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Lesson: Role of Biotechnology in Agriculture

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Introduction

Biotechnology employs biological systems to create useful products. It is a welcome technology in agriculture as it provides agricultural industry relevant varieties for unique environmental conditions. According to Food and agriculture organization (FAO) 2009, world population is expected to reach 9.1 billion by 2050. To meet the increasing demand of world population, food production has to increase by 70%. Agriculture is affected greatly by changes in climatic conditions as adverse changes in environment leads to abiotic stress to plants. There is a need for sustainable food security, and even to increase nutritional quality of food.

Genetic modification of plants was initially carried out using traditional methods of plant breeding. This was achieved by crossing two different plant varieties to develop a cultivar which possesses the desirable characteristics of both parent plants. But the problem in using this method is that along with desirable characteristics, many undesirable traits often appear in the progeny. These undesirable traits can be removed by time consuming additional breeding experiments. Breeders can further select and multiply the progeny with the desired traits.

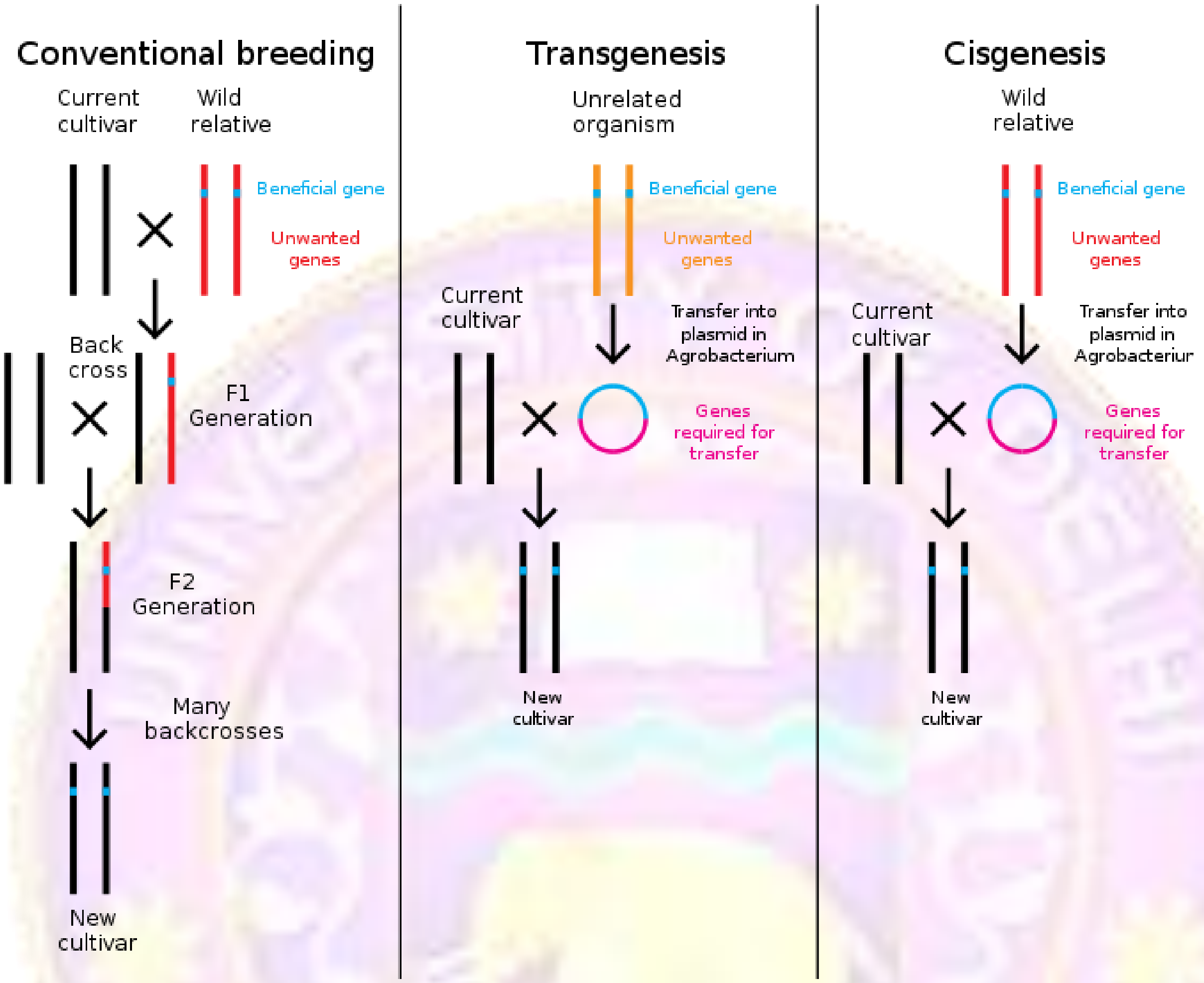


Figure: Generation of new cultivars using conventional breeding vs. transgenic approach. In transgenic approach, new genes may be incorporated from related species or unrelated organism.

Source: http://en.wikipedia.org/wiki/Cisgenesis (cc)

Genetic engineering techniques enhance the capacity to access potentially important genetic resources present in other species or even organisms for producing more useful plants possessing novel or improved characters. Organisms carrying a fragment of foreign DNA in their genome are called genetically modified organisms, GMOs. These genetically modified organisms are also called transgenics. The first transgenic plant was developed in tobacco by Fraley et.al. (1983). Now there are more than 50 plant species (cotton, tomato, corn, brinjal, potato, sunflower, mulberry, grapes, carrot etc.) for which transgenic plants have been made. The transgenic crops covered 170 million hectares acreage in 2012.

Table: Distribution of transgenic crops in five major transgenic crop growing countries in \the world in the year 2013.

Country	2013– GM planted area (million hectares)	Biotech crops
Canada	10.8	Canola, Maize, Soybean, Sugarbeet
India	11.0	Cotton
Argentina	24.4	Soybean, Maize, Cotton
Brazil	40.3	Soybean, Maize, Cotton
USA	70.1	Maize, Soybean, Cotton, Canola, Sugarbeet, Alfalfa, Papaya, Squash
Total	175.2	----

Source: http://en.wikipedia.org/wiki/Genetically_modified_crops (cc)

Traditional plant breeding is being supported today by genetic engineering programs, especially for production of crop plants with resistance against abiotic or biotic stresses or other improvement traits. The success of biotechnology relies on the hypothesis that resistance to pest, herbicides, diseases and stress can be obtained by inserting genes from foreign sources to cultivated plants. Genetic engineering techniques provide useful solutions to problems of agriculture.

The agricultural crops have following desirable properties for which they can be engineered:

- Crops with multiple resistant traits against biotic and abiotic stress,
- Crops which are high yielding and
- Crops which have improved nutritive qualities.

This chapter highlights the potential applications of biotechnology in agriculture. Some of the important examples are:

- Bt cotton offers a vastly improved method for delivering *Cry*-insecticides to target insects, compared to traditional Bt sprays. It can be effectively used in insect pest management.

- Herbicide resistant plants are genetically modified crops that harbor genes that enable them to degrade the active component in the herbicide, making them harmless to herbicide spray. With herbicide resistant crops, farmers can spray broad spectrum herbicides without killing valuable crops.
- A large number of genes (LEAs, dehydrins, chaperons, osmoprotectants) provide resistance against abiotic stresses.
- Nutritional value of crop plants can also be improved by genetic engineering of crop plants. Golden Rice produced through genetic engineering is capable of synthesizing beta-carotene, a precursor of Vitamin A in the endosperm of transgenic rice. The Flavrsavr tomato is the first genetically engineered tomato with increased shelf life.
- Remarkable progress has been done in the field of horticulture. Moondust” and “Moonglow” are lilac and blue colored carnations created by the Australian company Florigene. Transgenic technology has been utilized for production of these ornamental plants and new varieties have been developed.

Biotic stress resistant plants

Pest resistant plants

Pests are a major threat to agriculture leading to reduced plant productivity. Insects can cause major yield losses, both in the fields and during storage. Breeders have also developed cultivars that can survive and produce higher yields in presence of insects. With the advent of new techniques crop plants have been genetically modified so that they can survive in presence of pests by being toxic to them. Cotton being an important cash crop plays an important role in the Indian economy. Millions of people are engaged in cultivation, processing and trade of cotton. India ranks third in cotton production after China and USA. Insect pests (cotton aphids, bugs, whitefly, caterpillars, spider mites, thirps) are responsible for low yields of cotton. These insects harm cotton plants in various stages of growth as sap-suckers, defoliators and tissue borers. Despite huge spray of chemical insecticides, bollworm infection was not being controlled leading to huge economic losses.

Figure: Cotton bollworm (*Helicoverpa sp.)* on cotton ball

Source: http://theconversation.com/worm-turns-for-cotton-pest-as-australia-breeds-in-resistance-15457 (cc)

Figure: Results of insect infestation on Bt (right) and non-Bt (left) cotton bolls.

Source: http://www.bt.ucsd.edu/gmo.html

***Bt* toxin**

The soil bacterium *Bacillus thuringiensis*, *Bt* is a gram-negative aerobic spore forming bacterium found world-wide. It produces proteins toxic to various herbivorous insects especially to the larvae of insect order lepidoptera, which includes cotton bollworms, moths, butterflies, beetles and flies. It is harmless to mammals including humans, birds, fishes, or other beneficial insects. The toxic protein is produced in inactive crystalline form and is converted to active toxic form called as delta endotoxin only when it is consumed by insects. It is known as cry proteins as it is produced as crystalline proteins inclusions during sporulation. The protein binds to certain receptors (the aminopeptidase N (APN) receptors and the cadherin-like receptors) in insect’s intestine and causes wounding of epithelial midgut cells. This toxin destroys the gut of insect, ultimately leading to death of insect. This toxic protein is also known as Bt protein as it is produced by bacterium *Bacillus thuringiensis.* InUSA, *Bacillus thuringiensis* was registered as a biopesticide but the performance of *Bt* insecticide on cotton plants was limited. The insecticide can be degraded by light, heat, UV, high pH and desiccation. Even the areas where these pests of cotton (bollworms) feed are difficult to treat. The insect must eat sufficient treated plant to accumulate lethal dose of toxin.

