Plant Cell Bioprocessing
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Lecture – 22
Genetic transformations in plant cell – Part I

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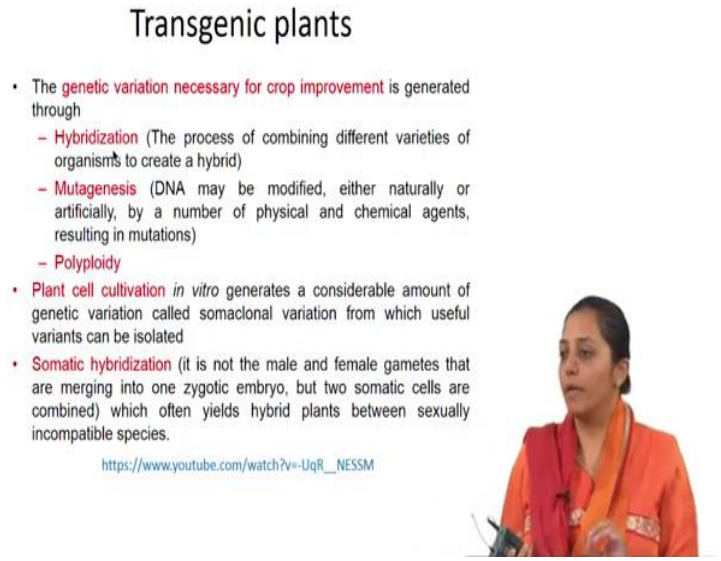

Genetic variation is necessary for crop improvement in agriculture. What are the different ways in which crop yields or the traits are improved? By hybridization. What is hybridization? It is the process of combining different varieties of the same plant. I will be talking about plant hybridization of the same plant such that you can select for the hybrid lines which will have the combination of the good traits of the both the varieties.

Although it is a random process, this will include a selection of the cell lines which will have all the positive traits of both the varieties together. Then the other way is mutagenesis, where DNA may be modified either naturally or artificially by a number of physical and chemical agents resulting in mutations after performing screening and selection. The third method is polyploidy, where you apply certain agents which can result in change in the ploidy in plants, which may lead to improvement in the traits desired.

Plant cell cultivation *in vitro* generates a considerable amount of genetic variation. Now we know that under *in vitro* conditions, these variations are called somaclonal variation which itself can be used to generate different cell lines of different genetic makeup because this can

lead to polyploidy change in the karyotopic nature of the cell. So, what is somatic hybridization? It is a well known technique we have learnt about it. Somatic hybridization is a method in which somatic cells are fused together such that the cytoplasm as well as the nucleus will get fused and therefore, you will end up in a single cell which will have traits of both the varieties of cells.

So, generally it is observed that these hybrid cells which come out will be more vigorous in nature; for crop improvements you will see that hybridization of plants is very commonly used. Seedless fruits for example, I do not know whether you have heard or seen seedless papaya or very bigger size fruits. These are all because of hybridization.

So, generally deliberate pollination is done; mixing is done such that the hybrids are generally observed to be more vigorous in nature in terms of their growth rates or in terms of yields.

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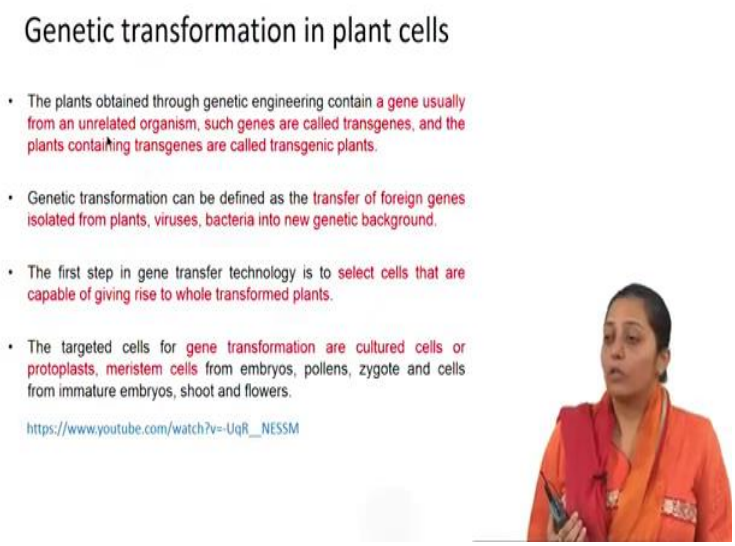

Now, the plants obtained through genetic engineering contain a gene usually from an unrelated organism. So, when we say transgene integration, it means that a gene which is not present in the plant, but has been transformed in the plant chromosome. Such genes are called transgenes and the plants containing the transgenes are called transgenic plants.

