin or antibiotics. It can also refer to transgenic animals or transgenic plants, such as Bt corn. Genetically altered mammalian cells, such as Chinese Hamster Ovary cells (CHO), are also used to manufacture certain pharmaceuticals. Another promising new biotechnology application is the development of plant-made pharmaceuticals [3].

VII. MOLECULAR BIOTECHNOLOGY IN NEW MEDICAL THERAPIES

It is also commonly associated with landmark breakthroughs in new medical therapies to treat hepatitis B, hepatitis C, cancers, arthritis, haemophilia, bone fractures, multiple sclerosis, and cardiovascular disorders. The biotechnology industry has also been instrumental in developing molecular diagnostic devices that can be used to define the target patient population for a given biopharmaceutical. Herceptin, for example, was the first drug approved for use with a matching diagnostic test and is used to treat breast cancer in women whose cancer cells express the protein HER2. Molecular biotechnology can be used to manufacture existing medicines relatively easily and cheaply. The first genetically engineered products were medicines designed to treat human diseases. To cite one example, in 1978 Genentech developed synthetic humanized insulin by joining its gene with a plasmid vector inserted into the bacterium *Escherichia coli*. Insulin, widely used for the treatment of diabetes, was previously extracted from the pancreas of abattoir animals (eg. cattle). The resulting genetically engineered bacterium enabled the production of vast quantities of synthetic human insulin at relatively low cost [9].

Modern biotechnology has evolved, making it possible to produce more easily and relatively cheaply human growth hormone, clotting factors for hemophiliacs, fertility drugs, erythropoietin and other drugs. Most drugs today are based on about 500 molecular targets. Genomic knowledge of the genes involved in diseases, disease pathways, and drug-response sites are expected to lead to the discovery of thousands more new targets[9].

VIII. MOLECULAR BIOTECHNOLOGY IN GENETIC TESTING

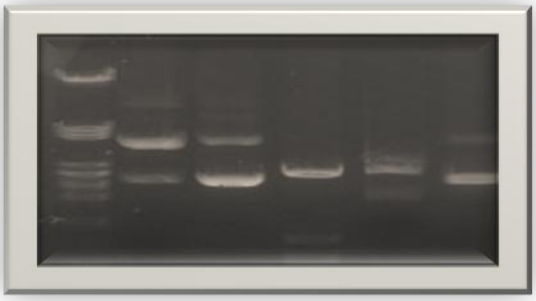

Fig. 5. Gel electrophoresis method in genetic test

Genetic testing (Fig. 5) involves the direct examination of the DNA molecule itself. A scientist scans a patient's DNA sample for mutated sequences. There are two major types of gene tests. In the first type, a researcher may design short pieces of DNA (probes) whose sequences are complementary to the mutated sequences. These probes would seek their complement among the base pairs of an individual's genome. If the mutated sequence is present in the patient's genome, the probe would bind to it and flag the mutation. In the second type, a researcher may conduct the gene test by comparing the sequence of DNA bases in a patient's gene to disease in healthy individuals or their progeny [1, 10]. At present genetic testing is used view to the Carrier screening, or the identification of unaffected individuals who carry one copy of a gene for a disease that requires two copies for the disease to manifest;

IX. CONFIRMATIONAL DIAGNOSIS OF SYMPTOMATIC INDIVIDUALS

The following topics are involved in confirmational diagnosis of symptomatic individuals: Determining sex; Forensic/identity testing; Newborn screening; Prenatal diagnostic screening; Presymptomatic testing for estimating the risk of developing adult-onset cancers; Presymptomatic testing for predicting adult-onset disorders [11].

Some genetic tests are already available, although most of them are used in developed countries. The tests currently available can detect mutations associated with rare genetic disorders like cystic fibrosis, sickle cell anemia, and Huntington's disease. Recently, tests have been developed to detect mutation for a handful of more complex conditions such as breast, ovarian, and colon cancers. However, gene tests may not detect every mutation associated with a particular condition because many are as yet undiscovered [11].

X. MOLECULAR BIOTECHNOLOGY IN GENE THERAPY

A new gene is inserted into an adenovirus vector, which is used to introduce the modified DNA into a human cell. If the treatment is successful, the new gene will make a functional protein. Gene therapy may be used for treating, or even curing, genetic and acquired diseases like cancer and AIDS by using normal genes to supplement or replace defective genes or to bolster a normal function such as immunity. It can be used to target somatic cells (i.e., those of the body) or gamete (i.e., egg and sperm) cells. In somatic gene therapy, the genome of the recipient is changed, but this change is not passed along to the next generation. In contrast, in germline gene therapy, the egg and sperm cells of the parents are changed for the purpose of passing on the changes to their offspring [12, 13].