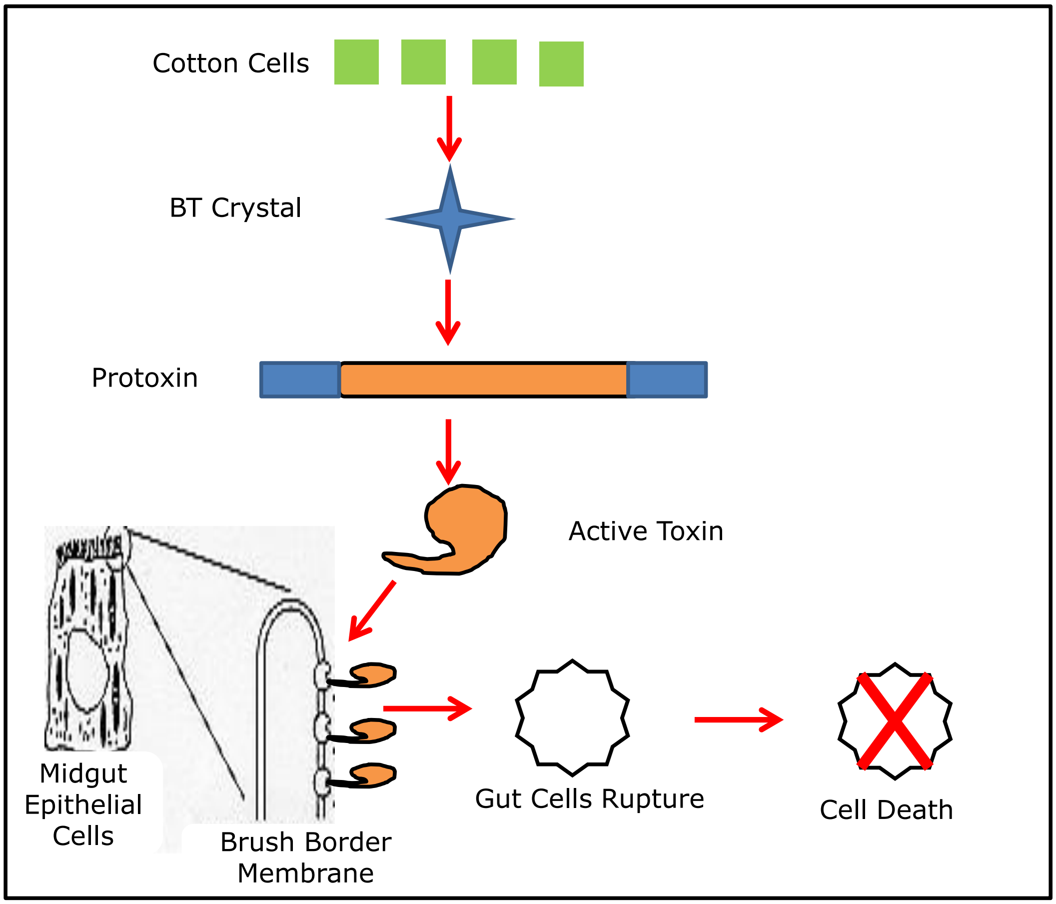


Figure: Mechanism of action of Bt toxin.

Source: Author

Genetic engineering techniques permits the scientists to isolate the bacterial genes required for production of *Bt* toxins and introduce them to plants. Recombinant plants overcome the limitations of insecticidal spray as *Bt* proteins are protected from environmental degradation. The proteins are produced in all the tissues of plant ensuring that larva will eat *Bt* protein wherever it feeds. These transgenic cotton plants are resistant to pests.

Almost 100 diverse forms of *Bt* toxin have been isolated from variable strains of *Bacillus thuringiensis.* The cry proteins and genes encoding them have been classified on the basis of their structure, activity spectrum and antigenic properties into four major groups.

- *CryI* genes are lepidoptera specific,
- *CryII* genes are lepidoptera- and diptera-specific,
- *CryIII* genes are coleoptera-specific and

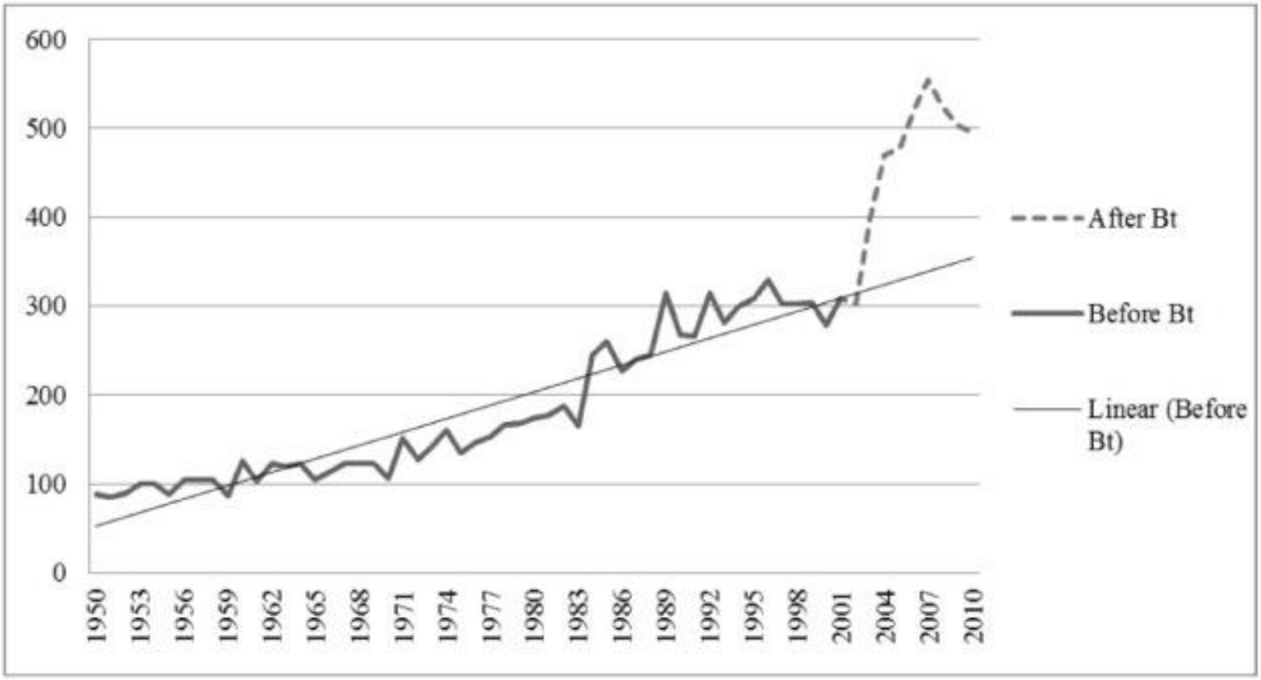
- *CryIV* genes are diptera-specific.

The toxicity of the protein changes with the molecular modification of the cry proteins.

Bt cotton

First Bt recombinant transgenic plants (Bt cotton) were registered in 1995, by the United States Environmental Protection Agency (EPA). Bt technology has been used for other crop as well. Apart from lepidopteran resistant cotton, other Bt crops include: European corn borer resistant corn, corn rootworm resistant corn, Bt eggplant, Colorado potato, beetle resistant Bt potato, potato tuber moth resistant,Bt potato, Bt soybean, and lepidopteran resistant tomato. Use of Bt plants has replaced the use of insecticides and led to global economic benefits from Bt cotton. Important agronomic traits like fiber quality, yield, harvestability, were maintained in recombinant cotton plants harboring *Bt* gene. There was tremendous increase in the yield and significant decrease in production cost. Bt cotton is the only GM crop permitted to be grown in India and China. The Economic Times survey showed that India's cotton yield in 1990-91 was 225 kg per hectare. In 2000-01 (bad monsoon year), it dropped to 190 kg per hectare. In 2002, Bt cotton cultivation began, and area under cotton cultivation increased from 0.29 million hectares in 2002 to 9.4 million hectares in 2011-12. Bt variety were spread in 90% of cotton acreage and therefore cotton yield was increased to 362 kg per hectare in 2005-06, and then rose to 510 kg per hectare in 2010-11.

Figure 2.1—Average cotton yields in India, 1950–2010



Source: Indian Cotton Advisory Board, obtained from International Cotton Advisory Committee.

Figure: Bt Cotton raised yields hugely in India

Time-line for the development and acceptance of *Bt*-Cotton in India

1995	Mahyco (Maharashtra Hybrid Seed Company) applied to Department of Biotechnology (DBT) for seeking permission to import a small seed stock of Bollgard® (*Bt* cotton) from Monsanto Company, USA. Acceptance given by DBT.
1996	100 gms seeds of cotton containing the Bollgard® *Bt* gene, *cry 1Ac,* were received by Mahyco from Monsanto, USA. Mahyco initiated crossing experiments so that the Indian cotton breeding lines harbor *cry 1Ac* gene. 40 elite Indian parental harbored *Bt* trait.
1996-1998	Regulatory studies for risk-assessment were conducted using *Bt*-cotton seeds from Indian parental lines containing *Bt* trait. - Pollen escape studies

	- Effect on non-target beneficial organisms - Aggressiveness and persistence studies - Biochemical analysis - Toxicological studies - Allergenicity studies
1999 – 2000	Field trials were repeated at 10 locations in 6 states. Data was again submitted to RCGM.
2000	The GEAC (Genetic Engineering Approval Committee), Ministry of Environment & Forests, Govt. of India, on the recommendation of RCGM gave approval to Mahyco for conducting large scale field trials and also for undertaking seed production.
2001	Field trials were also conducted by All India Coordinated Cotton Improvement Project of the Indian Council of Agricultural Research (ICAR).
2002	On 26 March 2002, GEAC approved Mahyco's three *Bt*-cotton hybrids, viz. MECH 12, MECH 162 and MECH 184, for commercial cultivation in India. This approval was initially valid for three years and also stipulated a few conditions.

