International Journal of Latest Engineering Research and Applications (IJLERA) ISSN: 2455-7137

Volume –06, Issue – 04, April 2021, PP – 17-25

Biotechnological Applications of Plant Genetic Engineering and Crop Improvement

Dr. Mandaloju Venkateshwarlu,
M.Sc. Ph.D. Botany, M.Sc. Biotechnology, FHAS, SFS, SFAB,CFE &(LLB.)
Department of Botany, UC, KU Warangal-09, T.S. India

Abstract: The first plant biotechnology products are emerging. Much research has focused on agronomic traits for controlling insects, viruses, bacterial and fungal plant diseases, and weeds. Other modifications include developing more nutritious food products, manipulating petal color of flowers. A very promising area of research is the production of specialty oils for detergents, cosmetics, and lubricants and the modification of the lipid. Composition of seed crops to reduce levels of saturated fats.

Examples of modified oils include those with increased levels of laurate and myristate for shampoos and soaps and those that solidify at room temperature without hydrogenation. Partially hydrogenated oils (such as margarine), which are added to many foods, have been shown to be unhealthy. Other types of specialty oils include cooking oil with reduced saturated fat, an oil substitute for cocoa butter in chocolate, oils for cosmetics, and liquid wax for lubricants. Because many oils are obtained from tropical plants, the production of oil substitutes from genetically engineered oilseed crops grown in the United States, primarily rapeseed, soybean, and sunflower, would decrease the dependency on tropical oils. Genetically engineered crop plants are beginning to be used for the production of industrially important chemicals and pharmaceutically active compounds.

Keywords: Biotechnology, Applications, Genetic Engineering & Crop Plants.

Introduction:

Biotechnology has produced six crops currently in the marketplace—soybeans, corn, cotton, papaya, squash, and canola—resulting in the production of 4 billion pounds of additional food on the same area of land, the improvement of farm income by $1.5 billion, and the decreased use of pesticides by 56 million pounds. The loss of prime farmland may one day require that crops be cultivated in areas that are less suitable or marginal for agriculture. Crops that can be grown in these regions are being developed. For example, stress-tolerant plants are being engineered that are able to live in colder, drier regions. A 2002 study compiled by the nonprofit research organization National Center for Food and Agricultural Policy documented through 40 case studies of 29 crops that the production of hardier crops through biotechnology will produce an additional 14 billion pounds of food, improve farm income by $2.5 billion, and at the same reduce the use of pesticides by 168 million pounds.

Genetically Engineered Traits:

Although numerous field trials are being conducted on genetically engineered plants, most are experimental and may never enter the commercial market. Prasad (2018) Rathore (2010) Rao *et al* (1998)Currently, six different traits have been engineered into crop plant species that have been approved for commercial production: herbicide, insect, and virus resistance; altered oil content; delayed fruit ripening and pollen control.

Herbicide Resistance: Because weeds growing with crop plants can significantly reduce yields by competing for nutrients, sunlight, and water, farmers must apply herbicides to control weeds, often mixtures of different herbicides. Herbicides are used annually in agricultural areas to decrease the impact of weeds on Crops. Rajashekaran *et al* (2006), Campestrina (2006); Venkateshwarlu *et al* (2019). Between 1966 and 1991, the use of herbicides in agriculture in the United States more than quadrupled, reaching an estimated 495 million pounds and a cost of $10 billion. Although farmers routinely applied more than 100 chemical herbicides, weeds still reduced crop productivity by approximately 12%. The sensitivity of traditional crops to herbicides limits the types that can be used. Because many herbicides do not discriminate between crop and weed, they must sometimes be applied early, before crop emergence. Often the chemical persists in fields when the crops are germinating and can kill them. Debay *et al* (1936), Murashig & Skoog (1962) MS Medium.

