academicresearchJournals

Vol. 7(3), pp. 150-159, April 2019
DOI: 10.14662/ARJASR2019.019
Copy©right 2019
Author(s) retain the copyright of this article
ISSN: 2360-7874
http://www.academicresearchjournals.org/ARJASR/Index.htm

Academic Research Journal of Agricultural Science and Research

Full Length Research

Application of Genetic Fingerprint/Recombinant DNA Technology in Plant Breeding: A review

Dasta Tsagaye Galalcha*

Ethiopian Institute of Agricultural Research, Kulumsa Agricultural Research Center, Asella, Ethiopia

*Corresponding author: Dasta Tsagaye Galalcha, Ethiopian Institute of Agricultural Research, Kulumsa Agricultural Research Center, Asella, Ethiopia; P.O. Box 489; E-mail: 6dasta2@gmail.com

Accepted 22 March 2019

Recombinant DNA technology, genetic engineering, genetic modification/manipulation are similar terms that are applied to the direct manipulation of an organism's gene. Recombinant DNA is artificially created from two or more DNA incorporated into a single molecule. This review paper was aimed to review the application of Genetic Fingerprint/Recombinant DNA Technology in Plant Breeding.Bases upon restriction enzymes that recognize certain sequences within the DNA, and cut the DNA at the particular site and living organisms, such as bacteria, virus, yeast and mammalian cells to achieve cloning. Recombinant DNA technology can be used for insertion of genes in plants not only from related plant species, but also from unrelated species such as microorganisms. In agriculture primarily for the production of transgenic plants with higher yield and nutritional values, increased resistance to stress and pests. Commercially important transgenic crops such as maize, soybean, tomato, cotton, potato, mustard, rice etc. have been genetically modified. The introduction, integration and expression of foreign genes in plants is to improve the crop with desired trait: resistant to biotic stresses; that means, development of pathogen resistant varieties, resistant to abiotic stresses, improvement of crop yield and quality

Key words: Genetic engineering, Recombinant DNA technology, Plant breeding

Cite this article as: Dasta, T.G (2019). Application of Genetic Fingerprint/Recombinant DNA Technology in Plant Breeding: A review. Acad. Res. J. Agri. Sci. Res. 7(3): 150-159

INTRODUCTION

Recombinant DNA technology, genetic engineering, genetic modification/manipulation are similar terms that are applied to the direct manipulation of an organism's gene. It is genetic alteration of cells or organisms by methods that require in-vitro modification of DNA. Recombinant DNA is artificially created from two or more DNA incorporated into a single molecule. This type of DNA will be formed by ligating together pieces of DNA that are not normally contiguous. The technologies involved in the construction, study and use of recombinant DNA molecule are collectively referred to as recombinant DNA technology (Benjamin, 2008).

Recombinant DNA technology bases upon restriction enzymes that recognize certain sequences within the DNA, and cut the DNA at the particular site. These enzymes are called molecular scissors. Many restriction enzymes exist that recognize many sequences of DNA bases. Some of the enzymes create a staggered cut, where one strand of DNA is longer than the complimentary strand. Because of the staggered cuts, treatment of different DNA with the restriction enzymes will generate regions, which complement one another.

These regions will anneal together. These behaviors allow foreign DNA to be inserted into target DNA. This is the basis of recombinant DNA technology. To be useful, the recombinant molecule must be replicated many times to provide material for subsequent experimental manipulations. Producing many identical copies of the same recombinant molecule is called cloning. Cloning can be done in the laboratory, using the technique called the polymerase chain reaction (James, 2010).Recombinant DNA technology also uses living organisms, such as bacteria, virus, yeast and mammalian cells to achieve cloning. The DNA is taken up by the cell when incorporated in a vector. Viral DNA and plasmids typically are utilized as vectors. The bacterium Agrobacterium tumefaciens is a popular means of transforming plants. The DNA shuttled by the vector to the host typically contains a gene that encodes resistance to an antibiotic or other feature, such as the ability to fluoresce when exposed to ultraviolet light (Lacroix*et al*., 2006).

These act as indicators to permit a means of monitoring the success of DNA integration and protein expression. For example, exposure of the bacteria Escherichia coli to vectors containing the recombinant DNA will produce some cells that have successfully incorporated and expressed the DNA. If the recombinant indicator is an antibiotic resistance gene, then the successful cells can be detected when they form colonies on a growth substrate containing the antibiotic. Each colony represents a clone of transformed cells. Selecting recombinant plants can be done in a similar fashion, with the recombinant insert containing an herbicide-resistance gene and the growth medium containing the herbicide (Lacroix*et al*., 2006).

Production of transgenic organisms has also created a great number of possibilities using plants. Transgenic plants have been obtained that express antibodies and recombinant pharmaceuticals. According to Martin *et al*., (1998),molecular techniques combined with the traditional techniques of plant breeding are being utilized by the forestry industry to generate trees of commercial interest that are capable of faster and straighter growth. Martin *et al*., (1998), added that transgenic crop plants, engineered to resist pests or targeted herbicides, have proved very popular and in 1999, the area planted to transgenic varieties was approximately half the U.S. soybean crop and about 25 per cent of the country's corn crop.

