RAMA UNIVERSITY

www.ramauniversity.ac.in

FACULTY OF ENGINEERING & TECHNOLOGY

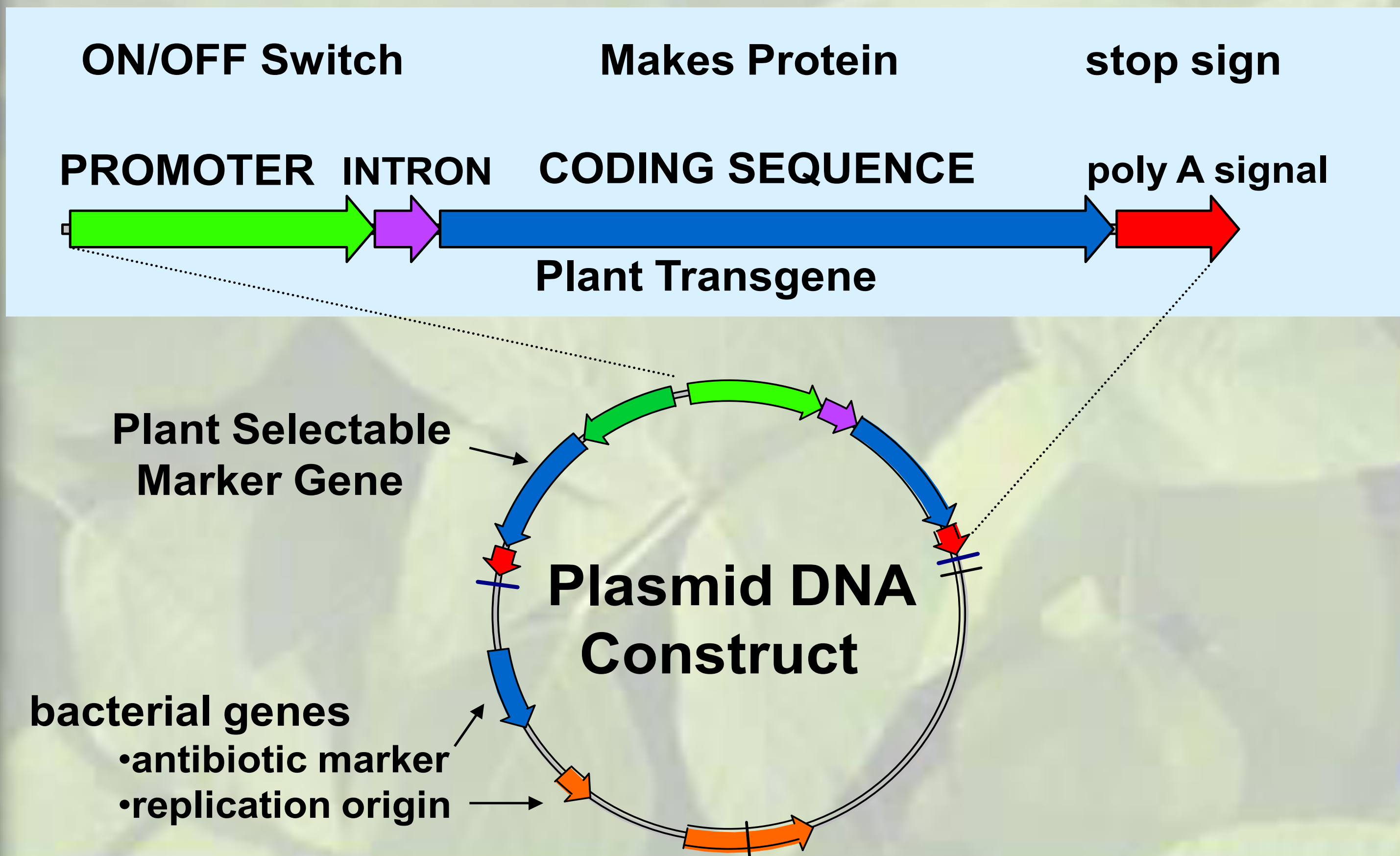
Dr. SIMRANJIT SINGH
Assistant Professor
Dept. of Biotechnology

GMO-Microbes, Plants and Animals

GMO

A genetically modified organism (GMO) or genetically engineered organism (GEO) is an organism whose genetic material has been altered using genetic engineering techniques. These techniques, generally known as recombinant DNA technology, use DNA molecules from different sources, which are combined into one molecule to create a new set of genes. This DNA is then transferred into an organism, giving it modified or novel genes. Transgenic organisms, a subset of GMOs, are organisms which have inserted DNA that originated in a different species.

