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Review Article

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Application of Recombinant DNA technology in Agriculture: A Review

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Abstract

Food is a very imported requirement of our life and its demand shall keep on increasing with the increase in population all over the world. But productivity is reduced because of many challenges of production. Only plant breeding methods cannot address the serious challenges. During the mid-1970s traditional methods of crop improvement like selection process, breeding in plants for yield, resistance to different diseases, and draught were in practice. But nowadays there are many new techniques are used in agriculture like gene transfer, cell, protoplast culture, etc. Due to this technique, transgenic plants are resistant to disease, predators, and drought and even can be grown without pesticides and fertilizer. 5.5 million Farmers are being benefitted by using transgenic plants in 148 million acres all over the world. Recombinant DNA which is also known as genetic engineering changes the natural genetic characteristics of an organism by inserting foreign DNA. It is widely used in agriculture to form genetically modified organisms which further help to produced genetically modified crops. Today, in the market, genetically modified foods are available in the vast majority. Recombinant DNA has increased the production of crops all over the world, as well as decreased the use of insecticides and herbicides by farmers.

Keywords: Biotechnology, Genetic engineering, Genetically Modified Organism, Recombinant DNA, Transgenic.

1. Introduction

1.2 Recombinant DNA technology

Recombinant DNA technology changes the genetic material of an organism to obtain the required feature in living organisms. In this technology there are several steps are involved like insertion of DNA from different sources, a desirable gene with their appropriate vector (Berk and Zipursky, 2000). Changes in an organism's genome are done with the help of the introduction of several new genes and blocking some expression of endogenous genes through recombining genes (Bazan-Peregrino *et al*., 2013). Restriction endonucleases enzyme is used as an enzymatic cleavage to obtain DNA fragments and DNA ligase enzyme used to join the fragments of DNA in vector. Now this vector is then introduced into the host living organism,

and this organism is grown to produce multiple copies of the incorporated DNA fragment in culture media, and DNA fragments are clones that are selected and harvested (Venter, 2007). In the agriculture and drugs field, r- DNA technology took longer as compared to anticipated because of many different and unexpected barriers and to solve this barrier and get desired results. There are many vaccines diagnostic tools hormones, etc. has been developed in the mid-1980s, to improve human health (Bazan-Peregrino *et al*., 2013).

1.2.1 Principle of Recombinant DNA Technology

The principle of Recombinant DNA Technology involves four steps,

a) Gene cloning and development of Recombinant DNA.
b) Transfer of vector into the host.
c) Selection of Transformed cell (host).
d) Transcription and translation of the inserted gene.
e) R-DNA provides the tools for studying the genetic makeup of the organism by isolating the DNA of any gene that's why they are so powerful. A particular gene can be isolated and produced in large quantities through cloning and its genetic information can be read by sequencing. The process of sequencing is based on a computer program. There are two methods of sequencing :

(i) Expressed Tag Sequencing: It reads icons only.
(ii) Sequence Annotation: It reads both icons and introns.

By the late 1970s, it became clear that those tools offered the fastest and surest route to understanding the molecular mechanism of the cells. There are certain tools to achieve rDNA technology.

1.2.2 Basic tools of recombination DNA technology:

Restriction Enzymes

Restriction enzymes are of two types endonuclease and exonuclease. The endonuclease is at a specific site (internal bonds) whereas exonuclease cuts the external bonds. Ligase enzymes help to bind the DNA molecule. Restriction endonuclease is also called a molecular scissor as it determines the specific site and is also the most important tool of genetic engineering. The restriction endonuclease identifies the specific seq. (palindromic sequence) at specific points and cut the DNA at that point called a restriction site. Thus sticky ends are created. The vector and GOI are cut through the same restriction endonuclease to obtain complementary sticky ends thus making the work of ligases easy to bind the GOI to the vector.