Applications of recombinant DNA technology include food crops engineered to produce edible vaccines. This strategy would make vaccination more readily available to children worldwide. Not only this, a recombinant protein was derived from recombinant DNA.The development of these new technologies have 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 extracellular proteins for use in either chronic replacement therapies or for the treatment of life threatening indications. Industrial biotechnology was developed to produce artificial enzymes that are similar to natural ones, and they are highly utilized in the in-vitro work (Shivanand *et al*., 2010).Therefore the objective of this paper is to review application of genetic fingerprint/recombinant DNA technology in plant breeding

History of Recombinant DNA technology

The recombinant DNA technique was first proposed by Peter Lobban, with A. Dale Kaiser at the Stanford University. The technique was then realized by Lobban and Kaiser; Jackson, Symons and Berg; and Sanley Norman Cohen, Chang, Herbert Boyer and Helling, from 1972 to 1974. They published their findings in papers including the 1972 paper "Biochemical Method for Inserting New Genetic Information into DNA of Simian Virus 40: Circular SV40 DNA Molecules Containing Lambda Phage Genes and the Galactose Operon of Escherichia coli", the 1973 paper "Enzymatic end-to-end joining of DNA molecules" and the 1973 paper "Construction of Biologically Functional Bacterial Plasmids in vitro"; all of which described techniques to isolate and amplify genes or DNA segments and insert them into another cell with precision, creating a transgenic bacterium(*wikipedia.org/wiki/Recombinant DNA*).

Recombinant DNA technology was made possible by the discovery, isolation and application of restriction endonucleases by Werner Arber, Daniel Nathans, and Hamilton Smith, for which they received the 1978 Nobel Prize in Medicine. Cohen and Boyer applied for a patent on the Process for producing biologically functional molecular chimeras which could not exist in nature in 1974. The patent was granted in 1980. The idea of producing food with desirable qualities paved the way for the development of genetically modified food worldwide. Scientists were able to isolate genes with favorable traits and insert them in crops to produce food that can resist drought, insects, and pesticides and even is more nutritious. It started by developing a tomato with a longer shelf life in 1994 called Flavr Savr (wikipedia.org/wiki/Recombinant DNA)

Methods of recombinant DNA technology

Cloning

The use of cloning is interrelated with recombinant DNA in conventional genetics, as the term "clone" refers to a cell or organism derived from a parental organism,

with modern biology referring to the term as a collection of cells derived from the same cell that remain identical. Therefore, gene or DNA cloning produces large numbers of copies of the gene/DNA being cloned. The step concerned with transformation of a suitable host with recombinant DNA &cloning of the transformed cells is called DNA cloning or gene cloning. (Benjamin, 2008)

Plasmids are extra chromosomal self-replicating circular forms of DNA present in most bacteria, such as Escherichia coli (E.coli), containing genes related to catabolism and metabolic activity, and allowing the carrier bacterium to survive and reproduce in conditions present within other species and environments. These genes represent characteristics of resistance to bacteriophages and antibiotics and some heavy metals, but can also be fairly easily removed or separated from the plasmid by restriction endonucleases, which regularly produce "sticky ends" and allow the attachment of a selected segment of DNA, which codes for more "reparative" substances, such as hormone medications including insulin, growth hormone, and oxytocin. In the introduction of useful genes into the plasmid, the bacteria's are then used as a viral vector, which are encouraged to reproduce so as to recapitulate the altered DNA within other cells it infects, and increase the amount of cells with the recombinant DNA present within them (Amulya*et al*., 2010)

The use of plasmids is also a key within gene therapy, where their related viruses are used as cloning vectors or carriers, which are means of transporting and passing on genes in recombinant DNA through viral reproduction throughout an organism. Plasmids contain three common features a replicator (origin of replication), selectable marker and a cloning site. The replicator or "ori" refers to the origin of replication with regard to location and where replication begins. The marker refers to a gene or DNA sequence with a known location on a DNA and associated with particular gene or trait. The cloning site is a sequence of nucleotides representing one or more positions where cleavage by restriction enzymes occurs. Most eukaryotes do not maintain standard plasmids; yeast is a notable exception. In addition, the Ti plasmid of the bacterium Agro bacterium tumefaciens can be used to integrate foreign DNA into the genomes of many plants. (Amy *et al*., 2002).

According to Benjamin (2008) the entire procedure of gene cloning may be classified into the following steps. The first course of action is production and isolation of the DNA fragments to be cloned. Next procedure is insertion of the isolated gene in suitable vector to obtain recombinant DNA. At this stage new genetic material (DNA) will be formed and bacterial plasmids can be used as cloning vector. The third step is introduction of the recombinant DNA into a suitable organism/cell by a process called transformation. Further, selection of the transformed host cells, and identification of the clone containing the desired gene/DNA fragment will continue. The last procedure is multiplication/expression of the introduced gene in the host. After multiplying, wherever and whenever needed, they will be transferred and expressed in the gene into another organism.

Isolation and construction of the desired gene

The identification and isolation of the desired gene (DNA fragment) is critical step in recombinant DNA technology. The DNA fragments used for cloning are generally obtained as follows: A mixture of fragments of genomic DNA is used to produce a genomic library. Genomic library contains DNA fragments that represent genes as well as those that are not genes. Chimeric DNA library contains only those genes that are expressed in the concerned tissue/organism. In both cases, a mixture of fragments is used for cloning to establish the library. The desired gene/ DNA fragment is then searched out from the library. When amino acid sequence/base sequence of a gene is known, it may be either synthesized chemically or amplified using Polymerase Chain Reaction (Benjamin, 2008).

