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Transgenic Plants and its role in crop improvement

Introduction

Transgenic is the area of biotechnology which mainly concerns itself with the manipulation of the genetic material of living organism, enabling it to perform specific functions. Food storage, population explosion and environmental stress have caused serious problems to mankind on globe.Agriculture contribute 17-18% in GDP, out of 5 the 1 people depends on agriculture. Now at the time agriculture industry is projected to suffer significant global setbacks(population growth, pest resistance, and burden on natural resources). The world population is increasing at very fast rate, and is projected to reach 8.5 billion by 2025. To fulfill the food demand of every individual with limited natural resources is not possible. So it will cause the food deficiency or malnutrition which is the major health problem in these days. The loss of arable land is also increase, In 2016 it is estimate that 0.242 ha/ person but in 2050 it is only 0.18 ha/ person. Decreases the arable land due to urbanization, rapid world population and increase demand of food resources. Transgenic crop reduces the pesticide usage up to 37% and impact of herbicide and insecticide on environment is also reduced up to 14%. So with the help of transgenics crop we increase our food production as well as qualitative and quantitative. Biotechnology for crop improvement also help to improve combat deficiencies in food by enhancing carbohydrates, vitamins, lipids and micronutrients composition (Zimmermann and Hurrell, 2002; Sun, 2008). Later 1990, the prime importance of agricultural biotechnology can be located on traits for improvement in crops related to insects and herbicide resistant, virus resistant, nutritional quality, biofuel production and self-life. In these traits number of genes are involved, so crop improvement by genetic engineering is not a simple process. Lack of fundamental knowledge of genetics and molecular biology of the plant species make this more exigent. Transgenic plant has been arising through different genetic engineering techniquesbut with a legal, political and social problems. For example, the world health organization has detected three main concerns with genetically engineering crops, specific GM food crops, including incorporation of modified genes in human body, crossing of transgenic plants with non-transgenic conventional plants, and generation of allergenic foods. Despite all of these barriers, many countries including China, USA, Brazil, India, Australia,Philippines, Myanmar, Pakistan, Vietnam, Bangladesh, and Indonesia (Table 5; ISAAA, 2018).The United States had the largest area of genetically modified crops worldwide in 2019, at 71.5 million hectares, followed by Brazil with a little over 52.8

million hectares. In USA the area under the transgenic crops is 70.9 million hectares and crops are maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, squash, potato. In Brazil the area under transgenic crops is 44.2 million hectares and crops are soybean, maize, cotton. In India only Bt cotton is cultivated. The area under BT cotton is 11.6 million hectares.Recently the government of the Philippines announced that it had approved golden rice, making it the first country to do so. Golden rice is a variety of rice that has been genetically modified to combat vitamin A deficiency.Gene can be transferred by biological methods, physical methods, and chemical methods. Biological methods includes Agrobacterium mediated gene transfer and plant virus vectors. In biological methods Electroporation, microprojectile, microinjection, liposome fusion and in chemical methods includes polyethylene glycol mediated and Diethylaminomethyl dextran mediated Agrobacterium-mediated transformation and gene gun is the most common technique used in plant transformation as it is efficient and effective in a wide range of plants.

