
Welcome

MSC BOTANY

GENETIC ENGINEERING

TRANSGENIC PLANTS

OBJECTIVES AND ACHIEVEMENTS

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OBJECTIVES AND ACHIEVEMENTS

- MAIN OBJECTIVE--TRANSGENIC PLANT PRODUCTION

IS THE TRANSFER OF DESIRABLE GENES TO PLANTS

- GENES CAN BE TRANSFERRED TO PLANTS FROM

OTHER PLANTS, ANIMALS, MICROBES

ACHIEVEMENTS:

1. **ENGINEERING FOR SECONDARY METABOLITES:**

Produced by cell suspension culture.

ADVANTAGES

1.Helps to study the biosynthetic pathway of secondary metabolites

2. Free from various environmental factors

3.Produced in controlled conditions

4.Qulaity of the product

5.Easy for extraction

6.Some plants are difficult to grow in field conditions or expensive to grow

7. Capacity for biotransformation

2. RESISTANCE AGAINST HERBICIDES-

- Herbicides are used for killing the weeds(unwanted plants)
- Herbicides disturb the metabolic activity of photosynthesis and amino acid synthesis
- So they affect the crop plants
- Mechanism of action of different herbicides differ
- Herbicide tolerance is a genetic trait
- The herbicide tolerance gene encodes enzyme which detoxify the herbicide and tolerate the effects.
- Some plants and number of microorganism possess such genes

2. RESISTANCE AGAINST HERBICIDES-

Using GE techniques transgenic plants have been produced by intoducing herbicide tolerant genes into crop plants. thus resistance agianst herbicides have been developed in many crops

Herbicide tolerant genes introduced into plants

1. **EPSPS *(5-enolpyruvate-shikimate-3phosphate synthase)* was transferred to Petunia and transgenic Petunia developed which was resistant against glyphosate.(a herbicide)**

2. Transgenic plant "round up ready " has been produced – tolerant to round up (glyphosate)


NOUVELLE MATIÈRE ACTIVE
FORMULE BIOCONTRÔLÉE
SPEED
ROUNDUP
PRÊT A L'EMPLOI
EFFETS EN 1H*
DÉSHERBANT
ACTION RAPIDE
Détruit les mauvaises herbes par contact
1,2 L

3. **Herbicide resistant gene *als* (*for enzyme acetolactase synthase*)of tobacco was introduced to tomato plant. Transgenic tomato was resistant against the herbicide (sulfonylurea)**

4. *Gox* gene of Achromobacter (resistant to Glyphosphate oxidase), *bar gene* of Streptomyces sps. (resistant to herbicide PPT), *bxn* gene of Klebsiella (resistant to bromoxynil) etc are transferred to various crop plants