Genetic transformation can be defined as the transfer of foreign genes isolated from plants, viruses, bacteria into a new genetic background. The first step in gene transfer is to select the cells that are capable of giving rise to whole transformed plants. Now, we will see what are the

different ways in which the selection is done and what are the different ways in which the transformation of plant cells is done.

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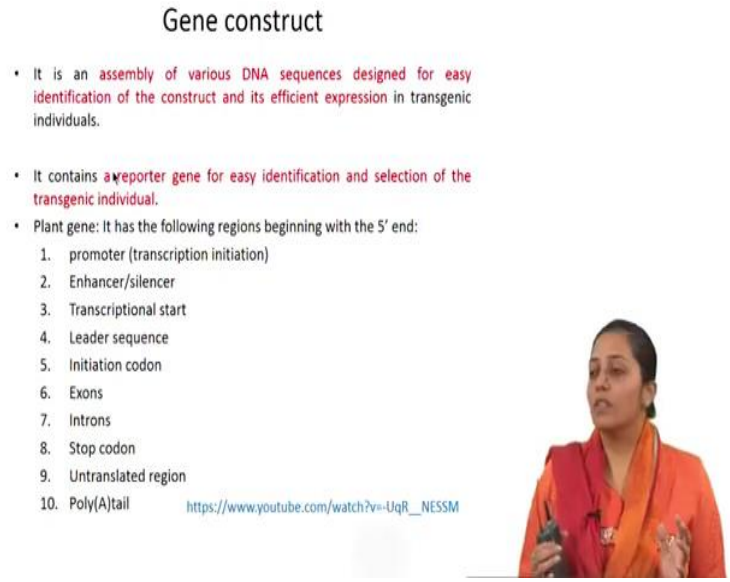

So, what is a gene construct? Gene construct for transgene integration will contain assembly of different DNA sequences. Generally, *Agrobacterium* is used as a tool for transferring this genetic material. There are other techniques also which we will be talking about. So, the whole transgene which is transferred will have these many sections as numbered here.

So, the construct will contain a promoter, it will contain an enhancer or silencer, transcriptional start, leader sequence, initiation codon, exons, introns. stop codon, untranslated regions and poly a tail. Have you heard about these terms? You have heard. So, for what promoter is used for?

Student: Transcription

Be loud.

Student: RNA polymerase binds

RNA polymerase?

Student: RNA polymerase binds and starts

The transcription of the gene, then enhancer and silencer?

Student: They regulate the promoter activity

They regulate something.

Student: The binding of the RNA polymerase

Right. So, they regulate as he said the promoter activity. When it is an enhancer it is enhancing the promoter. When it is a silencer, it will silence the promoter. This can have a certain sequence or this can lead to selective induction of the promoter or by exogenous addition of certain agents. So, this is how it may lead to regulation of the promoter activity.

Then, transcriptional start, leader sequence? These are the regions of mRNA for the regulation of translation at the ribosome level because once the mRNA is formed it will attach to the ribosome for translation. There this leader sequence regulates that. Then initiation codon, exons, introns. Which part of the gene is responsible for expression, introns or exons?

Student: Exons.

Exons. RNA splicing removes introns, but regulatory role has been found in the intronic regions as well. Stop codon is the transcriptional stop, and it is followed by untranslated regions. There are 5' untranslated region 3' untranslated region. 3' untranslated region also has poly A tail which is required for stability of the mRNA during the processing.

