PAPER – 302 PLANT BIOTECHNOLOGY

UNIT 1

Topic: BASIS OF TUMOR FORMATION , TI-RI PLASMID &DNA TRANSFER

INTRODUCTION

- Agro bacterium is a pathogenic bacteria that causes tumor in plants .
- Ti- plasmid causes crown gall disease in plants.
- Small part of Ti- plasmid (T- DNA) is transferred into plant genome.
- Ri – plasmid cause hairy root diseases in plants.
- DNA transfer – The foreign DNA transformation of bacterial cells and its subsequent integration into the genetic material of host.

BASIS OF TUMOR FORMATION

- Agro bacterium tumefactions causes tumor in plants i.e. crown gall disease.
- Agro bacterium rhizogenes causes hairy root disease.
- The tumor formation results from the transfer of particular set of Ti & Ri – plasmid into plant chromosome.
- Tumor formation occur by transfer of small segment of Ti – plasmid. (T-DNA).

AGROBACTERIUM

There are two main species of agro bacterium :-

1. Agrobacterium tumefaciens -

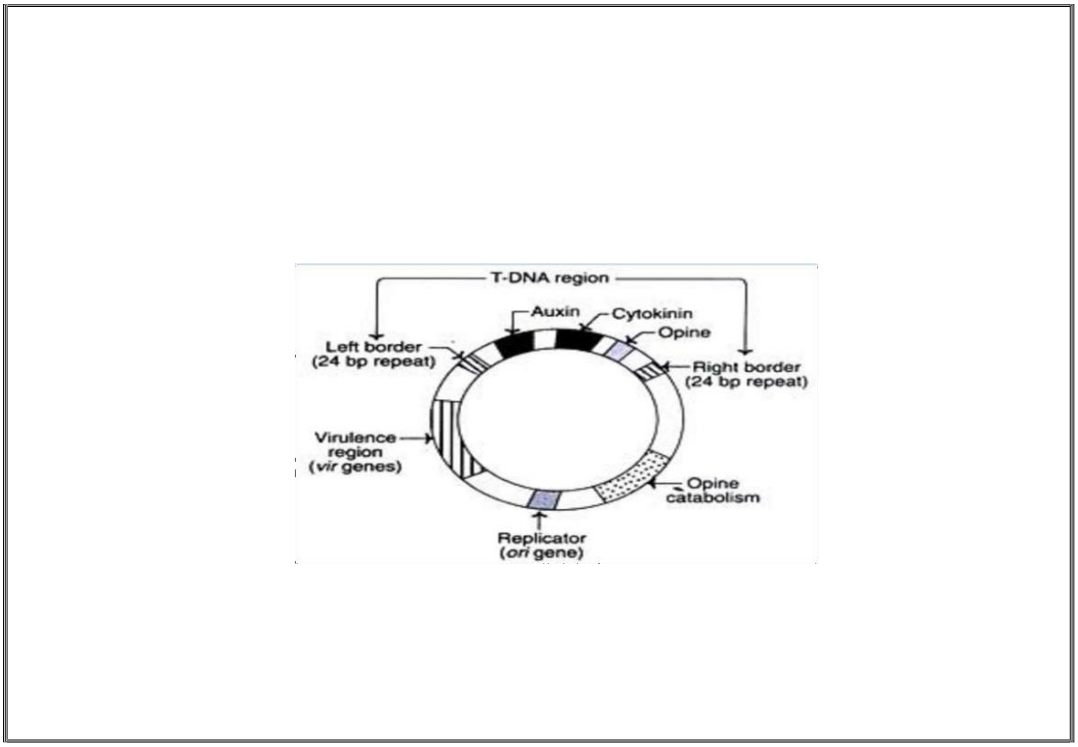
- Infects damaged plant tissue which is induced plant tumor i.e. crown gall.
- Crown gall occurs when bacterium releases Ti – plasmid into cytoplasm.

2. Agrobacterium rhizogenes –

- Induces hairy root disease Bacterial genes transfer its T- DNA from its Ri- plasmid into plant throughwound

Ti- PLASMID

- Extra chromosomal , double stranded circular DNA molecule.
- Vir genes helps in transfer of T-DNA into plant cell.
- Small segment of Ti- plasmid transferred to host plant & integrated with genome i.e. T-DNA.
- Agro bacterium induced tumors synthesize variety of unusual compounds-opines.



OPINES

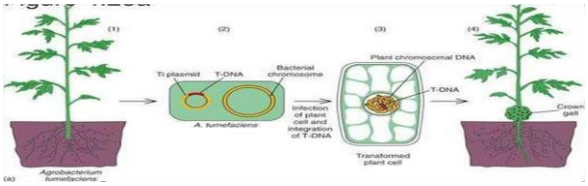
- Agro bacterium induce tumors synthesize a variety of unusual compounds called opines.

Types of opines:-

- Octapine

  Nopaline

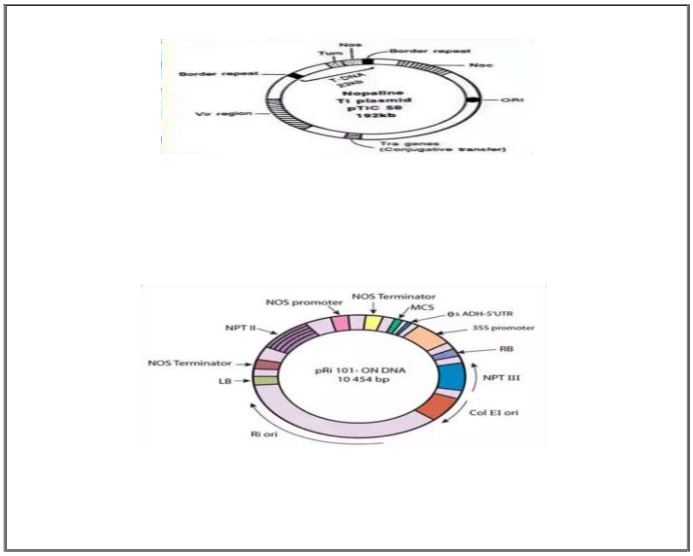
  Agropine
- These opines are catabolized by Agro bacterium to obtain energy



T- DNA

Only a small segment of Ti- plasmid is transferred to the host plant cell & gets integrated with genome.

- It contains genes for tumor formation (Tum) & nopaline biosynthesis(Nos).
- T-DNA is bordered by 25 bp repeats , required for excision & transfer ofDNA.



Ri- PLASMID

- The virulence plasmid of A.rhizogenes is commonly known as Ri- Plasmid(pRi).
- Agro bacterium rhizogene is a soil borne , gram negative bacterium.
- It causes hairy root disease in plants .

GENE TRANSFER TECHNIQUES

- Gene transfer techniques in plants
- Gene transfer techniques in plants
- Transgenicplantaretheplantthatcarrythestablyintegratedforeigngenes. These plants may also be called
- trans formed plants.

ThetransferredDNAmaybeexpressedforonlyshortperiodoftimefollowingtheDNAtransferprocess and this is called transient expression.

Stable transformation occur when DNA is integrated into the plant nuclear genome expression occurs in regenerated plant and is inherited in subsequent generations.

STEPS IN TRANSFORMATION

Identification of useful genes; desirable genes located in

Wild species, un related plant species, unrelated organism and animals.

Designing gene for insertion: The gene of interest is isolated from the donor source and cloned in the laboratory. The cloning is done generally using plasmid.

Insertionofgeneintotargetplant:Theclonedgenei.emultiplecopiesofthegeneofinterestareinserted into host plant or the recipient plant.

Identification of transgenic cells :Transformed cells are identified using selectable marker and are regenerated into whole plant in nutrient medium.

Regenerate plant compared with plant variety. It should look like parent variety except gene of interest.

GENETRANSFERMETHODS

1. Vector Mediated Gene Transfer

2. Vector less or Direct Gene Transfer Methods

VECTOR MEDIATED GENETRANSFER

Plant gene vectors being exploited for transfer of genes are plasmids of Agro bacterium viruses &transposable elements.

Vectors: Small circular DNA molecule occurring in bacteria ,which can exchange between different cells under natural condition.

Plasmids: Plasmids are the extra chromosomal self replicating &double stranded, closed &circular DNA molecule present in the bacterial cell.

AGROBACTERIUMMEDIATED TRANSFORMATION

Agro bacterium system historically first successful plant transformation system, breakthrough in1983.

Break through in gene manipulation in plants came by characterizing and exploiting plasmids carried by the bacterial plant pathogens Agrobacteriumtumefaciens&Agrobacteriumrhizogenes.

Bacteria of the genus Agro bacterium–gene vectors for plant cells. Agro bacterium–gram negative long to bacterial family Rhizobiaceae

Agrobacterium–near soill evelat junction of plant stem& root. Agrobacterium tumefactions : Induces crown gall disease

Agro bacterium rhizogenes:Induces hair root disease Agro bacterium radio bacter:An a virulent strain

Large Plasmids in theses agrobacteria are called Tumour inducing plasmids(Ti)and root inducing plasmid(Ri)

Diseases result from transfer and function alintegration of particular set of Ti or Ri plasmid in plant chromosome

Features of Ti plasmid which make them attractive gene vector

Ti plasmid integrates into plant genome and stably transmitted through division of mitosis and meiosis.

Genes like no palinesynthase encoded by T-DNA possess promoter that function in plant cells.

Foreign gene/DNA inserted into'T-DNA'region is integrated into plant genome.

Agro bacterium has broad host range hence the gene of interest in the"T-DNA"canbe transferred to wide range of plants.

Transformation Technique Using Agrobacterium

Important requirement for Agrobacterium mediated enter transfer in plants: Plant explants s must produce aceto syring one/

Agro bacterium may be pre induced with synthetic aceto syring one.

Agrobacteriashouldhaveaccesstocellthatarecompetentfortransformation.Transformationcompetentcells/tissue should be able to regenerate into whole plant Infection of wounded plant

Seedlings decapitated and freshly cut surface wound is inoculated with over night culture of Agro bacterium

Tumour produced excised out and grown as callus culture.

Transforming callus are picked off& regenerated

Co cultivation

Protoplast isolated during cell reformation stage

Incubated for24-40hrs in as suspension of Agro bacteria at about 100 bacteria per protoplast.

Transformation occur during subsequent few days of co cultivation and exposure to selective agent.

VIRUS MEDIATED GENETRANSFER

Vectors based on virus desirable –high efficiency of gene transfer can be obtained by infection and amplification of transferred genes that occurs via viral genome replication.

Viral infection of cell result in addition of new genetic material which is expressed in the host.

Additional genetic material incorporated in the genome of plant virus might be replicated and expressed in the plant cell along with viral genome.

Replicating genomes of plant viruses are non-integrative vectors as compared to those vectors based on the T-DNA of Agro bacterium tumefactions which are integrative gene vectors.

Non-integrative vectors as plant virus vector do not integrate into the host genome ;rather they spread systematicallywithinaplantandaccumulatetohighcopynumbersintheirrespectivetargetcells.

VectorsfortransferringgenesintoplantarebasedonDNA/RNAmoleculethatnaturallyexpresstheir genetic information in plant cells

ELECTRO PORATION

Use of short electric impulse of high field strength.

Electric impulses increases the permeability of protoplast and allow entry of DNA molecule into the cells,if DNA is indirect contact with the membrane.

If host cell has cell walls, enzymes are used to dissolve the walls, leaving only theses protoplast and the foreign DNA is introduced via electroporation.

Electroporation pulse generated by discharging acapacitora cross the electrodes in a specially designed

electro porator chamber

Protoplast exposed to short electric pulse which open the transient membrane channels through which DNA can pass target cells &then cultured in vitro on appropriate media.

ProtoplastinionicsolutioncontainingthevectorDNAaresuspendedbetweenelectrodes&duetohigh

Voltage ,pore sare made on the walls of protoplast which facilitate the entry of DNA

PARTICLE BOMBARDMENT /BIOLISTIC/PARTICLE GUNMETHOD

Biolistic is process of bombarding cells with microscopic projectile coated with DNA. Shot at high velocity from particle gun into cells/tissue.

Promising for plant which cannot be infected by Agro bacterium.

DNA delivery to plant cell made possible when heavy micro particle or micro carrier(tungsten/gold)coated with the DNA of interest are accelerated to very high initial velocity are made to bombard the living plant cell.