XI. MOLECULAR BIOTECHNOLOGY IN DNA CLONING

DNA cloning involves the removal of the nucleus from one cell and its placement in an unfertilized egg cell whose nucleus has either been deactivated or removed. In detailed, DNA cloning is the production of a lineage of cells all of which contain one kind of DNA fragment of interest derived from a population of many kinds of DNA fragments. Operationally by: inserting (recombining) a population of DNA molecules, known to contain the DNA of interest, into a population of vector DNA molecules in such a way that each vector molecule contains only a single DNA molecule from the original population; transforming a population of host cells with the vector DNA recombinants such that each host cell takes up only one vector; growing single host cells separately (cloning) by plating at low density to form a collection of separate colonies; screening the colonies (clones) formed for the presence of the DNA of interest [1, 14].

A) There are two types of cloning:

Reproductive cloning. After a few divisions, the egg cell is placed into a uterus where it is allowed to develop into a fetus that is genetically identical to the donor of the original nucleus.
Therapeutic cloning. The egg is placed into a Petri dish where it develops into embryonic stem cells, which have shown potentials for treating several ailments [1, 14].

XII. MOLECULAR BIOTECHNOLOGY IN FRUIT, VEGETABLE AND FLOWER PRODUCTION

Using the techniques of modern biotechnology, one or two genes (Smartstax from Monsanto, Dow, 2010) may be transferred to a highly developed crop variety to impart a new character that would increase its yield. However, while increases in crop yield are the most obvious applications of modern biotechnology in agriculture, it is also the most difficult one. Current genetic engineering techniques work best for effects that are controlled by a single gene. Many of the genetic characteristics associated with yield (e.g., enhanced growth) are controlled by a large number of genes, each of which has a minimal effect on the overall yield. There is, therefore, much scientific work to be done in this area. As for example, genetically modified soybean [15].

XIII. GENETIC MODIFICATION IN SOYBEAN

Genetically modified soybean is a soybean (*Glycine max*) that having DNA introduced into it using genetic engineering techniques. Soy is widely planted genetically modified crop that is used to produce genetically modified food [15]. To develop

a soybean's genetic makeup, the gene is to be introduced into the soybean then gene is to be isolated. If the gene does not display an obvious phenotype, or visible characteristic, a marker gene must be linked to it so the modified cells and unmodified cells can be distinguished. Once the gene is to be inserted into the soybean then DNA is to be isolated. There are several ways to insert the gene, though the most popular are using *Agrobacterium* and electroporation.

A. Agrobacterium mediated gene(T-DNA)

Agrobacterium tumefaciens is a type of bacteria that transfers its DNA via horizontal gene transfer to build tumors in plants. This is very useful to genetic engineering. Gene transfers using a restriction enzyme used to cut non-virulent plasmid DNA derived from A. tumefaciens and thus make an insertion point, into which the gene can be ligated. The engineered plasmid is then put into a strain of A. tumefaciens, which contains a helper plasmid and plant cells are treated with the recombinant bacterium in culture [16].

.B. Electroporation

Electroporation is exactly the process of the creation of pores by using electricity. Specifically, it is when a pulsed magnetic field is used to create pores in plant cells, "through which genes can be taken up, and in the form of naked DNA incorporated into the plant genome [17].

XIV. GENETICALLY MODIFICATION IN POTATO

A. T-DNA transformation using Agrobacterium by tissue culture

Solanum chacoense is a species of wild potato. Transformation with *Agrobacterium* can be achieved in two ways. Protoplasts or alternatively leaf-discs can be incubated with the *Agrobacterium* and whole plants regenerated using plant tissue culture. *Agrobacterium* is used as a vector to transfer the T-DNA into the plant cells where it integrates into the plant genome (Fig. 6). This method can be used to generate transgenic plants carrying a foreign gene [18, 19].

Fig. 6. Photograph shows Agrobacterium transformation in wild potato (S. chacoense). Plant (S. chacoense) transformed using Agrobacterium. Transformed cells start forming calluses on the side of the leaf pieces.

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