Vectors

Vectors are the ultimate vehicles that carry forward the GOI into the host organism so they are a very important tool for rDNA technology. The most common vectors in rDNA technology are plasmids and bacteriophages as they have high copy no. and carrying capacity.

Host Organism

The organism into which rDNA is introduced is called the host. The host must be compatible. They are the ultimate tool of rDNA technology because they take up the rDNA. There are multiple ways to inject rDNA into the host, microinjection, biolistic / gene gum, alternate cooling and heating, use of calcium ions, etc.

Gene Cloning and Development of Recombinant DNA

The foreign DNA (gene of interest) from the source is cleaned by restriction enzymes (exonucleases/endonuclease) and ligated to another DNA molecule i.e. cloning vector (plasmid, phagemid etc.) by DNA ligase to form recombinant DNA.

Transfer of Vector into the Host

This cloning vector with recombinant DNA is transferred into and maintained within the host cell. The introduction of r-DNA into a bacterial

host cell is called transformation. The transformation experiment was first performed by Federal Griffith (in 1960) in e-coli bacteria. He told that transformation is nothing but automatic take up by the cell.

Selection of Transformed Cells

Those host cells that take up the r-DNA are identified and selected from the pool using the selectable markers. Selectable markers select the transformed cell and reject the non-transformed cell.

Transcription and Translation of Inserted Gene

Once the transformed cell is selected then if require transcription and translation of inserted gene are done to get the desired protein.

(a) Transcription means to convert double standard DNA into single-stranded mRNA by RNA polymerize enzyme, which recognizes the binding site of a DNA called a promoter. The process of transcription is terminated by a terminator codon. This means the region from promoter to terminator codon is transcribed only.

(b) Translation means conversion of mRNA to protein. This process is carried out by the enzyme called DNA polymerase. There are three types of DNA polymerase (DP1, DP2, DP3). All have different functions. DNA polymerase synthesizes a new strand over m-RNA. Firstly, it reads the codon sequence over m-RNA in the 3' to 5' direction. Then it synthesizes the new strand in a 5' to 3' direction.

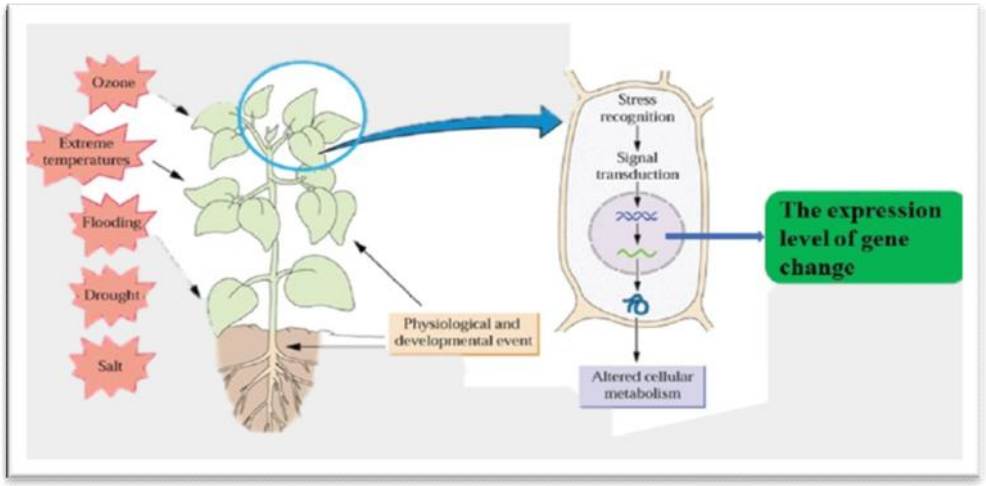

Figure: The model of gene expression information for depiction of plant growth status (Fang *et al.*, 2016).