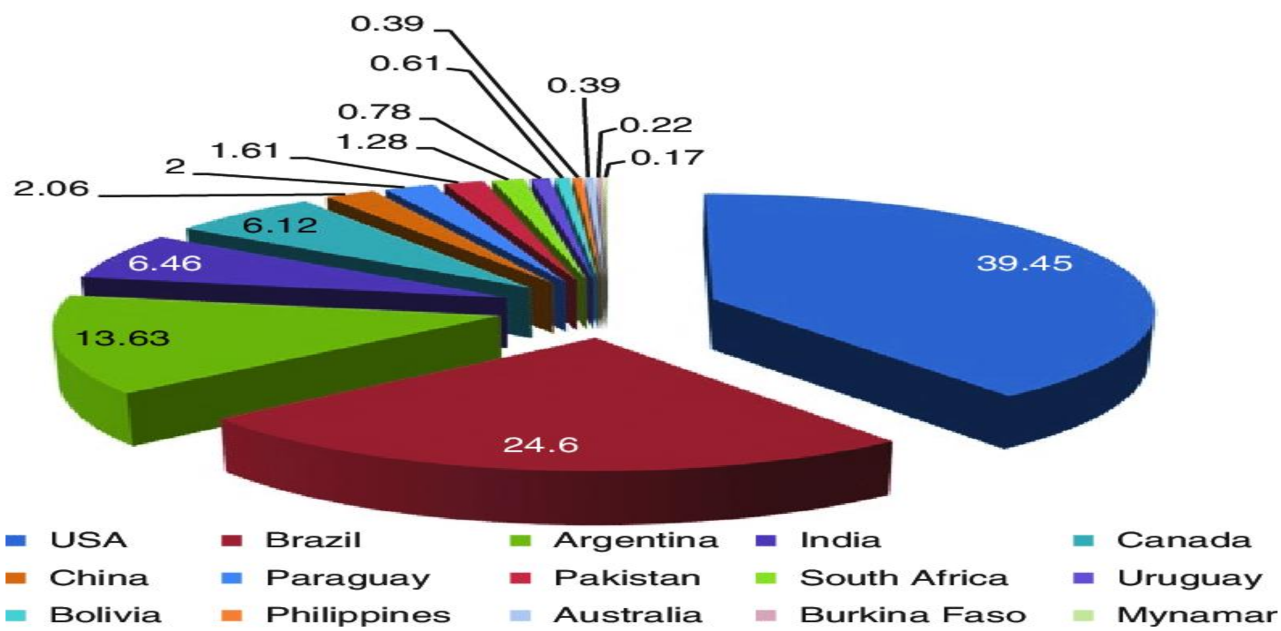

Figure 1 Percentage area of the top 15 countries (total 179.7 million hectares) under commercial genetically modified crop cultivation as of 2015 (Data from gene watch 2016)

What are transgenic plants

Transgenic plants are plants that have had their genomes modified through genetic engineering techniques either by addition of a foreign gene or removal of a gene.

Or

Transgenic plants are plants into which one or more genes from another species have been introduced into the genome, using genetic engineering processes. Techniques includes the biolistic method- in which a heavy metal is coated with plasmid DNA is shot into cells and Agrobacterium tumefaciens mediated transformation

History

The concept of transgenic is not new, about 1000 year back the human ancestors practiced " Selective Breeding and artificial selection". This concept gained development in 1946, when scientists first discovered that genetic material was transferable between twodifferent species. This was followed by DNA double helical structure discovery.

The first genetically modified crop was Tobacco which was reported in 1983. It was developed creating a chimeric gene that joined an antibiotic resistant gene to the T1 plasmid from *Agrobacterium*.

In 1984, 1st successful plant genetic engineering experiments using **caulimovirus** vector. CaMV is the member of the caulimoviruses that are the only plant viruses known to contain double-stranded DNA. Cloned viral DNA can be introduced directly into plants by rubbing the DNA onto leaves with an abrasive, provided that the bacterial plasmid used to propagate CaMV in *Escherichia coli*has been excised.

The first genetically modified crop approved for the sale in U.S., in 1994, was the "**FLAVR SAVR"** tomato. The FLAVR SAVR tomato was developed through the use of antisense RNA to regulate the expression of the enzyme polygalacturonase (PG) in ripening tomato fruit. The enzyme is one of the most abundant proteins in ripe tomato fruit and has long been through to be responsible for softening in ripe tomatoes.

In 1995, BT Potato was approved by the US environmental protection Agency. The Bt potato is a genetically engineered potato that has genes added so it will produce the insecticide (Bacillus thuringiensis or Bt toxin) in every cell. Two different types of Bt potato exist. Potato tuber moth resistant bt potato and Colorado potato Beetle resistant Bt potato.

