BIOTECHNOLOGY AND NUCLEAR AGRICULTURE

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Abstract
Biotechnology is enhancing the world day by day. Since ancient period, it was used in daily culture but new techniques are introduced to get a new version of the existing one. It is based on Human genome, Plant genome, etc. Cultivation of new species which are previously termed as an GMO (Genetically Modified Organisms).
Isolation of genome or the genetic material is done by different methods such as DNA electrophoresis, spooling method, PCR techniques (such as, Digital PCR, Multiple PCR, Reverse *transcription* PCR, Nested PCR) etc. It may be Organic extraction, Inorganic extraction, Adsorption method, Chromatography, etc. Assisted reproductive technology (ART) is the medical procedure which frames a Nuclear Agriculture. In includes In-vitro fertilization (IVF), Intracytoplasmic Sperm Injection (ICSI), Zygote Transfer (ZIFT), Embryo transfer, etc. others like Recombinant DNA Technology, Mutagenesis is used in protein engineering which results in the modification of the protein structure. By using several technique Fertility Rate has been enhanced, like embryo rescue is the condition in which the embryo is given a suitable environment to grow outside in the culture. As it may be blastula stage or morula stage. The only chance is to grew or die. The dead species are then again used in Plant modification. Biotechnology is creating a great culture ahead.
Keywords: *Biotechnology, Prototypes, Isolation techniques of genetic material, Recombinant DNA technology, PCR, Protein engineering, Embryo rescue.*

INTRODUCTION

Biotechnology and nuclear agriculture is a innovation to improve the quality of life. It uses the living part or any other of its element to get an outcome with a specific aim. It is a scientific field which eventually ensures a nuclear strategy to improve the nutritional value, crop yield, infestation-free organisms. Molecular breeding is an application of biotechnology and nuclear agriculture in which DNA markers or genetic information at a desired state is used to get more precise crop yield. It is done overall at molecular levels, whereas it overcomes traditional breeding practices. Another application is transgenic plants, in these two or more genes from the different species are introduced together so as to give a desired yield. Biotechnology helps in creation of product by solving problem and implementing in various biological entities. The term comes to as Genetically modified organisms (GMO), these are created by introducing genes from different organisms into a specific variety of crop. Another method also includes radiation induced mutagenesis where gamma rays are passed over the desired gene.
Tissue culture is a technique based on the totipotency also referred as micropropagation. In this field of biotechnology had found out the broad and effectual application. It involves development of cell culture techniques which is eventually important for the success of the gene transfer and growing them on the nutrient media. It is used in Meristem culture for propagation of virus free plant, Cell suspension culture, Tissue and Organ culture, Pollen culture for producing haploid plant, where an explant is prepared for culture and later incubated on appropriate media for further growth and development.
Isolation of Genetic material is done via., various techniques. Electrophoresis: Electrophoresis is employed to sort the DNA, RNA, Protein, carbohydrates, etc. It can be used to visualize the PCR results; it may also be separated based on the charge or conformation. Used in molecular biology and alongside chromatography and spectroscopy. Spooling method**:** It is the physical method of isolation of the genetic material by using the Alcohol(ethanol) by precipitation. Spooling is done by a glass rod or thin stick which is twirled into the solution of ethanol, the mass obtained is the pure extraction of DNA.

Recombinant DNA technology involves the insertion of a gene encoding the pathogen or immunoglobin sequence of a choice in a specific cell. Protein engineering determines the modification of a protein structure with the help of rDNA (recombinant DNA) technology to form the desired protein structure.

PCR technique**:** The polymerase Chain reaction is a molecular technique that amplifies the DNA sequence, it helps in the diagnostics of crucial research. One of them is COVID-19 tests, other like Genetic analysis and Forensics. PCR includes 3 Main steps: Denaturation, Annealing and Elongation. In the denaturation double stranded DNA is separated into single stranded DNA by disrupting the hydrogen bonds between the nucleotide bases (done at 94°C-98°C). In the Annealing temperature is lowered to 50°C-65°C by placing the short synthetic primer to bind the complementary sequences on the single stranded DNA, marking the starting material. Lastly, Elongation(extension) enzyme namely., Taq. Polymerase synthesis new DNA strands by adding nucleotide to the primer-bound region at nearly equal to 72°C

Embryo culture is a technique for the cultivation an embryo under the aseptic condition in the nutrient media. Method aims to rescue the abnormal cleaved embryo.