Building the Transgenes



History of GMO

- Genetic engineering was made possible through a series of scientific advances including the discovery of DNA and the creation of the first recombinant bacteria in 1973, i.e., *Escherichia .coli* expressing a Salmonella gene. This led to concerns in the scientific community about potential risks from genetic engineering which has been thoroughly discussed at the Asilomar Conference in Pacific Grove, California. Herbert Boyer'company, Genentech, in 1978 announced the creation of an E. coli strain producing the human protein insulin.
- In 1986, field tests of bacteria genetically engineered to protect plants from frost damage (ice-minus bacteria) at a small biotechnology company called Advanced Genetic Sciences of Oakland, California, were repeatedly delayed by opponents of biotechnology. There onwards started the advent of genetically engineered microbes.

Application of GMO

GMOs have widespread applications. Genetically modified microbes can be used for the following applications:

1. **Bioremediation**
2. **Industry**
3. **Agriculture**

❑ Genetically engineered microorganism (GEM) for detecting PAHs in the soil

- One of the areas, where genetically engineered organisms have been used and are likely to be used include biodegradation of polyaromatic hydrocarbons (PAHs) in soil. These PAHs include naphthalene, phenanthrene, and anthracene, whose occurrence in the soil is due to spills or leakage of fossil fuels or petroleum products. In USA, *Pseudomonas fluorescens* isolated from PAH contaminated soils, was genetically engineered with lux genes from *Vibrio fischeri*, a bacterium that lives in the light generating organisms of certain deep sea fish. The lux gene was fused with a promoter normally associated with the naphthalene degradation pathway. These lux genes do not need any independent substrate for light production. The modified strain, *P. fluorescens* HK44 responds to napththalene by luminescence, which can be detected with the help of light sensing probes. This will allow the detection of PAHs in the contaminated soils, so that the biodegradations can now be optimized by altering moisture content and level of different gases in the soil.

Genetically engineered microorganism for treating oil-spills

- The first genetically engineered organism for bioremediation was actually produced by Dr. Ananda Mohan Chakrabarty in USA. This GEM was a Pseudomonas, which was capable of degrading 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). the strain contained two plasmids, each providing a separate hydrogen degradative pathway, and therefore was claimed to be effective in treating oil spills. Several other microbes have been developed through genetic engineering for treatment of oil spills.

❑ Genetically engineered microorganism for sequestering of heavy metals

- A new approach for bioremediation that was suggested recently, involved engineering of microorganisms to enhance their ability of sequester heavy metals in the soil. In this approach, the toxic metal within the soil remains bound to the GEM, so that it is less likely to be taken up either by the underground part (roots) of the terrestrial plants, or by other plants or animals living in the soil. The enhanced ability to sequester heavy metals (e.g. cadmium) was achieved by transfer of a mouse gene, encoding metallothionein of a *Ralstonia eutropha* (a natural inhabitant of soil). Metallothionein in this GEM was expressed on the outer surface of the cells to help in sequestering of cadmium.

1.	synthesis and export of MTβ	**2.**	Genetically engineered *Ralstonia eutropha* soil
3.	inoculation	**4.**	cd-sensitive plant

Issues involve in application of GMO in bioremediation

Many issues remain to be resolved before this method is adopted widely. Priority areas of research include the following:

- ✓Improving microbial strains;
- ✓Improving bioanalytical methods for measuring the level of contaminants
- ✓Developing analytical techniques for better understanding, control and optimization of environmental and reactor systems

Using Genetically Engineered Microbes in Industry

- In recent years, micro organisms have found their application not only in the production of a variety of metabolites but also in the bio-transformation of several chemicals. The genetically engineered micro organisms are also being used for the commercial production of some non microbial products such as **insulin, interferon, human growth hormone and viral vaccines.** Microbes are also being used to meet effectively the crisis in both environment and energy sectors. They can reduce environmental pollution through a variety of processes and other means including the following:

i. Recovery of metals from polluted waterways-

ii. Elimination of sulphur from metal ores and coal fired power and

iii. Use of biofertilizers and biopesticides

iv. In the energy sector, they can be used for production of single cell proteins (SCP) to meet food and fodder problems, and for biogas production to provide energy to electrify villages.

