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BO- 242 Plant Biotechnology

Topic: Plant Genetic Resources

Plant Genetic Resources

- Historical perspectives and need for PGR conservation;
- Importance of plant genetic resources;
- Gene pool: primary, secondary and tertiary Centres of origin and global pattern of diversity;
- Basic genetic resources and transgenes. Principles, strategies and practices of exploration, collection, characterization, evaluation and cataloging of PGR;
- Plant quarantine and phytosanitary certification;
- Principles of *in vitro and cryopreservation.*
- *Germplasm conservation- in situ, ex situ, Registration of plant genetic resources and importance of NBPGR.*

- **Basic genetic resources and transgenes:**

Basic genetic resources

The sum total of hereditary material i.e. all the alleles of various genes, present in a crop species and its wild relatives is referred to as germplasm. This is also known as genetic resources or gene pool or genetic stock.

- **Plant genetic resources** are plant genetic materials of actual or potential value.
- They describe the variability within plants that comes from human and natural selection over millennia. Their intrinsic value mainly concerns agricultural crops.
- According to the 1983 revised International Undertaking on Plant Genetic Resources for Food and Agriculture of the Food and Agriculture Organization (FAO), plant genetic resources are defined as *the entire generative and vegetative reproductive material of species with economical and/or social value, especially for the agriculture of the present and the future, with special emphasis on nutritional plants*.

Important features of plant genetic resources :

- Genetic pool represents the entire genetic variability or diversity available in a crop species.
- Germplasm consists of land races, modern cultivars, obsolete cultivars, breeding stocks, wild forms and wild species of cultivated crops.
- Germplasm includes both cultivated and wild species and relatives of crop plants.
- Germplasm is collected from centres of diversity, gene banks, gene sanctuaries, farmer's fields, markers and seed companies.
- Germplams is the basic material for launching a crop improvement programme.
- Germplasm may be indigenous (collected within country) or exotic (collected from foreign countries)

Conservation of genetic resources:

The conservation of genetic resources is impossible to provide due to lack of funds. For this reason, attention is given to commercially important plant species.

Genetic variability of these plant species, as well as of those that are not subjected to conservation, depend on their distribution, status in the ecosystem and the presence of the pollen distribution vectors. Because of that, there is an analogy in predicting the population structure of many plant species on the basis of which can be designed state of genetic resources in a given time.

- **The importance of genetic resources in plant breeding:**

Modern agricultural technology and the introduction of new high-yielding varieties are largely eliminating the wide range of crop genetic diversity that has evolved during the five to ten thousand years since food plants were first domesticated. Related wild species are also on the decline because of new land use policies. These gene pools (or what is left of them) are generally spoken of as genetic resources, and are vitally needed in the creation of new crop varieties by plant breeders. Wild species and land races often furnish genes conferring resistance to diseases and pests and adaptation to environmental stresses which cannot be found in the modern crop varieties.

- The study of genetic diversity of crops, its storage in gene banks or in natural reserves, its evaluation and enhancement, are briefly described. The genetic resources work of the Food and Agriculture Organisation of the United Nations (FAO) and other international agencies such as the International Board for Plant Genetic Resources (IBPGR) is outlined.

- **STRATEGIES FOR GENETIC RESOURCE CONSERVATION:**

There exist two main approaches to genetic resources conservation:

- offsite (ex situ) conservation, by which is meant the maintenance of the resources in a site or facility which is not their natural or native habitat, and onsite (in situ) conservation, by which is meant the preservation of the resources in their native habitats. Four strategy levels for conservation can be distinguished.

- Conservation of cloned genes, gametes, embryos, seeds, tissues, or whole organisms in a quiescent state.

- Conservation of plants, animals, or microorganisms in a confined or controlled environment, such as plantations, gardens, zoological parks, reserves, or on host organisms in the case of obligate parasites. Conservation of plants, animals, or microorganisms in their natural habitats where population size and structure are managed. Conservation of plants, animals, or microorganisms in their natural habitats without regard to population size or structure. The successful conservation of any given genetic resource may involve combinations of two or more of these strategy levels, employing both onsite and offsite methods.