Micro particle penetrate the cell wall &get integrated into the plant genome.

High frequency of integration of multi copy insertion; no regeneration protocol necessary

DNAcoatingissophisticatedtechnology&requiresprecisepreparationofDNAcoated gold/tungs

Ten particle.

Gold-uniform size &shape ,less toxic to cells.

Coating of micro pellet with DNA by precipitation is important step.

1.25to18mgmicroparticlesaremixedwith0.5to70µgofplasmid DNAinCaCl2(0.25.2.5Mzandspermidine(0.1M)

solution.

Mixture continuously vortexed to ensure uniform coating.

After DNA precipitation, micropellets palced on macro carrier membranes &allowed to dry and immediately used.

PARTICLE GENEOME BOARDMENT

•It i›•asdevelopedbyPro1.Santordandcon or kers of Cornell University (USAJ in 1987

• As the telot denotes it shoots foreign DNA intoplantcellsortissueataie1a•highspeed.

•This techniques is also known as particle bombardment, particle gun method, bio listic process ,Croprojectile bombardment or particle acceleration

This technique1smost suitable for those plants which hardly re generate and do not shot ›sufficient responseto gene transfer through Agicicicciinri for example,

ADVANTAGES

It is clean and safe.

Transformation of organized tissue Universal delivery system.

Transformation of recalcitrantt species Study of basic plant development process.

DISADVANTAGES

In plant, gene transfer leads to non-homologous integration into chromosome and is characterized by multiple copies and some degree of rearrangement.

Emergence of chiameral plant.

Lackofcontroloverthevelocityofbombardment,whichoftenleadtosubstantialdamagetothetargetcell

- MICROINJECTION
- Direct mechanical introduction of DNA under microscopic control in specific target.
- Micro injection is able to penetrate intact cell wall.
- Host trans gene in dependent and does not require a protoplast regeneration system.
- Cells/ protoplast –glass micropipette of 0.5-10.0µm diameter ipsareused for transfer of macro molecule into the cytoplasm/nucleus of recipient cell/protoplast.

- Recipient cells can be immobilized by using methods such as agarose embedding, agar embedding,poly-lysine treated glass

Once injection achieved, the injected cell must be cultured properly to ensure its continued growth and development.

Disadvantage-production of chimeric plant with only a part of plant is transformed.

- Processs low, expensive,requires highly skilled and experienced personnel
- LIPOSOMEMEDIATEDGENETRANSFER
- Lipo some are small lipid bags ,in which large number of plasmids are prepared artificially.
- They can be induced to fuse with protoplast using devices like PEG ,there fore have been used for gene transfer.
- Liposome mediated transformation has been achieved by including positively charged agent such as cation s in the mixture or using the cationic liposome preparation.
- Advantage :Protection of DNA/RNA from nuclease digestion.
- Low cell toxicity.
- Stability and storage of nucleic acid due to encapsulation in liposome.
- High degree of reproducibility.
- Applicable to wide range of cell type

PEG MEDIATED GENETRANSFER

First conclusive demonstration of uptake &integration of isolated Ti plasmid DNA into plant proto plast was reported in Petunia& tobacco in the presence of PEG/Poly L-or nithine.

Protoplast are isolated-particular concentration of protoplast suspension taken in tube-followed by addition of plasmid DNA.

To this 40%PEG4000(w/v)dissolved in mannitol & calcium nitrate solution added slowly because of high viscosity &mixture incubated for few minutes.

Advantage :form of the DNA applied to the protoplast controlled entirely by the experimenter and not by an intermediate biological vector.

Disadvantage: system requires protoplast &a functional system for regeneration of the protoplast callus whole plants

TOPIC : MOLECULAR MARKERS AND TYPES OF MOLECULAR MARKERS

Molecular marker:

- Molecular marker is identified as genetic marker.
- Molecular marker is a DNA or gene sequence within a recognized location on a chromosome which is used as identification tool.
- In the pool of unknown DNA or in a whole chromosome, these molecular markers helps in identification of particular sequence of DNA at particular location.
- **Applications:**
    - It plays a crucial role in gene mapping by identifying the position of linked genes in the chromosome which inherited together
    - It also detect any alteration in a sequence of DNA or any genetic oddity. It ascertains genes involved in genetic disorders.
    - It is used to determine different characters in a DNA sequence which is used to distinguish between individuals, populations or species.
- Different types of genetic polymorphism can be used as Genetic markers. On the basis of polymorphisms detected in the genetic makeup of individuals that may vary in the length of a DNA sequence or in the identity of nucleotides located at specific position in chromosome, some of the common genetic markers are- RFLP, SSLP, etc
- Genetic markers can be classified as PCR based and hybridization based.
    - **PCR based genetic markers**: RAPD, ISSR, EST-SSR, microsatellite, CAPS etc.
    - **Hybridization based genetic markers**: RFLP, VNTRs, in which targeted gene is digested with restriction enzymes and then hybridized with RFLP probe.

Quality for a good genetic marker:

- Genetic markers should be largely polymorphic in nature
- They should be selectively neutral
- Assay for detecting markers should be simple and rapid
- Genetic markers should occur frequently within genome
- The genetic marker (gene) should show codominant inheritance pattern.
- They should be highly reproducible
- They should not interact with other markers while using multiple markers at a same time

Types of genetic markers:

1. Random Amplified Polymorphic DNA (RAPD):

- RAPD was developed by Welsh and McClelland along with Williams in 1990.
- It is pronounced as 'rapid'.
- It is based on PCR assay and it doesn't need require any prior sequencing of DNA.
- This procedure uses short arbitrary primer of 8-12 bp that randomly amplifies the region of DNA.
- This primer serves as both forward and reverse primer.
- This reaction proceeds when a single primer anneals to the genomic DNA at two distinct sites on the complementary strand of DNA template.
- The amplification of segment of DNA depends on the positions complimentary to the primers' sequence.
- The fragments obtained from RAPD are between 0.2 to 5.0kb and can be viewed by using agarose gel electrophoresis stained by ethidium bromide or with the help of polyacrylamide gel electrophoresis.
- If any mutation occurs in the primer binding region then no any PCR product will be produced, yielding a distinct pattern of amplified DNA segments on the gel.
- **Application:**
    - Distinct pattern of amplification is seen in different samples. This is why RAPD can be used for studying polymorphism.
    - RAPD is applicable for the mapping of genome, analyzing linkage, and individual specific genotyping.
    - RAPD markers are dominant in nature so it has restrictions for mapping purpose.
    - RAPD is strictly laboratory dependent so it requires sensitivity.
-

- **Demerits:**
  - It has demerits as poor reproducibility, yields faint products, problems occur in band scoring.

2. Restriction Fragment Length Polymorphism (RFLP):

- It was one of the first methods used for the analysis of DNA in various fields such as forensic science.
- It is a hybridization based technique.
- It was invented by **Alec Jeffreys,** an English scientist in 1984 during his research in genetic diseases.
- RFLP uses particular restriction endonuclease enzymes that cuts at its specific site yielding fragments of various lengths along with the fragment of interest.
- The length of the distinct fragments is determined by using blotting, now replaced with sequencing.
- RFLP markers are largely locus-specific and are co-dominant in nature due to the nature of restriction endonuclease used.
- **Steps for RFLP are as follows:**
  - DNA extraction is done from saliva, blood or other samples and is purified.
  - Restriction endonucleases digests the purified DNA resulting restriction fragments.
  - Now the restriction fragments are examined using gel electrophoresis.
  - The gel is now treated with luminescent dyes for the visibility of DNA bands.
- **Applications:**
  - RFLP was one of the first techniques applied for genetic fingerprinting/profiling.
  - It is used for identification of inherited diseases, carrier of that diseases, genetic mapping, and heterozygous detection.
  - The molecular basis of the RFLP is that any point mutations as such deletions, substitutions and insertions or alterations like duplications, inversions within the genome can eliminate or form new restriction sites. These alterations in genome can be detected by analyzing fragments of variable length, digested with restriction endonuclease enzyme
- **Demerits:**
  - requires relatively large DNA sample
  - laborious and tedious process
  - sensitivity and more precautions for contamination required

3. Amplified Fragment Length Polymorphism (AFLP):

- Zabeau and Vos invented the AFLP technique in 1993.

- AFLP was originally developed by the KeyGene in 1990.
- It is a PCR based technique for fingerprinting. It includes both PCR and RFLP.
- The basis of AFLP is the amplification of selected fragments followed by restriction digestion of whole genomic DNA of specific organism.
- The steps for the AFLP are as follows:
- DNA extraction and its restriction digestion followed by ligation with the short adaptor sequences.
- Amplification of restricted fragments by PCR
- Analysis of results in gel electrophoresis or PAGE followed by autoradiography.
- **Applications:**
    - AFLP has its ability for rapid generation of marker fragments for any organism without prior sequencing of DNA is required.
    - Also, it needs only small fragments of starting template DNA relative to RAPD and ISSR (inter-simple sequence repeats) and has much higher reproducibility.
    - AFLP is largely used for crop improvement programs, parentage and genomic interpretation of various crop species.
- **Demerits:**
    - AFLP require large DNA samples and require purification

4. Inter Simple Sequence Repeat (ISSR) markers:

- Inter simple sequence repeat (ISSR) technique is PCR based method.
- It was reported by Ztetikiewicz et al. in 1994.
- The ISSR markers are developed by PCR amplification of DNA segments between 2 similar microsatellites repeat regions by use of single primer consisting of microsatellite core regions.
- The primers can be usually 16-25 bp long, and unattached or attached at 3′ or 5′ end.
- **Applications:**
    - It is simple, rapid and economical like the RAPD technique and has higher reproducibility compared to RAPD because of longer primer length.
    - ISSR doesn’t require previous knowledge of genome for analysis and is highly polymorphic marker.
    - ISSR are used for identification of genetic diversity, phylogenetic analysis, to detect proximity of cultivars and to determine soma clonal variations in plants.

- Due to the simple set up of ISSR, it is applicable for studying gene mapping, gene tagging, distinct strain identification, and parental recognition.

- **Demerits:**
  - ISSR has less reproducibility and non-homology of identical sized fragments due to multi locus feature.

5. Microsatellites or simple sequence length polymorphisms (SSLPs):

- Microsatellite was termed by Jeffery et al. in 1985.
- Microsatellites or simple sequence repeated (SSR) loci are PCR based markers which needs previous knowledge of gene sequence.
- In literature it is referred to as **variable number of tandem repeats (VNTRs)** or **simple sequence length polymorphisms (SSLPs**) or **sequence tagged microsatellites (STMS).**
- They are dispersed throughout the nuclear genomes in eukaryotes and to a few extent in prokaryotes.
- Microsatellite primers are short tandem repeats (STRs), or simple sequence repeats (SSRs), having 1-6 base pair long sequences repeated several times.
- Usually microsatellites are repeated less than 100 times.
- Microsatellites can be recognized by constructing a small-insert genomic library followed by screening of library and sequencing of positive clones.
- Microsatellites are used as markers for studying gene mapping, closeness among the species, and population genetics.
- The amplification of tandem arrays followed by visualization in gel helps to detect variation in DNA length.
- The main cause for the variation in DNA length is polymerase diminution during DNA replication, or slipped strand mispairing.
- **Applications:**
  - Microsatellite consists of co-dominance of alleles and requires low quantities of DNA templates.
  - It has high reproducibility and is economical in nature.
  - The screening of microsatellite variation can be automated.
- **Demerits:**
  - Assay is costly if sufficient primer sequences for the species of interest are not available.
  - Errors in genotype scoring occurs if alterations are seen in primer annealing sites.

- chances of homoplasy (some characters are present in more than one species but not present in their common ancestor because of convergence evolution)

6. Single nucleotide polymorphism (SNP):

- SNP was invented by Lander in 1996.
- SNP is formed when any alteration/mutation occurs in single nucleotide (A, T, C, or G).
- The point mutation as such substitutions, insertions or deletions in single nucleotide it represents SNP.
- SNPs are based on hybridization of detected DNA fragments with SNP chips (DNA probe arrays) and the SNP allele is named with respect to the hybridization results.
- **Applications:**
  - SNPs are widely used in biomedical research for comparing the case and control groups of disease.
  - It is also used in studying phylo genetics, genetic variation etc.
- **Demerits:**
  - The information obtained is low as compared to microsatellites and therefore large numbers of markers and complete genome sequencing is needed

UNIT-II

Topic: **Herbicide Resistant plants**

Weeds are unwanted and useless plants that along with the crop plants.