Using Genetically Engineered Microbes in Agriculture

To date the broadest and most controversial application of GMO technology is in agriculture especially in patent-protected food crops which are resistant to commercial herbicides or are able to produce pesticidal proteins from within the plant, or *stacked trait* seeds, which do both. The largest share of the GMO crops planted globally is owned by the US firm Monsanto.

Different application of GMO in production of crops which resist different types of viral, bacterial and insect pest :

- Potato - modified to produce a beetle killing toxin
- Yellow squash – modified to contain viral genes that resistant to the most common viral diseases
- Develop foods that contain vaccines and antibodies that offer valuable protection against diseases such as cholera, hepatitis, and malaria
- Canola – modified to resist one type of herbicide or pesticide

Some Approved Agricultural Biotech Products

Canola
LibertyLink® Canola

InVigor® Hybrid Canola

Roundup Ready® Canola

Corn
Attribute™ Bt Sweet Corn

CLEARFIELD Corn®

DeKalbBtTM Insect-Protected Hybrid

DeKalb Brand Roundup Ready®

Gray Leaf Spot -Resistant Corn Hybrids

StarLink Corn

YieldGardTM Insect-Protected Corn

Soybeans
High Oleic Acid Soybeans

Low Linolenic Soybean Oil

Low Saturate Soybean Oils

Peanuts

High Oleic Peanuts

Papaya
Rainbow and SunUp

Cotton
Bollgard® Insect-Protected Cotton

Roundup® Ready Cotton

Milk Production
Chymogen®

Posilac® Recombinant Bovine Somatotropin

ChyMax®

Potatoes
NewLeaf® Insect-Protected Potato

NewLeaf® Plus

New-Leaf® Y Insect- and Virus-Protected Potatoes

Tomatoes

FreshWorld Farms® Tomato

FreshWorld Farms Endless Summer®

FreshWorld Farms® Cherry

Sunflowers
High Oleic Sunflower

High Oleic Sunflower Oil

WHAT ARE THE DANGERS OF USING GMO TECHNOLOGY?

Following issues are of great concern regarding GMO

1. Fundamental weaknesses of the concept
2. Health hazard and environmental hazard and related food safety
3. Increased corporate control of agriculture and unintended economic consequences

Fundamental Weaknesses of the Concept

- **Imprecise Technology**—A gene can be cut precisely from the DNA of an organism, but the insertion into the DNA of the target organism is basically random. As a consequence, there is a risk that it may disrupt the functioning of other genes essential to the life of that organism. (Bergelson 1998)
- **Side Effects**—Genetic engineering is like performing heart surgery with a shovel. Scientists do not yet understand living systems completely enough to perform DNA surgery without creating mutations which could be harmful to the environment and our health. They are experimenting with very delicate, yet powerful forces of nature, without full knowledge of the repercussions. (Washington Times 1997, The Village Voice 1998)
- **Widespread Crop Failure**—Genetic engineers intend to profit by patenting genetically engineered seeds. This means that, when a farmer plants genetically engineered seeds, all the seeds have identical genetic structure. As a result, if a fungus, a virus, or a pest develops which can attack this particular crop, there could be widespread crop failure. (Robinson 1996)
- **Threatens Our Entire Food Supply**—Insects, birds, and wind can carry genetically altered seeds into neighboring fields and beyond. Pollen from transgenic plants can cross-pollinate with genetically natural crops and wild relatives. All crops, organic and non-organic, are vulnerable to contamination from cross-pollinatation. (Emberlin et al 1999)

Health and environmental hazard and related food safety

Health Hazards

No Long-Term Safety Testing—Genetic engineering uses material from organisms that have never been part of the human food supply to change the fundamental nature of the food we eat. Without long-term testing no one knows if these foods are safe.

Toxins—Genetic engineering can cause unexpected mutations in an organism, which can create new andhigher levels of toxins in foods. (Inose 1995, Mayeno 1994)

Allergic Reactions—Genetic engineering can also produce unforeseen and unknown allergens in foods. (Nordlee 1996)

Decreased Nutritional Value—Transgenic foods may mislead consumers with counterfeit freshness. A luscious-looking, bright red genetically engineered tomato could be several weeks old and of little nutritional worth.