The tools used for gene cloning

Gene cloning utilizes certain biological products and biological agents for achieving its objectives. These items are termed as gene cloning tools. Four different types of enzymes are used for the purpose. Nucleases are used to cut DNA at specific sites. DNA ligases are utilized as molecular glue to seal cuts that remain in the recombinant DNA molecule. The third enzyme is DNA polymerase used add nucleotides to single stranded DNA in that case, the DNA will be cloned. The last enzymes are called DNA modifying enzymes. Examples of these enzymes are alkaline phosphates; which adds phosphate group to an end having free 5'-OH, S1 nucleases which are used for removing single stranded extensions from the ends and exonucleases that remove nucleotides from the ends((Benjamin, 2008).

The other tools or instruments in gene cloning are vectors. Vectors are suitable DNA molecules capable of self-replication in the selected host cell. The DNA segment to be cloned is integrated into these tools. Cloning vectors are used for propagation of DNA inserts in a suitable host. As principle, such vectors contain at least the regulatory sequences having optimum function in the chosen host. They should have promoters, operators, ribosomal binding sites, etc. It is desirable that all cloning vectors have relaxed replication so that they can produce multiple copies per host cell. Cloning vectors allow amplification of inserted DNA fragments.

According to Benjamin, (2008) they can be developed

from naturally occurring bacterial plasmids and can also be introduced into competent bacterial cells by transformation. Among bacteria, E. coli occupies a prominent position. The third tools are host organisms. These are suitable organisms that serve as hosts for propagation of the recombinant DNA (DNA insert). These organisms should be easy to be transformed and should support the replication of recombinant DNA. Further, to be a good host, they should be free from elements that interfere with replication of recombinant DNA and should lack active restriction enzymes (Amy *et al*., 2002).

Genetically Modified (GM) Plants

The term GM plants or GMOs (genetically-modified organisms) is most commonly used to refer to crop plants created for human or animal consumption using the latest molecular biology techniques. These plants have been modified in the laboratory to enhance desired traits such as improved shelf life, disease resistance, stress resistance, herbicide resistance, pest resistance, production of useful goods such as biofuel or drugs, and ability to absorb toxins, for use in bioremediation of pollution. Due to high regulatory and research costs, the majority of genetically modified crops in agriculture consist of commodity crops, such as soybean, maize, cotton and rapeseed. Recently, some research and development has been targeted to enhancement of crops that are locally important in developing countries, such as insect-resistant cowpea for Africa and insect-resistant brinjal (eggplant) for India (James, 2010)

The enhancement of desired traits has traditionally been undertaken through breeding, but conventional plant breeding methods can be very time consuming and are often not very accurate.Genetic engineering, on the other hand, can create plants with the exact desired trait very rapidly and with great accuracy. Critics have objected to GM crops parse on several grounds, including ecological concerns, and economic concerns raised by the fact these organisms are subject to intellectual property law. GM crops also are involved in controversies over GM food with respect to whether food produced from GM crops is safe and whether GM crops are needed to address the world's food needs (Sinha and Sunita, 2005)

Genetically engineered plants are generated in a laboratory by altering their genetic makeup. This is usually done by adding one or more genes to a plant's genome using genetic engineering techniques. Most genetically modified plants are generated by the biolistic method (particle gun) or by Agrobacteriumtumefaciens mediated transformation (William *et al*., 2007).

Application of Recombinant DNA Technologies in Plant Breeding

The genetic manipulation of plants has been going on since prehistoric times, when early farmers began carefully selecting and maintaining seeds from their best sow for the next season. Plant breeders have cross fertilized related plants to provide next generation plants with new characteristics such as higher yield, resistance to diseases and better nutrient content long before the science of genetics was developed. Recombinant DNA technology can be used for insertion of genes in plants not only from related plant species, but also from unrelated species such as microorganisms (Amulya*et al*., 2010). This process of creation of transgenic plants is far more precise and selective than traditional breeding. Application of recombinant technology in agriculture is primarily for the production of transgenic plants with higher yield and nutritional values, increased resistance to stress and pests (William *et al*., 2007).

Several commercially important transgenic crops such as maize, soybean, tomato, cotton, potato, mustard, rice etc. have been genetically modified. During the last decades, considerable progress has been made to understand the function of genes, isolation of novel genes and promoters as well as the utilization of these genes for the development of transgenic crops with improved and new characters. There are many potential application of plant genetic engineering. The ultimate goal of introduction, integration and expression of foreign genes in plants is to improve the crop with desired trait: Resistant to biotic stresses (insects, viruses, bacteria and fungi); that means, development of pathogen resistant varieties, Resistant to abiotic stresses (herbicide, temperature, drought, salinity and etc.), Improvement of crop yield and quality (e.g. storage, longer shelf life of fruits and flowers, etc.), transgenic plants with improved nutrition, and used as bioreactors for the commercial products (E.g. protein) (James, 2010)

Furthermore, James (2010) reported that, by 2009 there were 2.2million hectares with GM maize, GM soybeans and GM cotton in South Africa. All three crops are available as herbicide-tolerant plants; for maize and cotton there are also insect-resistant varieties. In the case of cotton almost the entire acreage (98%) was cultivated with GM varieties, for soybeans the share was 85 percent and for maize 78 percent.