Weeds compete with the crops for light and nutrients, besides harboring variouspathogen.

So it is estimated that the worlds crops yield is reduced by **10-15%** due to the presence of weeds.

Herbicides are broad spectrum as they can

kill wide range of weeds

What are Herbicides

Herbicides are chemicals that are sprays on the garden used to kill weeds. They are generally a last resort for home gardeners.
They have several advantages and disadvantages.

An ideal herbicide is to posses the following characters:

Capable of killing weeds with out affecting crop plants. Not toxic to animals and microorganism
.Rapidly trans located with in the target plant
Rapidly degraded in the soil

Effects:

when herbicides are sprayed on fields, they cannot distinguish from crops andweeds.

Scientists have developed GM crops that are resistant to Herbicide resistant crops. Several classes of herbicides are effective for broad spectrum weed control.
Act by inactivating vital enzymes (involved in photosynthesis). To face this problem, herbicide resistance plants are generated

Herbicide resistant plants:

Herbicide resistant plants are the plants having the ability to reduce the herbicide- sensitive target in the plant which binds to the herbicide.

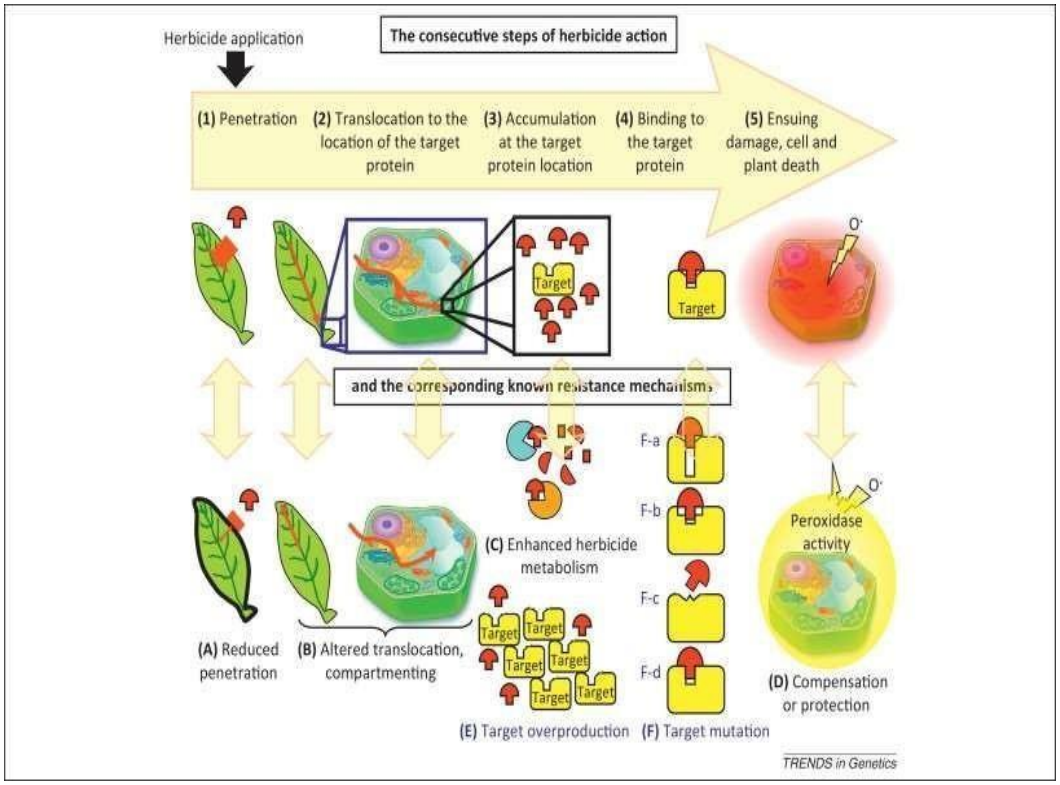
Genes for resistance against certain herbicides have been introduced into crop plants so they can thrive even when exposed to herbicides.

Objectives:

Modification of plant enzyme target of herbicidal action to render it insensitive toherbicide.

Overproduction of the unmodified target protein permitting normal metabolism to occur even in herbicide presence.

Introduction of an enzyme to degrade herbicide prior to its action.



Examples:

The Most common forms of herbicide resistant crops are; Soybean, Maize, cotton, tomato, rice, potato, tobacco ,sugar beet, Wheat.

Mechanism:

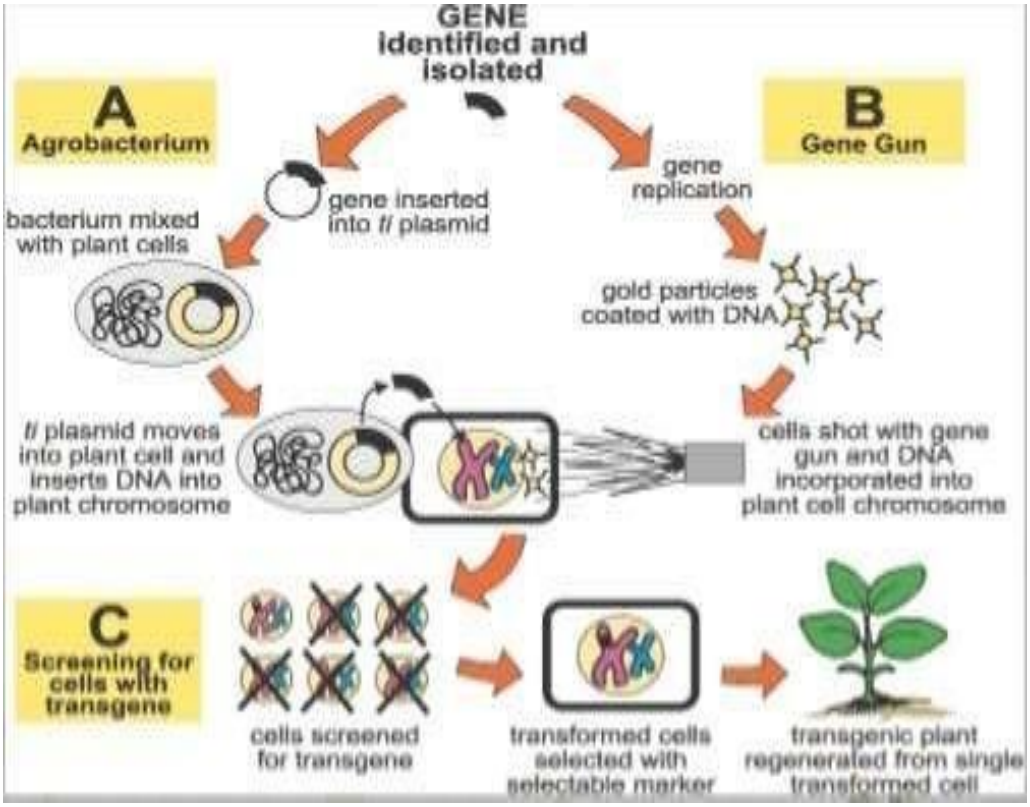
Introduction of a gene coding for an herbicide detoxifying enzymes.

Introduction of gene coding for a herbicide insensitive form of a normal functioning enzyme or over expression of the genes coding for a herbicide target enzyme such that the normal metabolic functioning is still achieved in the planteven though some of the enzyme is inhibited.

Modification of the herbicide target enzyme is such a way that the herbicide molecular does not bind to it and.

The more recently described engineering of active herbicide efflux from plantcells.


Unsprayed
Roundup
READY
Sprayed



Herbicide resistance:

Glyphosate resistance:

Glyposate=**"**Roundup",

"Tumbleweed**"**=systemic herbicide. Marketed under the name Roundup, glyposate inhibits the enzyme **EPSPS (5- enolpyruvylshikimate-3 phosphate-** involved in chloroplast amino acid synthesis), makes aromatic amino acids.

Ex: cotton, corn, soya beans.

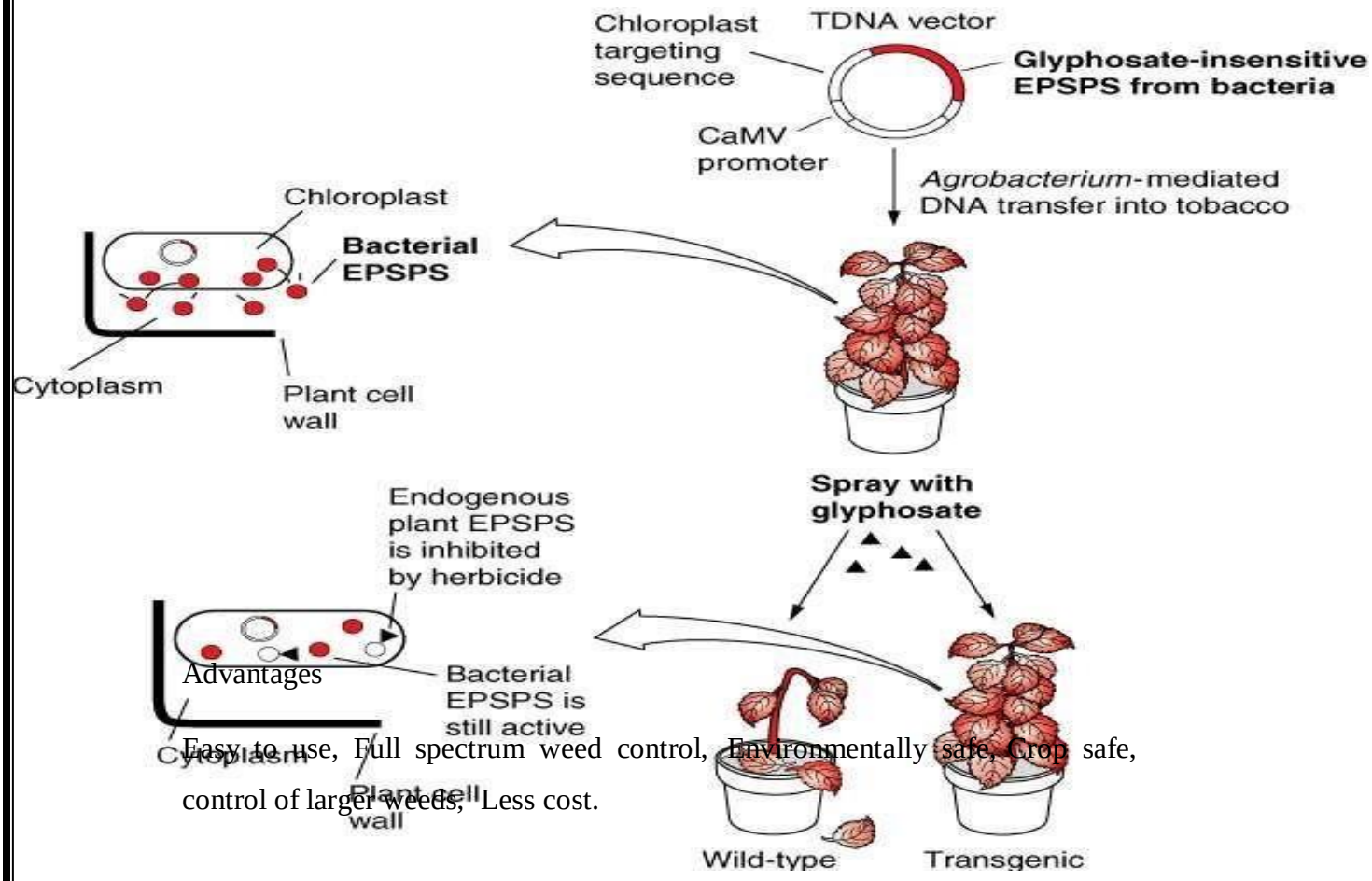
Glyphosate resistance in crop plants:

An over expression gene of EPSPS was detected in petunia.Gene from petunia was isolated and introduced in to other plants.The transgenic plants can tolerate glyphosate 2-4 times higher than thatrequiredtokillwildtypeweedplants

Glyphosate competitively inhibit EPSPS and plants die due to a **lack of aromatic amino acids** required for their survival.

