

## Role of Recombinant DNA Technology in Agriculture

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### ABSTRACT

Food is an essential requirement, and the demand for food shall keep on increasing with the increase in population. The classical breeding programs have contributed enormously to the improvement of various crops and subsequently molecular genetics which today constitutes the basis of genetic engineering research has added new direction to crop improvement. Genetic engineering is the process of genetic modification of organisms through transferring genetic material from one organism to another organism in order to change an organism's characteristics to the desired traits. A genetically modified organism (GMO) is an organism (plant, animal or microorganism) whose genetic material has been altered using gene or cell techniques of modern biotechnology. Genetic engineering is the improvement program which enhances the efficiency of crop improvement relative to conventional phenotypic selection by changing the focus from the paradigm of identifying superior varieties to a focus on identifying superior combinations of genetic regions and management systems. Plant biotechnology facilitates the farming of crops with multiple durable resistances to pests and diseases, particularly in the absence of pesticides. Likewise, transgenes or marker-assisted selection may assist in the development of high yielding crops, which will be needed to feed the world and save land for the conservation of plant biodiversity in natural habitats. Hence, crops should be engineered to meet the demands and needs of consumers. The genetic base of crop production can be preserved and widen by an integration of biotechnology tools in conventional breeding. Similarly targeting specific genotypes to particular cropping systems may be facilitated by understanding specific gene-by environment interaction(s) with the aid of molecular research. High quality crops with improved nutritional and health characteristics as well as other aspects of added-value may be obtained through multidisciplinary co-operation among plant breeders, biotechnologists, and other plant scientists. Generally, the world population is increasing alarmingly, but productivity is reduced because of several production challenges. Hence, conventional plant breeding method alone cannot address the serious challenges that world facing. Therefore, in order to overcome the food security problems, conventional plant breeding should be assisted and integrated with various biotechnology developments to hasten the crop genetic improvements. To ensure the rapid and advanced agricultural developmental within short period of time, the incorporation of genetic engineering in plant breeding is very relevant in the future world. Genetic engineering is not about the replacement of conventional breeding rather than integrating with it in order to make further improvement. There are various challenges in relation to the application of genetic modifications because of its costs in large scale utilization especially in developing countries. The integration of genetic engineering into conventional breeding program is an optimistic strategy for crop improvement in the future.

**Keywords:** Recombinant DNA; Gene cloning; Vectors; Host organism; Gene therapy

### INTRODUCTION

Genetic engineering is the process of genetic modification of organisms through transferring genetic material from one organism to another organism in order to change an organism's characteristics to the desired traits. A genetically modified organism (GMO) is an organism (plant, animal or microorganism) whose genetic material has been altered using gene or cell techniques of modern biotechnology (Ssekyewa, C. and Muwanga, M.K, 2009). 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. Genetic modification (GM) is the area of biotechnology which concerns itself with the manipulation of the genetic material in living organisms, enabling them to perform specific functions (Zhang C *et al.*, 2016). Genetic engineering is using recombinant DNA technology to change the genetic make-up of an organism for the production of intended

organisms with desired traits. Genetically modified crops are known as genetically engineered or bio-engineered crops and these are crops produced from organisms that have had changes introduced into their DNA using the methods of genetic engineering. Genetic engineering techniques allow the introduction of new traits from one organism to another organism for significant improvement over the previous existing traits and greater control over traits than previous methods such as selective breeding and mutation breeding (Lawlor, D.W, 2013).

The era of recombinant DNA began in the early 1970s, when researchers discovered that bacteria protect themselves from viral infection by producing enzymes that cut viral DNA at specific sites. When cut, the viral DNA cannot direct the synthesis of phage particles. Scientists quickly realized that such enzymes, called restriction enzymes, could be used to cut any organism's DNA at specific nucleotide sequences, producing a reproducible set of fragments. This set the stage for the development of DNA cloning, or making large numbers of copies of DNA sequences. By 1996, the commercialization of transgenic crops demonstrated the successful integration of biotechnology into plant breeding and crop improvement programs (Koziel *et al.*, 1993; Delannay *et al.*, 1995).