Development of stress tolerant plant

Plant resistant to Abiotic stress

Plants need to cope up with abiotic stresses such as drought, cold, heat and soils that are too acidic or salty to support plant growth. While plant breeders have successfully incorporated genetic resistance to biotic

stresses into many crop plants through crossbreeding, their success at creating crops resistant to abiotic stresses has been more limited, largely because few crops have close relatives with genes for resistance to these stresses. Therefore recombinant DNA technology is being increasingly used to develop crops that can tolerate difficult growing conditions (Glenn *et al*., 1999).
According to Gabrielle *et al*. (1999), genetically modified tomato and canola plants, that tolerate salt levels 300 percent greater than non-genetically modified varieties have been developed and other researchers have identified many genes involved in cold, heat and drought tolerance found naturally in some plants and bacteria.

Herbicide Resistance

Many effective broad spectrum herbicides do not distinguish between weeds and crops, but crop plants can be modified to make them resistant to herbicides, so as to eliminate weeds more selectively. For example, the herbicide Roundupcontains the active ingredient glyphosate, which kills plants by binding to the active site of enzymes called enolpyruvalshikimate phosphate syntheses (EPSP syntheses). This enzyme is critical for the synthesis of aromatic amino acids. Roundup is an extremely effective herbicide but it kills almost all species of plants, including most crop plants (William *et al*., 2007). .

On the other hand, it is very safe for humans and animals because they do not have EPSP syntheses. By using rDNA technology, modified EPSP syntheses gene (that produced enzymes that were still functional but were not inhibited by glyphosate) have been introduced into crop plants such as cotton and soya bean. These genetically modified plants were found to be highly resistant to treatment with Roundup. Genes that provide resistance to other herbicides such as sulfonyl urea's gluphosinates etc. have also been developed and transferred to produce various transgenic plants (William *et al*., 2007). Another approach of producing herbicide resistance plant is production of crops that aretolerant to other herbicides: Phosphinothricin or bialaphos-based herbicides (glufosinate) are broad-spectrum and non-selective organic phosphate herbicides that break down rapidly in the soil. These herbicides strongly inhibit glutamine synthase activity in plants, resulting in the accumulation of toxic ammonium in the cells that kills the plants. To engineer glufosinate-tolerant crops, two approaches can be used, either over-express the glutamine synthase gene, or introduce a gene to deactivate the herbicide.

The enzyme phosphine thricinacetyltransferase (PAT or BAR) modifies phosphinothricin into an inactive form through acetylation. The pat gene was isolated from Streptomyces viridichromogenes while the bar gene from S. hygroscopicus. Using either of these two genes, phosthinothricin-tolerant transgenic cotton, corn and canola were developed (De Block *et al*. 1987 as cited in William *et al*., 2007).

Accordingly, Bromoxynil herbicides inhibit electron transport in photosynthesis. Introduction of nitrilase can detoxify bromoxynil. A gene encoding BXN nitrilase from Klebsiellapneumoniae(Stalker *et al*., 1988) was introduced into cotton and canola to generate resistance. However, transgenic crops with tolerance to these two classes of herbicides, especially the bromoxynil herbicides, are not as popular as those that are glyphosate tolerant since glyphosate costs less and controls more weed species (Castle *et al*. 2006 as cited in William *et al*., 2007).

According to William *et al*. (2007), using the same molecular approaches, tolerance has been generated in plants against the sulphonyl urea and imidazolinone herbicides, which inhibit the branched-chain amino acid biosynthesis pathway, by introducing a mutant acetolactate synthase (ALS) gene that is resistant to the herbicides. For the herbicide atrazine, which inhibits photosystem II, resistance can be engineered by introducing a mutant gene for Q8 protein, or by introducing the gene encoding glutathione-S-transferase to detoxify the atrazine.

Drought resistance

Water deficit is the most prominent abiotic stress that severely limits crop yields, thereby reducing opportunities to improve livelihoods of poor farmers in the semi-arid tropics where most of the legumes, including groundnut and chickpea, are grown. Sustained long-term efforts in developing these legume crops with better drought tolerance through conventional breeding have been met with only limited success mainly because of an insufficient understanding of the underlying physiological mechanisms and lack of sufficient polymorphism for drought tolerance-related traits. The transgenic approach has been used to speed up the process of molecular introgression of putatively beneficial genes for rapidly developing stress-tolerant legumes. Nevertheless, the task of generating transgenic cultivars requires success in the transformation process and proper incorporation of stress tolerance into plants (John and Sons, 2006).
Drought tolerance is an extremely important agricultural trait. According to John and Sons, (2006), one way of engineering drought tolerance is by taking the genes from plants that are naturally drought tolerant and introducing them to crops. The resurrection plant, native to dry regions processes a gene for a unique protein in its cell membrane. Hence plants given this gene are less prone to stress from drought. Some genes have been found that control the production of the thin, protective cuticle found on leaves. If crops can be grown with a thickened

waxy cuticle, they could be better equipped for dealing with dryness.