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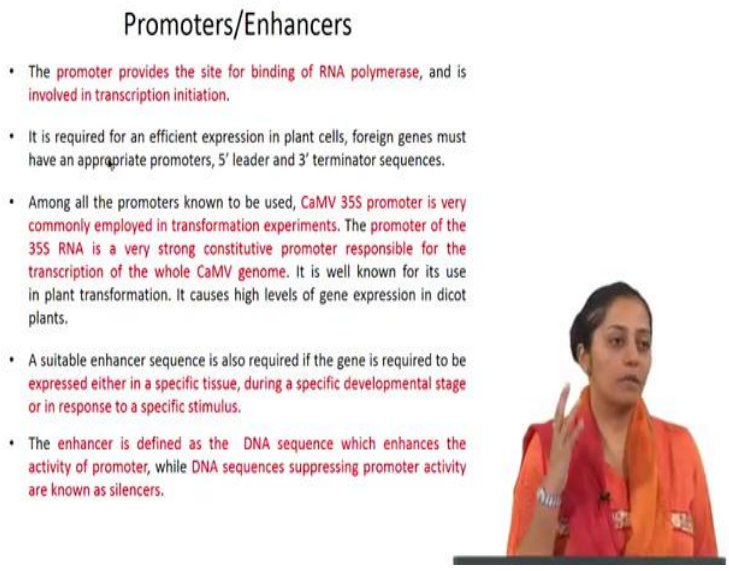

So, the promoter provides the site for binding of RNA polymerase and is involved in transcription initiation. It is required for an efficient expression in plant cells. So, generally the promoters used are either inducible promoters or constitutive promoters. What is an inducible promoter? Inducible means the promoter activities are regulated; in the presence of certain chemicals the promoter will be active.

Constitutive promoter irrespective of the conditions, it is active all the time. So, CaMV 35S promoter. Have you heard about CaMV? It is a very widely used cauliflower mosaic virus promoter for genetic transformation in plant cells. Now is this a constitutive promoter or an inducible promoter? This particular promoter is responsible for the entire gene transcription of mosaic virus. It is a constitutive promoter and has very high activity and it is well known to be infecting the plant cells.

So, this particular promoter being a constitutive promoter or strong promoter is used for genetic transformations in plant cells. Among all the promoters known, CaMV35S promoter is very commonly employed in transformation experiments. The promoter of the 35S RNA is a very strong constitutive promoter that leads to the transcription of the whole CaMV35S. So, it is well known for its use in plant transformation.

A suitable enhancer sequence is also required to control the activity of the promoter. Now, how do you think this enhancer sequence is useful? If you want to express it at a particular developmental stage or you want an expression in a particular tissue, then you need to regulate the activity of the promoter. That can be done using your enhancer or your silencer sequence.

The enhancer is defined as the DNA sequence which enhances the activity of the promoters and the DNA sequence suppressing the activity of the promoter is known as silencer.

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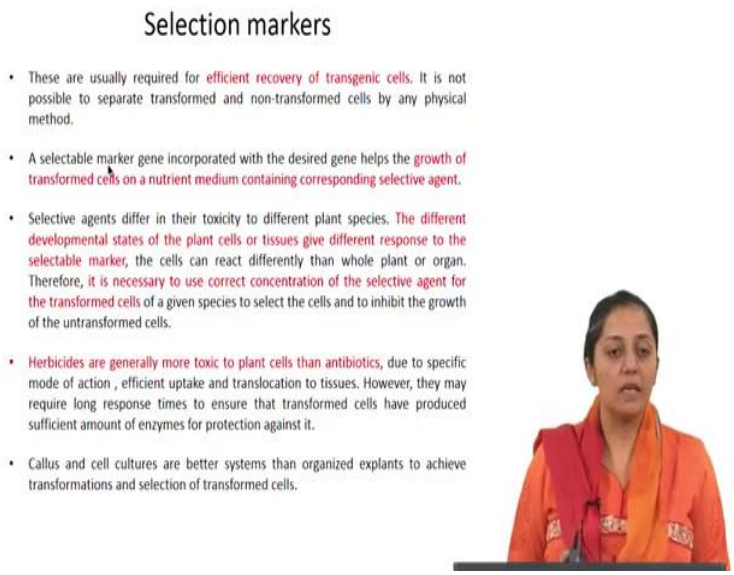

So, how do we recover transgenic cell lines? Suppose we have done transformation using this construct, how do you find out which particular cell line is transformed or not? How do you select? You would be needing a marker system. Now, what is the marker system used? So, generally antibiotic resistance is used. So, it is a selective marker system. Now what does it mean? It means because it is present in the transgene which is integrated into the plant chromosome; it will get expressed in the plant chromosome, then only it will be able to show antibiotic resistance.