By modifying crop plants so they are resistant to a broad-spectrum (kills all or most plants) herbicide, a single chemical is effective without killing the crop plants and the number of applications may be reduced. A larger selection of biodegradable or less-toxic herbicides can be made available to the farmer. Companies that develop herbicides also are engineering herbicide-tolerant plants to accompany their chemicals (Monsanto is an example). Critics, however, claim that if a chemical approach to weed control continues, chemical use will most likely increase. In this case, instead of creating a safer and cleaner environment, biotechnology will have perpetuated our dependence on toxic chemicals. This dependence may generate more herbicide-resistant weeds through increased abundance of chemical applications or perhaps by accidental crossing of herbicide-resistant crops with neighboring related wild plants. There are several different types of genetically engineered herbicide resistance. One way that herbicides kill plants is by binding to a specific target in a plant's biochemistry. Scientists study how specific herbicides affect a plant and then can modify the target (usually a protein) so that it no longer binds the herbicide. These plants are herbicide resistant and tolerate exposure to that particular herbicide. Another way that plants become resistant is to produce a new protein that inactivates or detoxifies the herbicide. Crop plants have been engineered to be resistant to four types of herbicides. M. Venkateshwarlu *et al* (2018&2017)

Herbicide	**Resistance-Modified Crops**
Glyphosate	Soybeans, corn, canola, cotton, and sugarbeets
Glufosinate	Soybeans, corn canola, cotton, sugarbeets, and rice
Bromoxynil	Cotton
Sulfonylurea	Cotton and flax

Glyphosate Resistance One of the most commonly used broad-spectrum herbicides is glyphosate (marketed as Roundup, Rodeo, and others). Most genetic engineering uses a single bacterial gene that confers resistance to an herbicide. For example, glyphosate-resistant plants have been engineered to readily degrade the herbicide Roundup (made by Monsanto) into nontoxic compounds. The herbicide normally, inhibits an important enzyme, EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), in the aromatic amino acid synthesis pathway (the shikimate pathway) in both plants and bacteria. The gene encoding EPSPS in a glyphosate-resistant *E. coli* strain was isolated, placed under control of a plant promoter, and transferred into: plant cells. Monsanto has marketed Roundup Ready crops that are resistant to glyphosate such as soybeans, corn, rapeseed (canola), and cotton. Monsanto also has developed Roundup Ready sugar beets not yet on the market.

Glufosinate Resistance The active ingredient (phosphinothricin) in glufosinate herbicide (for example, market names Basta and Liberty) mimics the structure of the amino acid glutamine. This compound binds to the plant enzyme glutamine synthase, which is required for nitrogen metabolism and blocks this metabolic pathway (by inactivating glutamine synthase). Herbicide-resistant plants have been generated by inserting a gene from the bacterium *Streptomyces,* whose protein product inactivates phosphinothricin in the herbicide. Genetically engineered glufosinate-resistant varieties of soybean, corn, and rapeseed have been approved and are on the market. Sugar beets and rice have been approved but are not marketed at this time. Roundup Ready soybeans, first grown in 1996, are the most widely cultivated genetically engineered plant on the market. Hassaiin (2000), Shahzad (1999).

Inactivates photosynthesis in plants. Bro-moxynil-resistant plants are produced when a gene encoding the enzyme bromoxynil nitrilase (BXN) is transferred into plants from the soil bacterium *Klebsiella pneumoniae.* Nitrilase inactivates bromoxynil before this herbicide can kill the plant. Only cotton has been engineered for bromoxynil resistance (Monsanto's BXN cotton). The EPA allows only approximately 10% of the cotton crop to be sprayed with bromoxynil because of exposure limits considered to be safe.

Sulfonlurea Sulfonylurea kills plants by blocking an enzyme required for the synthesis of three amino acids, valine, leucine, and isoleucine. Herbicide resistance was obtained by first modifying the enzyme by gene mutation in tobacco, and then transferring the mutated gene into crop plants. Monsanto's cotton is the only crop currently on the market. Ugender & Venkateshwarlu M (2010), Edmond (1977).