The term recombinant DNA has two meanings in genetics. The more specific of the two is a DNA molecule formed in the laboratory by joining together DNA sequences from different biological Sources. Such recombinant DNA molecules are artificial laboratory creations and are not found in nature. The term recombinant DNA is also used more loosely to refer to the technology that is utilized to create and study these hybrid molecules. The power of recombinant DNA technology is astonishing, enabling geneticists to identify and isolate a single gene or DNA segment of interest from the thousands or tens of thousands present in a genome (William S. Klug, *etal.*, 2009).

Recombinant DNA technology is the technique used in genetic engineering that involves the identification, isolation and insertion of gene of interest into a vector such as a plasmid or bacteriophage to form a recombinant DNA molecule and production of large quantities of that gene fragment or product encoded by that gene. Recombinant DNA technology alters the phenotype of an organism (host) through a

genetically altered vector. This cloning vector is introduced and integrated into the genome of the organism. So, basically, the process involves the introduction of a foreign piece of DNA into the genome which contains our gene of interest. The gene which is introduced is the recombinant gene and the technique is called the recombinant DNA technology. Inserting the desired gene into the genome of the host is not as easy as it sounds.

It involves the selection of the desired gene for administration into the host followed by a selection of the perfect vector with which the gene has to be integrated and recombinant DNA formed. This recombinant DNA then has to be introduced into the host. And at last, it has to be maintained in the host and carried forward to the offspring. A recombinant DNA technology can be complete and achieved with the help of some elemental tools. (Bhatnagar, R, 2006). The advances in recombinant DNA technology have occurred in parallel with the development of genetic processes and biological variations. The development of new technologies has resulted into production of large amount of biochemically defined proteins of medical significance and created an enormous potential for pharmaceutical industries. The biochemically derived therapeutics is large extra cellular proteins for use in either chronic replacement therapies or for the treatment of life-threatening indications (Pandey Sh. and Suba N, 2010).

Recombinant DNA technology (rDNA) is technology that is used to cut a known DNA sequence from one organism and introduce it into another organism thereby altering the genotype (hence the phenotype) of the recipient. The process of introducing the foreign gene into another organism (or vector) is also called cloning. Sometimes these two terms are used synonymously. Recombinant DNA technology comprises altering genetic material outside an organism to obtain enhanced and desired characteristics in living organisms or as their products. This technology involves the insertion of DNA fragments from a variety of sources, having a desirable gene sequence via appropriate vector (Berk A. and Zipursky S.L, 2000). Manipulation in organism's genome is carried out either through the introduction of one or several new genes and regulatory elements or by decreasing or blocking the expression of endogenous genes through recombining genes and elements (Bazan-peregrine M *et al.*, 2013). Enzymatic cleavage is

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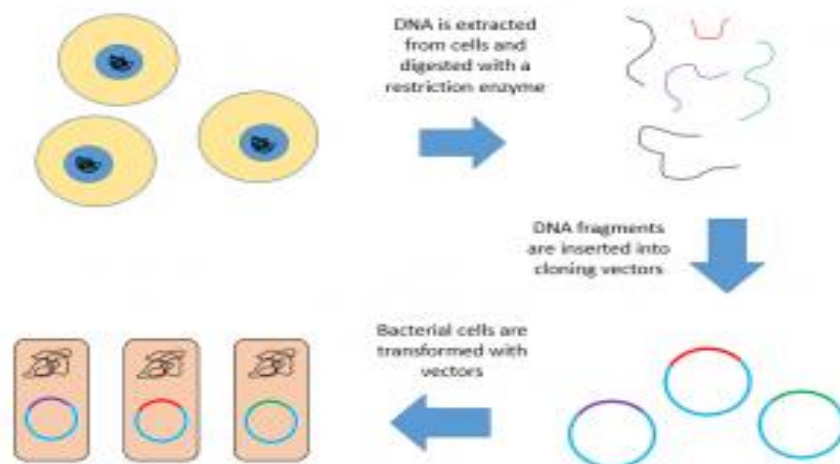
applied to obtain different DNA fragments using restriction endo nucleases for specific target sequence DNA sites followed by DNA ligase activity to join the fragments to fix the desired gene in vector. The vector is then introduced into a host organism, which is grown to produce multiple copies of the incorporated DNA fragment in culture, and finally clones containing a relevant DNA fragment are selected and harvested (Venter, 2007).