- **Methods of conservation and proper use of genetic resources:**
- It can be concluded that conservation and preservation of nature and genetic resources presents the preservation of future.
- The goal of conservation is to enable sustainable development by protecting and using biological resources without compromising the wealth of genes and species.
- There are two basic methods of genetic resources conservation: in situ and ex situ.
- **In situ conservation** is the preservation and maintenance of the plant population in its natural environment. Evolutionary processes and plant population adaptability are present. It can be considered as conservation of ecosystems and the natural environment and the recovery of existing populations of species in their natural environment. This type of conservation is very sensitive and, for example, can be endangered by fires, extreme weather conditions, etc.
- **Ex situ conservation** is the preservation of genetic resources outside of the environment and is mainly used for saving endangered species mainly used for saving endangered species.

- This type of conservation methods includes: seed storage, DNA storage method, pollen storage, in vitro conservation, botanical gardens, cryoconservation (freezing plant material mainly in liquid nitrogen at -196° C), molecular marker technology.
- Seed storage is one of the simplest methods for long-term preservation of plant genetic material. For long term storage of vegetative plant material favourable method is cryoconservation .
- In the past, access and transfer of genetic material was limited, because the old varieties were kept solely as in situ collections. The data indicate that in situ conservation is now less used, and that far more research is done by ex situ methods.
- It is necessary to supplement the in-situ conservation measures by maintaining ex-situ locations and implementation of ex-situ conservation measures. Often ex situ conservation will be used as a complement to, or substitute for, in situ conservation of unique populations that are threatened in their natural habitat.

- **Germplasm Conservation:**

Conservation refers to protection of genetic diversity of crop plants from genetic erosion.

- There are two important methods of germpalsm conservation or preservation.
- i) In-situ conservation and
- Ii) ex situ conservation.
- **i) In - situ conservation:**
- Conservation of germplasm under natural conditions is referred to as in situ conservation. This is achieved by protecting the area from – human interference, such an area is often called natural park, biosphere reserve or gene sanctuary. NBPGR, New Delhi, established gene sanctuaries in Meghalaya for citrus, north Eastern regions for musa, citrus, oryza and *saccharum*.

- **ii) Ex - situ conservation:**

It refers to preservation of germplasm in gene banks. This is the most practical method of germplasm conservation. This method has following advantages.

- It is possible to preserve entire genetic diversity of a crop species at one place.
- Handling of germplasm is also easy.
- This is a cheap method of germplasm conservation.

- This type of conservation can be achieved in the following 5 ways.
- **1) Seed banks:**
- Germplam is stored as seeds of various genotypes. Seed conservation is quite easy, relatively safe and needs minimum space. Seeds are classified, on the basis of their storability into two major groups.
- 1) Orthodox and 2) Recalcitrant

- **Orthodox seeds:** Seeds which can be dried to low moisture content and stored at low temperature without losing their viability for long periods of time is known as orthodox seeds. (eg.) Seeds of corn, wheat, rice, carrot, papaya, pepper, chickpea, cotton, sunflower.
- **Recalcitrant:** Seeds which show very drastic loss in viability with a decrease in moisture content below 12 to 13% are known as recalcitrant seeds. (e.g) citrus, cocoa, coffee, rubber, oilpalm, mango, jack fruit etc.
- **Seed storage:** Based on duration of storage, seed bank collects are classified into three groups. (1) Base collections. (2) Active collections and (3) Working collection.
- **Base collections:** Seeds can be conserved under long term (50 to 100 years), at about -20OC with 5% moisture content. They are disturbed only for regeneration.
- **Active collection:** Seeds are stored at 0OC temperature and the seed moisture is between 5 and 8%. The storage is for medium duration, i.e., 10-15 years. These collections are used for evaluation, multiplication, and distribution of the accessions.

- **Working collections:** Seeds are stored for 3-5 years at 5-10OC and the usually contain about 10% moisture. Such materials are regularly used in crop improvement programmes.

- **2. Plant Bank: (** Field or plant bank)is an orchard or a field in which accessions of fruit trees or vegetatively propagated crops are grown and maintained.
- **3. Shoot tip banks:** Germplasm is conserved as slow growth cultures of shoot-tips and node segments. Conservation of genetic stocks by meristem cultures has several advantages as given below.

- Each genotype can be conserved indefinitely free from virus or other pathogens.
- It is advantageous for vegetatively propagated crops like potato, sweet potato, cassava etc., because seed production in these crops is poor
- Vegetatively propagated material can be saved from natural disasters or pathogen attack.

- Long regeneration cycle can be envisaged from meristem cultures.
- Regeneration of meristerms is extremely easy.
- Plant species having recalcitrant seeds can be easily conserved by meristem cultures.