Insect Resistance Biopesticides, pesticides pioduced by living organisms, have had a bright beginning in agriculture. One type of biopesticide is produced by the Bt toxin gene found in the bacterium *Bacillus thuringiensis.* Microbial Biotechnology, discusses the use of the Bt gene to generate insect-resistant plants. Bt-based insect resistance has been engineered in varieties of corn and cotton that are on the market. Most Bt-corn varieties produced by Monsanto are also glyphosate resistant. Aventis marketed a variety of Bt corn called *SlarLink* that also included glyphosate resistance. Potato varieties have been produced, but they have not been marketed since 2001. Hanan (2010) Wayne *et al* (2011), Venugopal (2005), Venkateshwarlu M (2020) In the 1990s many experiments were conducted to explore the use of plant protease inhibitors as biopesticides. Insect pests attack stored cereal grains, peas, and beans and result in huge losses, and evidence showed that protease inhibitors might be able to protect plant products, especially in storage. Protease inhibitors, naturally produced by plants, are produced in response to wounding. There are several classes of protease inhibitors that correspond to insect gut proteases that aid in digestion.

After protease inhibitors are ingested by insect larvae, their digestive enzymes are inhibited, and starvation results. Examples of successful laboratory experiments include the transfer of the cowpea trypsin inhibitor gene into tobacco and the potato protease inhibitor into rice. Garden pea seeds also, have been engineered that resist attack by two species of weevils that damage crops in storage. A protein, CC-amylase inhibitor, blocks the action of the enzyme OC-amylase, which the insects use to digest the starch in seeds. In studies, most of the weevils that fed on pea seeds either died or suffered inhibited development. The OC-amylase inhibitor is expressed only in the seeds of peas. Other experiments have shown that a cow-pea protease inhibitor gene effectively protects oil palms against attack by bagworm larvae. Although Bt toxins have been very successful in combating insect pests, results with protease inhibitors have been mixed. Because plant pests have co-evolved with plants, they have long been exposed to plant protease inhibitors, and it is thought that many insect have developed mechanisms to tolerate these enzymes. Therefore, not all crops or even varieties of the same crop are protected by protease inhibitor genes.

Virus Resistance Many crops are lost to viral diseases, resulting in losses of millions of dollars each year. Chemicals are widely used to help control the insect vectors (for example, aphids) that spread viral diseases from one plant to another. However, controlling viral diseases is very difficult. Viral diseases cause symptoms such as yellowing and mottling of leaves, deformed fruits, and stunted growth. When a virus infects a plant, its DNA enters a plant cell (the protein coat is removed). The virus reproduces in the plant cell by making copies of the virus and packaging each DNA molecule into a protein coat. Thousands of virus copies are made and leave the cell to infect other cells of the plant. Pathak (2014), Ugender & Venkateshwarlu (2019)

Genetic engineering may provide a more desirable alternative to chemicals. Research has focused on isolating genes involved in resistance to diseases caused by viruses, bacteria, and fungi. Genetically engineered virus resistant plants harbor a viral coat protein gene whose protein product is overproduced. In this way, plants are immunized against the virus because the virus is unable to reproduce in the plant host cell. The plant shuts off the gene of the overproduced coat protein, and at the same time, the coat protein gene of the invading virus is shut off. This prevents the virus from reproducing. This type of resistance is called coat protein—mediated viral resistance.

Numerous plant viruses have been identified, and many of them are host specific. The coat protein genes of a number of viruses have been used to genetically engineer resistance in crops. Among the viruses are cucumber mosaic virus, alfalfa mosaic virus, tobacco streak virus, tobacco etch virus, tobacco rattle virus, potato virus Y and potato virus X, and potato leaf roll virus. Resistance genes for a variety of diseases—not just viral infections—have been isolated from plants: a gene isolated from *Arabidopsis thaliana* (which serves as a model for the study of plant molecular genetics) and tomato confers resistance to the bacterial pathogen *Vseudomonas syringae;* a tomato gene is involved in resistance to the fungal pathogen *Cladosporiumfulvum;* a flax gene has been isolated that confers resistance to fungal rust disease; and a tobacco gene protects against tobacco mosaic virus. One day these genes may be transferred to other agronomically important crops. To date, yellow squash, potatoes, and papaya have been genetically engineered to be virus resistant. Virus resistance was transferred from yellow squash to zucchini by traditional breeding.