Generally you would like to choose an antibiotic resistance against an antibiotic to which the plant cells are highly sensitive. So, what kind of antibiotics is chosen? We have seen kanamycin in bacterial transformations. Generally hygromycin is used because plant cells are found to be highly sensitive to hygromycin because of their uptake and their reach availability to plant cells and affect them. So, the hygromycin system is used as a reporter system or as a marker system. So, selective agents differ in the toxicity to different plant species. The different developmental states of plant cells or tissues give different responses to the selectable marker. It is necessary to use the correct concentration of the selective agent. Herbicides are generally more toxic to plant cells than antibiotics. Now, callus and cell cultures are better systems than organised explants to achieve transformations and for the selection of transformed cells.

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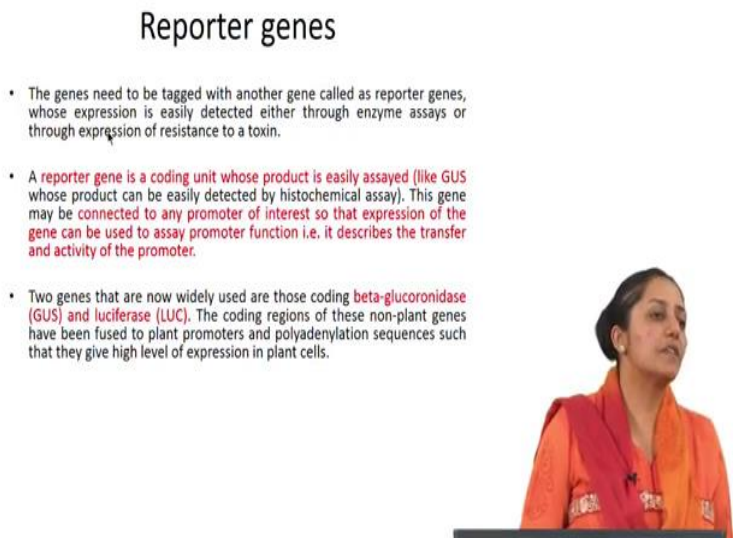

Now, how is the reporter system different from your marker system? Generally you have heard about GUS and GFP; why is it used?

Student: You want to know what levels of protein are being expressed. So, we can correlate it with.

It can be quantified as well as qualified. If it is qualitative in nature, what will it tell you?

Student: whether it is expressed or not

Expressed or not. So, generally these systems in plant cells, GUS assays are very frequently used which is glucuronidase assay or luciferase assay. In these assays blue coloured product come on the cells once they are exposed to X-Gluc substrate. So, this particular enzyme if it is expressed properly in the transgenic cell lines then it will be able to break down the substrate X-Gluc into a product which is blue in colour that is visible and this becomes an indication of successful transgene integration.

Now, the genes need to be tagged with another set of genes called as reporter genes. Now I was saying that you can confirm whether a transformation is successful or not using reporter systems. What else can be the use of a reporter system? It can also be used to check promoter activity and whether the promoter is active or not.

So, if you use the same promoter which you have used for your desired transgene integration for this particular GUS or luciferase activity and if that gets expressed then there is a high chance that the promoter activity is intact and is able to show good activity in this particular plant species. So, a reporter gene is a coding unit whose product is easily assayed like GUS and whose product can be easily detectable. So, it is called a histochemical assay.

So, this gene may be connected to any promoter of interest so that the expression of the gene can be used to assay promoter function i.e, it describes the transfer and activity of the promoter. Two genes that are widely used are beta glucuronidase (GUS) and luciferase (LUC). The coding regions of these non plant genes (transgenes) have been fused to plant promoters and polyadenylation sequences (you need a 5' UTR and 3' UTR).

