Transgenic plants

Transgenic plants are plants in which one or more genes from another species have been introduced into the genome using genetic engineering processes.

The aim is to introduce a new trait to the plant which does not occur naturally in the species.

A transgenic plant contains a gene or genes that have been artificially inserted. The inserted gene sequence is known as the transgene, it may come from an unrelated plant or from a completely different species.

Techniques include the biolistic method—in which a heavy metal is coated with plasmid DNA is shot into cells—and *Agrobacterium tumefaciens* mediated transformation.

Applications of transgenic plant

Herbicide Resistance

- **Weeds are unwanted & useless plants that grow along with the crop plants .**
- **Weeds compete with the crops for light & nutrients, besides harboring various pathogens . So it is estimated that the worlds crop yield is reduced by 10 – 15 % due to the presence of weeds.**
- **To tackle the problem of weeds , modern agriculture has developed a wide range of weed killers (herbicides).**
- **Herbicides are broad spectrum as they can kill wide range of weeds.**

Types of Herbicides by Chemical Families

Chemical Family	Affected System	Target Protein	Spectrum
Triazines (atrazine, ametryne, cyanazine, prometryn, simazine)	Photosystem II, electron transport from Q_A to Q_B	D-1 protein, product of *psbA* gene	Total
Sulfonylureas, imidazolinones, triazolopyrimidines	Amino acid synthesis	Acetolactate synthetase (ALS)	Selective
Aryloxypenoxypropionates (AOPP), cyclohexanediones	Lipid synthesis	Acetyl coenzyme A carboxylase (ACCase)	Selective
Glyphosate (N-phosphonomethyl)glycine	Amino acid synthesis	5-enoylpyruvyl-shikimate-3-phosphate synthetase (EPSPS)	Total
Bromoxynil	Photosystem II	D-1 protein	Total
Phenoxycarboxylic acids (eg 2,4-D)	Unknown	Unknown	Selective
Glufosinate (Phosphinothricin, PPT)	Amino acid synthesis	Glutamine synthetase (GS)	Total
Biprydiliums, praquats, diquats	Photosystem I	Electron transfer system	Total

Problems in the application of herbicides

- Lack of tolerance to the chemical by one or more of the major world crops, eg rice, maize, soybean, wheat, rapeseed.
- Use of multiple types of herbicides to broaden the spectrum of the affected weeds, which in turn increases the possibility that the crop is injured also.

How to prepare a transgenic herbicide plant

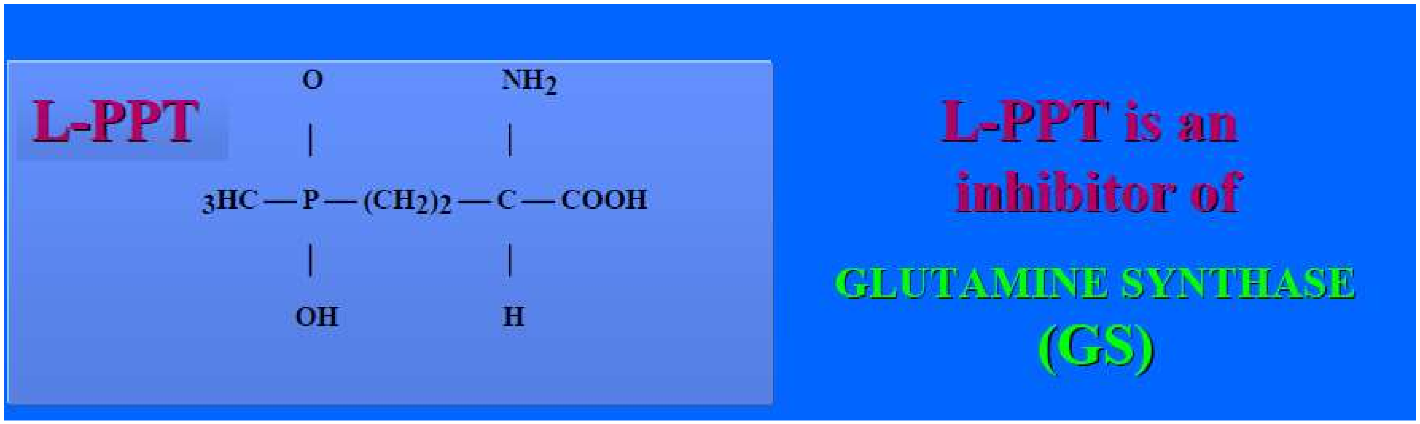
(1)Modification of target protein

(2)Overproduction of target protein

(3)Detoxification of active ingredient

(4)Production of antibodies against active ingredient

Phosphinothricin (PPT)