Yellow Squash and Zucchini As grow Seed markets several different squash varieties that are resistant to three devastating viruses that cause huge losses in squash-related crops such as zucchini, pumpkins, squash, watermelon, and cucumber: watermelon mottle virus 2, zucchini yellow mosaic virus, and cucumber mosaic virus.

Potato Although no longer marketed (as of 2019) because of poor sales, Monsanto developed two varieties of potatoes resistant to the potato leaf roll virus and potato virus Y, called NewLeafPlus and NewLeafY, respectively. These varieties also contain a Bt-resistance gene that was first marketed as NewLeaf to resist Colorado potato beetle larvae. Two factors caused the demise of NewLeaf varieties (those with both the Bt gene and virus resistance): large chain restaurants such as McDonald's and Burger King and manufacturers of potato products such as Procter and Gamble and Frito-Lay do not use genetically engineered potatoes because of public pressure, and a newly developed insecticide controls insect pests.Meena (2010), Kar *et al* (2006)

Papaya Two varieties of genetically engineered virus-resistant papaya were developed by Cornell University and researchers in Hawaii after an outbreak of papaya ring spot virus that reduced crop productivity by 50% from 1992 to 2016, these varieties, called Rainbow and SunUp, have been provided to papaya farmers at no cost.

Altered Oil Content: Oils from plants (extracted from the seeds) serve many functions, from use as food additives such as salad and cooking oil and margarine, as well as additions to processed foods, to commercial applications such as in detergents, soaps, cosmetics, lubricants, and paints. Plants produce different types of oils, the properties of which are determined by the types of fatty acids (long carbon—hydrogen chains) the plant makes. Each type of plant oil can be used for a specific industrial purpose. For example, peanut and canola oils are used for cooking, while other types of oils are good for cosmetics (for example, jojoba oil). Several varieties of soybean and canola *have* been genetically engineered to produce oils with better cooking and nutritional properties. Imported tropical coconut oil and palm oil is generally used to make soap and detergent, but genetically engineered canola oil (called high laurate canola) may eventually substitute. The fatty acid type is changed by genetically modifying an enzyme in the fatty acid synthesis pathway. Changes vary from changing carbon length of the fatty acid chain to varying the degree of saturation (number of carbons on the carbon chain). Venugopal & Kaviraju (2005), Venkateshwrlu M (2020).

Delayed Fruit Ripening: The only genetically engineered tomatoes that have been marketed are those with delayed-ripening genes. This desirable trait allows more time to pass between removals from the vine to the market and, thus, increases the shelf life of the tomato. Although a typical ripe tomato tastes better, it becomes too soft (during ripening) and rots before or during shipment. Typically, tomato varieties are picked before the onset of ripening to avoid over ripening and softening by the time they reach the market. Although the green, unripe tomatoes are gassed with ethylene (a plant growth hormone that induces ripening) to turn them red, this process does not allow them to develop any flavor. Some genetically engineered tomatoes produce a reduced amount of ethylene so that these tomatoes can fully develop on the vine but stop just before ripening and turning red. In this way, the tomato can develop more flavors on the vine before shipment to markets. An example of this type of genetically engineered delayed ripening tomato is Endless Summer by DNA Plant Technologies. At this time, there are no genetically engineered tomatoes on the market.

Flavr Savr Tomato: The first genetically modified food product was the Flavr Savr tomato developed by Calgene, Inc. (now part of Monsanto), a biotechnology company that was in California. The bioengineered tomato generated much discussion and controversy but offered many benefits, including a garden-fresh taste year round. Flavr Savr tomatoes were produced by blocking the expression of the polygalacturonase (PG) gene. Fruit softens because the PG enzyme degrades pectin, a major component of plant cell walls. When the amount of PG is decreased, the fruit can remain on the plant longer without becoming soft and is redder and riper by the time it is picked for distribution. In addition, shelf life is increased and tomato products are thicker because of a higher pectin-to-water ratio. Tomato plants were transformed with the antisense PG gene, which encodes mRNA that is complementary to PG mRNA. The two messenger RNAs (anti-PG mRNA and PG mRNA) may bind to one another so that they are degraded and translation of PG protein is prevented. Antisense technology is being developed to block the production of many other proteins that will have application in agriculture and medicine. Rathore (2010), Rajendra prasad *et al* (2018), Sudarshan *et al* (2018)