HISTORY

Biotechnology is grown prominently since 1970's and emerged from the complex constellation phenomenon by means of representing manipulating the molecular building of life. (molecular biotechnology). Since the development of cascade novel technique was introduced. Scientific decision making is termed as a cascade method. In this the community is placed to rapidly collaborate the data. It made more easier to the development of biotechnology. In the 1830's and 1840's, the scientist namely Mattias Schledien and Theodor Schwann introduced to the cell totipotency on the basis of tissue culture and in 1960s the researchers like Reinert, Steward, and Vasil and Hildebrandt introduced the regeneration of the entire flowering plant from cell isolated cell. Plant hormone Auxin in 1930's, Kinetin in 1955 and cytokinin by Folk Skoog led to the development of effective nutrient media. This was used widely Murashige and Skoog medium in 1962. In 1980's the gene gun an alternative method to transform crops was put forth. Since 1980's, PCR has revolutionized molecular biology, with application in molecular diagnostics, forensic research, etc. Before, biotechnology researchers, scientist, or investors were worried whether the court would permit patents on the organisms; although they were not allowed. Later, the U.S supreme court in year 1980 resolved the matter by ruling that "A live human made micro-organism is patentable." Since this about many further discovered in the recombinant DNA technology. In the year 1978, the recombinant insulin was a great achievement in the genetic engineering with was employed by David Goeddel and their colleagues using the E.coli bacteria.

BIOTECHNOLOGY

Biotechnological techniques had Biotechnology is the biomolecular processes to develop the technology, new techniques are employed to get a new consequence. It is done to improve our lives and the health of overall planet. Since, 6000 years biological processes of micro-organisms are used to make useful food products like, cheese, bread and other preserved dairy products. Multicellular organisms like, corn, potato, tobacco plant, rats, sheep, etc have genetically engineered to produce the substances which proves medically useful for the human beings. Currently, all over 250 biotechnological vaccines and health supplies Biotechnology helps in producing a precise tool for the disease detection and fighting over serious illness, with leads to confronting the developing world. This help in reducing the rates of causing the infectious diseases. Biotechnology has significantly transformed diagnostics, reshaping how disease are identified, evaluated and managed by merging biological science with advanced technology. A major breakthrough in biotechnology is driven diagnostics is the development of molecular technique such like PCR which amplifies minute quantity of a DNA and RNA to identify specific genetic mutagen or pathogen. Although, PCR has revolutionized molecular biology with application in medical diagnostics and the forensic Research. More than 10 years, the biotechnology was dominant by the recombinant DNA technology or Genetic (protein) engineering. It includes the splicing of the specific gene for a beneficial protein into the production of cells (like; mammalian cells, yeast, bacteria) with results in beginning the protein in volume. The technique of elimination, addition, or modification of genes of an organism to make the changes in the protein structure is concluded as a genetic engineering.

Hence, all the advancements have concluded to the various aspects like, new specific ways to diagnose the diseased caused, different fermentation techniques in the large scale as per the requirement, improvement in the quality of the traits in the agricultural plants, to the more efficient crops, etc. Likely, biotechnology has created a huge sector and thoroughly boosted the economy all over the world.