In 2002 the Bt cotton was introduced in India. Bt cotton with the trade name Bollgard-I, developed by Mahyco-Monsanto Biotech Ltd. Bt cotton has been genetically modified by the insertion of one or more genes from a common soil bacterium, Bacillus thuringiensis. These genes encode for the production of insecticidal proteins, and thus genetically transformed plants produce one or more toxins as they grow. The production of Bt cotton is very good as compare to non Bt cotton. The area under Bt cotton has increased from 0.29 lakh hectares in 2002-2003 to 117.47 lakh hectares in 2019-20, according to Directorate of Economics and Statistics. Bt cotton remains the only GM crop approved for cultivation in India.

In 2014 March GEAC (Genetic Engineering Approval Committee) approved field trails for 11 crops, including maize, rice, sorghum, wheat, groundnut and cotton. In 2016 genetic engineering approval committee gave green signal to GM mustard for field trails, but supreme court stay the order and sought public opinion on the same. There are 20 GM crops already undergoing trails at various stage.

Gene transfer methods

The gene to be transferred may be introduced into plant cells through one of the following methods: 1. Use of Ti or Ri plasmid of *Agrobacterium* species as vectors, 2. By employing caulimovirus or Gemini virus vectors, 3. Direct DNA uptake up cells, 4. Polyethylene glycol-induced DNA uptake, 5. Microinjection, 6. Particle gun, 7. Electroporation, 8. Calcium phosphate precipitation, 9. Liposome-mediated DNA transfer. Of these, *Agrobacterium* mediated gene transfer have been the most successful, followed by particle gun.

Agrobacterium tumefaciens

Ti (tumour inducing) plasmid is found in *Agrobacterium tumefaciens*, which produces crown gall (tumour) in a large number of dicot species. Uniformly the root inducing plasmid occurs in A. rhizogenes, which causes hairy root diseases of many dicots and monocots plants. Both the plasmids are basically similar, they both carry the genes for the production of tumours and roots. T-region (the region transferred into host genome normally called T-DNA) and vir (virulence) region are present in both the plasmids. In T-DNA four important genes are present, out of four genes two genes, *iaaM* and *iaaH* control the synthesis of IAA; one gene, *ipt*govern the production of cytokinin and remaining one *os*is concerned with opine. Opine is the nitrogenous compound). The genes *iaaM*, *iaaH*and *ipt*are involved in tumour production and are called oncogenes. The oncogenes can be removed from T-DNA of Ti plasmid before it can be used as a vector. The process of removing these genes from T-DNA is called disarming of Ti plasmid.

*Agrobacterium tumefaciens*is a rod shape and gram-negative bacteria which is found in the soil. It causes the crown gall diseases in plant. The bacteria go in the plant through wounds present in root or stem. After entering in plant the bacteria insert the DNA and stimulate the plant to grow swollen galls. Agrobacterium tumefaciens is effective to DNA transfer and it is a potential vector in the production of transgenic plants. In plant wounds amino acids, sugar, organic acids, are released. Agrobacterium tumefaciens is attracted to amino acids, sugar, and organic acids. It attach the wounded tissue by polar attachment mechanism. During this attachment switches on genetic operons critical to start of gene transfer expression. The vir regulon is regulated and starts expression during the attachment stage. The wound phenolics and monosaccharides directly or indirectly cause the autophosphorylation of the virA transmembrane receptor kinase. This in turn activates the soluble cytoplasmic transcriptional factor virG through another phosphorylation event. Turn on *vir* G begin the transcription of individual vir operon. These gene result produced in the process transfers the DNA fragment into plant using T-DNA present in tumor induced plasmid. Nearly all laboratory experiments use a binary system consisting of two plasmids. Once the plant cell has been introduced the new DNA in a stable manner, the next step is to regenerate plant from the transformed cells (Sunil Kumar B., Immanuel Selvaraj C. (2019).