The Flavr Savr tomato was the first genetically engineered food to be reviewed by the U.S. regulatory network. The Food and Drug Administration (FDA) approved the Flavr Savr tomato in May 1994, making it the first genetically engineered whole food to gain government approval in any country. The FDA ruled that the Calgene tomato was as safe as a conventional tomato and did not require labeling in markets to indicate which gene was added or that it was genetically modified. Calgene identified their tomatoes as Mac Gregor's brand. In the summer of 1994, the tomatoes were first sold in Chicago-area markets. Labels identified them as genetically modified. They were a success; however, the tomatoes were delicate and many were damaged during shipping. In addition, the cost of developing the Flavr Savr had been high, die tomatoes did not grow well in Florida, and tomato prices were low. To add to Calgene's financial problems, Monsanto filed a patent-infringement lawsuit against Calgene. To settle the suit, Calgene sold 55% of its shares to Monsanto. The Flavr Savr tomato was sold through 1996, including in a small market in Canada. By 1997, Monsanto purchased the remaining shares, and the Flavr Savr tomato was no longer, marketed. Ugender *et al* (2010)

In the mid-1990s, Monsanto, DNA Plant Technologies (DNAP), and Agritope developed and received regulatory approval for genetically engineered delayed ripening tomatoes using a different approach from Calgene's Flavr Savr. None of these varieties have been marketed. Although genetically engineered tomatoes are not on the market, numerous companies (for example, Calgene, Agritope, Seminis, Aventis, and DNA Plant

Technologies) are still developing genetically engineered varieties of delayed-ripening tomatoes. Venkateshwarlu M (2018), Rao (1998).

Pollen Control Hybrid crops are primarily used in agriculture today because they often have improved traits over the parent plants. A hybrid is made by crossing two distantly related varieties of the same crop plant. For example, two varieties of soybean are crossed. The resulting hybrid may be taller, produce more seeds and higher yields, and may be more resistant to environmental pressures. The mixing of pollen (male) and ovule (female) for fertilization must occur in a controlled manner to ensure that the correct parental plants are used in the cross. Because many types of crop plants have both male and female reproductive structures on the same plant, methods must be applied to prevent the plant from fertilizing itself. Plant breeders use different methods to control pollen transfer: One way is to remove the male flower parts by hand before pollen is released. An example is removing the tassel (male part) on a corn stalk (female part is the ear). Fig-1

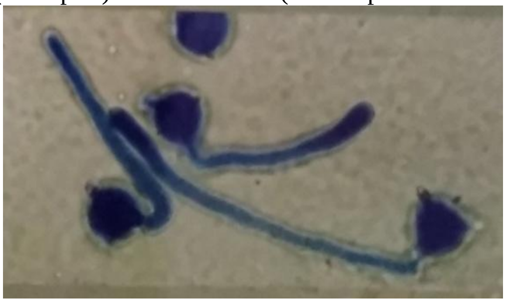

Fig-1 (Pollen Germination)

To simplify this process, scientists have engineered male-sterile plants by inserting in one variety of a particular crop plant a gene that comes from the bacterium *Bacillus amyloliquefaciens* that produces a protein (barnase) in pollen-producing tissues. Barnase blocks pollen production and renders the male reproductive structure sterile. In a different plant variety of the same crop plant, a gene encoding a protein that blocks barnase (called *barstar)* is inserted. When pollen from the barstar plant variety is used to fertilize the male-sterile barnase plant variety, a hybrid plant is produced that is fertile because the barstar and barnase genes will mix. In nature *Bacillus amyloliquefaciens* produces barnase to degrade the RNA of a foreign invader, while barstar is produced to inactivate barnase by binding to it. Two crops, corn and chicory, have been genetically modified with the barnase-barstar genes to make hybrids. Table-1 (Plate-I *In vitro* production of Crop Plants)