A version of the enzyme that both was resistant to glyphosate and that was still efficient enough to drive adequate plant growth was identified in an **Agrobacterium** strain called CP4.

This version of enzyme, **CP4 EPSPS**, is the one that has been engineered into several genetically modifies crops.



Advantages

Easy to use, Full spectrum weed control, Environmentally safe, Crop safe, control of larger weeds, Less cost.

Disadvantages: rates

The increased use of glyphosate on glyphosate resistant crops could lead to increases in human health problems.

Glyphosate formulated herbicides have been linked to numerous health problems including **cancer**. Short term health effects include **lung congestion** and increased **breathing**

Topic: Abiotic stress tolerance plants

Environmental abiotic stresses, for example, high temperatures, low water accessibility, mineral insufficiency, high salt levels, and lethality, are serious threats to the harvest survival which someway influence the harvest yield. A few traditional strategies are used for sustainable harvest efficiency; however, with the expanding abiotic stress because of changing climatic conditions and enhancing pressure of populace, the conventional procedures of overcoming abiotic stress are not ready to meet the demands. Biotechnology is the best ways by which the productivity of crops can be improved by enhancing their ability to resist or tolerate biotic and abiotic stresses. In biotechnology different strategies are involved for the improvement of harvest yield and quality. This chapter concentrates on the traditional and new enhanced biotechnological strategies for the betterment of a biotic stress tolerance in plants.

A biotic stresses affect crop plants and cause decreases in plant quality and productivity. Plants can overcome environmental stresses by activating molecular networks, including signal transduction, stress perception, metabolite production and expressions of specific stress-related genes. Recent research suggests that chemical priming is a promising field in crop stress management because plants can be primed by chemical agents to increase their tolerance to various environmental stresses. We present a concept to meet this objective and protect plants through priming of existing defense mechanisms avoiding manipulation of the genome. In addition, recent developments in plant molecular biology include the discovery of genes related to stress tolerance, including functional genes for protecting cells and regulatory genes for regulating stress responses. Therefore, enhancing a biotic stress tolerance using a transgenic approach to transfer these genes into plant genomes has attracted more investigations. Both

chemical priming agents and genetic engineering can enhance regulatory and functional genes in plants and increase stress tolerance of plants. This review summarizes the latest findings of chemical priming agents and major achievements in molecular

approaches that can potentially enhance the abiotic stress tolerance of plants

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Biotechnology is the best way by which the productivity of crops can be improved by enhancing their ability to resist or tolerate biotic and a biotic stresses. In biotechnology different strategies are involved for the improvement of crop yield and quality. In this chapter, we will focus on the impact of environmental conditions such as extreme temperature, salinity on crop plants, and water accessibility. Figure 1 shows a number of physical factors that may impose an abiotic stress on plants and adversely affect their quality and yield. These stresses also affect physiological, biochemical, and developmental processes of crop (Amit kumar and Sengar 2013). These include a number that can be gathered together as temperature stresses (heat, chilling, and freezing) which in turn belong to a bigger subgroup that can be arranged as stresses that result in water shortfall. The figure likewise accentuates the point that most abiotic stresses straightforwardly or indirectly lead to the generation of free radicals, creating oxidative stress and reactive oxygen species

Types of environmental stress

The impact of a biotic stresses on crop yield compared with biotic stresses (weed, pest, and disease effects) is shown in Table 1. One of the first things to notice is the large difference between the average yields of crops and the record yields. It is clear from these data that the major difference between record yield and average yield is accounted for by abiotic stress. Thus, the variation in environmental conditions from 1 year to the next produces such a variation in yield for wheat in which the average yield is only 13% of the maximum. In contrast, the control of biotic stresses in industrialized farming is such that they tend to reduce the annual yield by a fairly stable proportion, which is generally less than the most adverse abiotic stresses. Improving the tolerance of crops to a biotic stresses could therefore enable them to maintain growth and development during the normal fluctuations of adverse conditions and consequently buffer crops against the large swings in yield experienced from 1 year to the next.

Table 1

Average and record yields of some major crops

Crop	**Record yield (kg/ha)**	**Average yield (kg/ha)**	**Average yield (% of record yield)**	**Average losses (% of record yield)**	
				Biotic	**Abiotic**
Wheat	14,500	1880	13.0	5.0	82.1
Barley	11,400	2050	18.0	6.7	75.4
Soybean	7390	1610	21.8	9.0	69.3
Corn	19,300	4600	23.8	10.1	65.8

In the long time, the predicted depletion of the ozone layer and climate changes related with a worldwide temperature alteration are probably going to add to the burden of environmental stresses on harvest plants and enhance the imperative to prepare stress-tolerant varieties. Moreover, there is increasing pressure to extend the area of harvest cultivation to environments that are not ideal for the development of significant harvests (high-salt condition). The preparation of stress-tolerant plant is therefore a major aim of agricultural biotechnology and onethat is likely to become increasingly important.

2 Nature of A biotic Stress

When discussing the subject of stress tolerance, it is necessary first to try to define stress in relation to plant physiology. Plants are subject to many types of fluctuation in the physical environment. Many of the strategies used by animals to avoid the effects of these fluctuations are not available to plants, because of the sessile nature of their growth habit. Plants therefore depend largely upon internal mechanisms for tolerating variations in the external environment. Not all such fluctuations present a stress to plants, since they can adapt to typical variety by virtue of their plasticity. Consequently, plants are adjusted to work in a fluctuating environment, and normal outside changes are countered by inward change without detriment to development and advancement. It is only acute or chronic extremes of environmental condition that lead to environmental stress that has the potential to cause physical damage to the plant.

A biotic stresses, for example, high temperatures, low water accessibility, high salt levels, mineral insufficiency, and toxicity, are regularly skirmish by plants in both

natural and agriculture frameworks. By and large, a few classes of abiotic stress challenge plants in blend; for instance, high temperature and shortage of water can be exacerbated by mineral toxicities that constrain root development. Over a range of cropping frameworks around the globe, abiotic stresses are estimated to shorten yields not as much as a half of that possible under perfect development conditions(Boyer 1982).

A biotic stress responses, particularly to water inadequacy and high level of salts, are unpredictable physiological and morphological phenomena in plants (Wang et al. 2003). At the cellular level, alterations in extracellular solute concentrations cause osmotic stress and finally inadequate water availability. This water deficiency causes an abatement in turgor pressure and an expansion in concentration of intracellular solutes, which gives a strain on layers and macromolecules.

Abiotic stresses exhibit a big challenge in our journey for sustainable food generation as these may lessen the potential yields by 70% in cultivates. Intense water insufficiency hinders photosynthesis (Gallagher et al. 1975). Changes in the worldwide atmosphere, remarkably in territorial spatial and temporal temperature designs, are anticipated to have vital outcomes for crop production (Parry and Duinder 1990); both plant development and improvement are influenced by temperature (Porter and Moot 1998).

Given the range of a biotic stresses to which plants are exposed, it might be thought that a wide range of different strategies would be required to engineer particular types of stress. This chapter will concentrate on the variety of different stresses.
These damages result from water deficit caused by various distinctive environmental conditions, including temperature, cold, drought, and salinity.

3 Tolerance to Drought Stress

Agriculture is a major client of water assets in numerous regions of the world. With expanding aridity and a developing populace, water will turn significantly scarcer in the future. Suboptimal accessibility of water for unlimited plant development and transpiration, i.e., drought, is a noteworthy constraint to agriculture yield (Boyer 1982 and Delmer 2005). Drought is a standout among the most well-known environmental stresses that influence development and advancement of plants through shifts in metabolism and expression of gene. It is changeless to agriculture creation in many developing nations and periodic reason for losses of agriculture production in created ones (Ceccarelli and Grando 1996).

In India, 29 percent of the total cultivable area faces drought condition, out of which 10 percent is under severe drought (Anonymous 2003).

Harvest plants developed under dry spell conditions are presented to a mix of stresses that are attributable from high temperatures, over the top irradiance, and soil resistance to root infiltration and low water potential. Drought is one of the main factors adding to a few yield losses of wheat developed in minimal land and to essentially diminish yields in temperate area (Morris et al. 1991; Trethowan et al. 2001). Drought is the most genuine abiotic stress restricting wheat production invarious parts of the world (Chaves et al. 2003).

. 4.1 Marker-Assisted Selection for Heat Stress

Molecular marker have been applied in quantification of genetic diversity, genotype identification, mapping and tagging of utilize genes, and MAS in cereals for biotic stresses, a biotic stresses, and quality traits. Many genes for those traits have been mapped, tagged, and cloned, and linked markers have beencreated. Those have been successfully used in marker-assisted breeding program to develop genotypes with resistance.

This approach involves the use of molecular markers associated with important agronomic traits for selection of desirable plants in the segregating generations. This is particularly desirable for traits, for which selection through conventional method of plant breeding is either difficult or cost/time ineffective. This has assumed significance in recent years due to the realization that improvement of traits like water use efficiency and nutrient use efficiency along with tolerance to a number of other abiotic stresses would be necessary to augment the productivity

In spite of the fact that it is not a crop plant, Arabidopsis has played an important role in the explanation of the essential procedures underlying stress tolerance, and the information acquired has been transferred to a specific degree to imperative food plants. Different types of the gene known to be required in stress resistance have been isolated at first from Arabidopsis. Two general procedures for the metabolic engineering of abiotic stress resistance have been proposed which expanded production of particular specific desired compounds, or the decrease in the enzymatic step is typically managed by the tendency of cell frameworks to reestablish homeostasis, thus limiting the potential of this approach (Fig. 2). The transgenic approach is a helpful innovation to beat reproductive isolation among species and use useful exotic genes.of all major food crops, as is required to meet the future demands of food and nutritional security

of all major food crops, as is required to meet the future demands of food and nutritional security.

In order to meet the demands of molecular breeding, one needs to have the knowledge either about the marker trait association, as determined in case of linkage-based QTL interval mapping and LD-based association mapping, or about the genomic estimated breeding values of individual markers as worked out in case of genomic selection. The merits and limitation of these three different approaches have been widely discussed, and improvements in the basic proposed initially have been suggested.

MAS and hereditary engineering are two most basic molecular methodologies for enhancing stress resistance in plants. With the appearance of molecular markers, MAS has turned into a fundamental component of new discipline named as molecular breeding with the assistance of which allelic variation among the genes' basic traits can be accurately and proficiently detected (Mohammadi et
al. 2008b). Various markers, for example, RAPDs, SSRs, RFLPs, and AFLPs, have been accounted for different abiotic stresses for accomplishing mapping of the QTL involved in stress tolerance (Korzun et al. 1999). Relatively, however, limited research has been directed to recognize genetic markers related with heatresistance in various plant species.

3.4 **QTL Mapping for Drought Tolerance**

Quantitative trait is represented by poly genes and is markedly influenced by the environment. Thus, it demonstrates a constant variation rather than the discrete variation that is characteristic of qualitative traits. Poly genes are those genes that have little however combined impact on the concerned traits, and a few poly genes influence a single trait. A quantitative trait locus is a position in a chromosome that contains at least one poly genes involved in the assurance of a quantitative trait.

Many of DNA marker maps are not adequately thick to accomplish satisfactory QTL mapping,

since inadequate marker maps extremely confine the power of QTL mapping. The mapping populace must be relatively huge in order to identify QTLs having minor impacts, and the biological relevance of the revealed QTLs relies upon the cutoff decided for statistical significance. In QTL mapping, natural variables and genetic background have a marked effect on the outcomes; as a result, some QTLs may be detectable in some but not in other environments. A standout among the most capable applications of QTL mapping is to analyze

gene x gene and gene x environment interactions, yet this requires some extensive, tedious trials to allow a thorough investigation of a system.