The first recombinant DNA (rDNA) molecules were generated in 1973 by Paul Berg, Herbert Boyer, Annie Chang, and Stanley Cohen of Stanford University and University of California San Francisco. In 1975, during “The Asilomar Conference” regulation and safe use of rDNA technology was discussed. Paradoxically to the view of scientists at the time of Asilomar, the recombinant DNA methods to foster agriculture and drug developments took longer than anticipated because of unexpected difficulties and barriers to achieve the satisfactory results. However, since the mid-1980s, the number of products like hormones, vaccines, therapeutic agents, and diagnostic tools has been developed continually to improve health (Bazan- peregrine M et al., 2013). A quick approach is offered by recombinant DNA technology to scrutinize the genetic expression of the mutations that were introduced into eukaryote genes through cloned insulin genes insertion inside a simian virus

fragment (Lomedico P.T, 1982). The objective/s of the paper was to review the application of recombinant DNA technology in agriculture and the efficiency of crop improvement relative to conventional breeding program to produce new superior varieties for desirable agronomic traits.

### BASIC TOOLS OF RECOMBINANT DNA TECHNOLOGY

Recombinant DNA techniques are so power full because of they provide the tools to study the genetics of the organism by isolating the DNA of virtually any gene. A particular gene can be isolated and produced in large quantities through cloning and its genetic information can be read by sequencing. The function of that gene can then be analyzed by using in vitro mutagenesis to make specific alteration in that information before re introducing the mutated DNA into the organism to determine the effects of the mutation. By the late 1970s as it became clear that those tools offered the fastest and surest route to understanding the molecular mechanisms of formerly intractable process such as development and cell division, they were seized eagerly by biologists in almost every field (Bhatnagar, R, 2006). A recombinant DNA technology can be complete and achieved with the help of some elemental tools. The different tools used for the purpose are discussed below



### Restriction Enzymes

The enzymes which include the restriction endonucleases – help to cut, the polymerases- help to synthesize and the ligases- help to bind. The restriction endonucleases used in recombinant DNA technology play a major role in determining the location at which the desired gene is inserted into the vector genome. They are of two types, namely endonucleases and

exonucleases. The endonucleases cut within the DNA strand whereas the exonucleases cut the nucleotides from the ends of the DNA strands. The restriction endonucleases are sequence specific which is usually palindrome sequences and cut the DNA at specific points. They scrutinize the length of DNA and make the cut at the specific site called the restriction site. This gives rise to sticky ends in the sequence. The

desired genes and the vectors are cut by the same restriction enzymes to obtain the complimentary sticky notes, thus making the work of the ligases easy to bind the desired gene to the vector.

### Vectors

The vectors help in carrying and integrating the desired gene. These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism. Plasmids and bacteriophages are the most common vectors in recombinant DNA technology that are used as they have very high copy number.

### Host Organism

Host organism is the organism into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA by the help of the enzymes. There are a number of ways in which this recombinant DNAs are inserted into the host, namely – microinjection, biolistic or gene gun, alternate cooling and heating, use of calcium ions, etc.

### Principle of Recombinant DNA Technology

The principle of recombinant DNA technology involved four steps. The four steps are: (1) Gene Cloning and Development of Recombinant DNA, (2) Transfer of Vector into the Host, (3) Selection of Transformed Cells and (4) Transcription and Translation of Inserted Gene.

### Gene Cloning and Development of Recombinant DNA

The foreign DNA (gene of interest) from the source is enzymatically cleaved and ligated (joined) to another DNA molecule i.e. cloning vector (plasmid, phagemid etc.) to form recombinant DNA. Any gene to be cloned must be inserted in a cloning vector (plasmid). A foreign gene (DNA fragment) introduced (by transformation) into a bacterium cell will not be replicated with bacterium. The reason for this is that the enzyme DNA polymerase, which is responsible for copying DNA, does not initiate the process at random. It is initiated at selected sites known as “origin of replication”. Generally, small fragments of DNA do not possess an origin of replication. Using rDNA technology, it is possible to insert the gene into a ‘cloning vector’, which in turn will make copies of the fragment (inserted DNA). A cloning vector is simply a DNA molecule

possessing an origin of replication’ and which can replicate in the host cell of choice. Most commonly ‘plasmids’, extra chromosomal, autonomously replicating, circular DNA molecules, are used as vectors. Sometimes, viruses are used as vector for gene insertion into micro-organisms, but they are better vectors for animal cells.