Adverse effects of Bt crops

Though Bt toxin is highly selective, it may lower the general abundance of some beneficial insects. It has been seen that decline in caterpillar population results in less food for predators that attack them. Recent studies have also shown that Bt resistant insects are evolving causing damage to plants. First Bt resistant corn pests were observed in 2011. Additionally genes from Bt crops may be transferred to other native crops by horizontal gene transfer. A report published in 2010, in journal of reproductive toxicology found that *Cry1Ab* can cross placenta to reach fetus and is detectable. The study revealed that the *Cry1Ab* toxin was found in 80% and 93% of fetal and maternal blood samples, respectively and was detected in 69% of tested blood samples from non-pregnant women. The authors speculate that the probable reason could be consumption of meat from animals that fed on Bt corn and had retained the Cry1Ab protein in their flesh.

Herbicide Resistant Crops

Genetically modified herbicide tolerant crops are one of the biotechnological inventions commercially accepted and exploited world-wide. Weeds grow with crop plants and compete for water and nutrients. They significantly decrease the crop yields and productivity and are one of the major problems in crop management. Herbicides are chemicals that kill weeds. Herbicide development and production is a tough task as herbicides cannot differentiate between weeds and crop plants. Therefore selective herbicides that can kill only a specific weed were used in crop management programs. These selective herbicides do not harm the crop plant, but are ineffective in killing all types of weeds. Non-selective herbicides often known as 'broad-spectrum' herbicides are effective at removing wide range of weeds but they can also kill valuable crop plants. Several crops have been genetically modified to make them resistant to non-selective herbicides. These genetically modified herbicide resistant crops harbor genes that enable them to degrade the active component in the herbicide, making the modified plant harmless to herbicide. Farmers can therefore easily control weeds during the entire growing season and have more flexibility in choosing times for spraying. There are four mechanisms of resistance to herbicide action:

A) Altered target site: An herbicide has a specific target site of action where it binds and disrupts a particular plant process. If this target site is altered, the herbicide can no longer bind to its specific site and is unable to exert its toxic effect. This is one of the most common mechanisms of herbicide resistance.

B) Enhanced metabolism: A weed can degrade an herbicide and detoxifies it before it can reach its site of action within the plant.

C) Compartmentalization: Some plants have the ability of sequestering the active compounds of herbicides within their cells or tissues to render the compounds ineffective. Herbicides can be inactivated by binding to a sugar molecule or sequestered to metabolically active regions of the cell like cell wall.

D) Over-expression of the target protein: If the target protein can be produced in significantly large quantities by the plant, then the effect of the herbicide becomes insignificant.

Visit the following link for animation of mechanism of herbicide resistance:

http://passel.unl.edu/pages/animation.php?a=TargetSiteResistance.swf

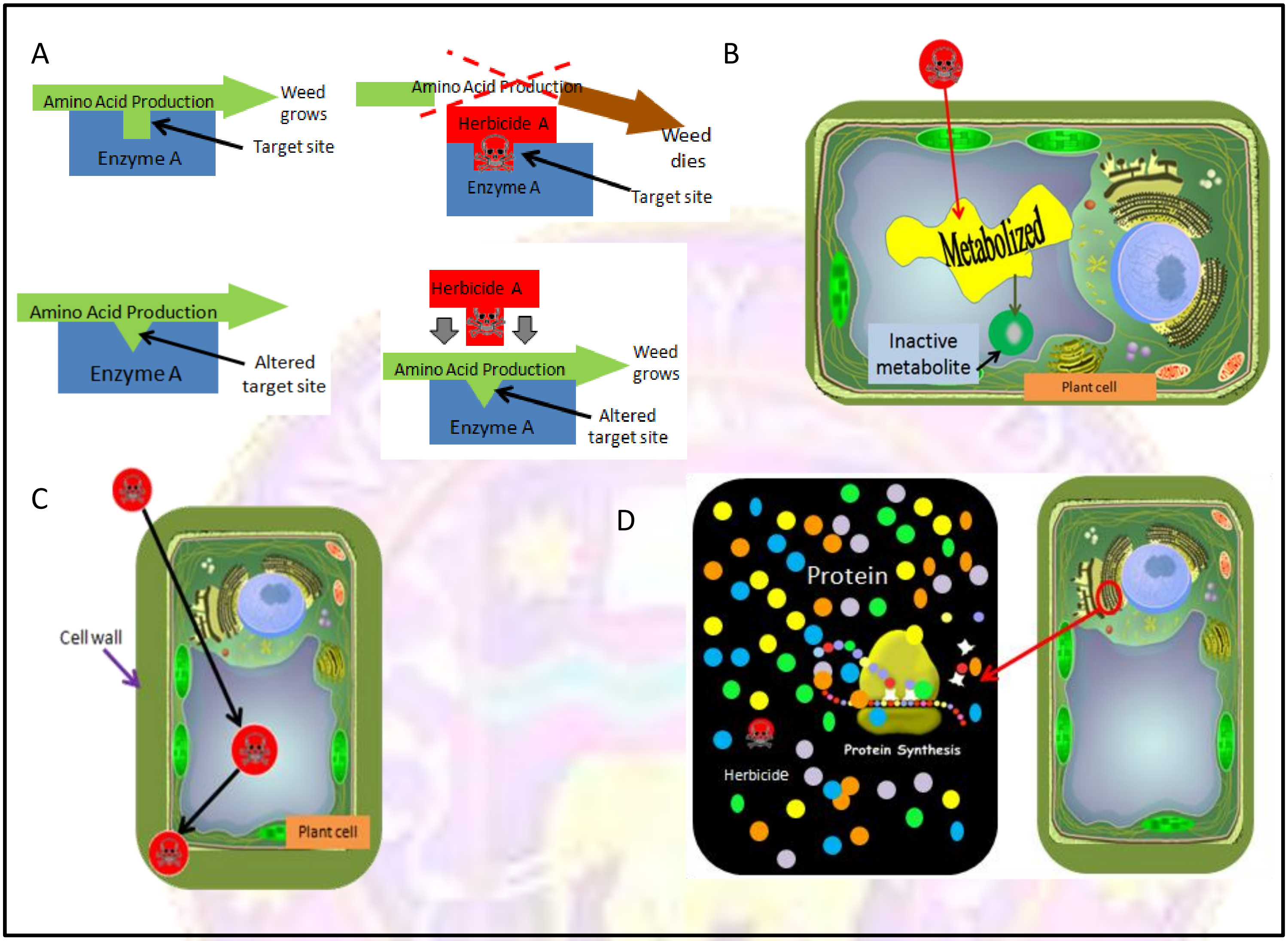


Figure: Mechanism of herbicide resistance

Source: http://pesticidestewardship.org/resistance/Herbicide/Pages/Mechanisms-of-Herbicide-Resistance.aspx

Two herbicide resistant cropping systems are routinely used for soybean, maize, rapeseed, and cotton:

- Monsanto’s *Roundup Ready* with active agent as glyphosate and
- Bayer's *Liberty Link* with active agent as glufosinate.

These are actually broad spectrum herbicides which are effective in killing all green plants except those which are protected as a result of the genetic modification against these components. Use of these herbicide resistant cropping systems enables effective weed control by herbicide application.

Figure: Roundup weatherMax herbicide and seeds of Roundup Ready Soyabens with active agent as glyphosate

Source: http://bio100bgmo.wikispaces.com/ (cc)

Roundup ready system

Roundup ready resistant Soyabean was developed in 1974 and commercialized in 1996. Scientists from Monsanto genetically modified soyabean to contain in-plant resistance genes to Roundup WeatherMAX herbicide. With roundup crops, farmers can spray broad spectrum herbicides without killing valuable crops. Glyphosate, the active agent of roundup ready, interferes with the synthesis of essential amino acids like phenylalanine, tyrosine and tryptophan. Plants and microorganisms can make these essential amino acids with the help of an enzyme called 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The enzyme catalyzes the penultimate step of the shikimate pathway for the biosynthesis of above mentioned essential aromatic amino acids. This enzyme is present only in plants and lower organisms but is absent in animals. Animals obtain these aromatic amino acids from their diet.

Roundup Ready Soybeans have been genetically modified to express a version of EPSPS. Scientists inserted the genetically modified plasmid containing necessary elements for EPSPS expression into soyabean germplasm. The plasmid harbors EPSPS from the CP4 strain of the bacteria , *Agrobacterium tumefaciens* known as CP4 EPSP synthase, 35S promoter (E35S) from cauliflower mosaic virus (CaMV), (CTP4) coding sequence from *Petunia hybrida* encoding a chloroplast transit peptide, and a transcriptional termination element called nopaline synthase (nos 3') from *Agrobacterium tumefaciens*.