2.1 Agriculture

Crops modified genetically in agriculture is done for two purposes, high yield and resistance of pests, and this is already used in a commercial context in several countries (Paolett*iet al.*, 1996)

Genetically modified crops are not only used for consumption but also in commercial contexts in various countries i.e., the first genetically engineered crop to be granted a license for human consumption is tomato CGN-89564-2 (in 1994) genetically modified (Bruening *et al.*, 2000). And its name was 'FlavrSavr', and it failed in the market. Further 93% of soybean and 88% of corn are genetically modified in the US (Winerip, 2003).

Brinjal in the first one to get GEAC approval but the introduction of pest resistance brinjal (eggplant) was showed bad performance in many countries. Bt-brinjal is a genetically modified food crop developed by the seed company. Many companies are ready for large-scale field trials and seed products but this GM food crop has major health risks. To overcome the initial unpopularity of *Bacillus thuringiensis* in many countries like India, (Choudhary and Gaur 2009). Bangladesh (Unnayan Bikalper Nitinirdharoni Gobeshona, 2015.) and the Philippines (Conrow, 2016).

2.2 Application of Recombinant DNA technology:

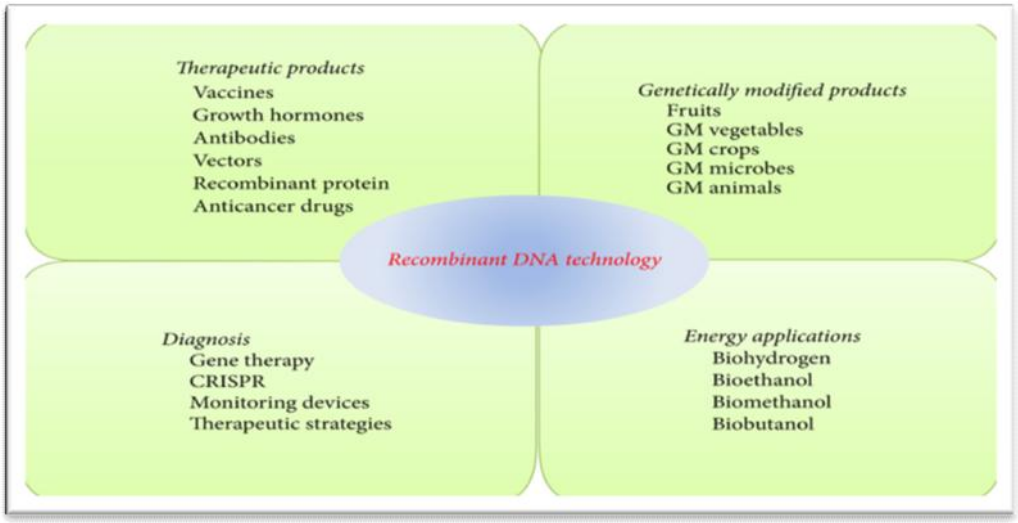

Figure: Application of RDT (Khan *et al.*, 2016).

In 1973, Paul Berg, Herbert Boyer, Annie Chang, and Stanley Cotien of Stanford University and University of California, San Francisco generated the first recombinant DNA molecule. During 'The Asilomar Conference' in 1975, regulation and safe use of r-DNA tech was discussed. At the Asilomar, the r DNA methods to faster agriculture and drug development took a longer time than anticipated by scientists because of unexpected difficulties and barriers to achieving satisfactory results. However, since the mid-1980's the no. of products like hormones, vaccines, etc. have been developed continually to improve health (Bargan Peregrino *et.al*., 2013). A quick approach is offered by r-DNA tech. to scrutinize the genetic expression of the mutations that were introduced into eukaryotic gives through cloved insulin genes insertion inside a simious virus fragment. (Lomedico, 1984). Forget genes disruption has been used to produce antitumor derivates in other hosts which were structurally similar for the production pathways (C. Mendez and J.A. Sales 2003). A new chimeric gene has been developed through their technique which contains the F & H B-submit cooling sequence and C-terminal peptide of the HCG B-submit coding sequences (Fauser *et al*., 2009).