Cutting and insertion of desired foreign gene into the plasmid require special enzymes known as restriction endonucleases or restriction enzymes. These enzymes cut large DNA molecules into shorter fragments by cleavage at specific nucleotide sequences called ‘recognition sites’. Therefore, restriction endonucleases are highly specific deoxy-ribonucleases (DNase). Both vector DNA and foreign DNA to be inserted is cut by the same restriction enzyme, generating complementary ends. Thus, ends of foreign DNA make perfect match with cut ends of vector and join to make again a circular molecule.

### Transfer of Vector into the Host

The next step in a recombinant DNA experiment requires the uptake by E. coli of the rDNA. The process of introducing purified DNA into a bacterial cell is called transformation. This is carried out by treating cells with calcium chloride and high temperature. A few transformed cells are obtained by this method. Extra chromosomal DNA that lacks an origin of replication cannot be maintained within a bacterial cell. Thus, uptake of non-plasmid DNA is of no significance in a recombinant DNA experiment. Suitable strain of E. coli is used which lacks capabilities to destroy plasmid DNA or carrying out exchanges between DNA molecules

### Selection of Transformed Cells

After transformation, it is necessary to identify the cells that contain plasmid-cloned DNA constructs. All cells are grown successively on media containing antibiotic, ampicillin or tetracycline and cells showing the recombinant DNA (depending upon the restriction enzyme site and loss of particular antibiotic resistance due to disruption of the gene). Selected recombinant bacteria are grown in bioreactor to obtain the gene product. Other methods for selection of recombinant bacteria are: If synthesized gene is used, selection is easy as compared to DNA fragments used from genomic library of an organism, which requires selection for a suitable character and by nucleic

acid hybridization technique. All the steps are required to be considered carefully in making a recombinant bacterium. Prokaryotic organism *E. coli* is the most frequently used bacterium for recombinant technology.

### *Transcription and Translation of Inserted Gene*

Transcription of DNA into mRNA is mediated through the enzyme RNA polymerase, which recognizes the binding site on DNA called promoter. The process of mRNA synthesis is terminated by a termination signal (terminator codon). This means, only gene lying between promoter and terminator will be transcribed. Gene isolated in certain ways such as cDNA cloning or artificial synthesis, do not have their own promoter, therefore they must be inserted into a vector close to promoter site. Even if a cloned gene carries its own promoter, this promoter may not function in the new host cell. In such circumstances, the original promoter has to be replaced. In the cell, transcription takes place inside the nucleus or close to the nucleoid in bacteria by the action of the RNA polymerase on DNA. This process can also be mimicked outside the cell, i.e. cloned DNA can be mixed with RNA polymerase and the four nucleotide in a tube and under appropriate conditions, RNA transcripts can be formed as it does inside the cell.

This is known as *in vitro* transcription. The cellular RNA polymerase, whether it is bacterial or from higher organisms, is an extremely complicated enzyme, containing several subunits. The cloned DNA, placed downstream of the above promoters, is then incubated with the purified RNA polymerase from the bacteriophage, along with the precursor ribonucleotide triphosphate, which synthesizes transcripts specific for the cloned DNA. *In vitro* derived transcripts are used extensively as probes for the detection of specific nucleic acid fragments both in Southern as well as in Northern hybridization. Translation of mRNA into proteins is a complex process which involves interaction of the mRNA with the ribosomes. For translation to take place the mRNA must carry a ribosome binding site in front (upstream) of the gene to be translated. Ribosome binds to this site and move along the mRNA and initiates protein synthesis at the first AUG codon, it encounters. Translation in a cell is carried out by the ribosomes, which synthesize polypeptides by decoding the information carried by mRNA. In addition, amino-acyl tRNAs and other proteinaceous accessory factors are also utilized.

Biochemically the process of translation is not yet fully characterized, which means that we do not know all the requirements for polypeptide synthesis and very few of the components required for the process of translation have actually been purified.

### **Applications of Recombinant DNA Technology**

#### *Food and Agriculture*

Recombinant DNA technology has major uses which made the manufacturing of novel enzymes possible which are suitable in conditions for specified food processing. Several important enzymes including lipases and amylases are available for the specific productions because of their particular roles and applications in food industries. Microbial strains production is another huge achievement that became possible with the help of recombinant DNA technology. A number of microbial strains have been developed which produce enzyme through specific engineering for production of proteases. Certain strains of fungi have been modified so that their ability of producing toxic materials could be reduced (Olempska Beer Z. S, 2006). Lysozymes are the effective agents to get rid of bacteria in food industries.