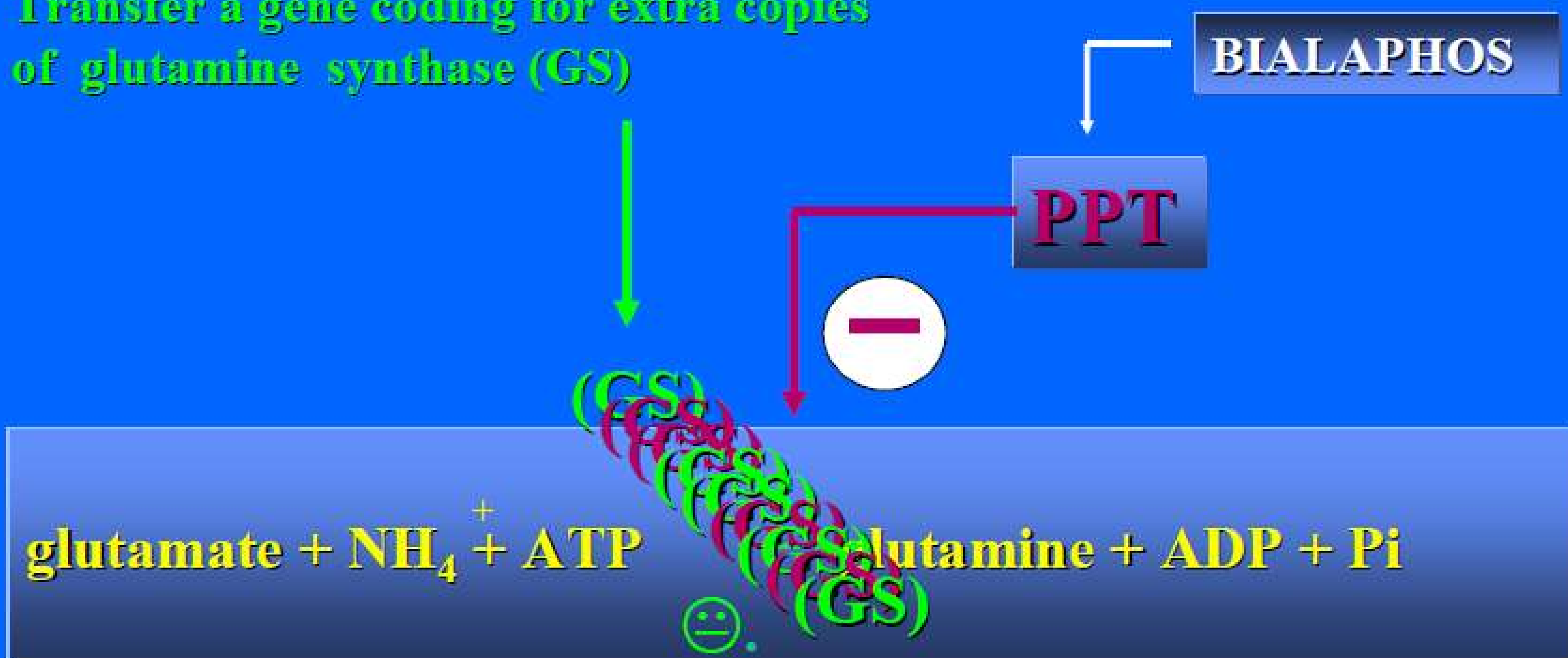


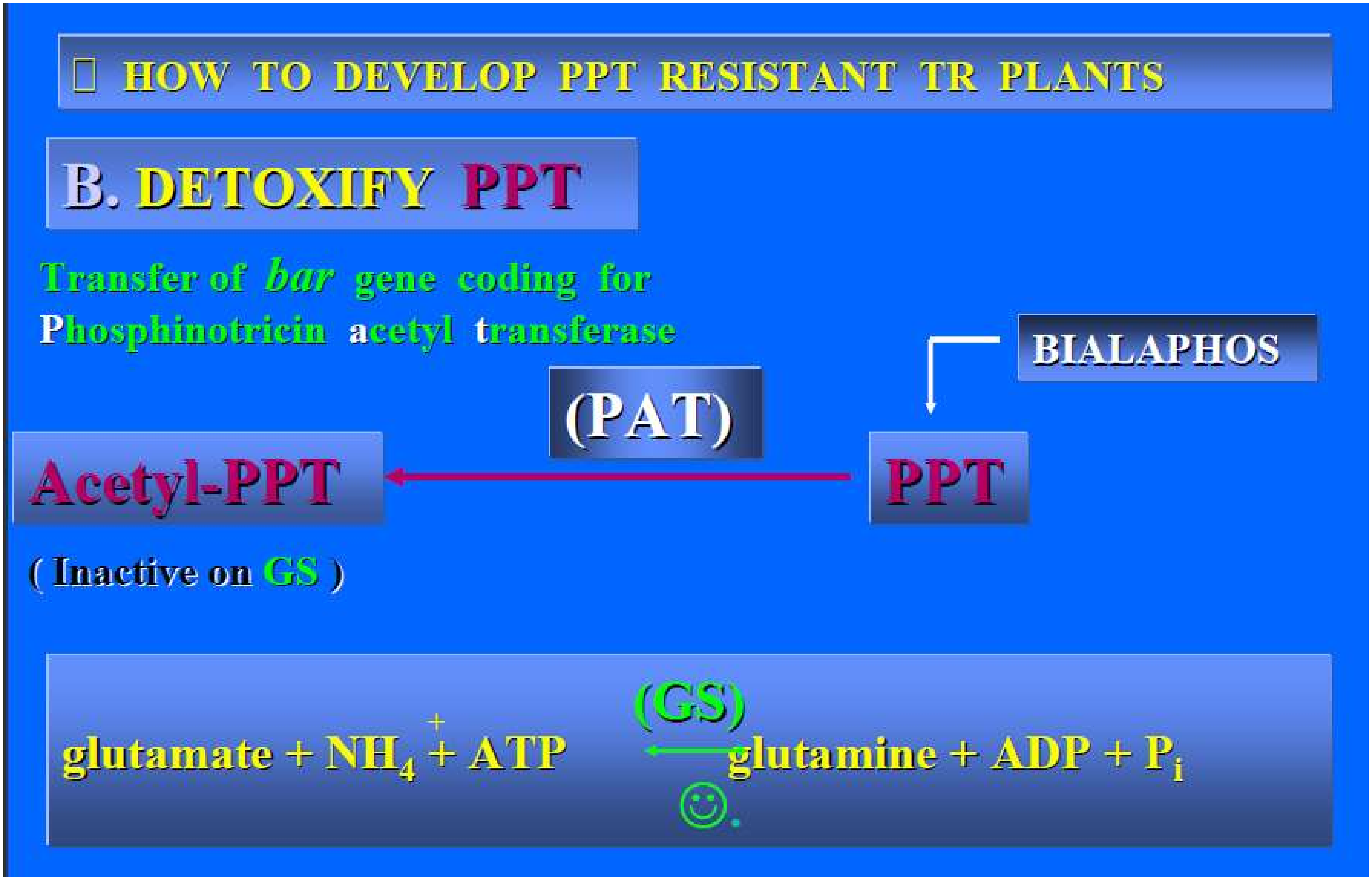
Inhibition of synthesis of glutamine, resulting in protein degradation