Salt resistance

Physiologically, salinity imposes water deficit that results from the relatively high solute concentrations in the soil. It causes ion-specific stresses resulting from altered K^+/Na^+ ratios and leads to buildup in Na^+ and Cl^- concentrations that are detrimental to plants. Plants respond to salinity using two different types of responses. Salt-sensitive plants restrict the uptake of salt and adjust their osmotic pressure by the synthesis of compatible solutes (proline, glycinebetaine, sugars etc.). Salt-tolerant plants sequester and accumulate salt into the cell vacuoles, controlling the salt concentrations in the cytosol and maintaining a high cytosolic Kþ/Naþ ratio in their cells (Glenn *et al.*, 1999). Clearly, ion exclusion mechanisms could provide a degree of tolerance to relatively low NaCl concentrations but would not work at high salt concentrations, resulting in the inhibition of key metabolic processes with concomitant growth inhibition.

There are three key processes that contribute to salt tolerance at the cellular level: The establishment of cellular ion homeostasis, the synthesis of compatible solutes for osmotic adjustment andthe increased ability of the cells to neutralize reactive oxygen species generated during the stress response (Glenn *et al.*, 1999).

Although Naþ is required in some plants, particularly halophytes (Glenn *et al.*, 1999), a high NaCl concentration is a toxic factor for plant growth. The alteration of ion ratios in plants is due to the influx of Naþ through pathways that function in the acquisition of Kþ. The sensitivity to salt of cytosolic enzymes is similar in both glycophytes and halophytes, indicating that the maintenance of a high cytosolic Kþ/Naþ concentration ratio is a key requirement for plant growth in high salt (Glenn et al., 1999). Strategies that plants could use in order to maintain a high Kþ/Naþ ratio in the cytosol include: (i) extrusion of Naþ ions out of the cell and (ii) vacuolar compartmentation of Naþ ions. A rise in extracellular Naþ concentration will establish a large electrochemical gradient favoring the passive transport of Naþ into the cells. Kþ outward rectifying channels (KORCs) could play a role in mediating the influx of Naþ into plant cells. KORC channels showed a high selectivity for Kþ over Naþ in barley roots and a somewhat lower Kþ/Naþ selectivity ratio in Arabidopsis root cells. These channels which open during the depolarization of the plasma membrane (i.e. upon a shift in the electrical potential difference to more positive values), could mediate the efflux of Kþ and the influx of Naþ ions.

Voltage-independent cation channels (VIC) in plant plasma membranes have been reported. These channels have a relatively high Naþ/Kþ selectivity, are not gated by voltage and provide a pathway for the entry of Naþ into plant cells (Maathuis and Amtmann, 1999).

Sodium ions can enter the cell through a number of low- and high-affinity Kþ carriers; among these is AtHKT1 from Arabidopsis, which was shown to function as a selective Naþ transporter and, to a lesser extent, to mediate Kþ transport. AtHKT1 was identified as a regulator of Naþ influx in plant roots. This conclusion was based on the capacity of hkt1 mutants to suppress Naþ accumulation and sodium hypersensitivity in a sos3 (salt-overly-sensitive) mutant background (Rus *et al.*, 2001), suggesting that AtHKT1 is a salt tolerance determinant that controls the entry of Naþ into the roots. Naþ extrusion from plant cells is powered by the operation of the plasma membrane Hþ-ATPase generating an electrochemical Hþ gradient that allows plasma membrane Naþ/Hþantiporters to couple the passive movement of Hþ inside the cells, along its electrochemical potential, to the active extrusion of Naþ.Recently, AtSOS1 from Arabidopsis has been shown to encode a plasma membrane Naþ/Hþantiporter with significant sequence similarity to plasma membrane Naþ/Hþantiporters from bacteria and fungi. The over expression of SOS1 improved the salt tolerance of Arabidopsis, demonstrating that improved salt tolerance can be attained by limiting Naþ accumulation in plant cells (Shi et al., 2003).

The paramount role of Naþcompartmentation in plant salt tolerance has been further demonstrated in transgenic tomato plants over expressing AtNHX1. The introduction of a vacuolar Naþ/Hþantiporter from the halophyte Atriplexgmelini conferred salt tolerance in rice. Most recently, the over expression of GhNHX1 from cotton in tobacco plants (Wu *et al.*, 2004) and the over expression of AtNHX1 in maize resulted in enhanced salt tolerance. Excess salinity is becoming a major problem for agriculture in dry parts of the world. In several cases, scientists have used biotechnology to develop plants with enhanced tolerance to salinity conditions. Plants with high tolerance to salt stress possess naturally high levels of a substance called glycinebetaine. Further, plants with intermediate levels of salinity tolerance have intermediate levels, and plants with poor tolerance to salinity have little or none at all (John and Sons, 2006).

Genetically Modified tomatoes with enhanced glycinebetaine production have increased tolerance to salty conditions. These tomatoes have been genetically engineered enabling them to grow in salty water. These tomatoes take up the salts from the soil and stores in the leaves. Another approach to engineering salt tolerance uses a protein that takes excess sodium and diverts it into a cellular compartment where it does not harm the cell. In the lab, this strategy was used to create a test plants that were able to flower and produce seeds under extreme salt levels (John and Sons, 2006).

Production of crop varieties for biotic stress

Insect resistance

The world population has topped 7 billion and is predicted to double in the next 50 years. Ensuring an adequate food supply for this booming population is going to be a major challenge in the years to come.

