ACHIEVEMENTS OF TRASGENICS

1. Pest resistant plants (BT cotton)

Carry Bt toxins genes

Bacillus thuringiensis, kill insects - 8-endotoxin protein disrupt digestive system of insects. Toxin not harmful to mammals. Toxin gene (bt2) from *B. thuringiensis* isolated and used for *Agrobacterium* mediated transformation of crop plants including tobacco, cotton and tomato plants, which shows 75-100% mortality of the larvae.

Advantages: Save cost and time, reduces health risks, provides ecological benefits, kill a broad spectrum of insects and nematodes.

Disadvantages: Machinery needed for application, application need to repeat, sunlight causes breakdown of active ingredient, water washes protein from plant, emergence of resistant strains.

BT cotton : A GMO or genetically modified pest resistant cotton variety, if eaten by Lepidopteron larvae, the pest killed by Bt protein. First approved for commercial use in US in 1995, In India introduced in 2002 and subsequently became largest producer of GM cotton in the world. By 2014, 95% of cotton grown in the India was genetically modified.

Herbicide resistant transgenic plants

Biodegradable herbicides, necessitated development of resistance in crop plants.

Two approaches for development of herbicide resistant plants:

1. Either target protein is overproduced or target molecules become insensitive to herbicide – e.g. transgenic petunia plants resistant to glyphosate (active ingredient of Roundup herbicide) developed by transfer of gene (*aro*A) for EPSPS (5-enol-pyruvyl-shikimat-3 phosphate synthase, involved in biosynthesis of aromatic amino acids) that overproduces this enzyme. *aro*A gene was also isolated from *Salmonella typhimurium* or *E. coli* and was transferred to tomato and/or tobacco.

2. A pathway is introduced that will detoxify the herbicide – glutathione-S-transferase or GST (in maize and other plants) detoxifies atrazine; nitrilase (coded by gene *bxn* in *Klebsiella pneumonia*), detoxifies bromoxynil, and phosphinothricin acetyl transferase or PAT (coded by *bar* gene in *Streptomyces spp*.) detoxifies PPT (L-phosphinothricin).

Transgenic tomato plants using both *bxn* gene and *bar* gene and transgenic plants in potato, oilseed rape (*Brassica napus*) and sugarbeet using only *bar* gene have been obtained and were found to be herbicide resistant.

Disease tolerant transgenic crop

Resistance against viral infection was first trait introduced using transgenic approach in tobacco, tomato and potato, to a broad spectrum of plant viruses. Six kinds of genes used –

Gene for Capsid Protein (CP) from Positive (+) strand RNA virus, genes for resistance against Geminiviruses, Gene for Nucleocapsid (N) Protein from Tomato Spotted Wilt Virus, Satellite RNA, Antisense SAHH gene, Mutant gene for protein for cell to cell movement.

Disease resistance against bacterial and fungal pathogen is controlled by resistance genes (*R* genes) in the host that specifically recognizes pathogen strains containing complementary avirulence genes (*Avr* genes). Infection induce signalling cascade, leads to hypersensitive reaction (HR) due to cell death in the host leading to inhibition of pathogen growth. HR depends on interaction between *R* and *Avr*. Regulating both gene disease resistant transgenic crop plants developed.

About two thirds of transgenic crops, confer resistance to biotic stress including herbicide tolerance, insect resistance and virus resistance.

Resistance against abiotic stresses

Stress (including drought, high and low temperature, salinity, metal toxicity, flooding etc) tolerance in plants can be achieved in two ways: 1. Enzymes like late embryogenesis-abundant (LEA) proteins, antifreeze proteins, chaperones and detoxification enzymes that are involved in the production of osmoprotectants, protect cells from dehydration. 2. In addition proteins like transcription factors, protein kinases, and enzymes involved in phosphoinositide metabolism induce transcription of stress responsive genes.

Genes with DRE (dehydration response element); Genes (example- betaine aldehyde dehydrogenase – BADH) involved in synthesis of osmoprotectants like Glycinebetaine, Trehalose, genes involved in synthesis of antioxidants (example- Superoxide Dismutase Genes); Early response to Dehydration (ERD) genes; Genes helping in cell membrane integrity; Genes involved in Ion homoeostasis; Genes encoding calcium binding proteins – are the main target genes which are of great interest during production of transgenic plants against abiotic stress tolerance.

Resistance against drought: Mechanism of drought avoidance and tolerance are considered. Examples- Trehalose-6-phosphate synthases (TPS 1) gene from budding yeast was engineered into tobacco and potato, which exhibited increased drought resistance. Transgenic tobacco carrying *betB* gene encoding betaine aldehyde dehydrogenase (BADH) from *E.coli* were drought tolerant.