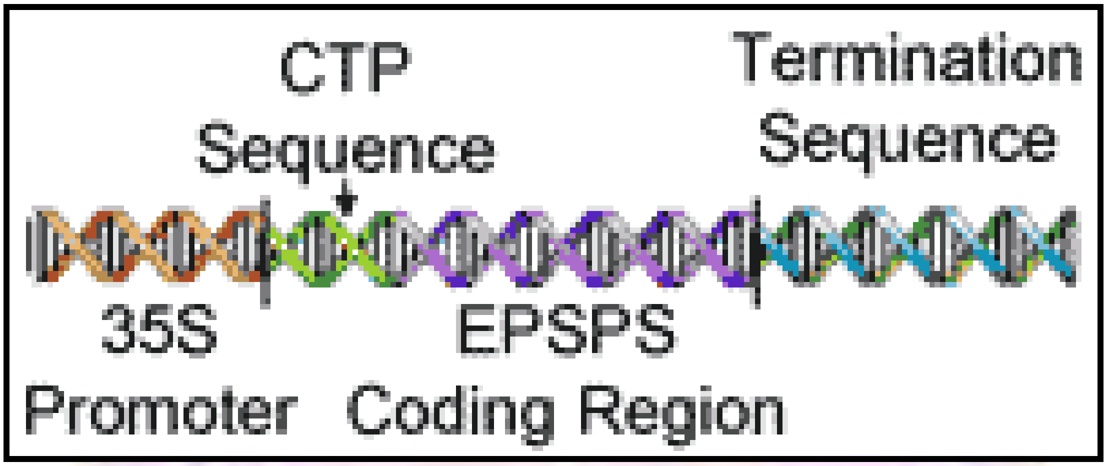


Figure: Necessary elements for EPSPS expression in soyabean germplasm

Source:http://plantandsoil.unl.edu/pages/printinformationmodule.php?idinformationmodule=959031259

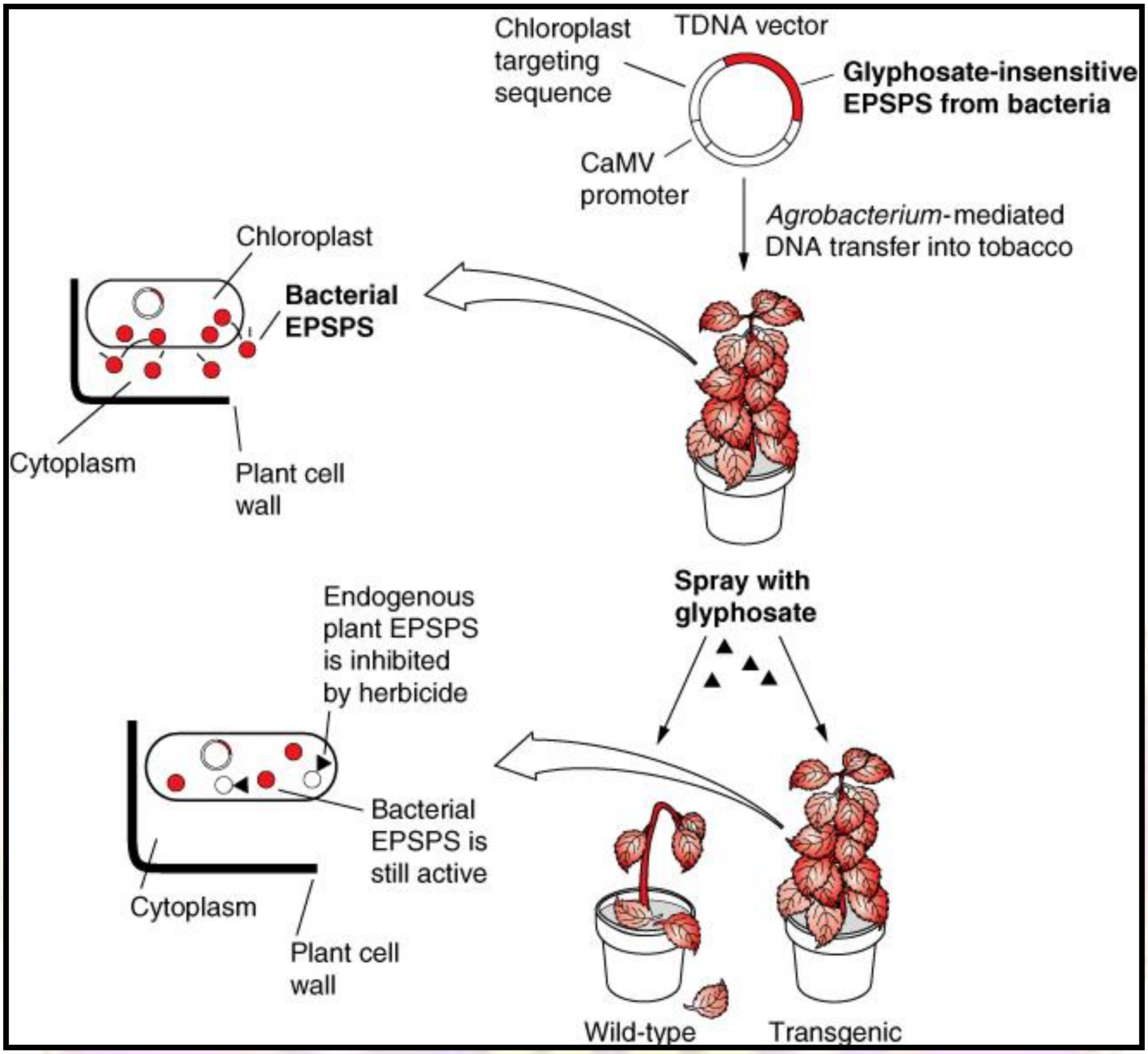


Figure: Process of modifying tobacco plants for glyphosate resistance.

Source:http://openwetware.org/wiki/840.119:Developing_Glyphosate_Resistance_in_Tobacco_Plants_(Jaime_and_Hanna) (cc)

Glyphosate is an inexpensive herbicide, and is environmentally friendly. Using the combination of glyphosate resistant crops (Roundup Ready crops) and glyphosate (Roundup Ready herbicide) has become widely popular.

Advantages of using herbicide tolerant crops

1. There is excellent weed control and hence higher crop yields are obtained.

2. It is possible to control weeds later in the plant's growth and hence there is flexibility in timing of herbicide application.

3. With herbicide resistant crops, there are reduced numbers of sprays in a season. Additionally because of less spraying there is reduced fuel use.

The use of herbicide resistant crops has some limitations as well. Their use is promoting the evolution of herbicide resistant super weeds (Refer to Chapter Biosafety concerns in plant biotechnology), which have multiple resistances. Extensive sprays of herbicides are required for eradication super weeds.

Virus resistant plants

Conventionally, resistance against viruses to the plants is provided through cross protection, where a susceptible variety of a crop is inoculated with a mild strain of virus, as a result susceptible variety develops resistance against more virulent strains. The method has been used in crops like tomato against tomato mosaic virus, in citrus against citrus tristeza virus and also in potato against potato spindle tuber viroid. The technique has many disadvantages:

- There is possibility of mutation in inducing mild virus strain.
- Also there is possibility of both the viral strains i.e. inducing virus and another unrelated virus, producing a combined effect greater than the sum of their separate effects.
- The mild virus may cause threat to the crop for future field losses.
- There could be some yield losses due to mild strain.

If a single gene responsible for cross protection is transferred, the above said disadvantages could be overcome and virus resistant transgenic crop can be produced. Using different genes, transgenic crop resistant to wide spectrum of plant viruses have been raised in potato, papaya and tomato. One such example is transfer of coat protein gene to papaya. The genetically modified papaya plants were resistant to infection and could be cultivated even when the virus was widespread.