QTL mapping involved testing DNA markers all through the genome for the probability that they are related with a QTL. Individuals in a suitable mapping populace are investigated regarding DNA marker genotypes and the phenotypes and the phenotype of interest. For every DNA marker, the individuals are split into classes as indicated by marker genotypes. These markers are being utilized to distinguish drought-related quantitative trait loci and their productive transfer into economically developed crop varieties of rice, wheat, maize, and millet. Great hereditary maps in view of molecular marker technologies are presently accessible for large cereal species (Snape et al. 2005; Langridge et al. 2006). In cultivated species with huge, complex genomes, QTL investigation is a vital tool in the recognizable proof of genetic markers to help breeding efforts. This approach is complicated in wheat on account of the polyploidy nature of the genome. Furthermore, the low levels of polymorphism, however, is straightforward in rice, barley, and maize (Snape et al. 2005)

Studies on the abiotic stress resistance of cereals incorporate the broad investigation of QTLs linked to the field assessment of stress tolerance (Langridge et al. 2006). Approach and improvement of molecular markers in quantitative genetics greatly incredibly encourage the investigation of complex quantitatively acquired inherited traits by the development of high-density genome linkage maps for harvests, for example, wheat (Xiao et al. 1996). This infers the utilization of molecular markers, and interim mapping is an intense approach, which allows the distinguishing proof and genetic mapping of loci controlling complex traits like grain yield and its contributing traits having extraordinary significance in plant breeding (Broman and Speed 1999). Lacking accessibility of water during drought may constrain the size of the plant and furthermore influence the improvement of different plant parts. Recent advances in plant genomics have prompted the identification of a vast number of potentially beneficial water stress-related gene, in addition to innovations for gene overexpression or silencing. Also, these can be brought into transgenic plants under the control of proper promoters and are transmitted to subsequent generations (Delmer 2005; Ma and Bohnert 2007).

Recently, Tuberosa and Saliva (2007) detailed that genomic-based methodologies give access to agronomically attractive alleles present at quantitative trait loci (QTLs) that influence such responses, accordingly enabling us to enhance the drought resistance and yield of crops in submerged restricted conditions more

effectively. QTLs for drought tolerance, one each situated on chromosome 7AL (Quarrie et al. 2005), and three QTLs for heat resistance, one each situated on chromosome arms 2DL (Mohammadi et al. 2008a), IBS, and 5BL (Mohammadiet al. 2008b) and their linked SSR markers, were utilized for validation for theirinevitable exploitation during amid MAS.

4.**Tolerance to Heat Stress**

For many years, it has been known that heat stress applied to a wide range of organisms induces a specific set of heat shock proteins (HSPs); they fall into five classes, four of which are highly conserved in prokaryotes and eukaryotes. These four are categorized according to size as the HSP 100, HSP 90, HSP 70, and HSP 60 classes whose members appear to function as molecular chaperones. Some of them are expressed constitutively and are involved in normal protein synthesis and folding. Those induced by heat appear to be involved in countering the effects of heat stress by protecting or refolding denatured proteins. Their expression is induced by heat treatment and, in some cases, can be correlated with the acquisition of thermo tolerance. The fifth group of several classes of small HSPs is particularly abundant in plants, but their function is not yet clear.

In a way analogous to strategies for engineering cold tolerance, individual HSPs have been transformed into plants to enhance heat tolerance. However, it is also known that the rapid heat shock response is coordinated by a heat shock factor. This protein is expressed constitutively but in normal conditions exists as a monomer bound to one of the HSP70 proteins. Heat binds to a heat shock element common to the promoters of HSP genes. Sequence NGAAN: five to seven of these repeats occur in the promoter close to the TATA box.

When the At HSF 1 gene was over expressed in Arabidopsis, the translation factor was not dynamic, and there was no impact on thermo tolerance. Nonetheless, combination of AtHSF1 to the N or C end of the gus A reporter gene created a fusion protein that could tri merize without heat. Transformation of this fusion protein into Arabidopsis created transgenic plants that expressed HSPs constitutively and showed upgraded thermo tolerance without requiring earlier heat treatment.

Marker-Assisted Selection for Heat Stress

Molecular marker have been applied in quantification of genetic diversity, genotype identification, mapping and tagging of utilize genes, and MAS in

cereals for biotic stresses, abiotic stresses, and quality traits. Many genes for those traits have been mapped, tagged, and cloned, and linked markers have been created. Those have been successfully used in marker-assisted breeding program to develop genotypes with resistance.

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In order to meet the demands of molecular breeding, one needs to have the knowledge either about the marker trait association, as determined in case of linkage-based QTL interval mapping and LD-based association mapping, or about the genomic estimated breeding values of individual markers as worked out in case of genomic selection. The merits and limitation of these three different approaches have been widely discussed, and improvements in the basic proposed initially have been suggested.

4.3 **QTL Mapping for Heat Tolerance**

One of the aims of molecular mapping is to produce a sufficiently fine-scale map to pinpoint the location of genes that play a role in determining importance agronomic traits. Many of these traits are described as quantitative; that is, they

are impacted by various hereditary and environmental factors. However, it is possible to map genes that have majorly affected quantitative traits by assessing the correlation between estimation of the quantitative trait and the allelic states at linked hereditary markers. A QTL (quantitative trait locus) is therefore a chromosomal location where there is considered to be a reasonable probability that practically unique alleles segregate and cause huge impacts on a quantitative

trait. QTL mapping requires a statistical analysis of molecular marker and phenotypic information from an extensive segregating populace to determine

those markers where allelic polymorphism correlates with the quantitative trait phenotype. This primary QTL mapping is coarse and locates the gene within a chromosome region of approximately 10–30 cm, which could contain several hundred genes. To identify the gene involved in the quantitative trait, two methods are available: positional cloning and association mapping.

Positional cloning requires further steps to the QTL to a much finer resolution and relates this map position to the DNA sequence. One strategy is to cross nearly isogenic lines in which the only allelic variation occurs in the short region of the coarse-mapped QTL. In the resulting populations, the QTL is described as a mentalized and fine map with more precise centimeter distance between the QTLs, and adjacent molecular markers can be produced. This is relatively straightforward when there are many polymorphic markers mapped in the region, but this is currently possible only for those plants whose genome has already been, or is in the process of being, sequenced. At this stage, the markers closest to the QTL are used to anchor the genetic map to the physical map, and it may then be possible to determine the gene responsible from candidate gene in the location by identifying the mutation responsible for the QTL effect. Alternatively, it may be necessary to test each predicted coding sequence in the region functionally, by overexpressing or downregulating the gene.

To date, the level of accomplishment in distinguishing hereditary markers related with high-temperature tolerance in wheat, and to be sure other crop species, has been limited. Dependable marker trait associations are essential for a viable marker-assisted breeding program , and these are most successfully settled by means of quantitative trait locus mapping QTLs for heat resistance have been reported by various researchers ; Barakat et al. 2011; Tiwari et al. 2013). At present, the good breeders' technique is to challenge the material by sowing late, in the information that this will expose the plants to high temperatures amid grain filling; after this, determination is regularly based on yield performance. Recent outcomes demonstrate that synchronous improvement of yield potential and thermotolerance is possible and that the CIMMYT strategy to grow high-yielding early developing wheat lines is promising for South Asia and Mexico (Mondal et al. 2013). The recognizable proof of QTLs for heat tolerance has given a chance to deploy MAS for the improvement of the high-temperature resistance wheat

(Paliwal et al. 2012).

4 Tolerance to Salt Stress

Salt resistance might be characterized as a differential impact on different life processes of a similar tissue concentration of salt in different genotypes of a species. There is considerable evidence that genotypes differ in resistance to a similar amount of salt in their tissues. However, the enzyme and cellular processes of halophytes are as sensitive to salt as those of glycophytes. Most of the crops grow under the saline environment by which yields are generally poor in the initial 3–4 years of the reclamation of saline lands through application of gypsum. Therefore, improving salt tolerance of crops may help to a great extent in increasing food production of the growing population of India. Therefore, cultivation of salt-tolerant varieties can play important role in rehabilitation of such lands besides reducing the requirement of input in the form of chemical amendment.

cells with large vacuoles may act as sinks for the accumulation of excess sodium by transport into the vacuole.

5.2 **QTL Mapping for Salt Tolerance**

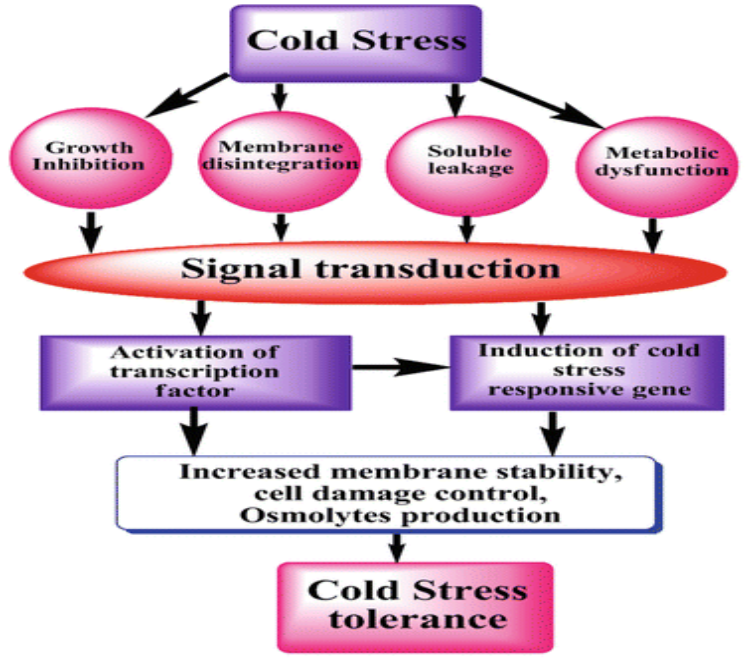
QTL is therefore a chromosomal location where there is considered to be a reasonable probability that functionally different alleles segregate and cause significant effect on a quantitative trait. QTL mapping requires a statistical analysis of molecular marker and phenotypic data from a large segregating population to determine those markers and phenotypic data from a large segregating population to determine those markers where allelic polymorphismcorrelates with the quantitative trait phenotype.

Molecular technology is a new technology for analyzed the quantitative trait such as salt tolerance and detect the chromosomal location, which is associated with such character, known as Quantitative trait loci. Few scientists have been reported that the QTL for salinity damage at early embryo stage in rice (Prasad etal. 2000).

Tolerance to Cold Stress

Diverse plants differ enormously in their capacity to withstand cold and freezingtemperatures (Fig. 3). Most tropical plants have essentially no ability to survive chilling conditions. Furthermore, plants can survive a scope of chilling

temperatures from −5 to −30 °C relying on the species. Plants from colder districts routinely withstand temperatures even lower than this. It is realized that plants are better ready to withstand colder or chilling stress in the event that they initially experience a time of chilly acclimation, at a low however nonchilling temperature. For instance, wheat plants developed at ordinary warm temperature are killed by chilling at −5 °C; however, after a time of cold acclimation when theplant develops at temperatures underneath 10 °C, they can survive chilling temperature down to −20 °C.



Cold stress response in plant

Plants differ in their ability to withstand cold or freezing condition, and cold tolerance is one of the traits that plant breeders have selected for over many centuries. However, there has been little improvement in the cold tolerance ofmajor crop species over the past two decades by conventional breeding, prompting the search for molecular solutions to this problem.

One approach has been to study the mechanisms of freezing resistance that existin some plant species. Amid the time of acclimation, plants produce various chilly instigated proteins that are expected to assume a part in the ensuing cold

tolerance. Around 50 frosty prompted proteins have been recognized in various plant species. These fall into few groups, but they all share the property of being to a high degree hydrophilic. A significant number of them additionally have relatively basic amino acid composition, with current motifs. Some of these groups had already been distinguished as late embryogenesis abundant (LEA) proteins, which seem to play a defensive role amid seed desiccation. Different groups of proteins encoded a class of genes assigned as chill-responsive gene as per their patterns of expression. The exact function of these chill prompted genes is not yet known, but rather it has been hypothesized that they may contribute specifically to chilling tolerance by mitigating the possibly harming impacts of lack of hydration related with freezing. Over expression of this chilly prompted protein could in this way be a possible route to be particular technology of cold orchilling stress resistance.

There is some case of the expression of chilly induced proteins in transgenic plants. For instance, constitutive expression of the little, hydrophilic, chloroplast- targeted COR protein COR 15a in Arabidopsis enhanced the chilling resistance of chloroplast frozen in situ or protoplast frozen in vitro. In any case, COR 15a expression has no perceptible impact on the survival of frozen plants. One clarification for this observation is that the cold-prompted proteins might be focused to various vulnerable cell segments and that they are altogether required to provide full protection to the cell. By implication, many COR genes would need to be changed into a transgenic crop to get in appreciable change in cold resistance.