Antibiotic Resistant Bacteria—Genetic engineers use antibiotic-resistance genes to mark genetically engineered cells. This means that genetically engineered crops contain genes which confer resistance to antibiotics. These genes may be picked up by bacteria which may infect us. (New Scientist 1999)

- **Problems Cannot Be Traced**—Without labels, our public health agencies are powerless to trace problems of any kind back to their source. The potential for tragedy is staggering.
- **Can Side Effects Kill Human Beings?**-37 people died, 1500 were partially paralyzed, and 5000 more were temporarily disabled by a syndrome that was finally linked to tryptophan made by genetically-engineered bacteria. (Mayeno 1994)
- **Environmental Hazards**
- **Increased use of Herbicides**—Scientists estimate that plants genetically engineered to be herbicide-resistant will greatly increase the amount of herbicide use. (Benbrook 1999) Farmers, knowing that their crops can tolerate the herbicides, will use them more liberally.
- **More Pesticides**—GE crops often manufacture their own pesticides and may be classified as pesticides by the EPA. This strategy will put more pesticides into our food and fields than ever before.
- **Ecology may be damaged**—The influence of a genetically engineered organism on the food chain may damage the local ecology. The new organism may compete successfully with wild relatives, causing unforeseen changes in the environment. (Metz 1997)
- **Gene Pollution cannot be cleaned Up**—Once genetically engineered organisms, bacteria and viruses are released into the environment it is impossible to control or recall them. Unlike chemical or nuclear contamination, negative effects are irreversible.

GM crops and food security

Arguments about whether genetically modified crops can increase food security for farmers and consumers in the developing world have been at the heart of debates about agricultural biotechnology for over a decade. Opponents of GM farming believe that the technology's failure to produce a decisive breakthrough on this front to date is proof that the technology's potential has been inflated by an overblown hype that has been built on a number of doubtful assumptions about the role of technology in "feeding the world". For their part, advocates of GM crops argue that important new benefits are just around the corner, and urge a quicker and more enthusiastic embrace of GM crop technology.
These debates about biotechnology and its potential contribution to food security revolve around issues of access and control – especially the roles played by public and private sectors, and the effects of intellectual property rights (IPRs), in shaping the types of biotechnologies that are developed and how they are made available.

Some critics argue that the enthusiasm for genetically modified crops reflects a fixation with the quick fix – technological "silver bullets" that can overcome problems which are actually rooted in social, economic and political institutions and structures. Others believe that obstacles to the free flow of knowledge and technology, which are imposed by restrictive IPRs, hamper the efforts of scientists working to develop "pro-poor" biotechnologies for farmers in the developing world.

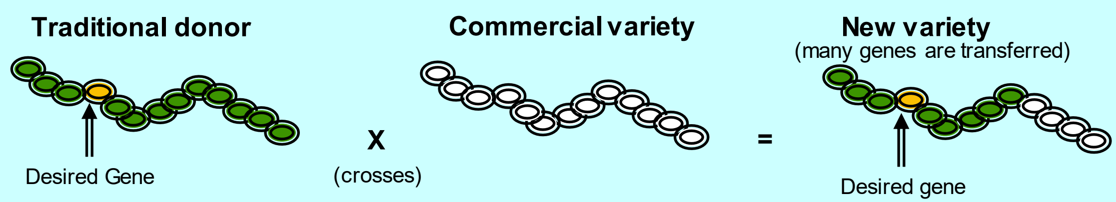
But many international organizations and aid donors take the position that, if the public and private sectors can work in complementary ways, in a context where IPRs are properly protected and technologies can be licensed for use, it will be possible to develop new types of GM crops and other biotechnologies that will more directly address the needs of farmers and consumers in the developing world.