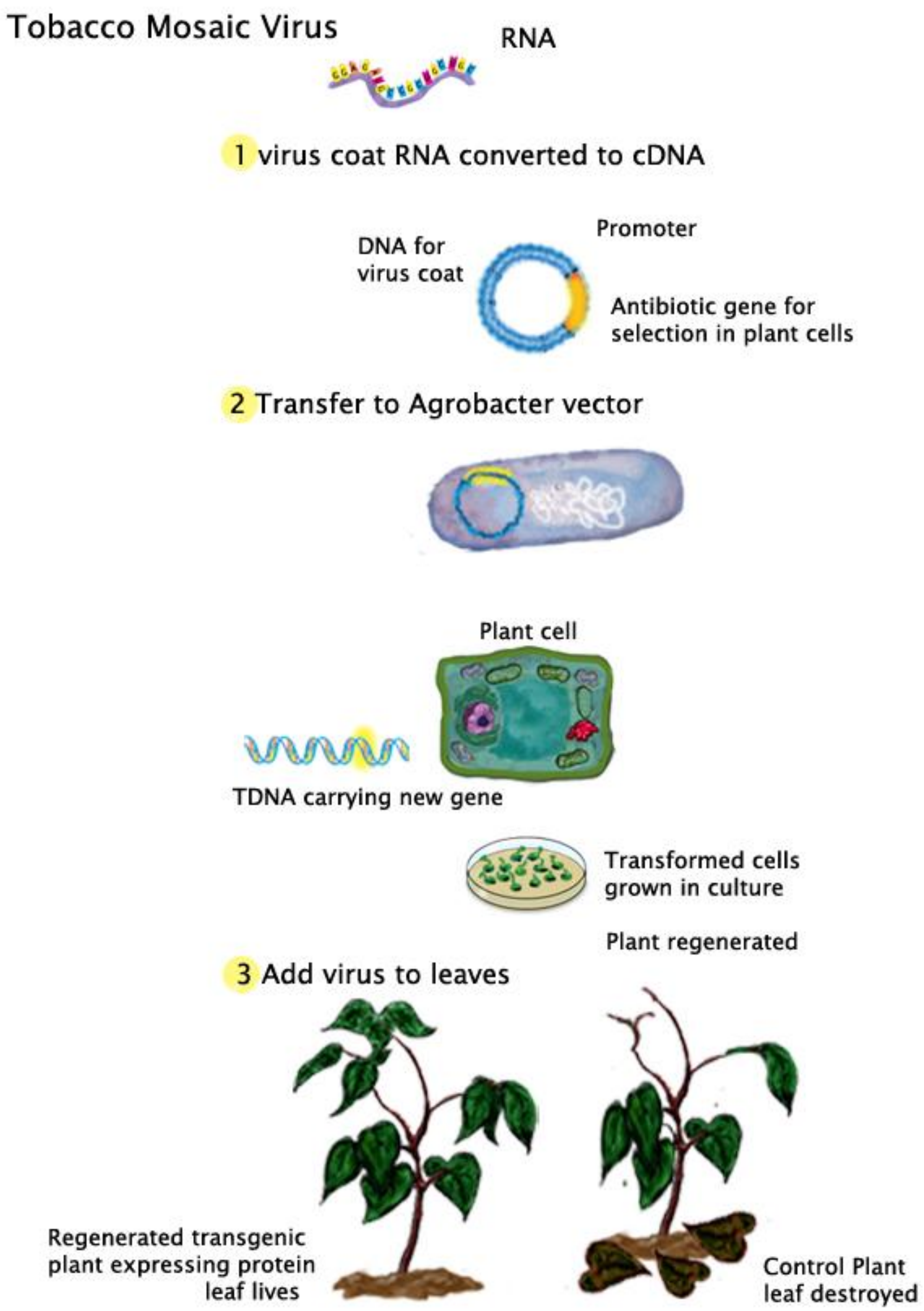


Figure: Method of producing virus resistant plants

Source: http://www.austincc.edu/awheeler/introtobiotech.php#

Resistance against bacterial and fungal pathogens

Advancement in plant molecular biology has helped biotechnologists to work out the molecular basis for resistance against several diseases in agricultural crop plants. It has been clearly understood that in many cases the disease resistance is under the control of single resistance gene present in the host recognizing specifically the pathogen strains with complementary avirulence gene. The complementary genes from both host and pathogen have been isolated in many plants and also cloned, facilitating the development of fungal and bacterial disease resistant transgenic crops.

Disease resistance is achieved by a chain of biochemical events involving induction of signaling cascade leading to hypersensitive (HR) reaction because of cell death in the host inhibiting the growth of pathogen. The HR reaction leads to broad spectrum resistance due to simultaneous or combined expression of R gene from host and Avr gene of pathogen in the same plant. Their expression has to be regulated in such a way that neither of them have a harmful effect on the host plant.

Two strategies have been used:

- In one approach, a pathogen-inducible promoter is attached with *Avr* gene. The *Avr* gene expressed when the pathogen attacked leading to hypersensitive reaction.
- In the second approach, a transposable element (TE) was inserted in R gene, so that the TE was excised by the pathogen and R gene expressed leading to hypersensitive reaction .

In a nutshell, many genes have been introduced in transgenic crops to confer tolerance to biotic stress which includes virus resistance, insect resistance, and also herbicide resistance. These developments will definitely promote increased food production as far as biotic stress tolerance is concerned.

Abiotic stress resistant plants

Abiotic stress like drought, temperature, salinity and metal toxicity etc. adversely affect plant growth and productivity worldwide. They are also serious threats to agriculture resulting in deterioration of environment. They induce a series of biochemical, physiological, morphological and molecular changes in plants. When a plant is exposed to any abiotic stress a number of genes get activated, altering the level of proteins, some of which provide protection to these stresses.

Efforts have been made to produce stress tolerant transgenic crops using genes which can alter the specific biosynthetic pathways. Stress induced gene expression involves genes which can be grouped into three categories:

(i) Genes having enzymatic or structural functions
(ii) Genes encoding proteins with unknown functions
(iii) Regulatory proteins

Initial attempts were made in most of the crops involving single action genes but recently a second stage of transformation with regulatory proteins has come up. Many genes responsible for stress tolerance can be simultaneously regulated by a single gene for stress inducible promoter or transcription factor, increasing tolerance for multiple stresses.

Table: Different types of abiotic stresses.

High Temperature (Heat)
Low Temperature (Chilling and freezing)
Excess water (Flooding and Anoxia)
Salinity (Salt stress)
Radiation (Visible, UV Radiation)
Chemical (Pesticides, Heavy Metals and air pollutants)

Source: Author

A large number of genes provide resistance against abiotic stresses which can be achieved by two different ways:

(i) Decreasing sensitivity to stress as in the case of salt tolerance through osmolyte protection and cold tolerance by polysaturated fatty acids
(ii) Protection from oxidative stress by superoxide dismutase (SOD)

Late embryogenesis abundant proteins (LEAs), antifreeze proteins, chaperones and detoxifying enzymes protect cells from dehydration. Osmoprotectants are a group of compounds which get accumulated in response to abiotic stresses and are low molecular weight toxic compounds. Some of these osmoprotectants include proline, glycine betaine and sugar alcohols.

Initial attempts were made in most of the crops involving single action genes but recently a second stage of transformation with regulatory proteins has come up. Many genes responsible for stress tolerance can be simultaneously regulated by a single gene for stress inducible promoter or transcription factor, increasing tolerance for multiple stresses. For example, Dehydration response element (DRE) is a promoter element which regulates the gene expression in response to temperature, drought and salinity. For instance Arabidopsis transformation with DREB1A gene driven by either the strong constitutive promoter of the cauliflower mosaic virus (35CaMV) or by a DRE- containing promoter from dehydration-induced gene (rd29A) resulted in a marked increase in tolerance to freezing, water and salinity stress.

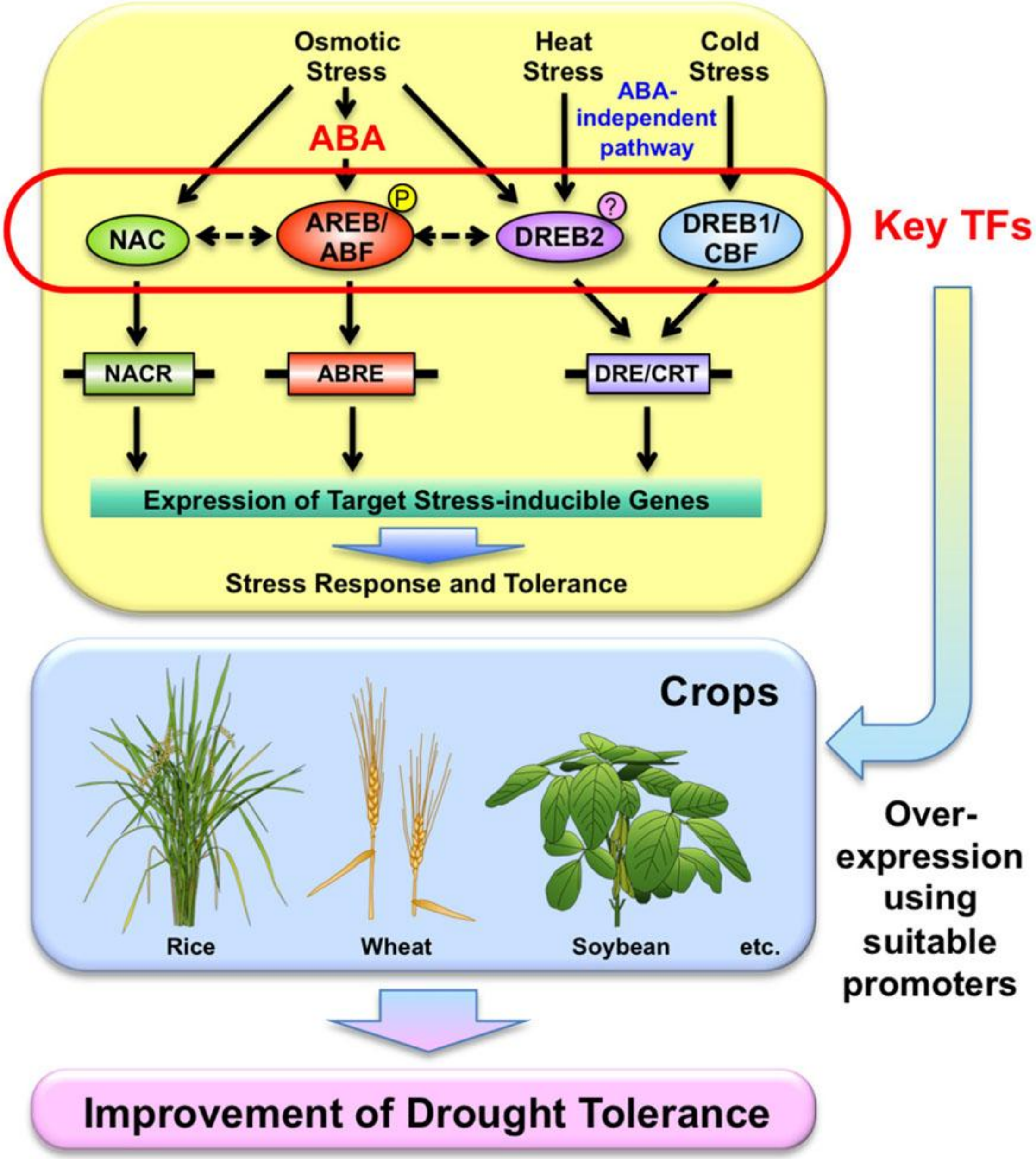


Figure: Various transcription factors (TFs) involved in stress-responsive pathways in stress responses can be used along with suitable promoters for the improvement of drought tolerance of crops.

Source: http://journal.frontiersin.org/Journal/10.3389/fpls.2014.00170/full (cc)

Transgenic crops for quality traits

Plants are the chief food of human beings. They not only provide calories but also fulfill the need for nutrients, essential amino acids, vitamins etc. required by our body. Around 870 million people suffer from hunger worldwide. In developing countries undernourishment and micronutrient deficiency are amongst common problems affecting people causing serious illness and death with increasing worldwide population. Staple crops rich in starch do not provide micronutrients, so physical and mental health of human population gets affected. The solution for this problem is production of staple crops with improved nutritional quality called 'biofortification' through genetic engineering and biotechnology. Transgenic crops are generated with improved nutritional traits beneficial for human beings. The examples are rare so far, but their number is increasing at a tremendous rate.