One answer for the issue of engineering a multigene trait has risen after the recognition that few distinctive chill resistance-related genes contain a comparative regulatory element in their promoter: the C-repeat (CRT) element/low-temperature response element (LTRE)/lack of hydration- or dehydration-responsive element (DRE). Besides, it has been discovered that the transcription factor CBF1 ties to the CRT/DRE/LTRE element and activates expression of this gathering of genes, which contain the COR regulon. Along these lines, the procedure is to overexpress the CBF1 gene, prompting the acceptance of this whole group of COR cold resistance gene. In

transgenic, Arabidopsis plants conveying a 35S promoter, CBF1 gene constructs have been developed. These plants express various COR genes without chill acclimation and have been appeared to be cold resistance without earlier cold acclimation. As a control, transgenic plants over expressing an individual COR protein, COR15a, were observed to be less cold tolerant than the CBF1 plants.

The interrelated nature of various stress reactions was shown in a similar

investigation. The expression of a CBFF1 homologue and DRE restricting protein DREB1A under the control of a stress-induced promoter in

transgenic Arabidopsis brought about plants that had enhanced drought, salt, and cold resistance.

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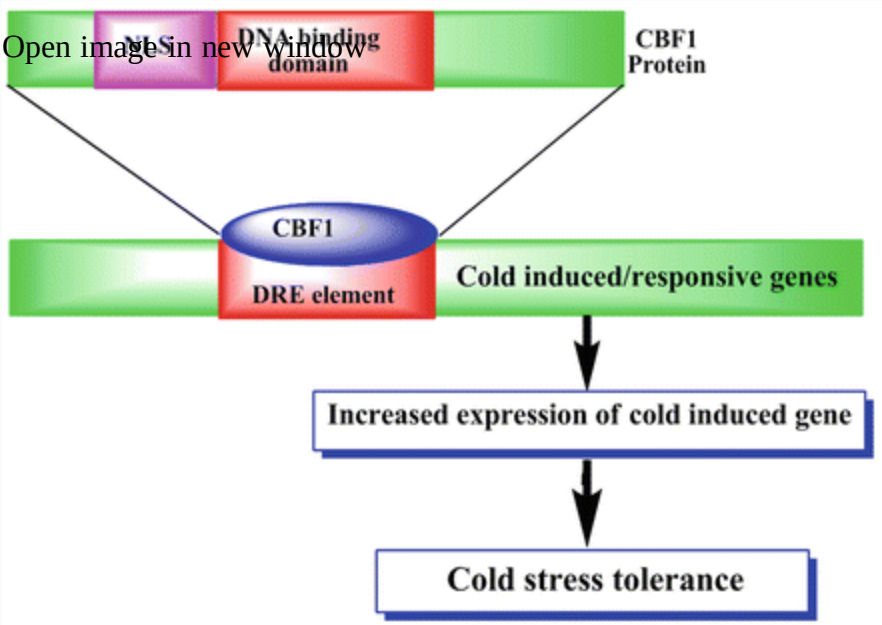

Cold-responsive gene contains CBF1 transcription factor and drought-responsive element (DRE) by which increased the expression of cold-induced gene

CBF1 is an individual from a small gene family member; CBF2 and CBF3 and also transcription factors and expression of all three CBF genes are induced rapidly by low temperatures. In addition, CBF3 overexpression results in several biochemical changes related with cold acclimation, such as elevated levels of compatible osmolyte, proline, and soluble sugar.

Although low-temperature-induced gene expression, mediated by the CRT element, appears to be well conserved in plants, not all cold-induced genes have the CCGAC element in their promoters. Other pathways of low-temperature gene expression, not mediated through CRT/CBF, appear to be present in plants, and another sequence element, CCGAAA, has been identified as conferring low- temperature inducibility in some genes.

Genetic engineering and molecular studies have recognized numerous genes which are incited by many stresses. Several stress-inducible genes encoding useful protein have been utilized for the improvement of stress resistance. Most of the reviews have been published on stress resistance (Christensen and Feldmann 2007; Umezawa et al. 2006; Valliyodan and Nguyen 2006). Numerous transcriptional activators, for example, DREB1/CBF (Kasuga et al. 1999; Liu et al. 1998; Kreps et al. 2002), which incite the stress-responsive gene, have been used to deliver the low-temperature-tolerant transgenic plants (Zhang 2003). The DREB/CBF qualities have been effectively used to engineer low-temperature stress resistance in different plant species, for example, rice (Dubouzet et al. 2003; Ito et al. 2006), pepper (Hwang et al. 2005), chickpea (Mantri et al. 2007), and potato (Rensink et al. 2005).

Screening for gene required in cold resistance is a critical introductory step. Vast quantities of studies have been accomplished for chilly stress resistance in plants. All the cold resistance-related genes contain a comparative regulatory element in their promoters, the C-repeat component and low-temperature-responsive component. A rundown of genes and transcription factor which are enhancing thechill resistance in various plants is given .

Unit-III

Topic; Chloroplast Transformation in Plants(With Diagram)

Chloroplast Transformation in Plants!

Thechloroplasts(plastids)andmitochondriaarebelievedtohaveevolvedfromprokaryotesduringthecourseof evolution.

Both these organelles have their own genome, although it is much simpler when compared to nuclear genome. Further, many of the proteins that function in chloroplast and mitochondria are encoded by nuclear genes-and then transported to the organelle.

Chloroplast Genome:

ADVERTISEMENTS:

Most of the higher plants have about 100 chloroplasts per leaf cell. Each chloroplast contains approximately 100 copies of chloroplast DNA genome. The chloroplast genome (the plastome) is a circular double-stranded DNA molecule (or chromosome) located in the stroma. Majority of chloroplast genomes are in the size of 120-1 60 kbp and contain about 120-140 genes. About 100 chloroplast genes are known to code for proteins. The protein synthesis in chloroplasts resembles that of prokaryotes.

Chloroplast Engineering:

Geneticengineeringofchloroplastthatleadstochloroplast(plastid)transformationisanimportantandexciting field in modern biotechnology as it offers the following advantages:

1. Chloroplastsarematernallyinherited;hencethereisnodangerofgenetransferthroughpollenorelatedweeds.Thisis because pollen does not contain transgenes.2.

2.gegenetransfercanbeconveniently carriedoutinchloroplastswhichisratherdifficultwithnucl ear genome.

ADVERTISEMENTS:

2. Chloroplastsgenomeisfunctionallycomparabletoprokaryoticgenome.Asinglepromotercan controltheexpressionof groupofgenes(transgenes).Itisthereforepossibletointroducedesirablemultiplegeneswhichcanb eexpressedunderthe control of a single promoter.

3. High level of transgene expression is possible with chloroplasts. There are about 100 chloroplasts per cell, each containing about 100 copies of genome. Thus, there is possibility of 10,000 copies of transgenes per cell! This is a tremendousnumberoftransgenescarriedbytransformedchloroplasts.Thereisatremendouspotent ialforaveryhighlevel of gene expression and large scale production of active proteins.

4. Chloroplasttransformationisnotassociatedwithgenesilencingwhichisamajorprob lemwithnucleargenome transformation.

5. Antibioticresistancegenesneednotbeusedasselectablemarkers.Evenifused,theycanbeeasily excised.

Toxicityassociatedwithforeignproteinproductioninchloroplastsismuchlesswhencomparedtonu clear-controlled foreign proteins

:

Design of Vectors for Chloroplast Transformation:

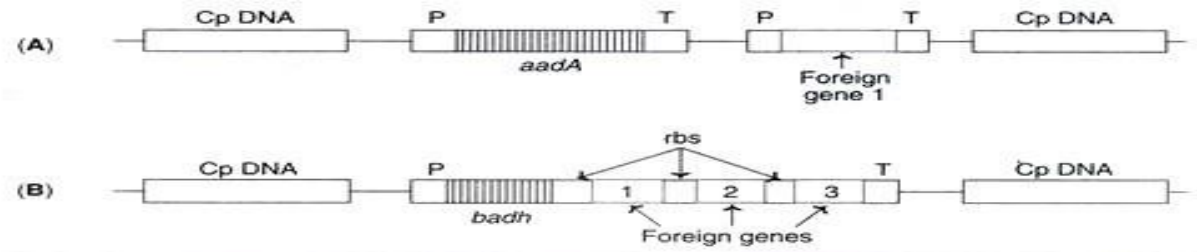

Fig. 49.13 : *A diagrammatic representation of vectors for chloroplast transformation* ***(A)*** *A construct designed for a single foreign gene* ***(B)*** *A construct designed for multiple foreign genes (Cp DNA-Chloroplast DNA; P-Promoter; aadA-A selectable marker gene that confers resistance to antibiotic spectinomycin; T-Terminator; badh-A selectable marker gene encoding betaine aldehyde dehydrogenase; rbs-Ribosome binding site).*

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1. A construct for expression of a single gene:

Thevectorforchloroplasttransformationisbasedontheselectablemarkergeneaad Athatprovidesr esistancetoantibiotic spectinomycin. The single foreign (desirable) gene is fused to regulatory sequences (promoter and terminator) which in turn is flanked on either side by chloroplast DNA (Cp DNA) (Fig. 49.13A).

:

2.A construct for expression of multiple genes:

Inthiscase,-theselectablemarkeristhebetaine-aldehydedehydrogenase(badh)gene.Itisflankedbyapromoterandthe multiple transgenes are flanked by a terminator. At both ends chloroplast DNA sequences are present. In between the transgenes, these are ribosome-binding sites (one between two transgenes) to ensure efficient translation

Introduction of Foreign Genes into Chloroplast Genome:

Most of the methods used for introducing the foreign genes into nuclear genome are not useful for chloroplast transformation.Themost successfulmethodforinsertingforeigngenesintochloroplastsisparticlegun bombardment.

After the bombardment, homologous recombination occurs between the chloroplast DNA sequences on the vector and thoseofonthegenome.Thisisasite-specificintegrationandthusavoidsthefrequentproblemsassociated withrandom insertionofforeigngenesintonucleargenome.Theregenerated plantsderivedfromthemodified plastome(chloroplast genome) are regarded as transplastomic plants.

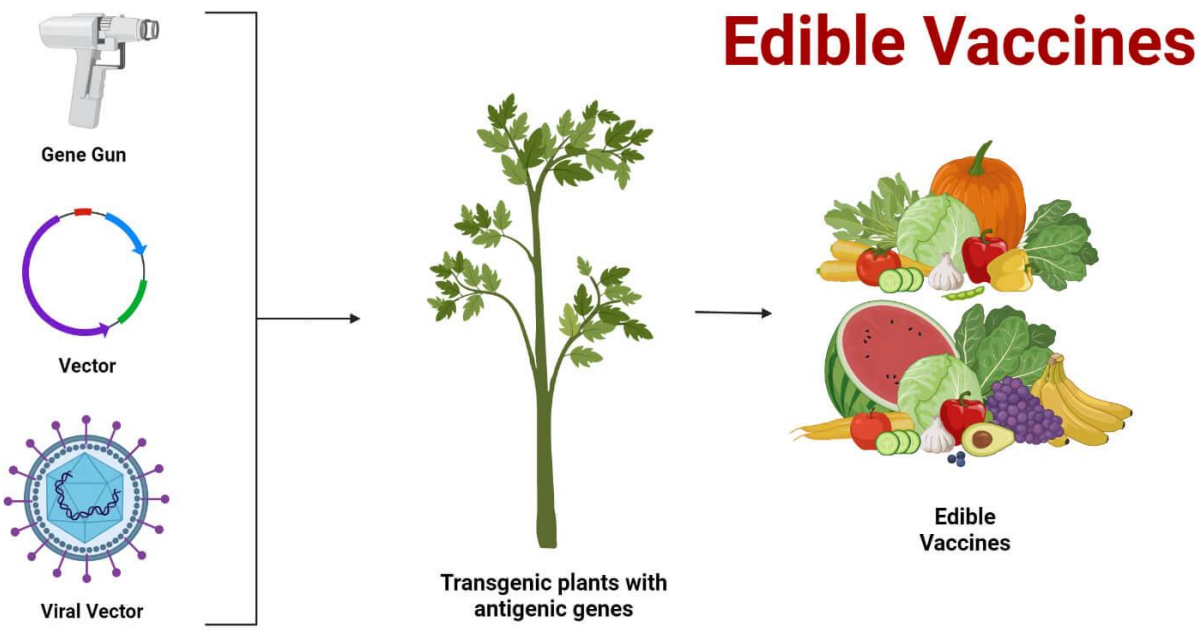
The Future of Chloroplas tTransformation:

The technology of chloroplast transformation is in the developing stages. In fact, it has not become as routine as transformation of nuclear genomes of plants. Chloroplast engineering, however, holds a great promise in plant biotechnologybeinganefficient,cleanandenvironmental-friendlyapproachfortheproductionoftransgenicplants.