For 5' UTR you are using the same promoter that is used for your desired gene and 3' UTR is used for the entire construct - GUS or beta glucuronidase or luciferase enzyme for expression.

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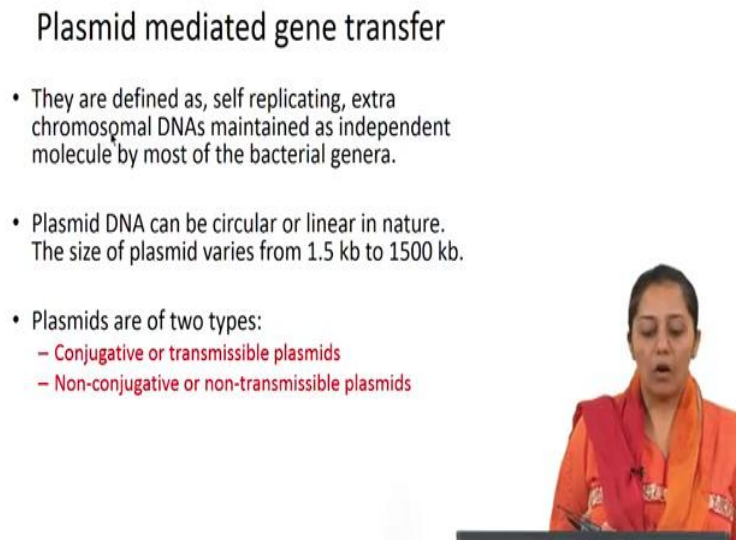

Now, plasmid mediated gene transfer. How many types of plasmids are used for gene transfer? There are 2 types - conjugative plasmid or transmissible plasmids, the others are non conjugative plasmids which are also called as non transmissible plasmids. What are plasmids? They are self replicating extra extrachromosomal DNA maintained as independent molecules and generally they are found in bacteria. Plasmid DNA can be circular or linear in nature and the size of the plasmids varies from 1.15 kb to 1500 kb.

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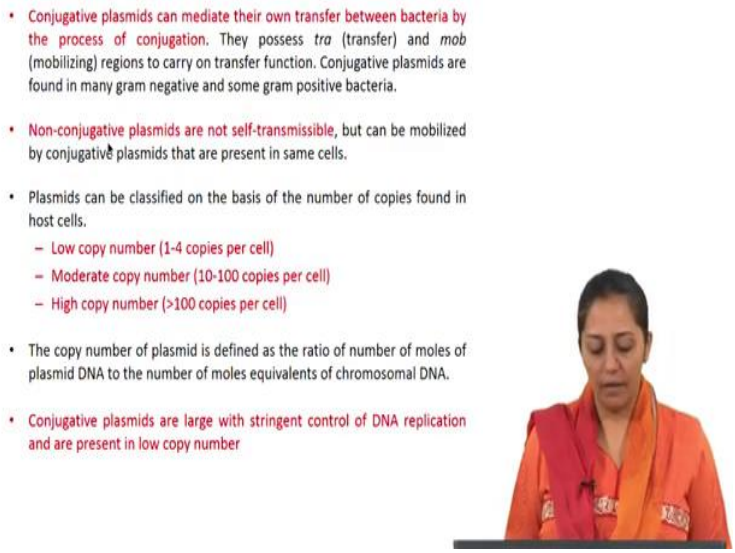

What are conjugative plasmids? They can mediate their own transfer between bacteria by the process of conjugation. There are appendages, which are formed and the two cells will come together to facilitate the transfer of the genetic material. Non conjugative plasmids on the other hand are not self transmissible. So, generally when we are using non conjugative plasmids their transfer is done with the help of conjugative plasmids.

Now, plasmids can be classified in terms of lower and high copy numbers; low copy numbers when it is nearly 1 to 4 copies per cell, high copy numbers when it is greater than 100 copies per cell. Thus conjugative plasmids are large with stringent control of DNA replication and are present in low copy numbers.