2.3 Application of RDT in field of agriculture:

GM crops improved in many fields like herbicides, resistance to plants, etc. **(**Paoletti *et al.*, 1996).In agricultural fields crops are modified according to the needs using RDT. The first genetically modified crop was tomato CGN-89546-2 in 1994. (Brueing *et al.*, 2000) gave its name as 'FlaourSanr Tomato'. It has qualities like prolonged flavor and delayed ripening etc. In the US 88% of corn and 93% of soybeans are genetically altered and much of this finds its way unlabelled into processed foods (Winerip, 2003). There are different types of GM crops such as B.T. cotton, B.T. Maize, B.T. Brinjal etc. *Bacillus thuringenesis* is a bacterium found in sail used in G.M. crops. The benefits of using B.T. toxin should be stressed in an attempt to overcome the

initial unpopularity of consuming BT-brinjals in India (Choudhary *el al*., 2009) Bangladesh (Unnayan 2019), and the Philippines (Camron , 2016).The advent of RDT revolutionized the world as it offered new opportunities for innovations to produce a wide range of products **(**Galambos and Sturchio, 1998; Steinberg and Raso, 1998**).** The pharmaceutical products synthesized through RDT changed human life in such a way that the U.S. Food and Drug Administration (FDA) approved more recombinant drugs in 1997 than in previous years, which includes anemia, aids, cancer, hereditary disorder, etc.(Liu *et al.,* 2013). Transcriptional regulation of endogenous genes, their effectiveness in the new location and the precise control of transgene expression are the major challenges in plant biotech. (Venter, 2007).

Food Quality.

Consumers accept food by its texture, taste, and aroma. This important attributes have proven to be very suitable in r-DNA technique in tomato, by introducing either a truncated "sense" (Smith, 1990) polygalacturonase gene or the "antisense" (Sheehy *et al., 1988*) during ripening pectin was breakdown. A enzyme known as ACC oxidase, which is involved in the conversion of ACC to ethylene (Hamilton *et al.,* 1990). Potential future strategies to influence the flavor properties of a plant have been demonstrated by results of the genetic transformation of a scented Pelargonium species, referred to as 'lemon geranium'. *Agrobacterium rhizogenes* help in transformation by increasing the production of essential oil and significantly changing the distribution of monoterpene alcohols (Pellegrineschi *et al*., 1994). Therefore, for improving food quality 'sense' and 'anti-sense' techniques are used. By appling "antisense" technology, it inhibit either the 1-amino-cyclopropane-1-carboxylate synthase (Oeller *et al.,* 1991) or the ACC oxidase.

Nutritional Quality.

If the nutritional value of a food is increased by r-DNA tech then it will attract the people in near future. The direction of fatty acid biosynthesis, in a favor of medium-chain fatty acid, can be achieved by the expression of a 12:0-acyl carrier protein thioesterase in transgenic oilseed plants (Voelker *et al*., 1992). Increasing the level of sculpture containing amino acids in soybean by introducing a gene from Brazil nut exemplifies the strategy to improve the balance of essential fatty acids in imp. Crops (Townsend, 1992). GM crop also modified for the allergenicity of the host plant (Nordlee, 1994). The modification of carbohydrate metabolism by r-DNA tech. has been demonstrated for starch in potatoes (Müller-Röber *et al.,* 1992; Stark *et al*., 1992). "Bioreactors" are used for the production of pharmacologically and industrially important substances such as proteins (Daleand Belanger, 1993). From transgenic potatoes, correctly processed human serum albumin can be obtained (Sijmons *et al.,* 1990).