HOW TO DEVELOP PPT RESISTANT TR PLANTS

A. OVERPRODUCTION OF GS

Transfer a gene coding for extra copies of glutamine synthase (GS)





bar gene encoding Acetyl -PPT

Glyphosate

It is a broad spectrum herbicide , effective against 76 of worlds worst 78 weeds .
Less toxic to animals , is rapidly degraded & short life span .
The american company (Monsanto) market it as round up .

Mechanism of Glyphosphate action

Capable of killing the plants in low conc .
Rapidly transported to growing tissues .
It is competitive inhibitor of EPSPS (a key enzyme of Tryptophan synthesis)
Glyphosate binds with EPSPS & blocks metabolism (sa).
Thus biosynthesis of tryptophan & other products are inhibited.
So cell division & plant growth are blocked.
This pathway doesn't occur in animals. So it is not toxic to animals

A no. of biological manipulations involved in genetic eng are in use to develop herbicide resistance plant

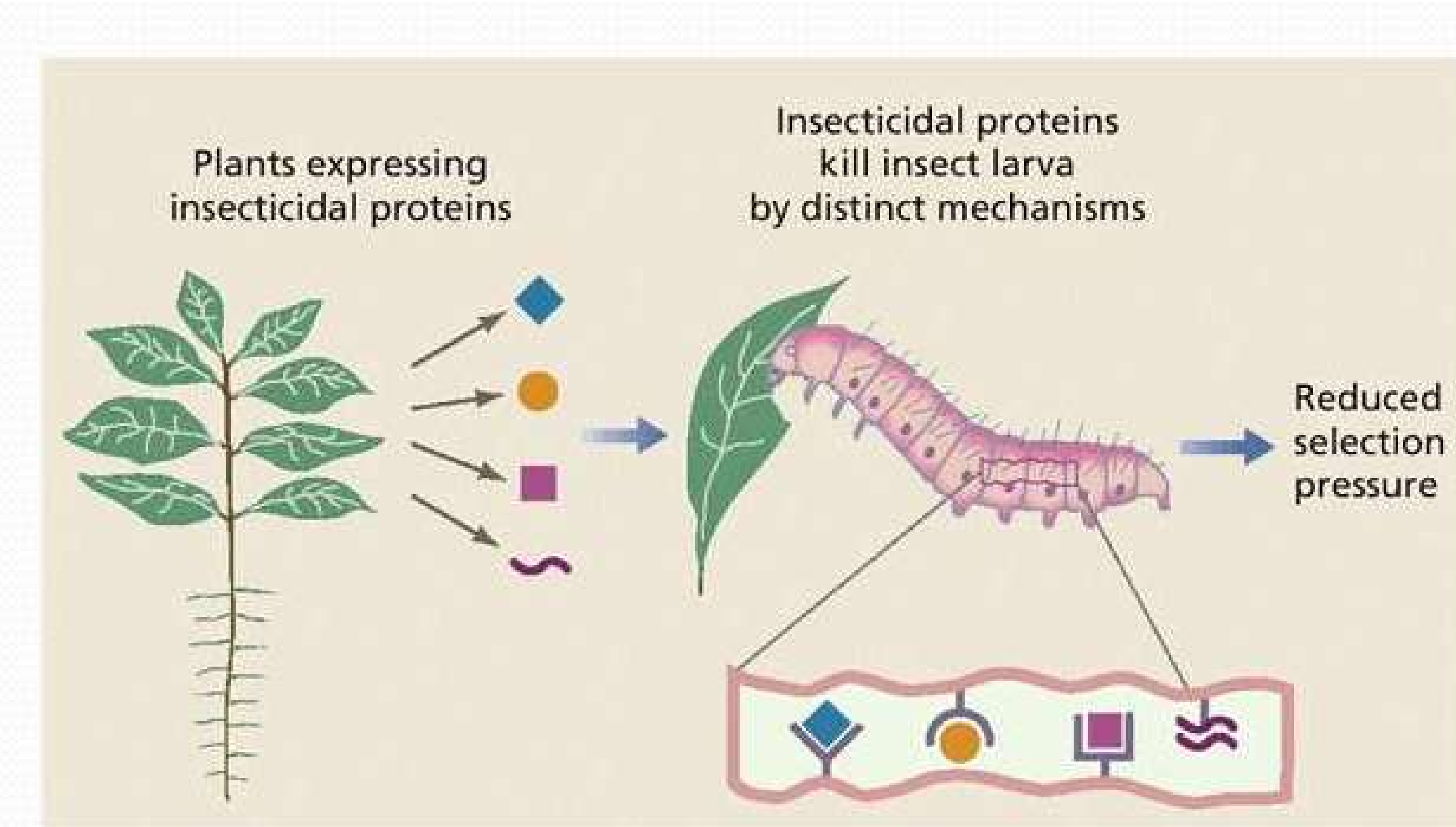
- Over expression of EPSPS gene
- Use of mutant EPSPS gene and inhibition of function of EPSPS
- Detoxification of herbicide by a foreign gene - glyphosate