GM vs. Mendel's Selective breeding

Selective breeding	GM
Slow	Very fast
Imprecise	Precise
Modification of genes that naturally occur in the organism	Can introduce genes into an organism that would not occur naturally

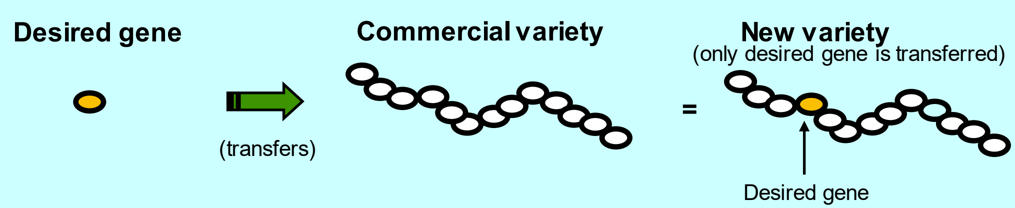
Traditional plant breeding

DNA is a strand of genes, much like a strand of pearls. Traditional plant breeding combines many genes at once.



Plant biotechnology

Using plant biotechnology, a single gene may be added to the strand.



Increased corporate control of agriculture and unintended economic consequences

Another concern associated with GMOs is that private companies will claim ownership of the organisms they create and not share them at a reasonable cost with the public. Use of genetically modified crops will hurt the economy and environment, because monoculture dominates over the diversity contributed by small farmers who can't afford the technology.

Possible Benefits of GM Foods

Easing of world hunger

Development of crops that can be grown in marginal soil

Reduced strain on nonrenewable resources

- Development of drought resistant crops.
- Development of salt-tolerant crops.
- Development of crops that make more efficient use of nitrogen and other nutrients.

Reduced use of pesticides and herbicides

- Development of pest resistant crops.
- Reduced herbicide use is better for the environment and reduces costs for farmers.

Improved crop quality

- Development of frost resistant crops.
- Development of disease resistant crops.
- Development of flood resistant crops.

Improved nutritional quality

- Development of foods designed to meet specific nutritional goals

Main controversies arises regarding GMOs

Safety

- Potential human health impacts, including allergens, transfer of antibiotic resistance markers, unknown effects
- Potential environmental impacts, including: unintended transfer of transgenes through cross-pollination, unknown effects on other organisms (e.g., soil microbes), and loss of flora and fauna biodiversity

Access and Intellectual Property

- Domination of world food production by a few companies
- Increasing dependence on industrialized nations by developing countries
- Biopiracy, or foreign exploitation of natural resources

Ethics

- Violation of natural organisms' intrinsic values
- Tampering with nature by mixing genes among species
- Objections to consuming animal genes in plants and vice versa
- Stress for animal

Labeling

- Not mandatory in some countries (e.g., United States)
- Mixing GM crops with non-GM products confounds labeling attempts

Society

- New advances may be skewed to interests of rich countries.

Act and Regulations on genetically modified organisms in India

In India, the Genetically Modified Organisms are regulated under the *Environment Protection Act 1986* (EPA).

In addition the Indian biosafety regulatory framework comprises:

- Rules for the "Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms, genetically Modified Organisms and Cells" (1989 Rules),
- Department of Biotechnology guidelines, the 1990 "Recombinant DNA Safety Guidelines" (1990 DBT Guidelines)
- Revised Guidelines for "Safety in Biotechnology" (1994 DBT Guidelines)
- Revised Guidelines for "Research in Transgenic Plants and Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant Parts" (1998 DBT Guidelines).
- Seed Policy, 2002

Objectives of regulations

- The objective of EPA is protection and improvement of the environment. The Act calls for the regulation of Environment Pollutants, defined as any solid, liquid or gaseous substance, present in such concentration that tend to be injurious to the environment.
- The 1990 and 1994 DBT guidelines recommend appropriate practices, equipments and facilities necessary for safeguards in handling GMOs in agriculture and pharmaceutical sectors. These guidelines cover the R&D activities on GMOs, transgenic crops, large-scale production and deliberate release of GMOs, plants, animals and products into the environment, shipment and importation of GMOs for laboratory research.
- The 1998 DBT guidelines cover areas of recombinant DNA research on plants including the development of transgenic plants and their growth in soil for molecular and field evolution. It also calls for the toxicity and allergenicity data for ruminants such as goats and cows, from consumption of transgenic plants. It also requires the generation of data on comparative economic benefits of a modified plant.