Prototypes

Plant biotechnology

Plant serves as an important source of primary and secondary metabolites used in pharmacy and biotechnology. Plant biotechnology has received an more importance on the basis of quality and quantity of a horticulture, agriculture, etc and aims to manipulating for the improved agronomic properties. Tissue culture is the most widely known form of biotechnology. Genetic engineering technique, tissue culture techniques are used to produce the genetically modified organisms (GMO), that exhibits the desired and improve quality of the existing one with better quality and better yield. It gives the resistance to the diseases, pest and other environmental adverse factors. Plant biotechnology also make possible in the production in plants of useful protein coded wit human gene or microbial animal. There are numerous of cases in plant biotechnology is used in producing crop plant with resist the certain plant pathogen. Some plants have been rendered resistant to the specific pathogen by genetic engineering(transforming) them with isolated specific gene that provides the resistance against the pathogen. Plant based biotechnology is important for development of renewable biofuels, enhancing bioremediation and improving food supply chain. Biotechnology plays a crucial role by integrating applied technology with biological system to enhance societal well-being. At a smaller scale, biotechnology facilities employ micropropagation technique to cultivate plantlets under in vitro environment, employing totipotency. Specifically, plant-based biotechnology is using transient and stable expression provides a rapid key to address this predicament through the production of low-cost antiviral drugs, immunotherapy and vaccines. One of the cases, like pandemic of COVID-19 has overcome on the basis of plant biotechnology, which acted as scalable bioreactors to rapidly produce diagnostic components such as recombinant protein (like, RBD's for antibody test) through the production of low-cost diagnostics and therapeutics. It provides cost-effective adaptable platform for producing critical reagent needed for rapid testing kits, thereby accelerating large scale diagnostics production during pandemic. Medicinal biotechnology has seen advancement driven by the integration of metabolic engineering, synthetic biology, genomics and precision agriculture technologies. In south Africa evolution of plant biotechnology has transmitted from conventional tissue method to more sophisticated biotechnological approaches such as molecular marker technology, genetic transformation and metabolic engineering, earlier research primarily focused on in vitro propagation technique to conserve endangered medical plant and meet commercial demand.

Medical Biotechnology:

The medical biotechnology includes numerous applications over the tissue engineering product, gene therapy, recombinant pharmaceutical products and others for better human life. Biotechnological tools produce purified bio-therapeutic agents on industrial scales which are further used as per the requirement. The vaccine containing the smallpox virus by the east countries was marked as the beginning of vaccination.

Industrial biotechnology

Industrial biotechnology is employed for the production of bio-based chemical. It includes shifting the resources base of chemical production from fossil feedstock to renewable raw material provides the exciting possibilities for the use of industrial biotechnology based on the specified tools. It is also known widely as a white technology. In Europe the industrial biotechnology relies on the use of whole cell or enzyme as a catalyst and such a process are already used for the manufacture of several commodity and special chemicals. Conventual strategies such as microscopic and molecular bio-process design are often insufficiency to exploit their full potential. It is used in specialized situations like food, pharmaceutical, nutraceutical, cosmetics and animal feed sectors, microbial biomass etc has been generated industrially. These are grown under closed buildings such as photo bioreceptors or an open structure. Microalgae is significantly used in recent practices. Innovation trend in industrial biotechnology includes microbial fermentation for the sustainable production of products at a large scale for the human purpose. It reminds in the advance and expand the production of sustainable chemical and natural products.

Molecular biotechnology:

Molecular biotechnology is a field of interdisciplinary at an interface of biology, chemistry, and engineering. It harnesses living cell and their component to develop new product and technologies that improve human health. It uses molecular and genetic tools such as DNA cloning, PCR and recombinant DNA technology to study and manipulate the biomolecules (Protein, DNA, RNA). Molecular

biotechnologist design and perform experiment at molecular level. They work in laboratory using different techniques. Molecular biotechnologist might introduce a gene of known organism into a bacterial cell so that the microorganism may form a useful protein structure. Its application includes the analysis of genetic material, developing new drug or vaccine (substance related to medicinal use). example of molecular biotechnology is recombinant Insulin. The scientists take off the human Insulin gene and insert it into a bacterium. This engineered bacteria then produce human Insulin protein. In agricultural molecular biotechnologist creates improved crops and livestock. Molecular biotechnology covers all the genome directed editing technologies and their application. Application ranges from energy, to environment, to food, and the agriculture. It also includes human health application which uses altered non-human DNA, genome and organism for human health purpose. As well as it also includes direct intervention into human genome such as gene therapy and human genetic enhancement.

Isolation techniques of genetic material

Electrophoresis

Technique of separation of DNA, RNA, or protein on the basis of their sizes. It is the separation on the basis of the charge on the material. Placing the material on the agarose gel as DNA and RNA are negatively charged due to the presence of phosphate group over, they the move over the positive charge (i.e electrode). Larger fragments can't move far away. EtBr (ethidium bromide) is added to the agarose gel because it is bright under the UV light. Orange bands are then visualized. The DNA of different sizes are been separated. Agarose is made up of repeating D and L-galactose and provides a lower resolution then polyacrylamide gel.