It is also considered one of the most important enzymes in food industry to kill wide range of foodborne pathogens (Thallinger B, *et al.*, 2013). Derivation of recombinant proteins being used as pharmaceuticals came into practice from first plant recently and many others are through to be used for more production of similar medically important proteins. Wide range of recombinant proteins have been expressed in different plant species to be used as enzymes in industries, some majorly used proteins in research are proteins present in milk which play a role in nutrition, and new polymeric proteins are being used in industries and medical field (Ma, k, *et al.*, 2003).

Plants have been used to produce several therapeutic protein products, such as casein and lysozyme for improving health of child and polymers of protein for tissue replacement and surgery. Furthermore, tobacco plants can be engineered genetically to produce human collagen. High yielding molecular proteins is one of the major tasks under consideration in field of recombinant DNA technology (Ma, k, *et al.*, 2003). Traditional breeding and quantitative trait locus (QTL) analysis assisted in the identification of a rice variety with protein kinase known as PSTOL1 (*phosphorus*

*starvationtolerance1*) help in enhancing root growth in early stages and tolerates phosphorus deficiency (Gamnyao R. *et al.*, 2012).

Over expression of this enzyme enables root to uptake nutrients in sufficient amount in phosphorus deficient soil which ultimately enhances the grain yield (Hiruma, K. *et al.*, 2016). Chloroplast genome sequences are important in plant evolution and phylogeny. Genetic modification is needed in facilitating gene by gene introduction of well-known characters. It allows access to extended range of genes from an organism. Potato, beans, eggplant, sugar beet, squash, and many other plants are being developed with desirable characters, for example, tolerance of the herbicide glyphosate, resistance to insects, drought resistance, disease and salt tolerance. Nitrogen utilization, ripening, and nutritional versatility like characters have also been enhanced.

### Health and Diseases

Recombinant DNA technology has wide spectrum of applications in treating diseases and improving health conditions. The following sections describe the important breakthroughs of recombinant DNA technology for the improvement of human health:

#### Gene Therapy

Gene therapy is an advanced technique with therapeutic potential in health services. The first successful report in field of gene therapy to treat a genetic disease provided a more secure direction toward curing the deadliest genetic diseases (Cavazanna-calvo, M. *et al.*, 2000). This strategy shows good response in providing treatment for adenosine deaminase-deficiency (ADA-SCID), which is a primary immunodeficiency.

#### Production of Antibodies and Their Derivatives

Plant systems have been recently used for the expression and development of different antibodies and their derivatives. Most importantly, out of many antibodies and antibody derivatives, seven have reached to the satisfactory stages of requirements.

#### Investigation of the Drug Metabolism

Complex system of drug metabolizing enzymes involved in the drug metabolism is crucial to be investigated for the proper efficacy and effects of drugs. Recombinant DNA approaches have recently contributed its role through heterologous expression, where the enzyme's genetic information is expressed in vitro or in

vivo, through the transfer of gene (Nicholson K. *et al.*, 2005).

### Development of Vaccines and Recombinant Hormones

Comparatively conventional vaccines have lower efficacy and specificity than recombinant vaccine. A fear free and painless technique to transfer adenovirus vectors encoding pathogen antigens is through nasal transfer which is also a rapid and protection sustaining method against mucosal pathogens. This acts as a drug vaccine where an anti-influenza state can be induced through a transgene expression in the airway. In vitro production of human follicle-stimulating hormone (FSH) is now possible through recombinant DNA technology. FSH is considerably a complex heterodimeric protein and specified cell line from eukaryotes has been selected for its expression. Assisted reproduction treatment through stimulating follicular development is an achievement of recombinant DNA technology. A large number of patients are being treated through r-FSH. Most interestingly r-FSH and Luteinizing Hormone (LH) recombination was made successful to enhance the ovulation and pregnancy (Assidi, M. *et al.*, 2008).