4. Cell and organ banks: A germplasm collection based on cryopreserved (at – 196OC in liquid nitrogen) embryogenic cell cultures, somatic/ zygotic embryos they be called cell and organ bank.

5. DNA banks: In these banks, DNA segments from the genomes of germplasm accessions are maintained and conserved.

- **Transgene:**

A **transgene** is a gene that has been transferred naturally, or by any of a number of genetic engineering techniques from one organism to another.

- The introduction of a transgene, in a process known as transgenesis, has the potential to change the phenotype of an organism.
- *Transgene* describes a segment of DNA containing a gene sequence that has been isolated from one organism and is introduced into a different organism. This non-native segment of DNA may either retain the ability to produce RNA or protein in the transgenic organism or alter the normal function of the transgenic organism's genetic code.
- In general, the DNA is incorporated into the organism's germ line.

- The construction of a transgene requires the assembly of a few main parts. The transgene must contain a promoter, which is a regulatory sequence that will determine where and when the transgene is active, an exon, a protein coding sequence (usually derived from the cDNA for the protein of interest), and a stop sequence. These are typically combined in a bacterial plasmid and the coding sequences are typically chosen from transgenes with previously known functions.
- Transgenic or genetically modified organisms, be they bacteria, viruses or fungi, serve many research purposes. Transgenic plants, insects, fish and mammals (including humans) have been bred. Transgenic plants such as corn and soybean have replaced wild strains in agriculture in some countries (e.g. the United States). Transgene escape has been documented for GMO crops since 2001 with persistence and invasiveness. Transgenetic organisms pose ethical questions and may cause biosafety problems.

- **Types:**

Transgenic plants have genes inserted into them, deriving from other species. The inserted genes can come from species within the same kingdom (plant to plant) or between kingdoms (bacteria to plant). In many cases, the inserted DNA has to be modified slightly in order to correctly and efficiently express in the host organism. Transgenic plants are used to express proteins, like the cry toxins from Bacillus thuringiensis, herbicide resistant genes and antigens for vaccinations.

- Cisgenic plants are made up of using genes, found within the same species or a closely related one, where conventional plant breeding can occur. Some breeders and scientists argue that cisgenic modification is useful for plants that are difficult to crossbreed by conventional means (such as potatoes). Those plants in the cisgenic category should not require the same level of legal regulation as other genetically modified organisms.

- **Advantages of transgenic plants:**

GM Technology has been used to produce a variety of crop plants to date. As the global population continues to expand, food remains a scare resource. Genetically engineered foods offer significant benefits by improving production yield, lowering transportation costs and enhancing the nutritional content.

- Significant benefits will include the following:
- (i) improved and more efficient weed control;
- (ii) decreased losses due to insect pests and viruses and decreased need of insecticide;
- (iii) decrease in post-harvest losses due to better shelf life and marketing flexibility (tomato) due to resistance against storage pests;
- (iv) increase in nutritional quality (oil in canola);
- (v) more effective production of hybrid seed.

1. Herbicide resistant plants:

Plants that can tolerate herbicides are called Herbicide Resistant Plants. Glyphosate is an active ingredient of many broad spectrum herbicides. Glyphosate resistant transgenic tomato, potato, tobacco, cotton etc are developed by transferring aro A gene into a glyphosate EPSP synthetase from *Salmonella typhimurium* and *E. coli* Sulphonylurea resistant tobacco plants are produced by transforming the mutant ALS (acetolactate synthetase) gene from Arabidopsis.

2. Insect resistant plants:

- Bacillus thuringiensis is a bacterium that is pathogenic for a number of insect pests. Its lethal effect is mediated by a protein toxin it produces.

Through recombinant DNA methods, the toxin gene can be introduced directly into the genome of the plant, where it is expressed and provides protection against insect pests of the plant.

3. Virus resistant plants

TMV resistant tobacco and tomato plants are produced by introducing viral coat proteins. Other viral resistant transgenic plants are (a) Potato virus resistant potato plants (b) RSV resistant rice, (c) YMV resistant black gram and (d) YMV resistant green gram etc.

4. Pest resistant plants

- There is clearly a benefit to farmers, if transgenic plants are developing a resistant into specific pest. For example, Papaya-ring-spot-virus resistant papaya has been commercialized and grown in Hawaii since 1996.[12] There may also be a benefit to the environment, if the use of pesticides is reduced. Transgenic crops, containing insect resistance genes from *Bacillus thuringiensis*, have made it possible to reduce significantly the amount of insecticide, applied on cotton in the USA. However, populations of pests and disease, causing organisms, adapt readily and become resistant to pesticides.