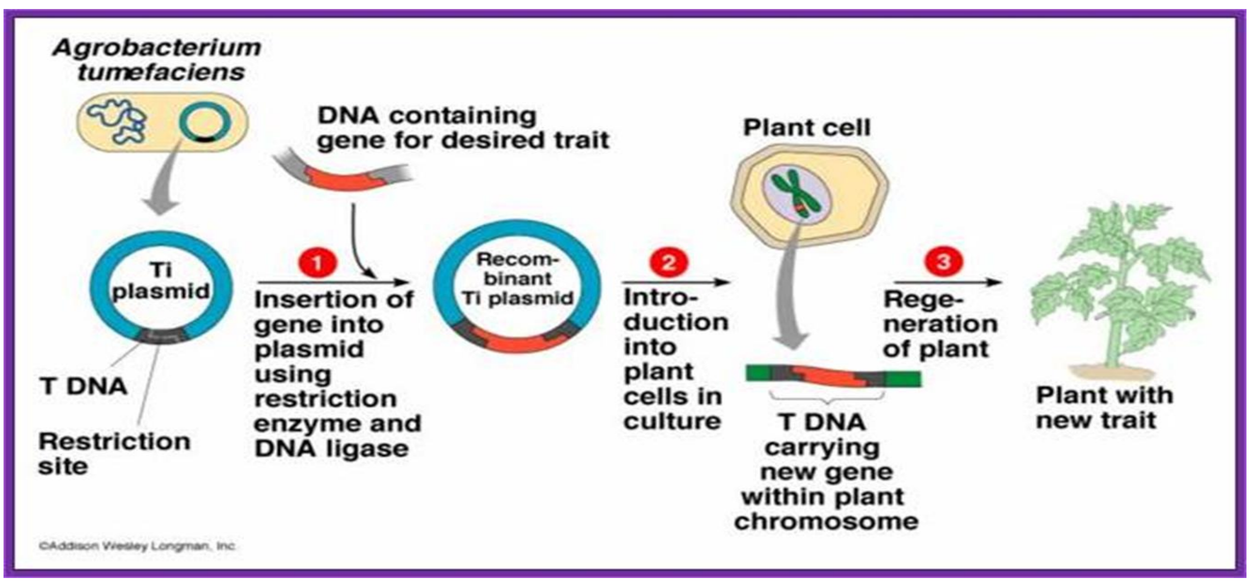

Figure 2 Agrobacterium mediated gene transfer reserachgate.net/figure/Agrobacterium -mediated-plant-transformation_fig1_348908271

Gene gun / Biolistic bombardment system

The first gene gun was the brainchild of John Sanford, a plant geneticist working at Cornell in the early 1980s. The invention was a crude but ingenious concept: use the principles of a normal gun to blast DNA-covered micro bullets at plant cells, thereby introducing foreign DNA and creating transgenic plants .Gene gun is mainly used in tissue culture cells or seedling of any species.In this the desired DNA is coated on microscopic gold/tungsten beads. These particle are fired by gun into plant tissue and penetrate the cell wall. After that the DNA unwinds from gold carrier particle and enter the nucleus (Gao, C., & Nielsen, K. K. (2013).