Microorganisms

For millennia microorganisms played an important role in food production. With the increase of knowledge, there have always been attempts to optimize the microorganisms in food production. Modern-day industries use microorganisms for the preparation of fermented foods (IFBC, 1990). The property of microorganisms can be changed with help of r-DNA tech (Geisen, *et al.,* 1990). Major goals are optimization of the production process, improvement of product quality, safety, and enlargement of product diversity (Muraoka and Imanaka, 1993). There are many micro-organisms available for industrial applications, one of them is *Saccharomyces cerevisiae.* This microorganism is used in the brewing industry for making bread and alcohol (Hollenberg and Strasser, 1990; Lang-Hinrichs and Hinrichs, 1992). GM yeast increased the activities of maltase and maltosepermease and an amylolytic brewer's

yeast (ACNFP, 1993) a genetically modified yeast, and used for food reviewed. Lactic acid bacteria play an important role in fermentation (Teuber, 1993). Many examples have been reported (Vos and W.M., 1992; Teuber, 1993). The constructions of safe, so-called 'food–grade' vectors have been extensively studied with these microorganisms (Venemma, 1993).

Current Research Progress

In agriculture, health, and the environment rDNA tech is growing very fast. E.g. (Lispro) (Humalog) in comparison to normal human insulin, is a well effective and fast-acting recombinant insulin**.** Epoetinalfa is a recombinant protein used in curing anemia (Masson *et al*., 2003). If a child's body is not producing a desired quantity of hGH then recombinant hGH is given. MPIF-1(cytokine myeloid progenitor inhibitory factor-1) a recombinant version which is approved by the FDA in 1997, was a great achievement. With the help of this mimic the division of immunologically cells (Patra *et al*., 2000; Macallan *et al*., 2008).The following part describes the recent developments of r-DNA technology. The solutions to several problems in different species have been brought about by (CRISPR) clustered regularly Interspaced short Palindromic repeats (Pennisi, 2013). The CRISPR of H-*hispanica* genome is capable of getting adopted to the nonlytic virus. The associated Cas operon encodes the interfering Cas 3 nucleases and other Cas proteins (Wang *et al*., 2016). Zinc finger nucleases (ZFNs) and Transcription activator-like effector nucleases (TALENs) are chimeric nuclease compounds. Recombinant protein fibroblast growth factor (FGF) has been developed which helps in the formation of new blood vessels in the myocardium.

The abovementioned new manufacturing structures beautify pipelines for the improvement of several vaccines and pills and so forth. Production of excessive exceptional proteins relies upon on physiology of a mobile phone and the prerequisites supplied to it. The expression of proteins will become retarded if a mobile goes below disturbing conditions, which may additionally want the manufacturing in some cases. Thus, similar upgrades are required for higher and secure manufacturing at genetic and metabolic levels. Microorganisms are viewed as the handiest hosts to produce molecular medicines. These cells enable the incorporation of overseas genes with much less resistant limitations and expression is effortlessly controlled. Compared to plant and mammalian cells to be taken as hosts, microbial structures supply less problematic equipment which subsequently enhances the overall performance and first-class of proteins production. The use of frequent microbial species, which include microorganisms and yeasts, is promising however the much less frequent lines have additionally been located promising as being mobile factories to produce recombinant molecular drugs

3. Conclusion

A recombinant DNA science can be entire and executed with the assistance of some elemental equipment like Enzymes, vectors, host organisms, etc. Much distinct equipment is on hand for growing and enhancing agricultural production. This equipment consists of strategies to improve new types such as classical breeding and biotechnology. Recombinant DNA science has fundamental makes use of in meals and agriculture. Genetically modified vegetation can mitigate several modern-day challenges in business agriculture. Traditional agricultural processes are experiencing some resurgence today, with a renewed hobby in natural agriculture; a method that does now not include the use of genetically engineered crops. Current market traits mission is one of the quickest developing and revolutionary international industries, which no longer solely advantage growers however additionally buyers and principal USA economies. However, it is integral that the agricultural enterprise and science neighborhood make investments in higher science verbal exchange and rules to address unethical lookup and misinformation In the future the

position of genetic engineering stands to play a sustainable agricultural improvement is a fascinating topic. As with the improvement of any new science, there are many issues about related risks, and agricultural biotechnology is no exception.

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