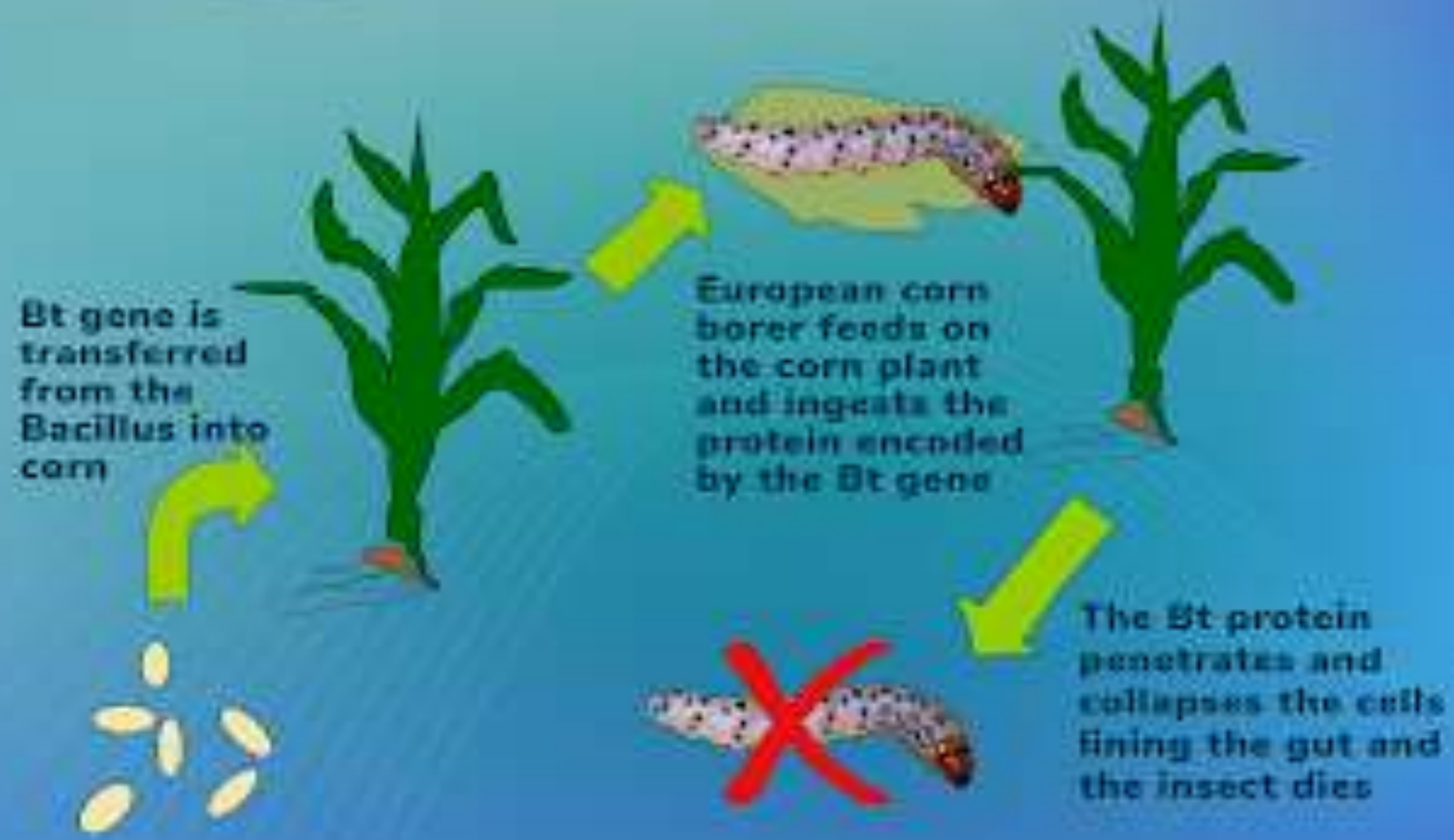
3. RESISTANCE AGAINST INSECTS

1. ***Cry* genes of *Bacillus thuriengiensis*-commonly called Bt gene**

- **Bt gene produces a proteinceous toxin (called endotoxin-beta endotoxin ,delta endotoxin)inside the bacterial cell**
- **When specific insects ingest the toxin , they are killed.**
- **Toxin denatures the epithelium of gut by creating minute holes(pore forming toxin)**
- **The insecticidal toxin of *Bacillus thuriengiensis* has been classified into 4 major classes –cryI,cryII,cryIII and cryIV, based on the insecticidal activities against many insects**

BACTERIA FOR AGRICULTURE

DARWIN



- The Bt gene has been successfully transferred to cotton plants
- The transgenic cotton (called Bt cotton) is resistant against bollworm,which is a notoroius pest of cotton
- The Bt gene has been transferred to other crops .
- Bt crops-tobacco, tomato, potato, cauliflower, brinjal, cabbage etc
- Bt cotton was released for commercial cultivation in India in March 2003


Bacillus thuringiensis
Bt Gene is inserted into crop
Crop is infected by European corn borer
Pest dies when feeding on any plant part

2.Cpti genes(cow pea trypsin inhibitor gene)of cow pea is insect resistant against the storage pest of seed called Bruchid beetle . Cpti gene has been transferred to tobacco