GM foods promise to meet this need in a number of ways: An extremely wide variety of modifications have been made, and the list is growing very rapidly. One of the applications that have received a lot of publicity is the production of plants that are resistant to insect attack (James, 2010).To minimize crop damage by insects, mites and nematodes; farmers use synthetic pesticides extensively which cause severe effects on human health and environment. The transgenic technology provides an alternative and innovative method to improve pest control management which is eco-friendly, effective, sustainable and beneficial in terms of yield. This involves genetic incorporation of toxic gene (product of which is lethal to insect) in to the plant. This kills the insects without use if dangerous insecticide thus has double benefit in crop improvement (Amulya *et al.,* 2010).

The first genes available for genetic engineering of crop plants for pest resistance were Cry genes (popularly known as Bt genes) from a bacterium Bacillus thuringiensis. These are specific to particular group of insect pests, and are not harmful to other insects. Transgenic crops (e.g. cotton, rice, maize, potato, brinjal, cauliflower, cabbage, tobacco, etc.) with Btgenes have been developed and such transgenic varieties proved effective in controlling the insect pests and it has been claimed worldwide that it has led to significant increase in yield along with dramatic reduction in pesticides use. The most notable example is Bt cotton (which contains Cry/Ac gene) that is resistant to a notorious insect pest Bollworm (Hellicoperpaarmigera) and Bt cotton was adopted in America and India (Benjamin, 2008)

Biotechnology has opened up new avenues for natural protection for plants by providing new bio pesticide, such as microorganisms, that are toxic to targeted crop pests but do not harm humans, animals, fish, birds or beneficial insects. As bio pesticide act in unique ways, can even control pest population that has developed resistance to conventional pesticides. Using recombinant DNA technology, the gene that makes these microorganisms lethal to certain insects can be transplanted into the plants on which that insect feeds. The plant that once was a food source for the insect now kills it, lessening the need to spray crops with chemical pesticides to control infestation (Amulya *et al*., 2010).

The spores of Bacillus thuringiensis(Bt) contain a crystalline protein (Cry) which breaks down to release a toxin, known as delta-endotoxin (protoxin) is highly toxic to lepidopteran larvae. This toxin binds the intestinal lining and creates pores resulting in an ion imbalance, paralysis of the digestive system, and consequent death of the insect. Bt toxin sprays and powders have been in use for many years (Benjamin, 2008).Different Cry genes, also known as Bt genes have been identified, cloned and characterized. Effective gene constructs have made it possible to deliver these genes into plant tissues so that they are expressed at levels high enough to kill the insects. The Bt genes are effective against different orders of insects. Bt cotton and maize which have increased resistance to boll worms have been developed and cultivated since 1996. Farmers get benefited by saving costs by using less of traditional pesticides. However, one of the major concerns about Bt based transgenic is the possibility of development of toxin resistant insects. Efforts are also underway to identify and transfer other genes from Bt, which can impart insecticidal properties to the plants (Amulya *et al*., 2010).

Disease resistance

Like weeds and insect pests, plant diseases also cause loss of the total world crop production. Plant virus infections lead to a range of diseases causing significant economic damage to most of the world's major crops. Since there are no effective chemical viricides available, effort has been made from the inception of plant biotechnology to apply this new technology to develop virus resistance crops. Several approaches have been demonstrated to confer virus resistance to target crops, including the genes encoding viral coat proteins (CPs), replicates, movement proteins, proteinases, defective interfering RNA, and satellite RNAs. Recent studies suggest that plant protection against the viruses is, in most cases, by an RNA-based post-transcriptional gene silencing mechanism (Waterhouse *et al*., 2001).

The use of genes or gene sequences derived from viral genomes to confer virus resistance in transgenic plants is known as pathogen-derived resistance. The first transgenic virus resistant crop commercialized was squash resistant to watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV) through the introduction and expression of the coat protein genes of WMV2 and ZYMV. Other crops with resistance to a variety of viruses have also been developed (Ferreira *et al*., 2002).Plants are susceptible to viral, bacterial and fungal diseases. Much progress has been made in evolving transgenic plants resistant to viruses. For example, expression of a gene that encodes the coat protein of tobacco mosaic virus (TMV) in transgenic tobacco plants has been shown to cause the plants to resist TMV infection. A number of other viral resistant plants species have been developed including squash and potatoes.

There are several strategies for engineering plants for

viral resistance and these utilizes the genes from virus itself (e.g. the viral coat protein gene). The virus-derived resistance has given promising results in number of crop plants such as tobacco, tomato, potato, alfalfa, and papaya. Some viral resistant transgenic plants like papaya resistance to papaya ring spot virus have been commercialized in some countries (Shivanand *et al*., 2010).

Genetic engineering of crop plants for resistance to fungal and bacterial infections has been more difficult. However, by studying the protective genes that are expressed in naturally disease-resistant plants, an encouraging progress has been made (William *et al*., 2007). The proteins encoded by these pathogenesis related proteins (PR proteins) can provide limited disease protection in transgenic plants. Plants respond to pathogens by inducing a variety of defense responses like pathogenesis-related proteins (PR proteins), enzymes that degrade/destroy fungal cell wall (chitinase), antifungal proteins and compounds, phytoalexins, etc (Amulya *et al*., 2010).