  converted into glyoxylate by glyphosate oxidase

TABLE 50.7 Selected examples of gene transferred herbicide resistant plants

Herbicide	*Gene transfer/mechanism of resistance*	*Transgenic crop(s)*
Glyphosate	Inhibition of EPSPS	Soybean, tomato
Glyphosate	Detoxification by glyphosate oxidase	Maize, soybean
Phosphinothricin	*bar* gene coding phosphinothricin acetyltransferase	Maize, rice, wheat, cotton, potato, tomato, sugarbeet
Sulfonylureas/imidazolinones	Mutant plant with acetolactate synthase	Rice, tomato, maize, sugarbeet
Bromoxynil	Nitrilase detoxification	Cotton, potato, tomato
Atrazine	Mutant plant with chloroplast *psb A* gene	Soybean
Phenocarboxylic acids	Monooxygenase detoxification (e.g. 2,4-D and 2,4,5-T)	Maize cotton
Cyanamide	Cyanamide hydratase gene	Tobacco

INSECTS RESISTANCE PLANT



- Progress in engineering insect resistance in transgenic plants has been achieved through the use of insect control protein genes of *BACILLUS THURINGIENSIS.*
- In crop damage is mainly caused by insect larvae and some adult insect.

  The majority of the insect that damage crops are : -

- *Lipidoptera (bollworms)* *Coleoptera(beetle)* *Orthoptera(grass hpper)*

Homoptera (aphids)

Resistant gene from microorganism

- Bt gene from *Bacillus thuringiensis*.
- Ipt (isopentenyl transferase) gene from *Agrobaterium tumefaciens*.
- Cholesterol oxidase from *Streptomyces* fungus

Resistance gene from higher plant

- Proteinase & amylase inhibitor
- Lectin

Resistant gene from mammals

- serine protinase inhibition.

BT TOXIN GENE :- Bt toxin gene are isolated from *Bacillus thuringinesis*

- It is gram negative soil bacteria produce parasporal crystalline protein (Cry protein).
- This protein are responsible for the insecticidal activity of the bacterial strain.
- This protein is known as insecticidal crystalline protein or crystalline protein.
- Cry protein are solublized in the alkali environment of insect midgut.
- They are converted to active form upon infection by susceptible tissue then killing the insect by disruption of ion transport across the membrane of susceptible insect.

CROP	GENE TRANSFERRED	INSECTS CONTROLED
TOBACCO	Bt from B. thuringiensis Truncated cryl from B. Thuringiensis CpTl	*Manduca sexta* *M. Sexta* *Heliothis armigera*
TOMATO	Bt	*Heliothis armigera*
POTATO	Crylll from B. thuringiensis Modified crylll gene Snowdrop lectin (GNA)	*Colorado potato beetle (Leptinotarsa decenli - neata)* *L. decenlineata* *Tomato moth*
COTTON	Bt Bt	*Spodoptera* *Cotton boll worm*

Ipt (isopentenyl transferase) gene

cytokinin biosynthesis gene is responsible for accumulation of secondary metabolites in leaves

Cholesterol oxidase gene

Cholesterol or the related sterol at the membrane of the boll weevil midgut epithelium seemed to be accessible to the enzyme and is oxidized by CHOx, causing lysis of the midgut epithelial cells resulting in larval death

Resistance gene from higher plant

➢ PROTEINASE INHIBITORS :-

- Plants contain peptides acting as protease inhibitors .
- The different proteinases are serine, cysteine, aspartic and metallo proteinases.
- They catalyse the release of amino acids from dietary protein, there by providing the nutrients crucial for development of insects.
- The proteinase inhibitors deprive the insect of nutrients by interfering with digestive enzymes of the insect. Two such proteinase inhibitor genes have been described.