Our diet is rich in phytochemicals which include proteins, carbohydrates and fats which are the major constituents present in grams per 100g of food and minor constituents namely vitamins, minerals and secondary metabolites found in mg per 100g of food. Qualitative changes have been more common in major constituents like proteins, carbohydrates and fats as quantitative changes are difficult to achieve. Knowledge of biosynthetic pathways involved in engineering of any metabolic trait is essential which can be elucidated through functional genomics and gene delivery methods. Progress has been made to engineer fatty acid synthase pathway and accumulation of polyunsaturated fatty acids in oil crops whereas both quantitative and qualitative alterations in minor constituents have been widely reported.

Carbohydrates are the main source of calories in the food. Manipulation of carbohydrates can be carried out so as to increase their nutritional value. For example,

- Amylose, which is a branched portion of starch, restricts digestion. The food products with high amylose also have higher resistant starch content. Resistant starch is the portion of starch which on bacterial fermentation in large intestine produces short chain fatty acids which help in maintaining gut health and also prevent intestinal cancer. Another important group of starches are those which get slowly digested in small intestine providing continuous flow of glucose to blood. They decrease the insulin requirement of our body and hence control diabetes. This correlation has been used for the improvement of rice and wheat varieties and transgenic lines of wheat and rice have been produced with increased amylose and resistant starch content.

- Uptake of inulin in diet, which is a fructan, increases a group of bacteria in the gut called Bifidobacteria which increases vitamin B production, reduces the growth of pathogenic bacteria and also decreases cholesterol.
- Vitamin deficiency is a common health problem worldwide. Most of the cereal crops are deficient in essential vitamins. Therefore, transgenic manipulations have been done for improvement of vitamin content of many crops of commercial value the focused vitamins being vitamin A, B, C, and E.
- Antioxidants are compounds which neutralize free radicals and reactive oxygen species protecting membranes, DNA and tissues from damage caused by oxidative stress. Fruits and vegetables rich in antioxidants can reduce the risk of cancer, cardiovascular diseases and delay ageing when consumed in diet. Vitamin C and E are important antioxidants derived from plants. These antioxidants have been successfully overexpressed in transgenic crops, thereby improving their nutritional quality.
- Out of twenty amino acids, humans can synthesize only ten amino acids. Remaining have to be obtained exogenously through diet and are called essential amino acids. Proteins obtained from cereals and legumes are poor proteins as they lack or have low levels of these essential amino acids particularly lysine and methionine. Cereals are poor source of lysine and legumes are poor in methionine. Therefore, improving the content of methionine is a target in case of cereal crops.
- Fatty acid modification is another area of interest of biotechnologists. To increase the content of unsaturated fatty acids in plants, one of the strategies is to alter the expression of desaturase genes, responsible for introducing double bonds in the fatty acids at specific locations. In several crops like maize, soyabean and oilseed rape, nutritionally improved oils are produced through transgenic methodology.

Golden Rice

Vitamin A deficiency (VAD) causes blindness in 5 million children annually resulting in death of most of these children. Globally, nearly 124 million children suffer from VAD. It is a matter of concern in Southeast Asia, Africa, Caribbean and Latin America. One to two million deaths may be avoided by providing Vitamin A enriched nutrition among children 1-4 years and 0.5 million during higher age group of children. One approach is providing vitamin A capsules to children and new mothers and alternatively provitamin A can be provided in the form of b-carotene in rice which is one of the best known examples of nutritional improvement of a food crop. Carotenoids are compounds belonging to a class of plant metabolites called terpenoids or isoprenoids. Carotenoids are produced from its precursor by a biochemical pathway located in plastids. The 40-carbon backbone of b-carotene (pro vitamin A) is known as phytoene and is assembled by two 20-carbon geranylgeranyl diphosphate (GGPP) molecules by the enzyme phytoene synthase. Double bonds in phytoene are added by desaturation steps producing an antioxidant compound called lycopene, giving red color to tomatoes which is later converted to b-carotene by the enzyme lycopene cyclase. With the aim of fortified food production, research was carried out to grow and consume variety of rice in areas where VAD was found to be common.

A variety of *Oryza sativa* was produced through genetic engineering capable of synthesizing beta-carotene, a precursor of Vitamin A in the endosperm of transgenic rice. This variety, known as 'Golden rice', differs from the parental variety by having two additional beta-carotene synthesis genes.

Golden rice was produced by transforming rice with two beta-carotene synthesis genes:

(i) *psy* (phytoene synthase) from Daffodil (*Narcissus pseudomarcissus*)
(ii) *crt I* (carotene desaturase) from a soil bacterium(*Erwinia uredovora*).

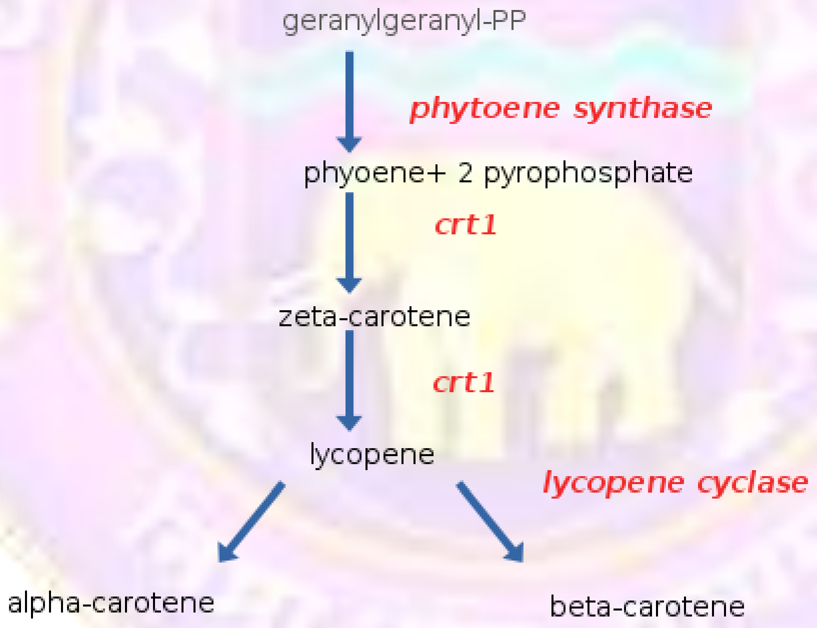


Figure: Carotenoid biosynthesis pathway in Golden Rice

Source: http://en.wikipedia.org/wiki/File:Carotenoid.svg (cc)

Both these genes were inserted into rice nuclear genome under the control of an endosperm specific promoter to express them only in endosperm. The end product of this pathway is lycopene, but plants do not accumulate it otherwise the rice would have been red. An endogenous enzyme inside the plant converts lycopene to beta-carotene giving golden yellow color after which it is named. The details of golden rice were first published in Science in 2000. This was product of an eight year project by Ingo Potrykus of Swiss Federal Institute of Technology and Peter Beyer of University of Freiburg. In the year 2005, a new variety of rice, called Golden rice 2 producing 23 times more beta-carotene in comparison to original Golden rice, was announced.

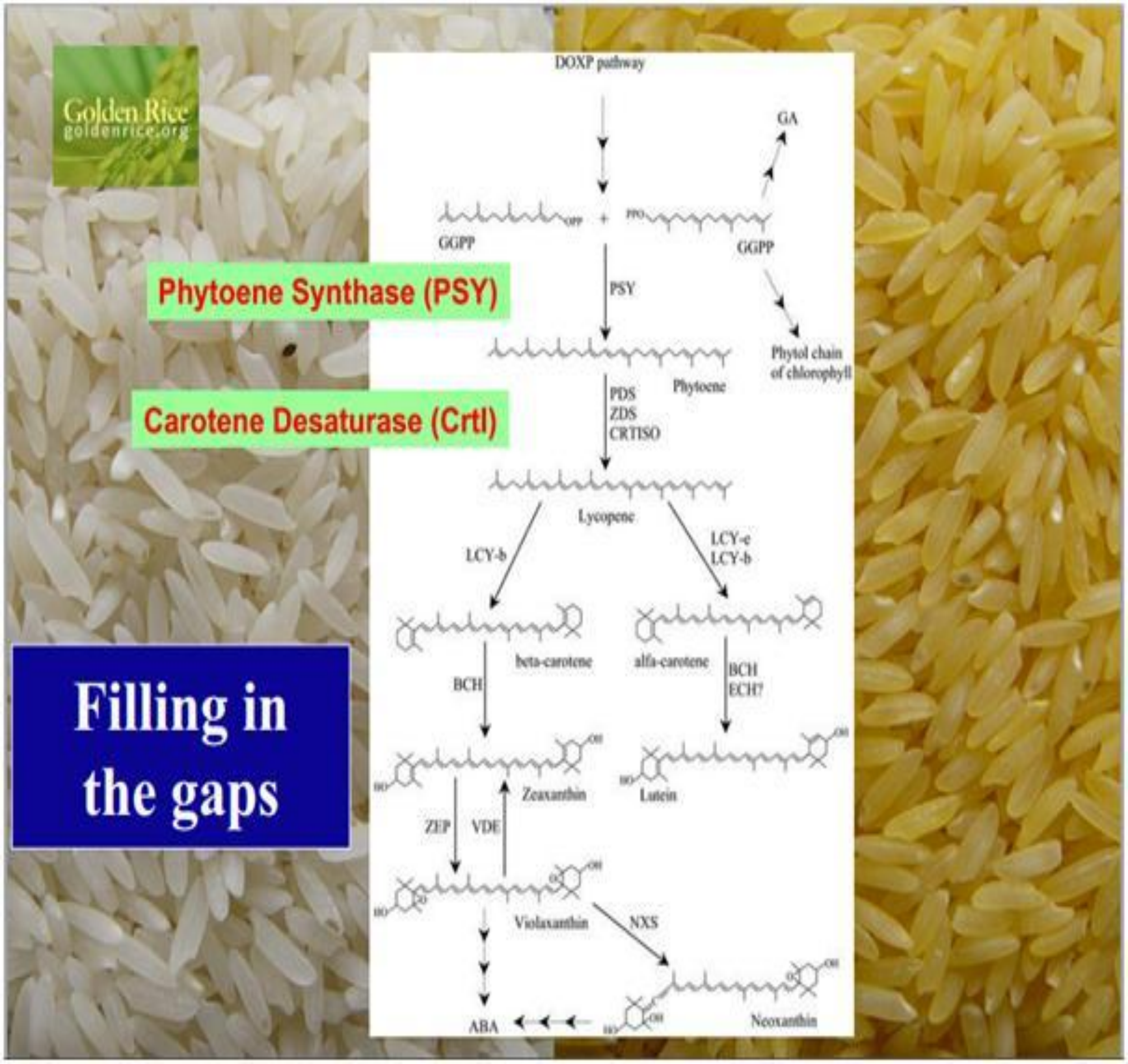


Figure: Flow diagram for chemical pathways in golden rice

Source: http://biol1020-2012-1.blogspot.in/2012/03/genetic-engineering-of-foods-to aid.html

Figure: Wild type rice and Golden rice

Source: http://www.goldenrice.org/Content4-Info/info5_sitemap.php

Various concerns have been raised by critics of genetically engineered crops. Green peace opposes the use of GMOs in agriculture. So technology transfer is the main hurdle for the golden rice to be in market on global scale.