(Plate-I *In vitro* production of Crop Plants)

Genetically Engineered Foods

More than 60% of processed foods in the United States contain ingredients that come from genetically engineered plants. Most of these plant ingredients are genetically engineered corn and soybeans, with the rest of the ingredients coming from canola or cottonseed oil. Although 12 different genetically engineered plants have been approved in the United States, with more than one variety of each type of plant, not all the approved varieties are on the market. Many approved genetically modified plant varieties were marketed only briefly and removed from stores, while some have never been marketed or are available in only some regions or food products. Campestrini *et al* (2006), Ram D (2002), Rajashekaran (2006)

Soybean In 2020, approximately 74% of the U.S. soybean crop was genetically engineered. Soybean products include vegetable oil, soy flour, and soy protein. Several soybean varieties have been modified to be resistant to broad-spectrum herbicides (see the previous discussion on soybeans and herbicides). In 2003, scientists at the U.S. Department of Agriculture (USDA), Pioneer Hi-Bred International, and Dupont removed a primary antigen from soybean. This protein, called P34, is a major soybean allergen and can cause a severe allergic response in sensitive individuals. The increased usage of soybean in processed foods poses a risk to people allergic to soybean; however, the elimination of a major allergen should allow people to ingest these foods without a major immune response. Although this research was experimental, it should have important implications for products that cause allergies such as peanut-containing processed foods.

Corn In 2016 approximately 40% of field corn in the United States was genetically engineered. Products include corn oil; corn syrup; corn flour; baking powder; corn starch; gluten; sweeteners such as fructose, dextrose, and sorbital; alcohol; and nutritional supplements such as vitamin C. Sweet corn makes up less than 3% of genetically engineered corn, and there is not a genetically modified popcorn. Bt insect resistance is the most commonly genetically engineered trait in corn and provides resistance to insect pests such as the European corn borer, a moth larva that burrows into corn stalks and damages the plants. Other genetically engineered traits include resistance to different herbicides. By 2000 approximately 25% of the U.S. corn crop was genetically engineered, with 72% being Bt corn, 24% herbicide-resistant varieties, and 4% a combination of both (Bt and herbicide resistant).

Canola Canola, extracted from the rapeseed plant, is grown primarily in Canada. More than 60% of the crop in 2016 was genetically engineered. Canola is found in many processed foods and is common cooking oil.

Cotton More than 71% of the 2016 cotton crop was genetically engineered. Genetically modified cottonseed oil is common in processed foods such as pastries, snack foods, fried foods such as potato chips, peanut butter, and candies, to name a few.

Other Crops Genetically engineered papaya (only in Hawaii) and squash (a small number of farmers plant these varieties) are grown only rarely in the United States. Other plants approved for commercialization include tomato, rice, sugar beet, flax, and red heart chicory, although these are not marketed in the United States and are not present in foods.

Nutritionally Enhanced Plant - Golden Rice: An International Effort

Much of the world's population suffers from malnutrition. It is the hope of many that biotechnology can provide the means to help increase the production of food, as well as to provide nutritionally enriched foods to those who need it. Because more than one-third of the world's population relies on rice as a food staple, one of the first projects was to enhance the quality of rice. Unfortunately, natural varieties of rice do not supply vitamin A. Vitamin A deficiency causes 500,000 cases of irreversible blindness in children each year and other vitamin A—deficiency related diseases, especially in the heavily populated areas of Latin America, Asia, and Africa.

Genetic engineering technology has been used to create a variety of rice, Golden Rice, with high levels of beta-carotene and other carotenoids. These carotenoids are precursors to vitamin A. Golden Rice was developed by two scientists, Dr. Ingo Potrykus of the Institute for Plant Sciences at the Swiss Federal Institute of Technology in Zurich, Switzerland, and Dr. Peter Beyer of the Center for Applied Biosciences at the University of Freiburg in Germany. This international effort was funded in part by the Rockefeller Foundation and the European Union in the hopes that Golden Rice can be distributed free of charge to farmers in developing countries. A number of agencies are now working to distribute Golden Rice worldwide. The inventors of this technology have sold the rights to Syngenta, one of the world's leading agricultural biotechnology companies, with sales in 1999 of approximately $7 billion. The recent surge in the healthy foods market in the industrialized world may pave the way for nutritionally enriched genetically modified plants.