5. Nutritional benefits

Vitamin A deficiency causes half a million children to become partially or totally blind each year. Milled rice is the staple food for a large fraction of the World's human population. Traditional breeding methods have been unsuccessful in producing crops, containing a high concentration of vitamin A. Researchers have introduced three genes into rice: two from daffodils and one from a microorganism. The transgenic rice exhibits an increased production of beta-carotene as a precursor to vitamin A and the seed is yellow in color. Such yellow, or golden, rice may be a useful tool to treat the problem of vitamin A deficiency in young children living in the tropics.

6. Use of marginalized land

A vast landmass across the globe, both coastal as well as terrestrial has been marginalized because of excessive salinity and alkalinity. A salt tolerance gene from Mangroves (*Avicennia marina*) has been identified, cloned and transferred to other plants. The transgenic plants were found to be tolerant to higher concentrations of salt. The gut D gene from *Escherichia coli* has been used to generate salt tolerant transgenic maize plants. Such genes are a potential source for developing cropping systems for marginalized lands (MS Swaminathan, Personal Communication, 2000).

11. Reduced environmental impact

Water availability and efficient usage have become global issues. Soils subjected to extensive tillage (plowing) for controlling weeds and preparing seed beds are prone to erosion, and there is a serious loss of water content. Low tillage systems have been used for many years in traditional communities. There is a need to develop crops that thrive under such conditions, including the introduction of resistance to root diseases currently controlled by tillage and to herbicides which can be used as a substitute for tillage.

7. Therapeutic proteins from transgenic plants

Proteins of therapeutic importance, like those used in the treatment, diagnosis of human diseases can be produced in plants, using recombinant DNA technology. Scaling-up of these transgenic plants to fields, results in industrial production of proteins. The area of research combining molecular Biotechnology and Agriculture is called Molecular farming or pharming. The proteins produced in transgenic plants for therapeutic use, are of three types – (i) antibodies, (b) proteins and (iii) vaccines. Antibodies directed against dental caries, rheumatoid arthritis, cholera, *E. coli* diarrhea, malaria, certain cancers, HIV, rhinovirus, influenza, hepatitis B virus and herpes simplex virus are known to be produced in transgenic plants. Vaccines against infectious diseases of the gastro intestinal tract have been produced in plants like potato and bananas. The another appropriate target would be cereal grains. An anti cancer antibody has recently expressed in rice and wheat seed that recognizes cells of lung, breast and colon cancer and hence could be useful in both diagnosis and therapy in the future.