Spooling

Manual method of extraction of genetic material. separation of isolated DNA in the form of white fibrous mass from a solution is termed as spooling. This is used for the easy and effective way of obtaining the large quantity of DNA extract. Cells are fragmented using certain enzymes to release the cellular content.it is precipitated out by use of isopropanol or chilled ethanol. Works as DNA is soluble in the salt solution but insoluble in alcoholic solution. So, the mass is precipitated and extracted. Spooling is done by a glass rod or thin stick which is twirled into the solution of ethanol, the mass obtained is the pure extraction of DNA. The spooled DNA is stored in a TE buffer solution for the further use.

Recombinant DNA technology

Recombinant DNA technology has thoroughly changed the modern biotechnology as it allows the scientists to manipulate genes and produce a Genetically Modified organisms (GMO's). The process starts by the isolation of DNA techniques (as mentioned above) and combining with other DNA sequence. Gene therapy is a form of biotechnology which aims to manage or treat the genetic disorders using traditional methods which are often difficult. Recombinant DNA technology has improved these treatments by making it possible to replace a gene by change in specific DNA bases. Firstly, the defective altered base is replaced by gene of interest which leads to creation of a specific protein molecule. Hence, cells can exhibit normal protein and overcome specific disease symptoms.

Polymerase chain Reaction (PCR)

PCR is a isolation technique which is used widely as an molecular technique that results in the amplification of specific DNA sequence.

- *Denaturation:* It is the first step in which the double stranded DNA is separated as a single stranded DNA molecule by disrupting the hydrogen bonding between the nucleotide bases at a temperature under 94°C-98°C.
- *Annealing:* The temperature at this step is lowered to 56°C-60°C which allows short primer to hind the complementary sequence on a single stranded DNA as a starting point.
- *Elongation:* The enzyme Taq. Polymerase synthesize the new DNA molecule at 72°C which enables the duplication of target sequence.

Innovation Of PCR In Technology

1. Digital PCR:

PCR in which the amplification is possible over a minute or small portions and number positive results are recognised under this influence. Digital PCR enables the direct and accurate quantification. This makes useful in application like gene expression analysis, copy numbers of research, rare mutation, detection and identification of low-level pathogens, etc.

2. *Multiple PCR:*

PCR technique is used to amplify multiple target DNA sequence simultaneously within a single reaction. It involves the use of multiple primer pairs that target different sequence, which enables detection and analysis of gene in one reaction. Each primer pair is designed with distinct sequence to ensure specificity and each product is typically distinguished by size or fluorescent label.

3. *Reverse-transcription PCR:*

In the diagnosis of Taura Syndrome virus (TSV) infection was made a histological examination in year 1955 and diagnostic method based on traditional RT-PCR. Real-time RT-PCR has advantage of speed sensitivity and specificity for the detection of RNA virus. By this study the Real-Time RT-PCR method was developed using TaqMan technique to detect TSV strip sample.

4. *Nested PCR:*

Nested PCR is a technique aimed at improving DNA amplification, specificity and sensitivity. In the initial stage outer primer are used to amplify target DNA segment. Then, in the next step inner primer which are designed to bind within the amplified product from the first round, target a more specific and narrower region of DNA for amplification.

5. **Protein engineering**

Protein engineering involves the creation of new amino acids to modify the protein molecule. All above this is done by recombinant DNA technique. It involves the synthesis of new amino acid or concluding with a minute change for the better outsource. From unlikely, sources Objective is to create superior enzyme, to get humanised antibodies with less immunogenicity and to change the substrate binding site to increase it specificity and efficiency. Protein engineering is used various sites like, in case of cancer treatment as pretargeting by radiation toxicity. The previous decade has seen a drastic increase in utilization of enzyme as a Green and sustainable catalyst in industrial and pharmaceutical application this sequence has been fueled by advance in scientist and engineering ability to customize native enzyme by protein engineering. Hence, generates enzyme variant with improved catalytic activity.

EMBRYO RESCUE

Assisted hatching of cleaved embryo or zygote is done over clinical embryo micromanipulation procedure. Embryo rescue is a specified technique in the embryo is cultivated under the aseptic condition in the nutrient media. Basically, it may be divided into 2 applications:

a. It is performed with mature embryo and help mainly shortening the period of germination by overcoming the seed dormancy condition.

b. The other is to perform with immature embryo and is called as an early embryo rescue. The purpose of this application is to focus embryo that would otherwise have been led to abortion.

It included the clinical manipulation techniques which brought rescuing the undeveloped cleaved embryo or zygote.

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