Resistance against salinity: Can be produced in three ways- 1. Synthesis of compatible solutes like proline, glycinebetaine or trehalose for osmotic adjustment – Example –transferring *TPS1* gene encoding Trehalose-6- phosphate synthases in Rice. 2. Establishment of ion homeostasis using ion transporters - Example – Transferring *AgNHX1* gene in Maize controlling Na^+ /H^+ antiporter and 3. Increased ability of plants to neutralize reactive oxygen species (ROS) during stress response using enzymes like superoxide dismutase and glyoxylase – Example – transferring *Gly1* gene encoding Glyoxylase in Tobacco.

Resistance against chilling: Presence of double bond in fatty acids found in the lipids, may lead to resistance against injury by low temperatures. Example- resistance against chilling was introduced in tobacco, by introducing gene for ‘glycerol-3-phosphate acyl transferase (GPAT) from chilling tolerant *Arabidopsis thaliana*.

Resistance against heat: By altering levels of heat shock proteins (HSPs). Examples – Transgenic tobacco plants, in which the gene encoding the chloroplast-localized fatty acid desaturase was silenced, were resistant to high temperature stress.

Resistance against heavy metals: Transgenic tobacco having cysteine synthase cDNA controlling O-acetyl-L-serine(thiol)lyase are tolerant to heavy metal like Cd, Se, Ni, Pb, Cu etc.

Transgenic crop with improved quality

Golden rice - Rich in vitamin A. Initially developed using two genes (gene *psy* encoding phytoene synthase and gene *lcy* encoding lycopene β-cyclase) from *Narcissus* and one gene (gene *Crtl* encoding phytoene desaturase) from *Erwinia* and designated as Golden Rice 1. Later Golden Rice 2 was developed (with 20 times higher vitamin A content than Golden Rice 1) using phytoene synthase gene (*psy*) from maize and the carotene desaturase gene (*crtl*) from *Erwinia uredovora*.

Flavr tomato –

Tomatoes exhibiting delayed ripening were developed by using antisense RNA against enzymes involved in ethylene production When fruits are allowed to stay on the plant longer, they accumulate higher amounts of soluble sugars, thus achieving better flavour. Tomatoes obtained thus named “Flavr Savr’.

Role of transgenic in pollution degradation (super-bug), Leaching of minerals

'Superbug' - *Pseudomonas putida* transformed with plasmids derived from 4 bacteria containing camphor, octane, xylene and naphthalene degrading plasmids - degrade oil, plastic.

Other examples - *Anabaena sp*. and *Nostoc ellipsosporum* by the insertion of *linA* (from *P. paucimobilus*) and *fcbABC* (from *Arthrobacter globiformis*) respectively. Gene *linA* and *fcbABC* controls biodegradation of lindane and halobenzoates respectively.

Genes involved in metal uptake, translocation and sequestration are transferred for genetic engineering of plants to improve phytoremediation traits; synthesis of metal chelators will improve the capability of plant for metal uptake.

Production of industrial enzymes

Scale up and cost effective production. Plant systems include tobacco, oiI seeds, barley and maize. First xenogenic proteins produced and sold is avidin (gene from chicken egg white introduced in Maize).

Industrial enzyme involve applications in feed, cleaning agents, processing reagents, wood products industry and biomass conversion - include xylanases, cellulases, laccase and trypsin; ProdiGene directed production of proteases in transgenic plants.

Edible plant-based enzyme - ProdiGene expressed sweet protein brazzein in transgenic maize. Non-food industrial enzymes are starch hydrolysis (amylases), textile desizing (amylases), leather production (proteases), detergent additives (proteases). New areas are in biomass conversion and the wood products industries.

These enzyme application replace chemicals that are damaging to environment.

Production of oil

Manipulation of long-chain and medium-chain fatty acid in common oil crops like soybean, oilseed rape, sunflower and maize.

By engineering Oilseed rape with a thioesterase gene from bay tree - much higher levels of beneficial fatty acids - suitable as replacement for palm and coconut oils.

By decreasing the levels of D12-desaturase in transgenic soybeans, the amount of oleic acid can be increased - high oleic acid soybeans have low levels of saturated fats and transfats - healthier product.

It also keeps the oil in a liquid form and makes it more heat-stable for cooking applications.

Production of edible vaccine

Gene encoding antigen from pathogenic organism are introduced in a food like potato, carrot, tomato, banana, alfalfa.

3 antigens investigated - malaria vaccine, merozoite surface protein (MSP) 4 and MSP 5 from *Plasmodium falciparum*, and MSP 4/5 from *P. yoelli*. The hepatitis B surface antigen (HbsAg) is used as a vaccine against hepatitis B virus in transgenic potato.

Paramyxovirus surface protein haemagglutinin in tobacco, potato, rice and lettuce against measles. Bacterial surface protein such as *Vibrio cholerae* B subunit in transgenic tobacco, tomato and rice plants elicit an anti-cholera immune response.

Advantage - simple and cheap way of mass vaccination programme.

Disadvantage - timing of administering the vaccine, dosage, biosafety and the ability of the protein to induce immunity on oral administration. Often recombinant protein is insufficient to stimulate immunity.