The regulations classify activities involving GMOs into four risk categories

- **Category I** comprises *routine* recombinant DNA experiments conducted inside a laboratory;
- **Category II** consists of both laboratory and greenhouse experiments involving transgenes that combat biotic stresses through resistance to herbicides and pesticides;
- **Categories III** and IV comprise experiments and field trials where the escape of transgenic traits into the open environment could cause significant alterations in the ecosystem.

The regulatory framework for GMO in India

The two main agencies responsible for implementation of the rules are the Ministry of Environment and Forests (MoEF) and the Department of Biotechnology (DBT), Government of India. The rules have also defined competent authorities and the composition of such authorities for handling of various aspects of the rules.

There are six competent authorities as per the rules:

- **Recombinant DNA Advisory Committee (RDAC)**
- **Review Committee on Genetic Manipulation (RCGM)**
- **Genetic Engineering Approval Committee (GEAC)**
- **Institutional Biosafety Committees (IBSC)**
- **State Biosafety Coordination Committees (SBCC)**
- **District Level Committees (DLC).**

Out of these, the three agencies that are involved in approval of new transgenic crops are:

- IBSC set-up at each institution for monitoring institute level research in genetically modified organisms.
- RCGM functioning in the DBT to monitor ongoing research activities in GMOs and small scale field trials.
- GEAC functioning in the MoEF to authorize large-scale trials and environmental release of GMOs.

Cartagena Biosafety Protocol

- The Cartagena Protocol on Biosafety, the first international regulatory framework for safe transfer, handling and use of living Modified Organisms (LMOs) was negotiated under the aegis of the Convention on Biological Diversity (CBD). The Protocol was adopted on 29th January, 2000. One hundred and forty three countries have signed the Protocol. India has acceded to the Biosafety Protocol on 17th January 2003. The Protocol has come into force on 11th September, 2003. As of date, 143 countries are parties to the Protocol.

Some Useful links regarding the details of biosafety regulations

- http://www.envfor.nic.in/divisions/csurv/geac/geac_home.html
- http://dbtbiosafety.nic.in
- http://www.igmoris.nic.in

Risk associated with genetically modified (GM) food

Sl. No.	Risk of genetically modified foods	Description
1.	Allergenicity	An allergic reaction is an abnormal response of the body's immune system to an otherwise safe food. Some reactions are life threatening, such as anaphyletic shock (a sever allergic reaction that can lead th death). To avoid introducing enhancing an allergen in an otherwise safe food, the biotechnolgy food industry evaluates genetically modified (GM) foods to determine wheather they are "as safe as" their natural counterparts. For example, in 1996 FDA reviewed the safety assessement for a GM soyabean plant that can produce heatlther soyabean oil. As part of a standard safety assessment, the GM soyabean was evaluated to see if it was safe as a conventional soyabean. Although soyabeans are a common food allergen and the GM soyabean remained allergenic, the results showed no significant difference between its allergenicity and that of conventional soyabeans. Specifically, serum (blood) from individuals allergic to the GM soyabean showed the same reactions to conventional soyabeans.
2.	Toxic reaction	A toxic reaction in human is a response to a posionous substance. Unlike allergic reactions, all humans are subject to toxic reactions. Scientists involved in developing a GM food aim to ensure that the level of toxicity in the food does not exceed the level in the food's conventional counterpart. If a GM food has toxic components outside the natural range of its conventional counterpart, the GM food is not acceptable. To date, GM foods have proven to be no different from their convetional counterpart with respect to toxicity. In fact, in some cases there is moreconfidence in the safety of GM foods because naturally occuring toxins that are disregareded in conventional foods are measured in the pre-market safety assessment of GM foods. For example, a naturally occuring toxin in tomatoes, known as "tomatine" was largely ignored until a company in the early 1990s developed a GM tomato. FDA and the company considered it important to measure potential changes in tomatine. Through an analysis of conventional tomatoes, they showed that the levels of tomatine, as well as othe similar toxins in the GM tomato, were the range of its convrentional counterpart
3.	Anti-nutritional effects	Anti nutrient s are naturally occurring compounds that interfere with absorption of important nutrients in digestion. If a GM food contains anti-nutrients, scientists measure the levels and compare them to range of levels in the food's conventional counterpart. If the levels are similar, scientists usually conclude that GM food is as its conventional counterpart. For example, in 1995 a company submitted to FDA a safety assessment for GM canola. The genetic modification altered the fatty acid composition of canola oil. To minimize the possibility that an unintendec anti-nutrient effect had rendered the oil unsafe, the company compared the anti-nutrient composition of its product to that of conventional canola. The company found that the level of anti-nutrients in its canola did not exceeds the levels in conventional canola. To ensure that GM foods do not have decreased nutritional value, scientists also measure the nutrient composition, or "nutrition profile", of these foods. The nutrient profile depends on the food, but it often includes amino acids, oils, fatty acids, and vitamins.