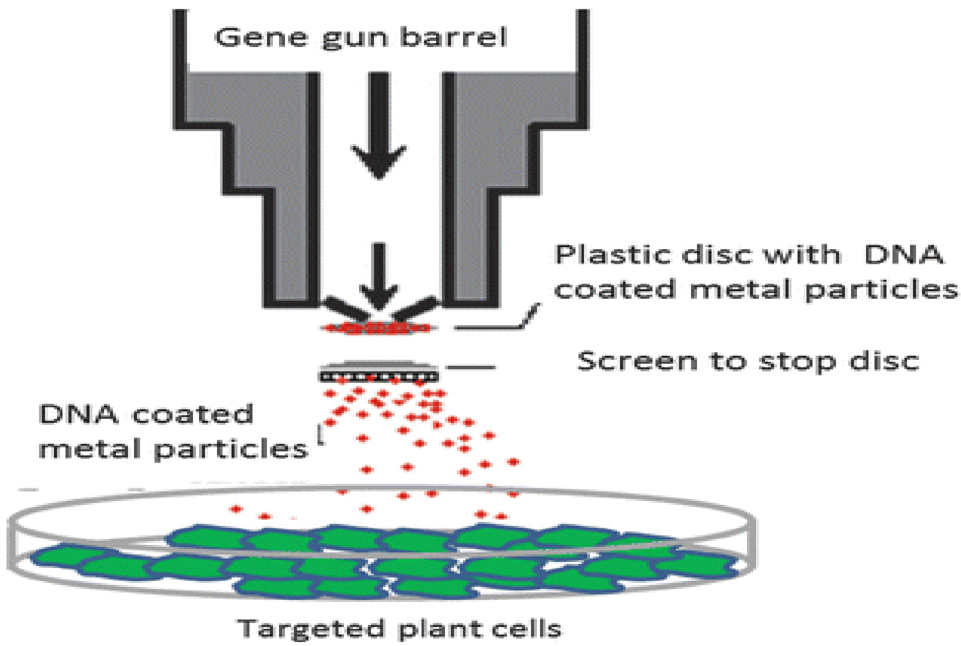

Figure 3 Gene gun https://link.springer.com/chapter/10.1007/978-3-319-63607-8_14

Benefits

1. Improved shelf life

Example -FlavrSavr tomato

- In FlavrSavr tomato Partial inactivation of the polygalacturonase gene increases the time between flavor development and spoilage of fruits (Martineau, B. (2018).

- Delays ripening of fruit and vegetables thus allowing an increased the length of storage.

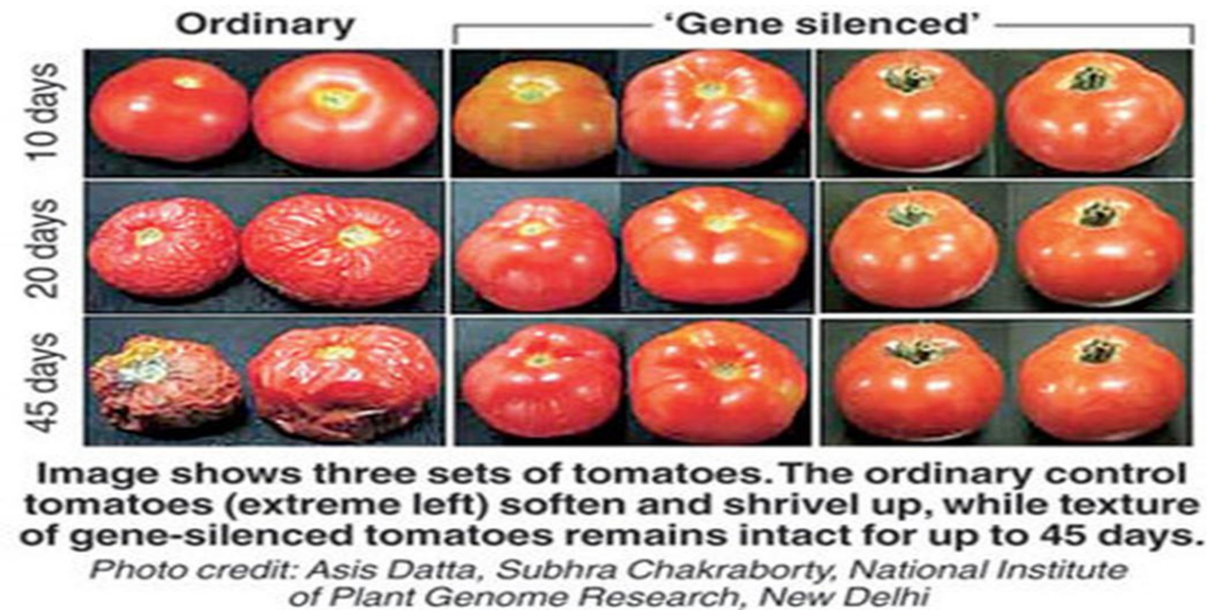

Figure 4 Difference between ordinary and gene-silenced tomatoes up to 45 dayshttps://flavrsavrtomato.weebly.com/pros-cons-and-my-views.html