Topic; Edible Vaccines

Traditional vaccine administration can be painful, but edible vaccines have come to our rescue.

Edible vaccines are produced from one edible part of plants, such as fruits and vegetables, that can be ingested orally rather than injected like traditional vaccines.



Edible Vaccines

- The edible vaccine concept involves using genetic engineering techniques to introduce genes that encode specific antigens from pathogens into plants.
- The traditional vaccine administration methods involve the delivery inactivated form of a virus or bacteria, a specific protein, or a genetically engineered molecule that resembles the pathogen into the body through injection or nasal spray.
- This exposes the immune system to a harmless form of the pathogen or its antigens, which the body recognizes as foreign and mounts an immune response against it.

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Overview of the concept of edible vaccines

The innovative approach to vaccination has the potential to revolutionize the field of immunization by offering several advantages over traditional vaccines.

Edible vaccines are produced by introducing the genes that encode specific antigens from a pathogen into the genome of a plant.

The plant produces the edible vaccine antigens in its edible parts, such as fruits or leaves. When the part of the plant is consumers, the human digestive system processes the vaccine antigens, triggering an immune response that produces protective antibodies against the pathogen.

The main advantage of edible vaccines is their potential to be more easily and cost-effectively produced than traditional vaccines. It also has the potential to overcome some of the limitations of traditional vaccines, such as the need for refrigeration and specialized storage and transportation infrastructure.

While they can eliminate painful needles, edible vaccines have some challenges with their development and use. Further, it includes concerns about the safety of genetically modified plants, the difficulty of controlling the vaccine dosage, and ensuring the vaccine is delivered consistently and effectively.

History of Edible Vaccines

People in developing countries may not have access to the vaccines they need as the traditional vaccines are costly, require skilled medical people for administration, and are less effective in persuading a mucosal immune response.

The first report of edible vaccine (surface protein from Streptococcus) in tobacco, at 0.02% of total leaf protein, appeared in 1990 as a patent application published under the international patent cooperation treaty.

Dr. Charles Arntzen and his coworker introduced the concept of transgenic plants as a product delivery system for subunit vaccines in which edible use of transgenic crop plants was used.

After that, the Boyce Thompson Institute for Plant Research researcher at Cornell University produced the world's first edible.

The concept of edible vaccines dates back to the early 1990s when researchers first demonstrated that plant cells could be genetically modified to produce antigens from a pathogen. In 1997, researchers at the Boyce Thompson

Institute for Plant Research at Cornell University produced the world's first edible vaccine by genetically engineering potatoes to produce the antigen for the Hepatitis B virus.

How Edible Vaccines Work

The immune system is a network of cells, tissues, and organs that defend the body against foreign substances.

Further, the immune system can be divided into two main categories: innate and adaptive. While the innate immune system is the first body line of defense, and the adaptive immune system targets specific foreign substances.

The edible vaccine introduces antigens into the body through a food product. The antigen is a substance that triggers an immune response for the production of antibodies to fight off the targeted diseases.

Mechanisms of action of edible vaccines

Edible vaccines are required to activate the mucosal immune response system(MIS), where human pathogens initiate infection.

Mucosal surfaces are found in the lining of the digestive, respiratory, and urinary reproductive tracts. Further, there are multiple ways by which antigens can enter the gut mucosal layer, called M cells and macrophages.

The macrophages are usually activated by interferon-gamma, macrophages presenting fragmented peptides to the helper T cells that produce antibodies.

M cells are another way the antigens are transported to the T cells. The antigenic epitopes are present on the surface of APC with the assistance of the helper of T cells, which then activate B cells.

The activated B cells migrate to the mesenteric lymph nodes, where they mature plasma cells, finally migrating to the mucosal membrane to secrete **immunoglobulin A** (IgA). IgA forms the secretory IgA transported to the lumen.

Producing secretory IgA is another complex since 50% of secretory IgA (sIgA) in the gut lumen is produced by B1 cells. These sIgA are polyreactive and recognize foreign antigens. While in the lumen, the sIgA neutralizes the invading pathogen by reacting with the specific epitopes.

Advantages of edible vaccines

- Edible vaccines are produced from inexpensive plants to cultivate, harvest, and process, which is more cost effective than traditional vaccines.
- It does not require specialized storage facilities and is easier to store and transport to remote areas.

- It can be administered orally, eliminating the need for needles and syringes to reduce the risk of infections and injury.
- Further, the edible vaccine is administered with the help of needles, reducing the risk of contamination from the blood-borne pathogen. Also, the potential for simultaneous delivery of multiple vaccines.

Disadvantages of edible vaccines

- It may not be as effective as a traditional vaccine as the immune response generated is less robust than the vaccine administered via injection.
- It is difficult to control the vaccine dosage delivered to each individual, which can lead to inconsistencies in the immune response generated by the vaccine.
- The edible vaccine may face regulatory hurdles that traditional vaccines do not.

UNIT-IV

TOPIC: BIOFERTILIZERS AND CLASSIFICATION

Definition:

TheBiofertilizerisasubstancecontainingMicroorganismsthathelpwithSoilFertility,andthishelpsin makingtheplantgrowinahealthyway.Thisalsohelpsinincreasingthestreamofvitalnutrientsintreesandplants. The Biofertilizers impart living organisms like mycorrhizal fungi, blue-green algae plus other types of bacterial Biofertilizers. The role of the bacteria as a form of Biofertilizers helps with the restoring of Soil's organic matter, and the regulation is done by natural cycle. he role of the Microbes in the Biofertilizers helps with the healthy growth ofplantsandalsothevalue ofthe Soilincreases. Biofertilizers providean eco-friendlyway ofsustainingthe crops and promoting organic agriculture. The less pesticides and synthetic fertilizersthat you use, the better it is for the health of the plants.

Loose Association of Nitrogen-Fixing Bacteria

Azospirillumisanitrogen-fixingbacteria thatlivearoundtherootsofhigherplantsbutdonotdevelopanintimate relationshipwithplants.Itisoftentermedasrhizosphereassociationasthesebacteriacollectplantexudateandthe same is used as food by them. This process is termed associative mutualism.

Symbiotic Nitrogen-Fixing Cyanobacteria

Blue-GreenalgaeorCyanobacteriafromthesymbioticassociationwithseveralplants.Liverworts,cycadroots,fern, and lichens are some of the Nitrogen-fixing cyanobacteria. Anabaena is found at the leaf cavities of the fern. It is responsible for nitrogen fixation. The fern plants decay and release the same for utilization of the rice plants. Azolla pinnate is a fern that resides in rice fields but they do not regulate the growth of the plant.

Free-LivingNitrogen-FixingBacteria

Theyarefree-livingsoilbacteriathatperformnitrogenfixation.Theyaresaprotrophicanaerobessuch as Clostridium beijerinckii, Azotobacter, etc.

AmongallthetypesofbiofertilizersRhizobiumandAzospirillumaremostwidelyused.

ComponentsofBiofertilizers

Thecomponentsofbiofertilizersinclude:

Bio Compost

Itisoneoftheeco-friendlyproductcomposedofwastematerialreleasedfromsugarindustrieswhichare decomposed. It is magnified with human-friendly bacteria, fungi, and various plants.

Azotobacter

It protects the roots from pathogens present in the soil and plays a crucial role in fixing atmospheric nitrogen. Nitrogen is a very important nutrient for the plant and about 78% of the total atmosphere comprises nitrogen. Azospirillum : Azospirillum is known to have a close associative symbiosis with the higher plant system. These bacteriahaveassociationwithcerealslike;sorghum,maize,pearlmillet,fingermillet,foxtailmilletandotherminor millets and also fodder grasses.

Rhizobium : Rhizobium is relatively more effective and widely used biofertilizer. Rhizobium, in association wit legumes, fixes atmospheric N. The legumes and their symbiotic association with the rhizobium bacterium result in the formation of root nodules that fix atmospheric N. Successful nodulation of leguminous crop by rhizobium largelydependsontheavailabilityofacompatiblestainforaparticularlegume.Rhizobiumpopulationinthesoilis dependent on the presence of legumes crops in field. In the absence of legumes the population of rhizobium in the soil diminishes.

Fungal based biofertillizers

MycorrhizaeMycorrhiza (fungus root) is the mutualistic association between plant roots and fungal mycelia. Frank (1885)gavethename"mycorrhiza" tothepeculiarassociationbetweentreerootsandectomycorrhizalfungi.95%of the plant species form mycorrhizae. It can act as a critical linkage between plant roots and soil. This association is characterized by the movement of plant produced carbon to fungus and fungal acquired nutrients to plants. Mycorrhizalfungiarethekeycomponentsoftherhizosphereareconsideredtohaveimportantrolesinnaturaland managed ecosystems. Types of mycorrhizaMycorrhizal associations vary widely in structure and function. Two main groups of mycorrhizae are recognized; the ectomycorrhizae and endomycorrhizae, although the rare group with intermediate properties, the ectendotrophic mycorrhizae.

1. EctomycorrhizaThe fungal hyphae form a mantle both outside the root and within the root in the intercellular spaces of the epidermis and cortex. No intracellular penetration into epidermal or cortical cells occurs, but an

extensivenetworkcalledtheHartignetisformedbetweenthesecells.SheathorMantleincreasesthesurfaceareaof absorbing roots and offers protection to the roots. Hartignet can act as storage and transport organ for P. Ectomycorrhizae are common on trees, including members of the families pinaceae (Pin, Fir, Spruce, Larch, Semlock), Fagaceae (Willow, Poplar, Chesnut), Betulaceae (Birch, Alder), Salicaceae (Willow, Poplar) and Myrtaceae.ThefungiformingEctomycorrhizal associationarecomingunderBasidiomycotinaandAscomycotina. eg: Laccaria laccata, Suillus, Rhizopogan, Amanita

2. EndomycorrhizaeEndomycorrhizaeconsistofthreesubgroups,butbyfarthemostcommonarethe

3. Arbuscular Mycorrhizal fungi. Fungi under AM are the members of Endogonaceae and they produce an internal network of hyphae between cortical cells that extends out into the soil, where the hyphae absorb mineral salts and water. This fungus do not form an external mantle but lives within the root. In all for ms, hyphae runs between and inside the root cells which includes, Ericoid mycorrhiza Orchid mycorrhiza ArbuscularmycorrhizaArbuscularMycorrhizal

fungiAssociatedwithsomespeciesofEricaceousplantsassociatedwithorchidplantsassociatedwith mostoftheplantfamiliesThemostimportantoneisAMAM,anendomorphicmycorrhizaeformedbytheaseptate phycomycetous fungi are associated with majority of agricultural crops, growing under broad ecological range. Class Order Family : : : Zygomycotina Endogonales Endogonaceae 150 species of AMF are known. Colonization Process Roots do not show visual morphological changes due to AM colonization. AM fungal infection into a host occurs by germination of spore, hyphal growth through soil to ho st roots, penetration of host roots and spread of infection inter and intracellularly in the root cortex. **Colonization occurs under two phases**:

(1) Extra matrical phase and(2) Intra radical phase. Extra matrical phase :Eventsoccurring outside the rootaf ter thegerminationofchlamydospores.Myceliumexploreslargersoilvolume.Fungalgrowthcanbe80130timesthe lengthofroot.Extramatricalhyphae(EMH)arelargerindiameter thaninnerhyphae.Oncethefungusrecognises the plant, appresorium is fo rmed in the host roots and penetration occurs via the appresorium. EMH ends with restingsporesinsoil.Intraradicalphase:Eventsoccurringinsidetherootcortex.Afterpenetratingthecortex,the fungus may produce intercellular as well as intracellul ar hyphae in the cortical cells. Forms two morphological structures namely arbuscules and vesicles inside the cortical cells.