Bt Crops Under Development

Sr. No.	Crop	Organisation(s)	Traits/Gene
1	Brinjal	Mahyco, Mumbai (Recommended or commercialization by GEAC in Oct. 2009 meeting)	Insect resistance /cry 1Aa nad cry 1 Asbc cry 1Ac cry 1Ac
2	Cabbage	Nunhems India Pvt. Ltd.	Insect resistance/cry 1Ba and cry 1CA
3	Cauliflower	Sungro Seeds Ltc., New Delhi nunhems India Pvt. Ltd.	Insect resistance/cry 1Ac, cry 1Ba and cry 1Ca
4	Cotton	Mahyco, Monsanto, Rasi, Nuziveedu, Amkur, JK Seed, CICR, UAS-D	Insect Resistance, herbicide tolerance cry 1Ac gene
5	Groundnut	ICRISAT, Hyderabad	Virus resistance/Chitinase gene
6	Maize	Monsanto, Mumbai	Shoot borer/cry 1Ab gene
7	Chickpea	ICRISAT	Insect Resistance/Pod borrer, Cry 1Ac
8	Mustard	UDSC, New Delhi	Hybrid seed, barnase/barstar gene
9	Okra	MAHYCO, Mumbai, Beejo Sheetal, Jalna	Borer cry 1Ac, cry 2Ab
10	Pigeon Pea	ICRISAT, MAHYCO	Pod borer and Fungal pathogene, Cry 1Ac and chitinase
11	Potato	CPRI, Shimla, NIPGR, New Delhi	Ama 1 and Rb gene derived from Solanum bulbocastanum
12	Rice	MAHYCO, Mumbai TNAU, Coimbatore	cry 1B-cry 1Aa fusion gene cry 1Ac, cry2Ab
13	Sorghum	NRCS, Hyderabad	Insect Resistance, Shoot borer
14	Tomato	IARI, New Delhi MAHYCO, Mumbai NIPGR, New Delhi	Antisense replicase gene of tomato leaf curl virus cry 1Ac

(Source: Dr. K.S. Charak, DBT)

CURRENT INDIAN FIELD TRIALS OF GM CROPS (CONTAINING NEW GENES/EVENTS: 2013

Sl. No.	Crop	Company Name	Trial	Trait	Gene/Event
1.	RRF Cotton	Maharashtra Hybrid Seeds Company Ltd.	BRL-I 2nd year	Herbicide tolerance	cp4epsps/ MON 88913
2.	Corn	Syngenta Biosciences Pvt. Ltd.	BRL-I	Insect Resistance and Herbicide Tolerance	events Bt11, GA21 and stack of Bt11 x GA21
		Syngenta Biosciences Pvt. Ltd.	BRL-I 2nd year	Insect Resistance and Herbicide Tolerance	Bt11, GA21 and stack event of Bt11 x GA21
		Syngenta Biosciences Pvt. Ltd.	Seed Increase	Insect Resistance and Herbicide Tolerance	Bt11 and GA21
		Monsanto India Ltd.	BRL-I 2nd year	Insect Resistance	cry2Ab2 and cry1A.105genes (Event MON 89034)
3.	Herbicide tolerant maize	Monsanto India Ltd.	BRL-I 2nd year	Herbicide tolerance	cp4epsps (Event NK603)
4.	TwinLink® Cotton	Bayer Bioscience Pvt Ltd	BRL-I	Insect Resistance	stacked events namely GHB119 (cry2Ae/PAT) & T304-40 (cry1Ab/PAT) containing cry1Ab, cry2Ac and bar
5.	Herbicide tolerant Glytol cotton	Bayer Bioscience Pvt Ltd	BRL-I (2nd season)	Herbicide tolerance	2mepsps(Event GHB 614)

Thank You