α – Amylase inhibitor

- **The larvae secrete a gut enzyme called α – amylase that digest the starch.**
- **Genes for three α – amylase inhibitors have been expressed in tobacco but the main emphasis has been on transferring the inhibitor isolated from adzuki bean (*Vigna angularis*).**
- **This α – amylase inhibitor protein blocks the larval feeding in the mid gut.**

Resistant gene from mammals

- Resistance genes involved are primarily serine proteinase inhibitors from mammals & the tobacco horn worm(*Manduca sexta*).
- Based on in vitro screening of inhibition of proteolysis by midgut extracts of a range of *lepidopteran larvae* Bovine pancreatic trypsin inhibitor (BPTI) α -antitrypsin (α_1 AT) & spleen inhibitor have been identified as promising insect resistance proteins have been transferred to a range of plants.

Abiotic stress tolerance

The plant growth and productivity of many crops are adversely affected by several abiotic stresses including salinity, drought, heat, flood, frost and mineral toxicities.

- A variety of abiotic stresses causing crop loss of about >50%
- Due to the declining water availability, by 2025, 30% crop production will be at risk
- World Bank projects that the climate change will reduce crop yield by 20% or more by the year 2050
- So for that reason transgenic plant have to be developed

Plant responds to drought and/or salinity in mainly two phases, primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell and oxidative stress which may cause denaturation of functional and structural proteins.

To protect from the negative consequences of abiotic stresses, plants have evolved many biochemical and molecular mechanisms.

These diverse environmental stresses trigger the cell signaling process, transcription controls and cellular responses such as the production of a number of stress proteins, expression and activities of antioxidants and accumulation of compatible solutes which activate stress-responsive mechanisms to reestablish homeostasis and protect and repair damaged proteins and membranes

Transgenic approaches

Transgenic approach is now a widely used procedure for introducing genes from distant genepools, ranging from prokaryotic organisms such as *E. coli* to halophytes, into many plant species for the development of stress tolerant plants

Mechanisms

1. Genes involved in synthesis of osmoprotectants like proline, betaine, sugars and sugar alcohol, and polyamines

2. Gene involved in membrane and protein protection functions such as heat shock proteins (HSPs) and late embryogenesis abundant (LEA) proteins

3. Detoxification or elimination of reactive oxygen species (ROS) such as various enzymes and non-enzymatic antioxidants

4. Genes involved in water and ion uptake and transport like aquaporins and ion transporters

5. Genes involved in signal transduction pathways (STPs) and transcriptional control

Engineering of Genes for Osmolyte biosynthesis

- Tolerance to abiotic stresses has mainly been achieved at cellular levels of osmotically-active solutes

  - Proline, glycine betaine
  - Sugars such as sucrose, trehalose and fructans
  - Sugar alcohols like sorbitol, mannitol, ononitol, pinitol and polyols

- These osmolytes are uniformly neutral with respect to cellular functions

Accumulation of these molecules helps plants to –

- Retain water within cells and protects cellular compartments from injury caused by dehydration
- Maintains turgor pressure during water stress
- Stabilize the structure and function of certain macromolecules
- Signalling functions or induction of adaptive pathways
- Scavenge reactive oxygen species

Proline

- Proline accumulates in many organisms in response to drought and salinity
- Proline is encoded by a nuclear gene ***Pyrroline-5-carboxylate synthetase (P5CS)***
- Proline may serve as a **hydroxyl radical scavenger** reducing the acidity of the cell

It may also function as osmolyte and protect protein integrity and enhance the activities of different enzymes

Transgenic plants for Proline

Gene	Molecular function	Source	Transformed plant	Performance of transgenic plant	Additional note
P5CS	*D1-pyrroline-5-carboxylate synthase*	*V. aconitifolia*	*O. sativa*	Drought & salt tolerance	Increased biomass (higher fresh shoot & root weight)
P5CS F129 A	*Mutated P5CS*	*V. Aconitifolia*	*N. tabacum*	Oxidative & osmotic stress tolerance	Removal of feedback inhibition resulted in 2-fold high proline than the plants expressing P5CS
PDH	*Proline dehydrogenase*	*A. thaliana*	*G. max*	Drought & heat tolerance	Rapid increase in proline resulted in least water loss under drought stress