Flavr Savr Tomato

The Flavr savr tomato is the first genetically engineered whole food which was introduced in 1994. It was produced by Californian company Calgene. Normally, the ripe tomatoes produce an enzyme called polygalactouronase (PG) which digests pectin. Antisense RNA technology was used to develop Flavr Savr tomato. For this purpose, PG gene was isolated and a complementary gene was made to synthesize a complementary mRNA that binds to the normal mRNA inactivating the normal mRNA for this enzyme Thus, their shelf life is increased by suppression of polygalacturonase (PG) gene.

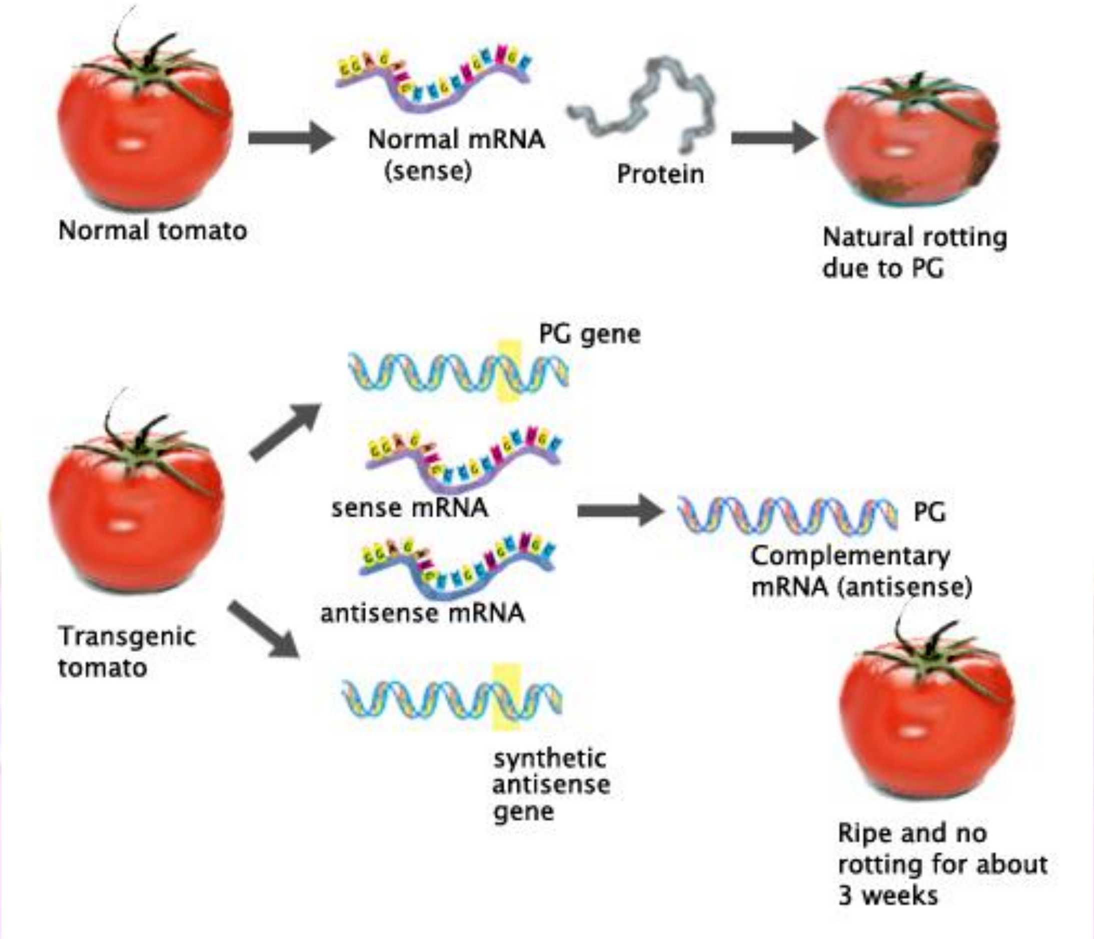


Figure: Normal vs transgenic tomato. Flavrsavr tomato is synthesized using antisense RNA technology.

Source: http://www.austincc.edu/awheeler/introtobiotech.php#

Improved Horticultural Varieties

Horticulture has emerged as an indispensable part of agricultural enterprise significantly affecting economy. The horticulture sector includes fruit crops, vegetables crops, tuber crops, ornamental crops, medicinal and aromatic crops, spices and plantation crops. Transgenic technology has made significant contributions for production of improved horticultural varieties. Genetically modified flowers are widely accepted and offer economic benefits.

Moon dust carnations

Rose, *chrysanthemum*, and carnations (*Dianthus caryophyllus*) are economically important flowers. Transgenic technology has been utilized for production of ornamental plants and new varieties have been developed. Moon dust carnations are the first genetically modified flowers sold in the world. Traditional breeding techniques cannot produce flowers with colors which do not occur in nature.

Pigments impart color to flowers and fruits. Flavonoids and carotenoids are widely distributed plant pigments. Carotenoids are responsible for bright yellow or orange color of flowers, while the flavonoids impart attractive red, pink and blue color. Anthocyanins, the most important group of flavonoids are responsible for scarlet to blue color of flowers, fruits, leaves and storage organs. Though they are universal in higher plants, the type of anthocyanin present may vary from plant to plant or fruit to fruit. Some fruits contain only one type of anthocyanin for example only cyanidin is present in peach, apple, cherry and fig; only delphinidin is present in pomegranate and eggplant where as some fruits like grapes contain multiple anthocyanins. Similarly some ornamental plants like *Petunia* and *Dianthus* contain only one main type of anthocyanin, i.e. delphinidin, whereas roses and tulips possess mixtures of anthocyanins. The delphinidin pigment imparts purple, mauve and blue color to flowers. F3'5'H (flavonoid 3',5'-hydroxylase) gene catalyzes the production of the blue-colored anthocyanin pigment delphinidin and its other derivatives. F3'5'H gene is absent in roses and carnations and hence delphinidin pigments have never been found to exist in roses or carnations. Therefore blue colored roses and carnations cannot be produced by traditional breeding experiments.

A team from an Australian company Florigene wanted to make a blue colored rose, but encountered problems while transplanting genes. The team extended their techniques to carnations, which are easier to manipulate. The petunia F3'5'H (flavonoid 3', 5′-hydroxylase) gene was overexpressed in carnations under the control of a constitutive promoter. Resulting carnation plants produced anthocyanins, 70% of which constitute delphinidin derivatives. These plants exhibited only a marginal color change towards blue. To further increase the concentration of delphinidin, it is important to reduce the competition between the two endogenous enzymes of the anthocyanin pathway, i.e. dihydroflavonol-4-reductase (DFR) and flavonoid 3'-hydroxylase (F3'H) gene for their substrate. Therefore, colorless carnation varieties, deficient in the DFR gene were selected and petunia F3'5'H gene (under the control of promoter from the snapdragon CHS gene) along with petunia DFR gene (under the control of a constitutive promoter) were introduced. This resulted in accumulation of delphinidin derivatives and color change in carnations. Carnations which were earlier colorless became blue colored. “Moondust” and “Moonglow” are lilac and blue colored carnations created by Florigene. These transgenic carnations were subjected to regulatory scrutiny and were declared safe for commercial use. They were first grown commercially in 1997. Since then these blue colored carnations are blooming in the global market. Australian Florigene is now part of Japanese group Suntory Ltd. In 1990 Florigene started a project with Suntory to develop blue roses. Genetically modified blue carnations pose no threat to the environment because these GM carnations are not related to any significant weed species in Australia. Secondly carnation varieties are infertile as seed set doesn't takes place in them. Thirdly carnation pollen is heavy and sticky and buried deep in the flower therefore it cannot spread by wind or bees. Even the humid environment of greenhouses where carnations are grown reduces the survival rate of carnation pollen. Lastly carnations cannot be propagated vegetatively, restricting their spread.

Figure: Moondust Carnations

Source: http://commons.wikimedia.org/wiki/File:Moondust-carnation.JPG (cc)

Drawbacks of Genetically Engineered Crops

1. Genetic engineering techniques modify plants to produce their own pesticide (Bt) or make them resistant to herbicides (Roundup-Ready). It is speculated that in short span of time insects will become resistant to Bt, and even to the herbicide.

2. Cross pollination between herbicide resistant crops with weeds of the same family, can create super-weeds which are herbicide-resistant. These super-weeds may outgrow native plants and spread beyond their natural habitat.