Syngenta is exploring commercial opportunities for Golden Rice in the United States and Japan, as well as providing expertise in making this product available at no cost to developing countries. Several other biotechnology companies also are helping in the endeavor by providing assistance to developing countries. For example, Monsanto will provide royalty-free licenses for its technology, which will aid in the development of Golden Rice and other vitamin A—enriched rice varieties. Although Golden Rice is not yet available for planting and consumption, plans are to have it ready for distribution in the near future after additional testing. Other nutritionally enhanced crops that are being produced by genetic engineering include iron-enriched rice and a tomato variety with three times the normal amount of beta-carotene. Conventional breeding methods are also being used to increase the vitamin and mineral content of rice, wheat, corn, beans, and cassava.

Molecular Farming

A new field is emerging whereby plants (and animals) are being genetically engineered to produce important pharmaceuticals, vaccines, and other compounds of value. This process is called molecular farming. In the not-too-distant future, plants will be used as living bioreactors (just as microbes already are being used) by inserting a foreign gene to produce proteins of therapeutic and industrial value. An inexpensive, readily available complex metabolite could be used as a nutrient source, and cells could then convert this compound into a valuable product, which accumulates until the cells are harvested. Whole plants or seeds would then be harvested and the product extracted, allowing producers to avoid complex *in vitro* biosynthetic reactions in the laboratory.

Soybean plants have been used as bioreactors to produce a variety of monoclonal antibodies with therapeutic value (such as for treatment of colon cancer). Other potential medical products from plants include corn, rice, and tobacco that produce clot-busting drugs and human blood products such as albumin and hemoglobin. In research designed to help slow the pace of HIV infection, Epicyte Pharmaceuticals has produced the first plant lines to produce human antibodies to HIV. Antibody production is a first step in the prevention of HIV, which currently infects 900,000 people in the United States and is predicted to infect as many as 25 million people in India and 15 million in China by the end of this decade. Epicyte is the first U.S. Company to begin Phase I clinical trials with a human herpes antibody produced in plants. This company also has planted a corn crop that produces an antibody to treat respiratory syncytial virus (RSV). The cost of producing foreign compounds in plants may be much lower than producing them in bacteria and mammalian cells for a number of reasons.

1. Commercial scale-up involves simply planting seeds rather than using costlier fermenters.
2. Proteins are produced in high quantity (for example, soybeans have a protein content of 40—45%).
3. Foreign proteins produced are active, unlike those produced by bacteria, which are denatured and must be renatured after isolation.
4. Foreign proteins in stored seeds are very stable (for example, in experiments, they have remained active after 5 years).
5. Pathogens, toxins, viruses, and other contaminants that are harmful to humans or animals are not likely to be present. In addition, because plants are eukaryotes, posttranslational processes (for example, glycosylation, phosphorylation, proteolytic cleavage) are present.

Biopolymers and Plants Recently, the advances made in plant genetic engineering methods have enabled multiple genes to be moved into plants. This provides opportunities for plants to acquire new multistep biochemical pathways. A promising area of plant biotechnology is in the area of biopolymers. As discussed in Microbial Biotechnology, a class of biodegradable thermoplastics called polyhydroxyalkanoate (PHA) polymers has much commercial value, and is produced by microbial fermentation technology. The production of biopolymers in plant seeds would be advantageous in that large amounts of product could be produced and readily extracted. Previous research demonstrated that a type of PHA polymer called *poly β-hydroxy butyrate* (PHB) is produced in the experimental plant model *Arabidopsis.* PHB is synthesized in a multistep pathway requiring three bacterial genes. Researchers have moved these genes from the bacterium *Alcaligenes eutrophus* into canola (rapeseed) for synthesis of PHB in seeds. They were successful in producing the polymer as 1% of canola seed weight. Other biopolymers also are being investigated. For example, the PHA polymer PHBV (poly β-hydroxybutyrate-co-3-hy-droxyvalerate) is currendy manufactured from fermentation of *Alcaligenes* and sold under the name *Biopol* by Monsanto. Plants are able to produce PHBV using genes from *Alcaligenes.*