- **Development of transgenic crops:**

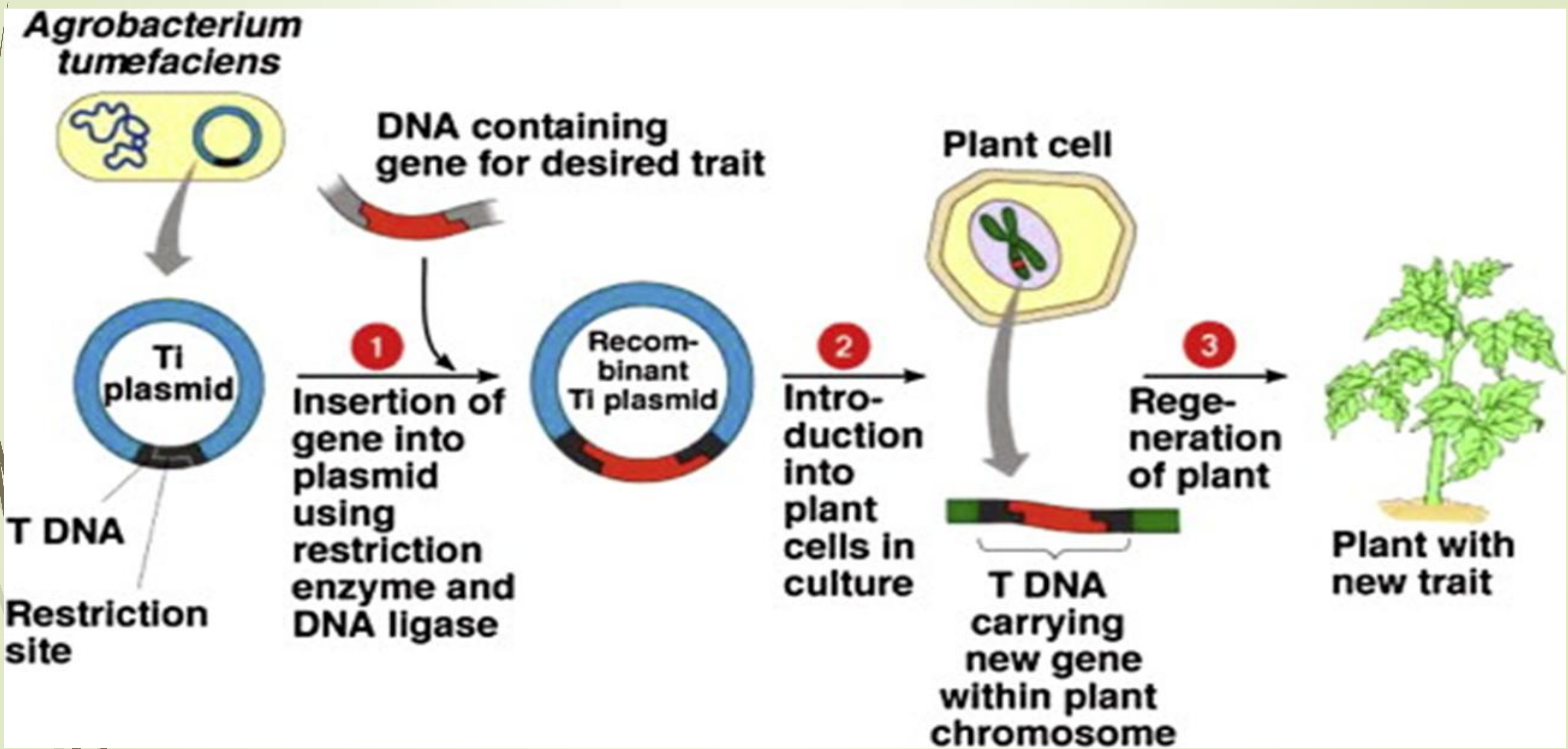
Genetically engineered plants are generated in a laboratory by altering the genetic-make-up, usually by adding one or more genes of a plant's genome. The nucleus of the plant-cell is the target for the new transgenic DNA. Most genetically modified plants are generated by the biolistic method (Particle gun method) or by *Agrobacterium* tumefaciens mediated transformation method.

- The "Gene Gun" method, also known as the "Micro-Projectile Bombardment" or "Biolistic" method is most commonly used in the species like corn and rice.
- In this method, DNA is bound to the tiny particles of Gold or Tungsten, which is subsequently shot into plant tissue or single plant cells, under high pressure using gun.
- The accelerated particles are penetrating both into the cell wall and membranes.
- The next method, used for the development of genetically engineered plants, is the "Agrobacterium" method

- The DNA separates from the coated metal and it integrates into the plant genome inside the nucleus. This method has been applied successfully for many crops, especially monocots, like wheat or maize, for which transformation using *Agrobacterium tumefaciens* has been less successful.
- This technique is clean and safe. The only disadvantage of this process is that serious damage can be happened to the cellular tissue.
- It involves the use of soil-dwelling bacteria, known as *Agrobacterium tumefaciens*. It has the ability to infect plant cells with a piece of its DNA.
- The piece of DNA, that infects a plant, is integrated into a plant chromosome, through a tumor inducing plasmid (Ti plasmid).
- The Ti plasmid can control the plant's cellular machinery and use it to make many copies of its own bacterial DNA.
- The Ti plasmid is a large circular DNA particle that replicates independently of the bacterial chromosome.

- The importance of this plasmid is that, it contains regions of transfer DNA (t DNA), where a researcher can insert a gene, which can be transferred to a plant cell through a process known as the “floral dip”.
- A Floral Dip involves, dipping flowering plants, into a solution of Agrobacterium carrying the gene of interest, followed by the transgenic seeds, being collected directly from the plant.
- This process is useful, in that, it is a natural method of transfer and therefore thought of as a more acceptable technique. In addition, “Agrobacterium” is capable of transferring large fragments of DNA very efficiently. One of the biggest limitations of Agrobacterium is that, not all important food crops can be infected by these bacteria.
- This method works especially well for the dicotyledonous plants like potatoes, tomatoes and tobacco plants.

Agrobacterium tumefaciens mediated transformation method:



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