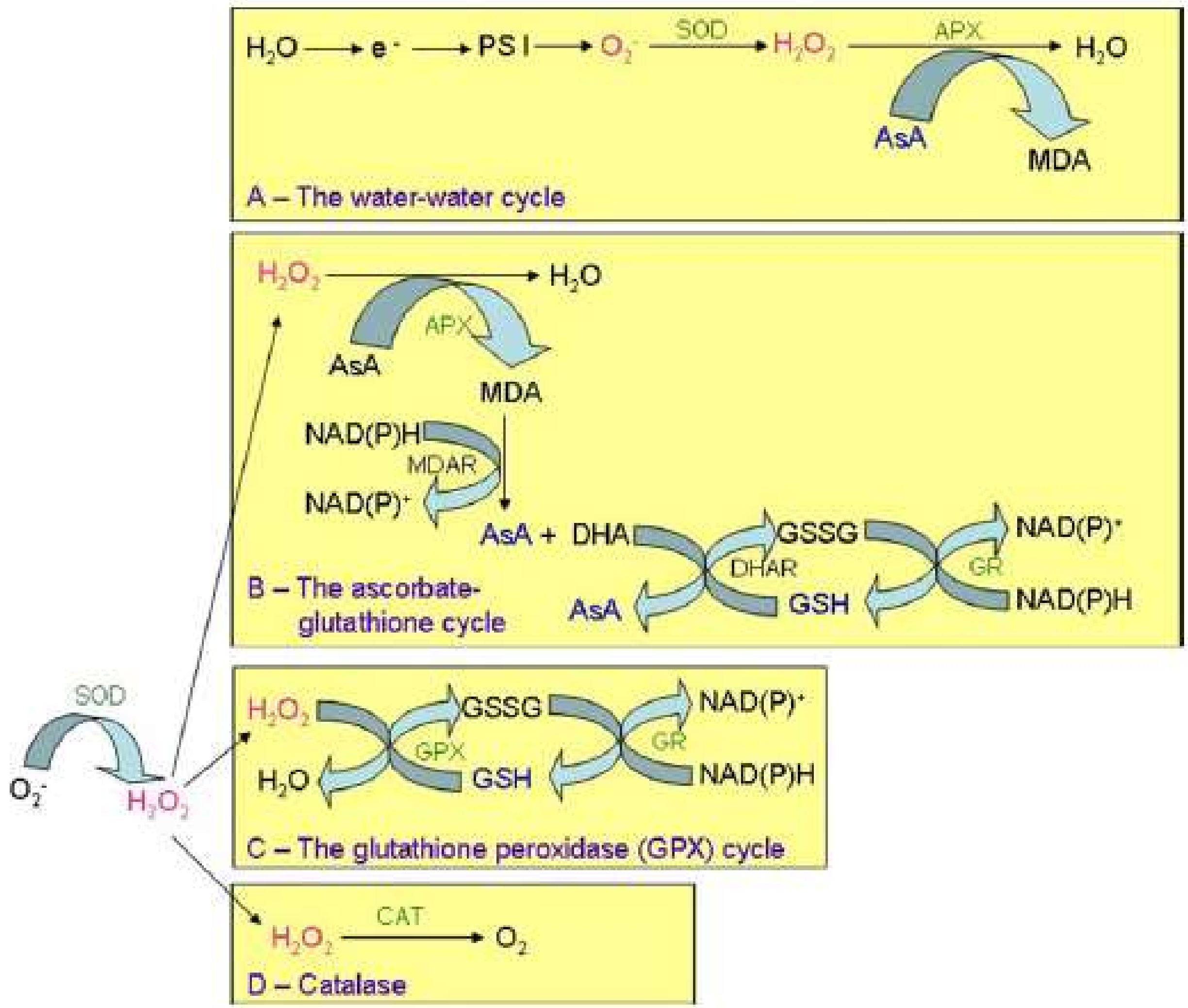
Transgenic plants for Glycine betaine

Gene	Molecular function	Source	crop	Function	Additional note
BADH	*Betaine aldehyde dehydrogenase*	*A. Hortens is*	*O. sativa*	Improved growth & salinity tolerance	10% transgenic plants set seed in 0.5% NaCl solution in greenhouse
BADH	*Betaine aldehyde dehydrogenase*	*H. Vulgare*	*N. tabacum*	Salt tolerance	Tolerance was judged by high biomass & photosynthetic
CHO	*Choline dehydrogenase*	*E. coli*	*O. sativa*	Drought & salt tolerance	Higher yield under stresses
betA	*Choline dehydrogenase*	*E. coli*	*Z. mays*	Drought tolerance at seedling stage	Grain yield was significantly higher compared with wild-type

Engineering of genes encoding enzymes for scavenging active oxygen

- Plants suffering from various stresses, which leads to overproduces active oxygen species in cells
- To minimize the damaging effects of active oxygen, plants produces antioxidant to detoxifying harmful oxygen
- Genes encoding enzymes with antioxidant capacity, such as ascorbate peroxidase, superoxide dismutase and glutathione reductase

M.K. Rai et al. / Environmental and Experimental Botany 71 (2011) 89–98



Pathways for ROS-scavenging in plant cell. (A) The water–water cycle. (B) The ascorbate–glutathione cycle. (C) The glutathione peroxidase (GPX) cycle. (D) CAT activity

Example:

- Transgenic alfalfa overproducing **superoxide dismutase (SOD)** showed reduced injury from water deficit and freezing stresses in field conditions

- Engineering chloroplastic **superoxide dismutase (SOD)** in tobacco led to an increase of chilling tolerance of photosynthesis

- Tobacco plants that overproduced cytosolic **ascorbate peroxidase** showed increased tolerance to oxidative stress

- Over expression of **glutathione S-transferase** or **glutathione peroxidase** provided some protection against cold and salt stress in tobacco

Genes encoding LEA Proteins

A genes encoding LEA (Late Embryogenesis Abundance) proteins is activated under osmotic stress

- LEA proteins play a role in desiccation tolerance during seed development and in response to dehydration, salinity and cold stress
  - **Maintenance of protein or membrane structure**
  - **Sequestration of ions,**
  - **Binding of water**

Rice plants transformed with barley LEA gene HVA1 possessed increased tolerance to water deficit and salinity

Heat shock proteins (HSPs)

- E.g: An antisense inhibition of the *HSP70* family prevented Arabidopsis plants from acquiring thermotolerance
- Heat shock proteins *HSP101* from soybean, corn, wheat and tobacco complemented a thermo tolerance defect in yeast caused by deletion of yeast *HSP104*

Ion Transporters and Abiotic Stress Tolerance in Plants

The osmotic component of salinity is caused by excess inorganic ions such as Na^+ and Cl^- in the environment that uptake by the plant root. In this scenario, plants can accumulate excessive amount of Na^+ in the cytosol which negatively affects many aspects of cellular physiology.