Plant growth regulators and concluded that BAP, Kn, 2,4-D the highest frequency of the well growing shoots. *In vitro* regeneration trails followed by *in vivo* plant leaf explants acclimatization. The results showed a variable shoot forming capacity depending on the combination of growth regulators used in the culture medium. The number of shoots produced increased with the concentration of BAP and Kn until 1.5 mg/l or 0.5 mg/ l of the cytokinin and showed high frequency of leaf explants exhibiting compact green callus with shoots (4-6). The present study demonstrates the successful shoot regeneration from the *in vitro* cultured Leaf explants of *Papaya & Luffa* and the efficacy of the plant growth regulators was assessed by counting the number of shoots per leaf callus as well showed that 3.0 mg/l NAA and 2.0 mg/l BAP was found best for callus induction and growth. But in the present experiment, a higher level of NAA (3.0 mg/l) and BAP (3.0 mg/l) was found best for callus induction, growth and also for shot induction. MS medium supplemented with 1.5 or 0.5 mg l^{-1} of BAP and Kn shoot regeneration was obtained within 15-20 days and proliferation was also observed in the same concentration of medium. Upadhyay *et al*. has also showed that 0.5 mg/l of cytokinin (BAP and Kn) was found best for shoot regeneration and shoot proliferation. (Plate-I, Table-I). A series of in vivo and in vitro plants were successfully produced and chemical analysis revealed contents of high frequency of shoots directly from leaf explants.

Table 1. Biotechnological Appications *in vitro* production of crop plants. (Papaya, Luffa)

Growth regulators (mg/ l)	Leaf explants showing callus response	No. of shoots explants
NAA (0.5) + BAP (0.5)	40	Callus
NAA (1.0) + BAP (1.5)	30	Callus
NAA (2.0) + BAP (2.5)	25	Shoots(1-3)
NAA (3.0) + BAP (3.5)	20	Shoots(2-4)
IAA (1.0) + BAP (2.0)	15	Shoots
2,4,D (2.0) + BAP (3.0)	10	Shoots (2-3)
Kn (1.0) + BAP (1.0)	25	Callus
Kn (2.0) + BAP (2.0)	30	Callus with shoot
Kn (3.0) + BAP (3.0)	20	Shoots (2-4)
Kn (4.0) + BAP (4.0)	15	Shoots (2-3)

Conclusion

Exciting new agricultural biotechnologies are providing solutions to important problems in agriculture. The wise use of modern biotechnology should embrace safer, less toxic agricultural practices as well as the conservation and use of germplasm. Genetically engineered plants can contribute in positive ways toward sustainable agriculture and should provide an alternative to poor agronomic practices that ultimately contribute to environmental degradation. Questions to be asked include how we can become less dependent on chemicals, hybrid seed, and other costly amendments, rather than encouraging high-input solutions that promote soil loss, increased use of chemicals, and the loss of germplasm diversity. Plant genetic engineering will offer sound alternatives that will help decrease our dependence on agronomic practices that promote environmental degradation. Nutrient-enriched plant products and heartier and higher-yield crops will help impoverished areas where malnutrition is high. Important characters such as drought tolerance and increased tolerance to other environmental stresses will enhance crop productivity. Plant-generated vaccines and pharmaceuticals will one day provide low-cost,; readily available, and safe medical products.

New technologies such as microarrays and DNA chips and the genome sequencing of *Arabidopsis*, rice, corn, and other important crop plants will have far-reaching implications for future agriculture. These new developments will revolutionize plant biotechnology and human health care. The future is bright for plant biotechnology.

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