2. Improved nutrition

Example – Golden Rice

- Golden Rice was developed in the late 1990s by German plant scientists Ingo Potrykus and Peter Beyer to combat vitamin deficiency, the leading cause of childhood blindness.
- Designed to produce beta- carotene, a precursor of vitamin A in the rice endosperm by transforming rice with 2 beta – carotene biosynthesis genes (Greedy, D. (2018).
- Reduce blindness and prevent malnutrition worldwide.
- Recently the government of the Philippines announced that it had approved golden rice, making it the first country to do so.
- In August 2012, tufts university published new research on golden rice showing that beta carotene produced by golden rice is as good beta carotene in oil at providing vitamin A to children

Figure 5Golden Rice (right) and normal rice. The yellow colour is the consequence of the presence of provitamin A (carotenoids) (Potrykus, I. (2013).

3. Insect resistance

Example – Bt cotton.

- Insect resistant cotton was first introduced which was commonly referred as BT cotton. Bt cotton is produced by Monsanto.
- Bt cotton produces an insecticidal protein (Cry1Ac) from the naturally occurring soil bacterium Bacillus thuringiensis (Bt) that protects the cotton plant from certain lepidopteran (caterpillar) insect pests(Kranthi, K. R., & Stone, G. D. (2020).
- The toxicity of each Bt type is limited to one or two insect's orders. Bt is nontoxic to vertebrates and many beneficial arthropods
- Bt cotton is ecofriendly and does not have adverse effect on parasites, predators, beneficial insecticides, and organism present in soil.
- In bt cotton the use of insecticide is very rarely so it helps to reduction to environmental pollution.

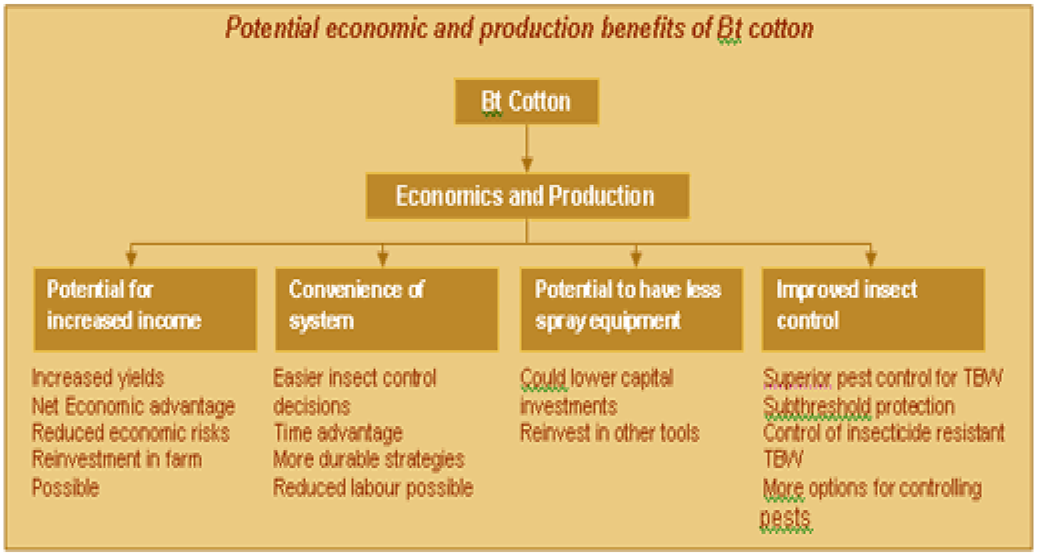

Figure 6 Potential economics and production benefits of Bt cotton
https://static.fibre2fashion.com/articleresources/images/1/12/images/12201.gif