### Chinese Medicines

As an important component of alternative medicine, traditional Chinese medicines play a crucial role in diagnostics and therapeutics. These medicines associated with theories which are congruent with gene therapy principle up to some extent. These drugs might be the sources of a carriage of therapeutic genes and as co-administered drugs. Transgenic root system has valuable potential for additional genes introduction along with the Ri plasmid. It is mostly carried with modified genes in *A. rhizogenes* vector systems to enhance characteristics for specific use. The cultures became a valuable tool to study the biochemical properties and the gene expression profile of metabolic pathways. The intermediates and key enzymes involved in the biosynthesis of secondary metabolites can be elucidated by the turned cultures (Ling C.Q. *et al.*, 2014).

### Environment

Genetic engineering has wide applications in solving the environmental issues.

### Phytoremediation and Plant Resistance Development

Genetic engineering has been widely used for the detection and absorption of contaminants in drinking water and other samples. Fine-tuning

enzymatic activity and knockout engineering together enhance the plant responses to toxic metals. Phytochelatin synthase, a heavy metal binding peptide synthesizing enzyme, revealed a way to enhance tolerance against heavy metals through enzymatic activity attenuation (Jez J.M. *et al.*, 2016). Recombinant DNA technology has proven to be effective in getting rid of arsenic particles that are considered as serious contaminants in soil.

### Energy Applications

Several microorganisms, specifically cyanobacteria, mediate hydrogen production, which is environmental friendly energy source. The specific production is maintained by utilizing the required enzymes properly as these enzymes play a key role in the product formation. But advanced approaches like genetic engineering, alteration in nutrient and growth conditions, combined culture, metabolic engineering, and cell-free technology (Ullah M.W. *et al.*, 2016) have shown positive results to increase the hydrogen production in cyanobacteria and other biofuels. The commercialization of this energy source will keep the environment clean which is not possible by using conventional energy sources releasing CO<sub>2</sub> and other hazardous chemicals (Tiwari A and Pandey A., 2012). Also, cyanobacteria can be engineered to make them able to convert CO<sub>2</sub> into reduced fuel compounds. This will make the carbon energy sources harmless to environment. This approach has been successful for vast range of commodity chemicals, mostly energy carriers, such as short chain and medium chain alcohols (Savakis P. and Hellingwerf K.J., 2015)

### SUMMARY AND CONCLUSION

The era of recombinant DNA began in the early 1970s, when researchers discovered that bacteria protect themselves from viral infection by producing enzymes that cut viral DNA at specific sites. Recombinant DNA technology is the technique used in genetic engineering that involves the identification, isolation and insertion of gene of interest into a vector such as a plasmid or bacteriophage to form a recombinant DNA molecule and production of large quantities of that gene fragment or product encoded by that gene. Recombinant DNA technology alters the phenotype of an organism (host) through a genetically altered vector. The gene which is introduced is the recombinant gene and the technique is called the recombinant DNA technology. A recombinant DNA

technology can be complete and achieved with the help of some elemental tools like Enzymes, vectors and host organisms. It involves different four steps. Recombinant DNA technology has major uses in food and agriculture, health and disease as well as in environment.

Genetically modified crops can mitigate several current challenges in commercial agriculture. Current market trends project is one of the fastest growing and innovative global industries, which not only benefit growers but also consumers and major country economies. However, it is imperative that the agricultural industry and science community invest in better science communication and regulation to tackle unethical research and misinformation. Imperfections and major genetically modified technology can also be combated by stricter regulation, monitoring and implementation by government agriculture bodies, a globally improved risk mitigation strategy and communication with growers, therefore ensuring greater acceptance. With key innovation in precision gene-integration technologies and emerging research in bio fortification and stress tolerance, genetically modified crops are forecasted to bring productivity and profitability.

The development of recombinant DNA technology (rDNA technology) permitting the transfer of genetic material between widely divergent species has opened a new era of research into the structure and function of the genome. Generally, the world population has topped 7 billion people and is predicted to double in the next 30 years. Ensuring an adequate food supply for this booming population is going to be a major challenge in the years to come. To meet growing need of ever-increasing human population, we need to enhance food production for sustaining food supply. Genetically modified foods promise to meet this need in a number of ways. Some of them are: Pest resistance, herbicide tolerance, disease resistance, cold tolerance, drought tolerance, salinity tolerance and nutritional improvement. The integration of conventional plant breeding with various biotechnological techniques advance crop genetic improvement and shortening the crop improvement cycle with desirable traits in order to satisfy the demand of people in both quantitative and qualitative.

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