Development of plants having improved characters

Increasing yield

Many attempts were made to use biotechnology to improve crop yields directly. Researchers at Japan's National Institute of Agro biological Resources added maize photosynthesis genes to rice to increase its efficiency of converting sunlight to plant starch and increased yields by 30 percent (Amulya *et al*., 2010). Other scientists are altering plant metabolism by blocking gene action in order to shunt nutrients to certain plant parts. Yields increase as starch accumulates in potato tubers and not leaves, or oilseed crops, such as canola, allocate most fatty acids to the seeds. In addition, the author reported that, crops that have better accessibility to the micronutrients and their need are also being developed.

Mexican scientists have genetically modified plants to secrete citric acid, a naturally occurring compound, from their roots. In response to the slight increase in acidity, minerals bound to soil particles, such as calcium, phosphorous and potassium, are released and made available to the plant. Nitrogen is the critical limiting element for plant growth and researchers from many scientific disciplines are tearing apart the details of the symbiotic relationship that allows nitrogen-fixing bacteria to capture atmospheric nitrogen and provide it to the plants that harbor them in root nodules (William *et al*., 2007).

Increase in quality of plant products

The quality includes several aspects that may be grouped into two categories: organoleptic properties and nutritious contents. Organoleptic quality involves color and texture of the fruit, but also taste and aroma, whereas nutritional quality refers to the content of metabolites contributing to the intake of nutritious such as Protein, sugars, carotenoids, flavonoids, ascorbic acid and so on (James, 2010). Most of the quality traits show a continuous variation, are attributed to the joint action of many genes and are strongly induced by environmental conditions. Beside their complex inheritance, fruit quality traits have often been engineered through approaches of reverse genetics, such as genetic transformation and mutagenesis, pointing at controlling the expression of single major genes involved in the regulation of a desirable phenotype. In addition, genetic transformation has often been successful in enhancing fruit quality-related traits (John and Sons, 2006).

In general, there are three main goals of engineering strategies in plants (Verpoorte *et al*., 2000): The enhancement of a desired trait, the decrease in the expression of a specific unwanted trait, and the development of a novel trait (i.e. a molecule that is produced in nature but not usually in the host plant, or a completely novel compound). Strategies aimed at inducing changes in the expression of a trait changing the synthesis of a specific metabolite are referred to as metabolic engineering. Approaches for achieving the redirection of metabolic fluxes include the engineering of single steps in a pathway to increase or decrease metabolic flux to target compounds, to block competitive pathways or to introduce short cuts that divert metabolic flux in a particular way. However, this strategy has only limited value because the effects of modulating single enzymatic steps are often absorbed by the system in an attempt to restore homeostasis (John and Sons, 2006).

Recently, strategies aimed at targeting multiple steps in the same pathway are gaining increasing interest because they help to control metabolic flux in a more predictable manner. This might involve up-regulating several consecutive enzymes in a pathway; up-regulating enzymes in one pathway while suppressing those in another competing pathway; or using regulatory genes such as transcription factors (TF) to establish multipoint control over one or more pathways in the cell. Since technical hurdles limits the number of genes that can be transferred to plants and pyramiding of transgenes by crossing transform ants for single targets is a highly time-consuming approach, researchers developed new transformation methods to introduce multiple transgenes into plants and express them in a coordinated manner (Navqvi *et al*., 2009).

In addition, the authors added that, controlling the expression of a single TF or a combination of TFs provides attractive tools for overcoming flux bottlenecks involving multiple enzymatic steps, or for deploying pathway genes in specific organs, cell types or even

plants where they normally do not express.One of the most successful research efforts to change the characteristics of a plant produce was carried out with tomatoes. Tomatoes need to be picked while still green so that they are firm enough to withstand mechanical handling and transport. Unfortunately, they do not develop the same flavor and texture of vine ripened tomatoes. Softening of tomatoes and other fruits is caused by the enzyme pectinase or polygalacturonase (PGA). This enzyme digests the pectin polysaccharide that cements the plant cells together. Softening of the fruit is caused, by this breakdown of pectin. In order to reduce the levels of PGA in ripening tomatoes, researchers placed the PGA gene in reverse orientation relative to the CaMV 35S promoter. This results in transcription of an antisense RNA that is complementary to the normal sense PGA mRNA (William *et al*., 2007).

Although the exact mechanism is unknown, antisense RNA is able to arrest the translation of the endogenous PGA mRNA in the tomato fruit. Transgenic tomato plants that express an antisense PGA gene only have about 5 to 10% of normal PGA levels. Fruits of these plants have normal color and flavor but they soften more slowly and can be picked and processed after they are ripe. They also have a higher content of soluble solids and are therefore better than normal tomatoes for processed tomato products. Transgenic lines of potato having increased levels of starch also have been developed by introducing a gene construct that expresses a gene from bacteria that produce an enzyme that enhances starch biosynthesis. A promoter from a potato gene that encodes the major protein in potato tubers has been used, so that the expression of the introduced gene is limited to the tuber. Tubers accumulate approximately 3 to 5% more starch than normal potatoes and when they are deep fried absorb less oil and yield chips having fewer calories (Charles *et al*., 1999).