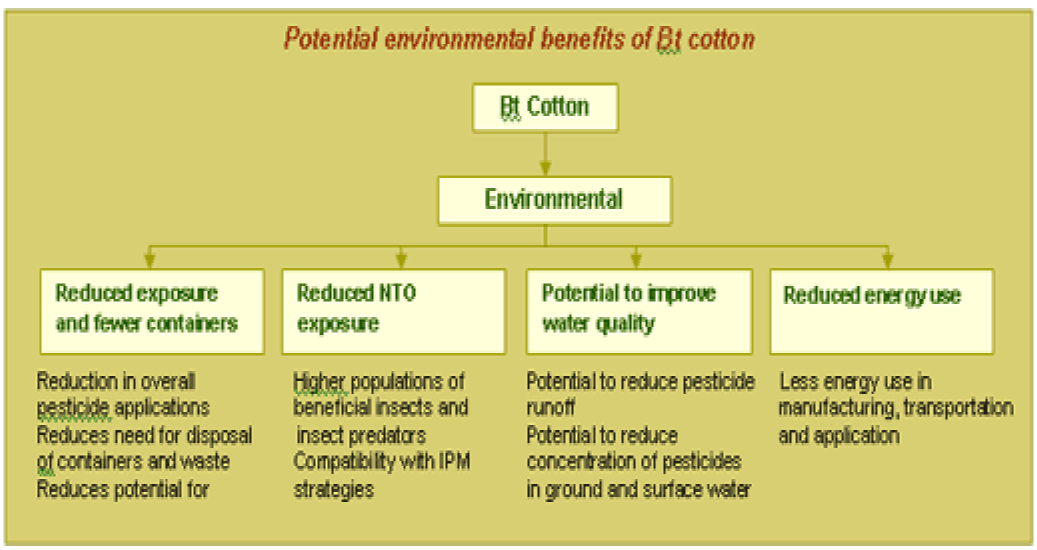

*Figure 7Potential environmental benefits of Bt cotton*https://static.fibre2fashion.com/articleresources/images/1/12/images/12202.gif

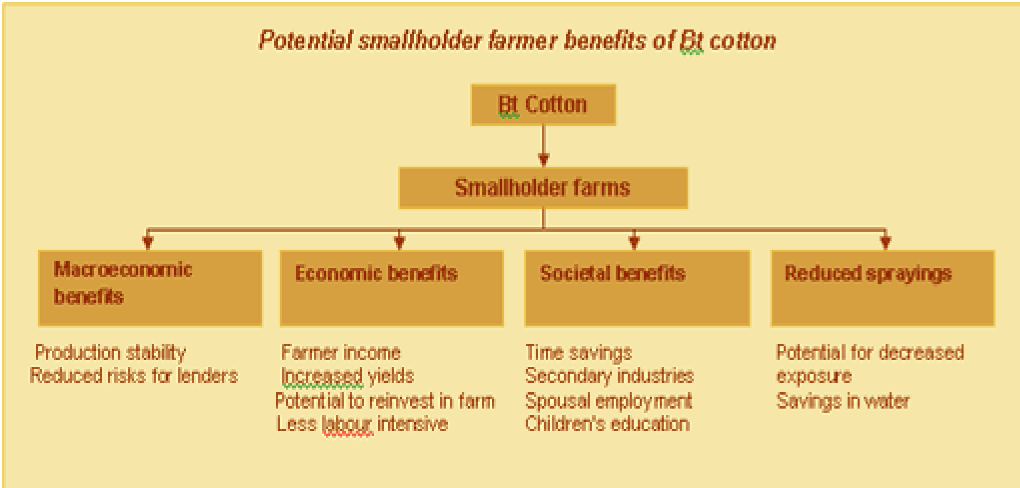

Figure 8 Potential small holder farmer benefits of Bt cotton
https://static.fibre2fashion.com/articleresources/images/1/12/images/12203.gif

4. Diseases resistance

Example – Transgenic PRSV (Papaya ringspot virus) resistant papaya

PRSV is the most dangerous viral diseases of the papaya. The PRSV is control by roughing and infected plant and spraying them with aphicides. Although roughing cannot stop the spread of diseases once it is occur in plant. Uniformly spraying with aphicides is often unsuccessful since the virus is transfer to the plants ahead of aphids are killed. This disease is only control to developing the resistant varieties of papaya.