3. Pollen from GE crops may drift and pollute neighboring plants growing in the vicinity.

4. Some beneficial insect species (Monarch butterfly, ladybugs and green lacewings etc.) are harmed by genetically modified plants. Studies have also shown that Bt toxin may leak into the soil and can potentially harm non-target organisms.

5. Genetically modified plants may contain hidden allergens and pose threat to human and animal life.

6. Some religious and moral considerations are associated with use of GM crops as genes from fish have been integrated into tomatoes to prevent freezing at low temperatures. Even chicken DNA has been incorporated to potatoes to increase disease resistance.

7. Transfer of DNA for genetically engineering a plant, is associated with transfer of antibiotic marker genes. This could lead to increased antibiotic resistance. The transgenic process can, however, affect other genes in a plant through interaction effects.

Summary

1. In the present scenario, a single crop with multiple resistance traits with reduced environmental impacts and providing sustainable nutritive food is desirable. Biotechnology is a welcome technology in agriculture as it provides agricultural industry relevant varieties for unique environmental conditions.
2. Bt cotton offers a vastly improved method for delivering Cry-insecticides to target insects, compared to traditional Bt sprays. It can be effectively used in insect pest management. Development of Bt cotton is one of the revolutionarily development in agriculture industry.

The soil bacterium *Bacillus thuringiensis* produces proteins toxic to various herbivorous insects. The toxic protein is produced in inactive crystalline form and is converted to active toxic form called as delta endotoxin only when it is consumed by insects. The protein binds to certain receptors in insect's intestine and causes wounding of epithelial midgut cells. This toxin destroys the gut of insect, ultimately leading to death of insect. This toxic protein is also known as Bt protein or cry protein.

3. Herbicide resistant plants are genetically modified crops that harbor genes that enable them to degrade the active component in the herbicide, making them harmless to herbicide spray. Two herbicide resistant cropping systems are routinely used: Monsanto's *Roundup Ready* with active agent as glyphosate and Bayer's *Liberty Link* with active agent as glufosinate. With roundup crops, farmers can spray broad spectrum herbicides without killing valuable crops. Glyphosate, the active agent of roundup ready, interferes with the synthesis of essential amino acids like phenylalanine, tyrosine and tryptophan. These essential amino acids are synthesized with the help of an enzyme called 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The enzyme catalyzes the penultimate step of the shikimate pathway for the biosynthesis of above mentioned essential aromatic amino acids.
4. Disease resistance is an important and effective mean of disease control. A large number of genes provide resistance against abiotic stresses which can be achieved by two different ways: Decreasing sensitivity to stress as in the case of salt tolerance through osmolyte protection and cold tolerance by polysaturated fatty acids and protection from oxidative stress by superoxide dismutase (SOD).
5. A variety of *Oryza sativa* was produced through genetic engineering capable of synthesizing beta-carotene, a precursor of Vitamin A in the endosperm of transgenic rice. This variety, known as 'Golden rice', differs from the parental strain by having two additional beta-carotene synthesis genes.
6. The Flavrsavr tomato is the first genetically engineered whole food which was introduced in 1994. The shelf life is increased by suppression of Polygalacturonase (PG) gene, resulting from antisense RNA against PG gene which depolymerizes the cell wall pectin of ripening fruit leading to skin softening.
7. Transgenic technology has been utilized for production of ornamental plants and new varieties have been developed. The delphinidin pigment imparts purple, mauve and blue color to flowers. F3'5'H (flavonoid 3',5'-hydroxylase) gene catalyzes the production of the blue-colored anthocyanin pigment delphinidin and its other derivatives. The petunia F3'5'H (flavonoid 3', 5'-hydroxylase) gene was overexpressed in carnations which resulted in

accumulation of delphinidin derivatives and color change in carnations. Carnations which were earlier colorless became blue colored. “Moondust” and “Moonglow” are lilac and blue colored carnations created bythe Australian company florigene.

Glossary

Antisense RNA Technology: An antisense construct of the target gene is integrated in the genome to suppress an endogenous gene.

Bacillus thuringiensis**:** A naturally occurring soil borne bacterium that produces crystal protein toxic to certain insects especially to larvae of insect order Lepidoptera.

Biofortification: Production of staple crops with improved nutritional quality through genetic engineering and biotechnology.

Coat Protein Gene: Viral gene encoding its coat protein.

Cry proteins: Several crystalline proteins produced in inactive crystalline form in Bt spores and are converted to active toxic form called as delta endotoxin only when insects consume them.

Delphinidin: An anthocyanin pigment that imparts purple, mauve and blue color to flowers.

EPSPS: Enzyme 5-enolpyruvylshikimate-3-phosphate synthasecatalyzes the penultimate step of the shikimate pathway for the biosynthesis of essential aromatic amino acids

Genetically modified organisms (GMOs): Organisms carrying one or more heterologous gene, i.e. genes that are not naturally present in their standard genome, are called genetically modified organisms.

Herbicide resistant plants: Genetically modified crops that harbor genes that enable them to degrade the active component in the herbicide, making them harmless to herbicide spray.

Hypersensitive Response (HR): Disease resistance is achieved by a chain of biochemical events involving induction of signaling cascade leading to hypersensitive (HR) reaction because of cell death in the host inhibiting the growth of pathogen.

Insect Resistance: Ability of some strains of a plant species to be less prone to insect attack or damage than other.

Larva: Immature developmental stage of insect species like moths, butterflies, beetles, caterpillars etc.

Lepidoptera: The order of insects including moths, butterflies, beetles etc.

Moondust: purple-mauve colored carnation flowers which get their purple color by transfer of DFR gene from petunia.

***Roundup Ready* Soyabean:** Herbicide resistant cropping systems with active agent as glyphosate.

Exercises

Multiple choice type questions

1. Starch content of potatoes can be increased by using a bacterial gene, known as

a. sucrose phosphate synthase gene

b.ADP glucose pyrophosphorylase gene

c.polygalactouranase gene

2. Approximately what proportion of the world's population suffers from some form of malnutrition:

a. one tenth

b. one half

c. one quarter

3. Which of the following is not a characteristic of a transgenic crop?

a. Increased methionine content
b. Herbicide resistance gene
c. Insect resistant toxinI
d. None of the above

4. Antisense transgenic plants produced fruits which got softened

- a. More rapidly than the non transgenic fruits
- b. More slowly than the non transgenic fruits
- c. Just like the non transgenic fruits
- d. None of the above

5. Tomatoes showing delay in ripening express antisense RNA against

    - a. Glycerol-1 phosphate acyl transferase
    - b. ACC deaminase
    - c. Sucrose Phosphate Synthase
    - d. Polygalactouranase

6. Which of the following organism was not used in the production of Golden Rice

- *a. E.coli*
- *b. Erwiniauredosvora*
- *c. A. tumefaciens*
- *d. Narcissus pseudonarcissus*

7. Name a commercially used, genetically modified cotton plant resistant to the Lepidoptera larva

- a. PR cotton
- b. HR cotton
- c. Bt cotton
- d. Ht cotton

8. Cry1 endotoxins obtained from *Bacillus thuringiensis* are effective against:

- a. Mosquitoes
- b. Nematode
- c. Bollworm
- d. Flies

9. *Bacillus thuringiensis* forms protein crystals which contain insecticidal protein. This protein

- a. Binds with epithelial cells of midgut of the insect pest ultimately killing it.

  b. Does not kill the carrier bacterium which is itself resistant to this toxin
  c. Is activated by acid pH of the foregut of the insect pest
  d. Is coded by several genes including the psy gene

10. What is *true* about Bt toxin?

    a. Btprotein exist as active toxin in the *Bacillus*.
    b. The inactive toxin gets converted to active form in the insect gut.
    c. The bacillus had antitoxins
    d. The active toxins enter ovaries of the pest and prevent their multiplication.

11. The Glyphosate, the active agent of roundup ready, interferes with the synthesis of

    a. Proline
    b. Phenylalanine
    c. Glycine
    d. Valine

12. Color change in Moondust carnations is due to accumulation of

    a. Cyanidin
    b. Delphinidin
    c. Malvinidin
    d. Peonidin

True or false

1. Golden rice is a genetically modified crop plant where the incorporated genes are meant for biosynthesis of beta carotene.
2. Proline and betaine are involved in stresstolerance.
3. Virus resistant genes are widely used to provide tolerance against plant virus.
4. Antisense technology selectively blocks expression of a gene.
5. Genetically engineered herbicide-resistant crops allow farmers to use a single broad-spectrum herbicide throughout the growing season.
6. Moondust carnations are colorless.
7. Use of herbicide resistant crops does not require application of herbicides to kill weeds.
8. Cry proteins save cotton plants from Bt toxins.

9. Blue colored roses cannot be produced by traditional breeding experiments.
10. The active component of roundup ready soyabean is glyphosate.

Subjective Questions

1. Give a brief account on applications of biotechnology in agriculture?
2. Discuss the different strategies used to produce virus resistant transgenicsplants. Briefly discuss their merits and demerits.
3. With the help of suitable examples discuss the approaches for the production of herbicide resistant varieties.
4. What do you mean by Hypersensitive response? Discuss the strategy used for the generating resistance in transgenic crops.
5. Discuss the environmental concerns with the use of pest and herbicide resistant plants
6. What is "Golden Rice" and why is it so named?
7. Explain the mechanism of production of Golden Rice and constraints in its commercialization.
8. Why blue colored roses and carnations cannot be produced by nature?
9. Carnations which were earlier colorless became blue colored. Comment?
10. What are herbicide resistant crops? Discuss their importance in agriculture.
11. Why genetically modified carnations pose no threat to environment?
12. Give a brief account of the achievements of Florigenecompany in horticulture?
13. Write short notes on:
    a. Roundup ready soyabean
    b. Moondust carnations
    c. Bt cotton
    d. Cry protein
    e. FlavsSavr tomato
    f. Golden Rice
    g. Role of antioxidants in abiotic stress
    h. Osmoprotectants and water stress

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Web Links

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