Some GM soybeans offer improved oil profiles for processing or healthier edible oils. GM plants are being developed by both private companies and public research institutions such as, the International Maize and Wheat Improvement Centre. Other examples include a genetically modified cassava with lower cyanogen glucosides and enhanced with protein and other nutrients, while golden rice developed by the International Rice Research Institute (IRRI), has been discussed as a possible cure for Vitamin A deficiency. An international group of academics has generated a vitamin-enriched corn derived from South African white corn variety M37W with 169x increase in beta carotene, proves that this can be done. Other vegetables and fruits with delayed ripening as well as modified flavor characteristics were also produced (http://en.wikipedia.org/wiki/Genetically_modified_crops#Improved_nutrition)

SUMMARY AND CONCLUSION

Genetically-modified foods have the potential to solve many of the world's hunger and malnutrition problems, and to help protect and preserve the environment by increasing yield and reducing reliance upon chemical pesticides and herbicides. Yet, there are many challenges ahead for governments, especially in the areas of safety testing, regulation, international policy and food labeling. Many people feel that genetic engineering is the inevitable wave of the future and that we cannot afford to ignore a technology that has such enormous potential benefits. However, we must proceed with caution to avoid causing unintended harm to human health and the environment as a result of our enthusiasm for this powerful technology.

REFERENCES

Amulya K. Panda 2010. National Institute of Immunology.JawaharLal Nehru University Campus ArunaAsaf Ali Marg, New Delhi.

Amy B. Vento and David R. 2002.Gillum Office of Environmental Health and Safety University of New Hampshire June 3, 2002.

Benjamin A. Pierce, 2008. Genetics Conceptual Approach, 3rd Ed. New York.

Charles W.Stuber, Mary Polacco, and M. Lynn Senior 1999. Synergy of Empirical Breeding, Marker-Assisted Selection, and Genomics to Increase Crop Yield Potential, crop science, Vol. 39, November-December 1999.

Ferreira SA, Pitz KY, Manshardt R, Zee F, Fitch M, et al. 2002.Virus coat protein transgenicpapaya provides practical control of papaya ringspot virus in Hawaii. Plant Dis 86:101–105.

Gabrielle J.Persley 1999. Application of biotechnology to crops: Benefits and Risks council for agricultural science and technology, USA.

Glenn, E.P., Brown, J.J. and Blumwald, E. 1999.Salt tolerance and crop potential of halophytes. Critical Reviews in Plant Science 18, 227–255.

James D. 2010. Recombinant DNA: Genes and Genomes: A Short Course. San Francisco: W.H. Freeman.

John Wiley and Sons.2006. Plant Biotechnology Current and Future Applications of Genetically Modified Crops, UK

Lacroix, B., Tzfira, T., Vainstein, A., andCitovsky, V. 2006. A case of promiscuity:Agrobacterium’s endless hunt for new partners. TRENDS in Genetics 22: 29-37.

Maathuis, F.J.M. and Amtmann, A. 1999.Kþ nutrition and Naþ toxicity: the basis of cellularKþ/Naþ ratios. Annals of Botany 84, 123–133

Naqvi, S.; Farré, G.; Sanahuja, G.; Capell, T.; Zhu C. andChristou, P. 2009. When more isbetter: multigene engineering in plants. Trends in Plant Science, Vol. 15, No. 1, pp. 48-56

PandeyShivanand SubaNoopur. 2010. Recombinant DNA technology and Genetic Engineering: a safe and effective meaning for production of valuable biological, International journal of pharmaceuticals sciences research and review.

Rus, A., Yokoi, S., Sharkhuu, A., Reddy, M., Lee, B., Matsumoto, T.K., Koiwa, H., Zhu, J.K.,Bressan R.A. and Hasegawa, P.M. 2001. AtHKT1 is a salt tolerance determinant that controlsNaþ entry into plant roots. Proceedings of the National Academy of Sciences USA 98,14150–14155.

Schütz. Martin 1998. 10th International Symposium of the European Federation of Defense Technology Associations, Biological Weapons: New Threats Geneva May 7th 1998.North Carolina A&T State University Policies & Procedures for the Use of Recombinant DNA And Infectious Agents 2005.North Carolina A&T State University.

Shi, H., Lee, B.H.,Wu, S.J. and Zhu, J.K. 2003. Overexpression of a plasma membrane Naþ/Hþantiporter gene improves salt tolerance in Arabidopsis thaliana. Nature Biotechnology 21, 81–85.

Sinha U. and Sunita S. 2005.Cytognetics, plant breeding and evolution, Vikas publishing house pvt.ltd, New Delhi.

Verpoorte, R.; van derHeijdenvan, R. &Memelink, J. 2000.Engineering the plant cell factory for secondary metabolite production.Transgenic Research, Vol.9, pp. 323-343

Waterhouse PM,Wang MB, Lough T. 2001.Gene silencing as an adaptive defense against viruses. Nature 411:834–842

William H. Kaye-Blake, Caroline M. Saunders, and Paul Dalziel.2007. Current Contribution of Four Biotechnologies to New Zealand's Primary Sector. Lincoln University, New Zealand, AgBioForum, 10(2): 65-70. ©2007 AgBioForum.

Wu, C.A., Yang, G.D., Meng, Q.W. and Zheng, C.C. 2004.The cotton GhNHX1 gene encoding a novel putative tonoplastNaþ/Kþantiporter plays an important role in salt stress. Plant and CellPhysiology 45, 600–607.

Websites

wikipedia.org/wiki/Recombinant_DNA

http://www.gmo-safety.eu/news/1242.disease-resistant-bananas-drought-tolerant-maize.html

http://en.wikipedia.org/wiki/Genetically_modified_crops#Improved_nutrition

www.jamesand thegiantcorn.com/2010/02/09/india-and-bt-brinjaleggplant