3. *Cholesterol oxidase* (against boll weevil) in Streptomyces has been engineered in tobacco

4.*Ipt* gene from Agrobacterium introduced to tobacco and tomato

5.α- amylase inhibitor gene of *Phaseolus vulgaris*

4. RESISTANCE AGAINST PATHOGENS

a. Resistance against fungus
b. Resistance against virus
c. Resistance against bacteria
d. Engineering toxin insensitivity
e. Resistance through phytoalexins

Resistance against fungus:

- The ability of hydrolytic enzymes to break chitin and glucan in the cell walls of fungal pathogens have been exploited to develop resistance against fungus
- The chitinase gene from a soil bacteria , *Serratia marcescens* have been transferred to tobacco
- Chitinase from *Phaseolus vulgaris* also used in transgenic

Resistance against virus

cross protection principle used- crop inoculated with mild strains so it develops resistance against virulent virus

- Power *et al* produced transgenic tobacco by transferring coat protein of TMV to tobacco. Transgenic tobacco showed resistance against TMV. Coat protein inhibits the virus replication in initial stages of infection
- Virus produce a protein (replicase protein) essential for replication and also provide high degree of resistance against viral infection. This replicase protein has been transferred to crop .so transgenic plants showed resistance

- Antiviral protein or ribosome inactivating protein(RIP) have been identified in many plants eg:Poke weed Antiviral protein (PAP)of *Phytolacca americana* . Trangenic tobacco and tomato produced using *PAP* gene

- Mammals produce interferons against virus .interferons induce the production of some proteins that leads to inhibition of viral multiplication eg. oligoadenylate synthase . Transgenic tobacco developed by transferring mammalian oligoadenylate synthase gene

RESISTANCE AGAINST BACTERIA

- Some organism may contain antibacterial protein.these proteins give resistance against bacteria
- Barley α thionine gene was transferred to tobacco . It showed resistance against *Pseudomonas syringae*

- Lytic peptides(cecropins, attacin etc) arre small proteins their effect is to form pores in bacterial membranes. Gene for cecropin has been transferred to potato and tobacco. Gene for attacin transferred to apple

ENGINEERING TOXIN INSENSITIVITY

Toxin inactivating enzymes have been succesfully used to engineer resistance

- *Pseudomonas phaseolicola (haloblight in bean)* produces a toxin phaseolotoxin. Phaseolotoxin inhibits enzyme ornithine transcarboxylase(OC). Bacteria have selected which contain phaselotoxin insensitive OC .this gene have been isolated ,cloned and transferred to tobacco

ENGINEERING TOXIN INSENSITIVITY(contd)

- Xanthomonas albilineans produce toxin albicidin. It blocks DNA replication and chloroplast development. Albicidin detoxifying enzyme (alb gene) from bacteria(*Pantoea dispersa*) has been tranferred to sugarcane

RESISTANCE THROUGH PHYTOALEXINS

- Phytoalexins –secondary metabolite– produced due to infection. Gives resistance.
- Reserveratrol –phytoalexin synthesised by enzyme resveratrol synthase.
- This gene from peanut transferred to tobacco

5.RESISTANCE AGAINST STRESS

- Almost all biotic stresses such as drought,low temperature,salinity,and alkalinity adversely effect growth and induce senescence leading to cell death and loss of crop yield
- Several abiotic stresses cause cellular water deficit or osmotic stress. In response to water deficit plants synthesise certain substances known as osmoprotectatsthey lower the osmotic potential and helps to maintain turgor.
- several plants possess genes that encode certain substances which provides resistance against stresss.