Therefore, adaptation of plants to salt stress requires cellular ion homeostasis involving efflux of Na+ and balance of Na+ and K+.

- plasma membrane Na^+/H^+ antiporter
- vacuolar Na^+/H^+ antiporter
- plasma membrane K^+/Na^+ symporter

Engineering of genes encoding Transcription Factors

- Responses of plants to abiotic stresses are multigenic and a single gene is not likely to induce the whole cascade

- Recently, transcription factors for abiotic stress-induced genes have been identified, cloned and used in transgenic experiments

- Simultaneous expression of downstream stress-inducible genes have been achieved with parallel increase in stress tolerance

Examples

- Over expression of the transcriptional activator ***CBF1*** ('C-repeat binding factor') induced the expression of four **COR** ('cold-regulated') genes and increased freezing tolerance of *Arabidopsis* plant

- By over expressing a single stress-inducible transcription factor (***DREB1A***), lead to plant tolerance to freezing, salinity and dehydration

Disease resistance

In the last two decades, considerable efforts have been made on the development of transgenic plants utilizing a broad range of genes to enhance disease resistance against fungal, bacterial and viral pathogens.

Ming et al. (2008) reported a draft genome sequence of 'SunUp' papaya, the first virus resistant transgenic fruit authorized for field cultivation and commercialization.

FUNGAL AND BACTERIAL RESISTANCE

For fungal and bacterial diseases, the following four strategies have been applied to make disease resistant transgenic plant: transgenic disease-resistant plants used

- **Genes encoding pathogenesis-related proteins (PR proteins), antimicrobial peptides or antimicrobial metabolites**
- **Genes that encode detoxification mechanisms**
- **Genes that have a role in pathogen recognition and**
- **Genes that regulate defense mechanisms**

The most widely used transgenic approach to enhance resistance against fungal diseases has been based on the over-expression of PR proteins.

The production of hydrolytic enzymes like chitinase and glucanase, best characterized class of PR proteins, capable of degrading the cell wall of invading pathogenic fungi is an important component of the defense response in plants against fungal pathogens.

The use of antimicrobial peptides constitutively expressed in plant tissues has been recommended for the genetic engineering of plants for disease resistance against fungal and bacterial pathogens

Defensins, one of the classical examples of small antimicrobial peptides, play an important role in the plant defense response against fungal targets. Ex. transgenic banana plants

Phytoalexins, an excellent example of antimicrobial metabolite, are an important component of plant defense and many studies demonstrated that phytoalexins contribute significantly to resistance against pathogens

Usually pathogens produce many toxins and cell-wall-degrading enzymes for successful infection. Detoxification of phytotoxins and degradation of phyotoxic metabolites by enzymes expressed in transgenic plants could provide an opportunity to enhance resistance to disease. However, due to health-risk this strategy to develop disease resistant plant is not approachable as some phytotoxins (i.e. mycotoxins) are harmful to mammals and the product of a detoxification reaction may still be toxic to the consumers

Virus resistant plants

For viral diseases, all viral molecules including genomes and three types of proteins: coat proteins (cp), movement proteins, and proteins involved in genome replication, represent potential targets for a genetically modified (GM) resistance strategy.

This approach is based on the concept of pathogen-derived resistance (PDR) in which pathogenic virus itself is used as gene source for developing genetically engineered viral resistant plant

Some of the earliest success stories for producing virus resistant plants by genetic engineering were using viral CP as a transgene. Virus-resistant plants have a viral protein coat gene that is overproduced, preventing the virus from reproducing in the host cell, because the plant shuts off the virus protein coat gene in response to the overproduction.

Ex. - Coat protein genes are involved in resistance to diseases such as cucumber mosaic virus, tobacco rattle virus, and potato virus X.

Golden rice

Golden rice is a variety of rice (*Oryza sativa*) produced from genetic engineering

Main purpose is to provide pro-vitamin A to third world, developing, countries where malnutrition and vitamin A deficiency are common

Mammals make vitamin A from β-carotene, a common carotenoid pigment normally found in plant photosynthetic membranes

Here, the idea was to engineer the b-carotene pathway into rice

HOW DOES IT WORK?

The addition of 2 genes in the rice genome will complete the biosynthetic pathway