Arbuscules : are the first formed structures after the hyphal entry into the cortical cells. Arbuscules are the fine dichotomously branched hyphal filaments look like little trees. Arbuscules start to form approximately 2 days after penetration. They are considered as the Arbuscules vesicles. Continued….. major site of exchange between the fungus and host root. They are short lived (4-13 days) and degenerate. Vesicles: Following the formation of

arbuscules, some species of fungi also form vesicles in the roots. Terminal or intercallery hyphal swellings of the hyphae called vesicles. Vesicles contain lipids and cytoplasm. They act as P storage organ and they ever be present in the root. Size of the vesicles is about 30-100 µm. In vesicles P can be accumulated as polyphosphates. EMH,

vesiclesandArbusculesplayakeyroleinnutrienttransferparticularlyinmobilisationofphosphorus.Mechanismof actionThe beneficial effect on plant growth and yields following inoculation with VAM is attributed to improvedmineralnutrition,especiallyP(P,Zn,Cu,K,S,NH4)

(ii)Mobilizationofnutrientsthroughgreater soilexploration.(

iii) Protectionofhostrootsagainstpathogeninfection.(

iv) Improvedwaterrelation

(v) Bettertolerance tostresslikesalinity,heavymetalpollution

(vi) Protection against trans plantations hock

Biomass Definition

Biomass Definition

Topic : Biomass productionomass Definition (Energy Source)

Biomass is the fuel developed from organic matter waste of living organisms like plant waste, animal waste, forest waste, and municipal wastes.

In biological terms, the word biomass refers to the organic plant matter, which is converted into fuel and used as an energy source. Biomass fuel is considered to be of great importance as it plays the role of a renewable and sustainable source of energy. For example, biomass is used for the production of electricity. Due to this, biomass is capable of replacing fossil fuels.

Organic materials which can be recycled like wood, agricultural wastes, and municipal wastes serve as excellent sources to produce biomass fuel. The biomass can be burnt directly and later converted into methane and ethanol biofuels. Biomass's chemical composition includes hydrogen, carbon, nitrogen, oxygen, certain alkali atoms, alkaline earth metals and heavy metals

Introduction to Biomass

Biomass energy refers to energy produced from organic matter. It is found in the form of living or recently living organisms, organic mass and waste. The energy produced from biomass is called bioenergy. Materials used to produce this bioenergy refers to feedstock which is mostly plants or animal material. Different types of feedstocks have different physical compositions but Carbon, water and organic volatiles are common in all.

Biomass can be defined as the organic life and mass means weight, so biomass means the total quantity or the weight of organisms in a given area or volume. Now, we are familiar with biomass and biomass definition.

Types of Biomass

Biomass comes from a variety of sources. Some of the different types of biomass example are:

1.Agricultural Residues

These are the Biomass sources or materials that are left in an agricultural field or orchard after the cro harvesting. The residues include stubble like leaves, stems, stalks, and seed pods. These residues are used as biomass for bioenergy production.

2. Animal Waste

Animal waste is an important source of nutrients and renewable energy and is a valuable biomass feedstock. Animal waste has chemical energy stored in it just like plants and when it is burnt, it releases bioenergy in the form of heat and fuel. Animal wastes are generally the excreted materials from living animals and can also include hay, straw, organic debris and wood shavings.

3. Forestry Residues

It is the residue which is left over from logging operations that may include branches, tree tops, sawdust and stumps. These can be obtained in two forms including primary forestry residues and secondary forestry residues.

Forest residues comprise of branches, tops and unmerchantable wood left after cleaning, final felling or thinning of forest stands. These are some of the important Biomass examples.

4. Wood Wastes

It is the portion of the waste stream which comprises discarded wood products, stumps, whole trees or pruned branches obtained during park or street maintenance. Therefore, a vast portion of wood waste can be collected to use as biomass and bioenergy production.

5. Industrial Wastes

It is defined as the waste which is generated by manufacturing or industrial processes. It includes a variety of waste including dirt, gravel, cafeteria garbage, concrete and masonry, scrap metals, oil solvents, trash, chemicals, wood, weed grass, trees, etc. A careful selection of the industrial waste to generate bioenergy is advised for prevention to bad impact on human health.

6.Municipal Solid Wastes and Sewage

Also known as trash or garbage, it is the everyday items that we use and throw away such as grass clippings, furniture, clothing, newspapers, appliances, paint, batteries, product packaging, kitchen waste, etc. Sewage sludge is a type of wastewater produced from a sewer or treatment plant. All of these are used as biomass feedstock for bioenergy production.

Biomass Conversion Process

For bioenergy production from biomass, multiple biomass conversion processes are used:

1. Combustion:

   Feedstock is burnt in the presence of air to release heat. Eg: heating wood, and steam heating to generate electricity

2. Gasification:

   It is the process of using heat, pressure and partial combustion to convert feedstock into combustible gas mixture called syngas (can be used as natural gas/electricity/other uses).

3. Pyrolysis:

   The process of heating feedstock in high temperature in the absence of oxygen. As oxygen is not present, organic material does not combust and it converts into 3 forms: bio oil (solid), bio-char (solid) and syngas.

4. Anaerobic Digestion or Biodigestion:

   Here, the feedstock is burnt which then gets converted into biogas with the help of bacteria in the absence of oxygen. The residue is called digestate and is a great fertilizer.

5. Fermentation:

   The process of converting feedstock or the plant glucose into an alcohol called ethanol by utilization of yeast. Ethanol produced is a biofuel that can be used in the automotive industry.

The usage of the specific process for a specific feedstock depends upon the availability of the resources and desired form of energy. Prior to the industrial revolution, biomass was the primary source of our energy. Now, it is a small percentage of the total energy usage. However, for approx 2.5 billion people, it still remains the primary source of energy for cooking and heating. As earlier stated, resources availability, availability of technology and economic viability are drivers of biomass use.

Disadvantages of Biomass

Biomass usage is highly environment-friendly and budget-friendly, also depending upon the feedstocks and technology type used. Some of the disadvantages of using biomass are discussed in the following points:

- Since the combustion process results in high carbon dioxide emissions leading to harmful impact on humans whereas waste energy biomass production process releases less carbon dioxide, being environment-friendly.
- Biomass production, due to lack of awareness and appropriate measures, especially among poor regions, may result in serious health hazards or risks to human health.

- Depending on the resources used, deforestation, land degradation and assaultation can be the major problems associated with biomass production.

Topic: Biopesticides in agricultural production

Introduction

Chemicals called pesticides are compounds used to eradicate pests. A pesticide is typically a chemical or biological agent such as a virus, bacterium, antibiotic, or disinfectant that inhibits, renders ineffective, or kills pests.

In the United States, The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) mandates that the Environmental Protection Agency (EPA) examine a pesticide's intended usage to ensure that there are no unreasonable hazards to human health and the environment. Only then can the pesticide be marketed and used in the country.

Weeds, plant diseases, and invertebrate pests are controlled with biopesticides. The microorganisms utilised include bacteria, yeasts, fungus, and viruses.

Biopesticides pose minimal dangers to individuals and the environment, making them a relatively safer alternative to chemical or chemical-derived pesticides. Environmental safety and host specificity are the main benefits of biopesticides in managing agricultural pests.

What Are Biopesticides?

The word "biopesticides" refers to compounds that, as opposed to general chemical pesticides, are used to control agricultural pests through specialised biological effects. Used to manage pests, biopesticides refer to products containing biocontrol agents, natural entities or chemicals produced from natural materials (such as animals, plants, bacteria, or specific minerals). These agents may also include their genes or metabolites.

The FAO defines biopesticides as passive biocontrol agents, compared to those that actively seek out the pest, such as parasitoids, predators, and numerous types of entomopathogenic nematodes.

Large numbers of greenhouse farmers in Michigan are learning that biopesticides can be employed in their integrated pest management (IPM) programs in addition to naturally occurring enemies that can be bought

commercially. Growers can benefit from several advantages provided by biopesticides such as lower employee risk, negligible (or no) re-entry and pre-harvest intervals, and compatibility with biocontrol programs.

Importance of Biopesticides

Most farmers attempting to establish a sustainable farming system know that the chemical shed is not their first line of defence against unwanted pests. A "softer" biopesticide or a conventional, synthetic treatment are the farmer's two options when a pest infestation gets too serious, and a chemical application is required. The Integrated Pest Management (IPM) program combines cultural measures, biological controls (such as predatory insects), and chemical control to keep pest populations under control.

Biopesticides are more environmentally friendly and do not harm the soil, water supply, or wildlife, including beneficial insects, which is one of the main advantages of introducing them into a sustainable agriculture system.

Biopesticides are typically used in rotation with conventional products rather than as a replacement, which reduces the amount of synthetic chemicals used. Insects and diseases develop resilience to synthetic chemicals over time. The effectiveness of the synthetic chemical is increased by alternating it with biopesticides.

- Some inoculants with bacteria are made using the fermentation method. Before planting, these inoculants are sprayed on the seeds, and some of them are released into the plants.
- In organic farming, a solution of Azotobacter and synthetic nitrogenase is used to control different insects, weeds, and nematodes.
- The use of biopesticides protects against fluoroacetamide and other chemicals from contaminating the soil. Additionally, they are less likely to affect both human and animal skin.
- Biotechnology enables the direct incorporation of bacterial and fungi toxins that can kill infections and pests into plants. Similar to bacteria, some fungus and virus species have pesticide properties. A biopesticide called spinosad is produced during fermentation.

Despite the potential benefits of using biopesticides, their usage has not been as popular as expected for the following reasons:

1. Expenses associated with creating, testing, and obtaining regulatory approval for new biological agents contribute to the high cost of pesticide manufacture.

2. Due to regional and climatic changes in humidity, temperature, soil conditions, etc., there is limited field effectiveness.
3. Farmers are hesitant about biopesticides because of their high specificity, which means that they only work against specific pathogens and pests.
4. Multiple biological agents are used in biopesticides to manage a variety of insects and pests in the field. These treatments are difficult to use, expensive, and inconvenient, and they are not suitable for many pests and pathogens.
5. Because biopesticides are sensitive to changes in temperature and humidity, they have a short shelf life.

Classification of Biopesticides

Biopesticides can be classified into the following classes:

- **Biochemical Pesticides:** Natural chemicals called biochemical pesticides use non-toxic ways to manage pests. Contrarily, conventional insecticides often consist of synthetic compounds, directly killing or inactivating the pest. Insect sex pheromones and other compounds that prevent mating, and different aromatized plant extracts attracting pests to traps are examples of biochemical insecticides.
- **Microbial Pesticides:** A microorganism (such as a bacterium, fungus, virus, or protozoan) serves as the active component of microbial pesticides. Although each active ingredient in microbial pesticides is specialised for its intended pest(s), they can control many pests. For instance, certain fungi kill particular insects, while others control specific weeds.

  Strains and subspecies of Bacillus thuringiensis (Bt) are the most widely utilised microbial pesticides. This bacterium creates several protein combinations, each of which mainly kills one or a small number of related insect larvae species. While some Bt components only target fly and mosquito larvae, other Bt components also control moth larvae in plants.

- **Plant-Incorporated Protectants (PIPs):** Plants can be manipulated to create pesticides called Plant-Incorporated-Protectants (PIPs) from genetic material, incorporated into the plant. For instance, researchers can insert the gene for the Bt pesticide protein into the plant's genetic makeup. The plant produces the pest-killing component rather than the Bt bacteria. EPA regulates the protein and its genetic makeup but not the plant itself.

Antifeedants are a term used to describe a variety of chemical substances that plants create to defend themselves against pests. In most cases, photosynthesis, growth, or other fundamental components of plant physiology are not known to be affected by biopesticides. They function against biological pests instead. These materials are renewable and biodegradable alternatives that may be cost-effective for practical use. Organic farming systems make use of this method of pest control.

Advantages of Biopesticides Usage

- When compared to traditional pesticides, biopesticides generally have lower intrinsic toxicity.
- Contrary to broad-spectrum, conventional pesticides, which can impact a variety of organisms, including birds, insects, and mammals, biopesticides often only affect the target pest and closely related organisms.
- Biopesticides work primarily in minimal doses and often degrade quickly, reducing exposure levels and mainly avoiding the pollution issues caused by chemical pesticides.
- Biopesticides can significantly reduce the use of chemical pesticides while maintaining excellent crop yields when utilised as a part of Integrated Pest Management (IPM) programs.