- corelation between Proline accumulation and tolerance to drought and salinity. P5CS pyroroline 5 carboxylate synthetase synthesizes proline. Transgenic tobacco produced by transferring P5CS . They showed 10-18 times proline hence enhanced growth against salt and drought

- *E.coli* gene mtlD mannitol 1 phosphate dehydrogenase involved in biosynthesis of mannitol. Introduced to tobacco showed salt resistances

- Transgenic tobacco plants were produced by introducing a bacterial gene for fructan biosynthesis. Transgenic plants accumulating fructan showed drought stress

- Barley lea gene HVA1 showed stress protection in transgenic rice

- Dehydration responsive transcription factors (DREB) were identified . They induce transcription of several genes in response to cold and water stress. DREB genes were used to produce transgenic plants tolerant to freezing, drought, and salinity

- High temperature stress tolerant transgenic plants were produced by altering the level of expression of heat shock proteins(HSP)

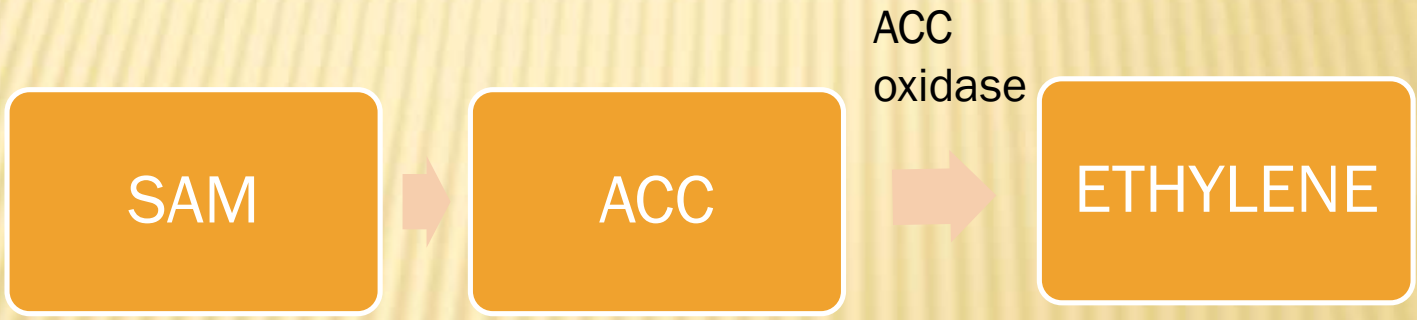
- Cold tolerance can be induced in trangenic tobacco by the expression of a gene for chloroplast W3 fatty acid desaturase

- Expression of fish antifreeze protein in transgenic plants has been demonstrated , but without a correlation to increased freezing tolerance

6. TRANSGENICS FOR QUALITATIVE CHANGES

A. TRANSGENICS FOR IMPROVED STORAGE:

- Flavr savr tomato
- Transgenic tomato –modifying ethylene biosynthesis. Ethylene regulates fruit ripening. In biosynthesis of ethylene **s- adenosyl methionine (SAM)converted to 1 amino cyclopropane 1 carboxylic acid(ACC)**



GM Tomato are produced by introducing a gene from Pseudomonas that encodes an enzyme capable of breaking down ACC into metabolites other ethylene . This causes delayed fruit ripening

B. TRANSGENIC FOR LENGTH OF FLOWER LIFE:

Petal senescence – controlled by ethylene. In carnation petal senescence "in rolling" behaviour

Acc oxidase converts ACC into ethylene. Transgenic carnation produced by introducing antisense ACC oxidase gene. The flowers produced little ethylene delayed senescence flower life extended by 200%

C. TRANSGENICS FOR FLOWER COLOUR AND SHAPE:

ANTHOCYANIN –major flower pigments. Delphindin an anthocyanin leads to blue pigmentation

- Florigene company Australia cloned the gene from *Petunia hybrida* coding for delphindin. Transgenic violet carnation were produced Flower colour has also been changed by introducing certain transcriptional factors that control the structural gene for biosynthesis of anthocyanin

- Intensity of colour has also been modified

- White and pink flower varieties have been achieved by introducing sense and antisense chalcone sythase gene in chrysanthemum,rose etc
- Size and shape has also been modified by altering the cytokinin : auxin ratio

D. TRANSGENICS FOR MALE STERILITY:

Mariani *et al* 1990 constructed a hybrid gene having *TA29 gene* of tobacco and *Barnase gene* of *Bacillus amyloliqufaciens*.