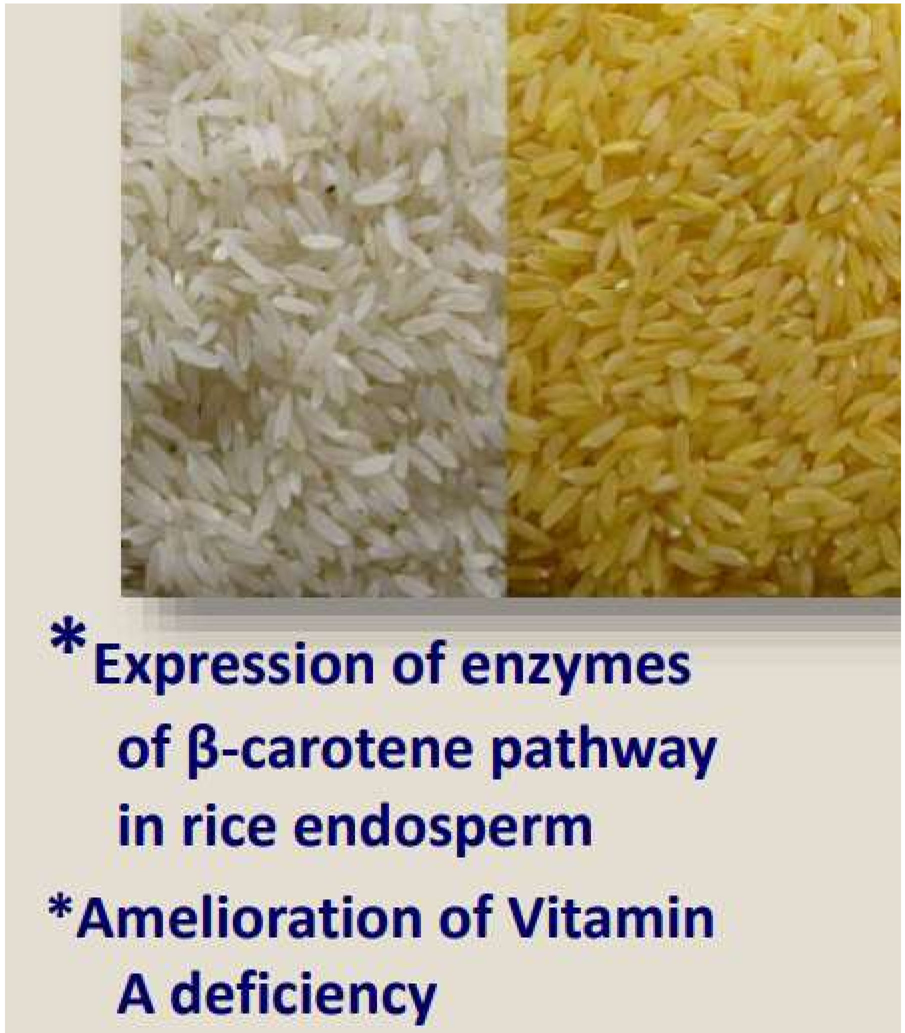
1. **Phytoene synthase (psy)** – derived from daffodils, Lent lily (*Narcissus pseudonarcissus*)

(Phytoene synthase is a transferase enzyme involved in the biosynthesis of carotenoids. It catalyzes the conversion of geranylgerany pyrophosphate to phytoene.)

2. Lycopene cyclase (crt1) – from soil bacteria *Erwinia uredovora*

Produces enzymes and catalysts for the biosynthesis of carotenoids (β-carotene) in the endosperm

The psy and crt1 genes were transformed into the rice nuclear genome and placed under the control of an endosperm-specific promoter, so they are only expressed in the endosperm.



The transgenic rice is yellow or golden in color and is called “golden rice”

ADVANTAGE

Golden rice give more quantity vitamin-A

Easy distribution when released to needy

Cheaper option to supply vitamin A requirement compared to other supplementary measures

Sustainable option as once released for common cultivation can be cultivated every growing season by farmer saved seeds, therefore no need of yearly budgetary investment for distribution

Disadvantage

May cause allergies or fail to perform desired effect

Loss of Biodiversity. May become a gregarious weed and endanger the existence of natural rice plants

Genetic contamination of natural, global staple foods

Edible Vaccine production

Edible vaccines are vaccines produced in plants that can be administered directly through the ingestion of plant materials containing the vaccine. Eating the plant would then confer immunity against diseases.

One focus of current vaccine effort is on hepatitis B. Transgenic tobacco and potatoes were engineered to express hepatitis B virus vaccine.

Potatoes have been studied using a portion of the E. coli enterotoxin in mice and humans and then transgenic potatoes were produced.

Other candidates for edible vaccines include banana and tomato

FLAVR-SAVR TOMATO

This is produced by antisense technology.

The polygalactouronase gene, which is responsible for fruit decay is silenced.

1st genetically modified crop approved for sale in U.S. was FlavrSavr tomato. 1994

Biopolymers and plants

a) Plant seeds may be a potential source for plastics that could be produced and easily extracted.

b) A type of PHA (polyhydroxylalkanoate) polymer called "poly-beta-hydroxybutyrate", or PHB, is produced in *Arabidopsis* or mustard plant.

c) PHB can be made in canola seeds by the transfer of three genes from the bacterium *Alcaligenes eutrophus*, which codes for enzymes in the PHB synthesis pathway.

d) A polymer called PHBV produced through *Alcaligenes* fermentation, which is sold under the name Biopol.

HISTORY

- The first genetically modified crop plant was produced in 1982, an **antibiotic-resistant tobacco plant.**

- In 1987, Plant Genetic Systems ,founded by Marc Van Montagu and Jeff Schell, was the first company to genetically engineer **insect-resistant (tobacco) plants** by incorporating genes that produced insecticidal proteins from Bacillus thuringiensis (Bt).

- The first genetically modified crop approved for sale in the U.S, in 1994, was the **FlavrSavr tomato** as it had a longer shelf life.

- In 1994, the European Union approved tobacco engineered to be resistant to the **herbicide bromoxynil,** making it the first commercially genetically engineered crop marketed in Europe.

In 2000, Golden rice with β- carotene developed with increased nutrient value