In transgenic PRSV (papaya ringspot virus) papaya(Hamim et al.,(2018)

- It contain a virus gene that encodes for the production of the coat protein of the virus.
- The coat protein’s primary function is to protect viral genetic information.
- Expression of this gene renders the Papaya resistant to the virus.

5. Pesticide resistance

Example- Bt corn

One of the major problem in maize is insects, pests. About 40% of world food supply is lost due to pest, diseases and spoilage. European corn borer is one of the major pest of maize. It is native of Europe. European corn borer is firstly reported in North American in 1917. The European corn borer caterpillar kills the ears of corn and stalks chewing tunnels causing the plant to fall over. So European corn borer directly affects the maize production. So with the help of *Bacillus thuringiensis* (Soil borne bacteria) Bt corn is developed.Bt prevents feeding of insects on the plant, which cause damage and produce opening to fungus spores to grow.Bt corn has been genetically modified to express the Cry1Ab protein of *Bacillus thuringiensis* to kill the lepidoptera pests. In bt corn lignin content in all hybrids whether grown in plant growth room and in the field, was significantly higher (33-97%) as compare to non Bt isolines (Saxena et al., 2001).

Figure 9 Difference between Bt cotton and non Bt cotton
https://www.apsnet.org/edcenter/apsnetfeatures/Pages/InsectResistantCorn.aspx

Economic effects

Pros

- Improved crop productivity in poor area
    1. Able to boost agricultural production.
    2. Insect-resistant and herbicide tolerant able to lower the risk of crop losses
- Potential to produce medicines inexpensively
    1. Transgenic plants offer the modest cost for pharmaceuticals.
    2. Example :- transgenic bacteria produce most of the insulin to treat the diabetics in the united states

Cons

- Poor farmers might depend only on international corporations for seeds

- Some transgenic crops are designed with terminator technology. So may have two negative impacts :
    1. Environmental
    2. Economics aspects
- farmers could become dependent on buying seed every year that require high costs.

Ethical issues

Adverse effect on environment:

- When herbicide resistant GMO's are used use of herbicide will be stressed adversely affecting environment.
- When using insect resistant GMO beneficial insect species may be adversely affected. E.g.:- Monarch butterfly affected by use of BT corn.
- The gene may be transformed by cross pollination to non GMO crops (which may have unpredicted consequences).
- Mass scale propagation of GMO's may lead to production of superweed and superpests.
- End result - ecosystem disturbed.

Adverse effect on human health

- Since GMO's will introduce in to our diet a protein that was earlier not a part of it, there are high chances of development of immune reaction like allergy.

  Example:
- When gene responsible for production of Met was transformed from Brazilian nut to soybean allergic reaction was developed in those people who were known to allergic to nut

Benefits and risks of GMOs

Benefits of GMOs	**Risks of GMOs**
Nutritional value of foods could be improved (e.g. by introducing proteins, vitamins or vaccines	New traits could causes adverse health reactions (e.g. new proteins may cause allergic responses)
Crops can be grow produced that lack known allergens	Removal of traits could have unknown effects
Crops can grow in arid conditions for better yield (e.g. by introducing drought resistant genes)	Crops may limit biodiversity of local environments (increased competition with native species)
GM crops can produce herbicides to kill pests	Cross pollination could lead to 'super weeds'
Improve food supply/agriculture in poor countries (GM crops can be engineered for improved yields)	Patents restrict farmers from accessing GM seeds (biotech companies hold monopolies over crop use)
GM crops may have longer shelf lives (less spoil)	Foods with GM components may not be labeled
Reduces economic costs and carbon footprint-less need for land clearing and pesticide usage	Different governments may have conflicting regulatory standards concerning safe usage

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