Using this gene transgenic tobacco,tomato, cotton *etc* have been produced

Kriete *et al* 1996 used *arg E* gene from *E.coli* to produce transgenic male sterile . This gene encodes N acetyl Ornithine deacetylase . This enzyme deacetylase converts the inactive herbicide acetyl bialaphos into active form . In active form it causes male sterlity by destroying anther tissue

Transgenic plants expressing *arg E gene* when sprayed with inactive acetyl bialaphos showed male sterility.

Advantage : *arg E* gene does not affect pollen development

7. TRANSGENIC PLANT AS BIOREACTORS:

Transgenic plants are used as bioreactors for the large scale production and inexpensive production of nutritional chemicals and pharmaceuticals. This is also called molecular farming

A. NUTRITIONAL QUALITY

I .CARBOHYDRATES

- **Cyclodextrins (cd)are produced from starch by cyclodextrin glucosyl transferase CGTase enzyme this enzyme is coded by CGT gene. This gene is isolated from Klebsiella and introduced to potato so potato produce high cyclodextrin**

- **Fructosyl transferase gene from bacillus has been transferred to potato ,,tobacco etc. helps in accumulating fructan**

- **Tobacco plants have been transformed with mannitol 1 phosphate dehydrogenase gene (mtld) from E.coli. Transgenic plant synthesise more mannitol**

ii. Vitamin A

Vitamin A is synthesised from carotenoids. Three genes are involved in the synthesis of carotenoids. GM rice was produced by introducing these three genes – for biosynthesis of carotenoids—transgenic rice yellow in colour- called golden rice---yellow colour due to high provitamin A

iii. Lipids

- **Rat Desaturase gene (remove H group from fatty acid)introduced to tobacco-increased level of oleic and palmitic acid**
- **Thioesterase gene from bay tree introduced to *Brassica napus* (rape seed) –accumulate lauric acid**
- **Gene for synthesis of linolenic acid present in cyano bacteria– transferred to tobacco. Linolenic acid helps to prevent coronary diseases**
- **Ricinoleic acid – used in paint, varnishes etc. castor oleate hydrolase gene- transferred to plants. Accumulate ricinoleic acid**

iv. Proteins

- 2S albumin gene of brazil nut . Introduced to tobacco – increased albumin production
- storage protein gene Ama 1 of Amaranthus transferred to potato increased protein
- Soyabean glycine gene introduced to rice- increase protein

v. Iron content

- To increase iron content a ferritin transgene from *Phaseolus vulgaris* is used

b. Immunotherapeutic drugs

i. Edible vaccines: Expressing antigens was successfully made. plants having Edible parts (consumed raw) can be used for production of edible vaccine . First edible vaccine produced in 1990

- ✓ a surface protein from streptococcus to tobacco plant.
- ✓ Cholera toxin- in potato and tobacco.
- ✓ Rabies virus coat glycoprotein in tomato.
- ✓ Hepatitis B surface antigen in tobacco

ii. Edible antibodies: Transgenic plants are used as a source of an source of antibodies. These plants provide passive immunisation by direct application. A hybrid antibody lgA/G has been used successfully against *Streptococcus mutans*, which causes dental problems. The gene for this antibody has been transferred to tobacco

iii. Edible interferons- transgenic maize and tobacco plants that secrete human interferon(IFNR) have been produced. It was produced by transformation of nucleus and chloroplast through particle bombardement method

8.Biodegradable plastics or phytopolymers-

Poly hydroxy butyrate (PHB) an aliphatic polyester with thermoplastic properties was synthesised in plants by introducing two genes – gene for acetoacetyl-coA reductase and gene for PHA synthase- from the bacterium *Alcaligenes euthophus* into a plant *Arabidopsis thaliana*.

Gene transfer worked but plant produced very less PHB. Problem was solved by adding a sequence to the gene. This results in storage of enzymes that transgenes produce in plastids . Rest of cell protected from harmful effect of PHB accumulation


Thank
You