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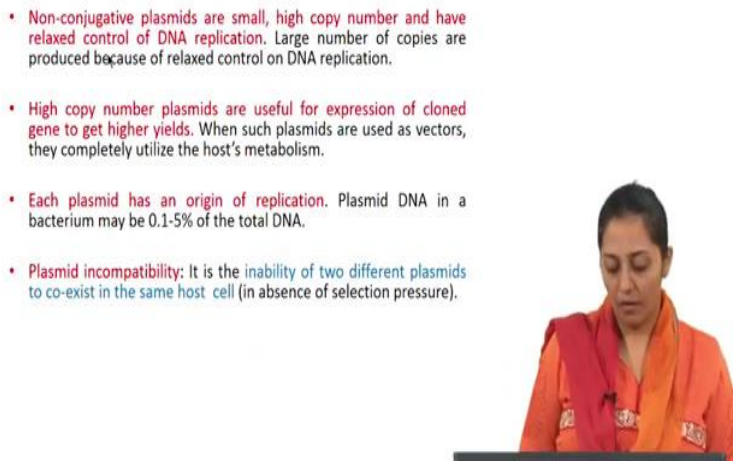

Non conjugative plasmids are small, have high copy number and have relaxed control of DNA replication. So, suppose you are working for cloning, what kind of plasmid you will prefer?

Non conjugative plasmids; large number of copies are produced because of the relaxed control of DNA replication. High copy number plasmids are useful for expression of cloned genes to higher yields. So, when I say binary vector these plasmids can get expressed in more than one type of organisms. For example, in plant transformation, binary vectors like pCambia, they can get expressed, multiply in *E. coli* as well as in *Agrobacterium*.

When such plasmids are used as vectors, they completely utilise the host metabolism. So, what is the demerit? They will utilise the host metabolism if they are expressed in an organism. Each plasmid has an origin of replication. Try to make out why we say any kind of transgene integration is a metabolic burden.

Each plasmid has an origin of replication and plasmid DNA in bacteria may be 0.1 to 5 percent of the total DNA.

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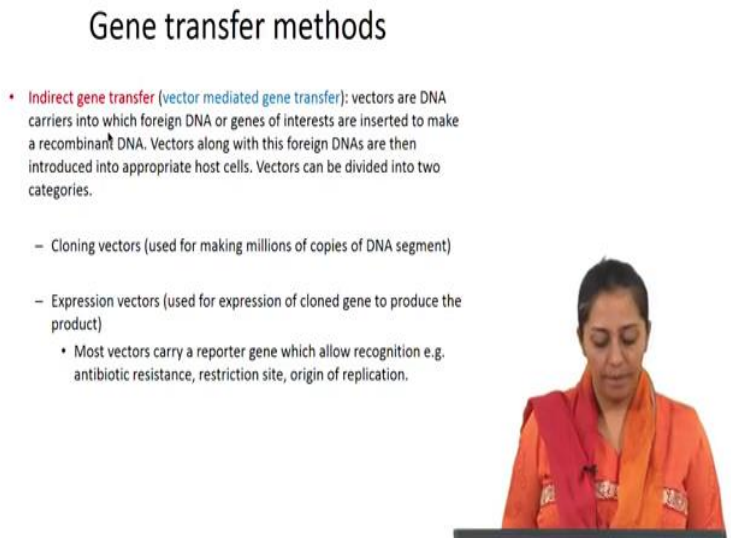

Now, gene transfer methods; one is indirect gene transfer and the other is direct gene transfer. We will take up the direct gene transfer later. The indirect gene transfer methods are the most frequently used in transgene integrations of plant cells. Indirect gene transfer is vector mediated gene transfer. Vectors are DNA carriers into which foreign DNA or genes of interest are inserted to make a recombinant DNA.

Now, what are the different kinds of vectors? Cloning vectors and expression vectors. Cloning vectors are required if you want to multiply and have a large number of copies. Expression vectors are used when you want to express the transgene into the plant. It is used for expression of the clone gene to produce the product.

What do most vectors carry? We have already studied this - A reporter gene which allows recognition for example antibiotic resistance.

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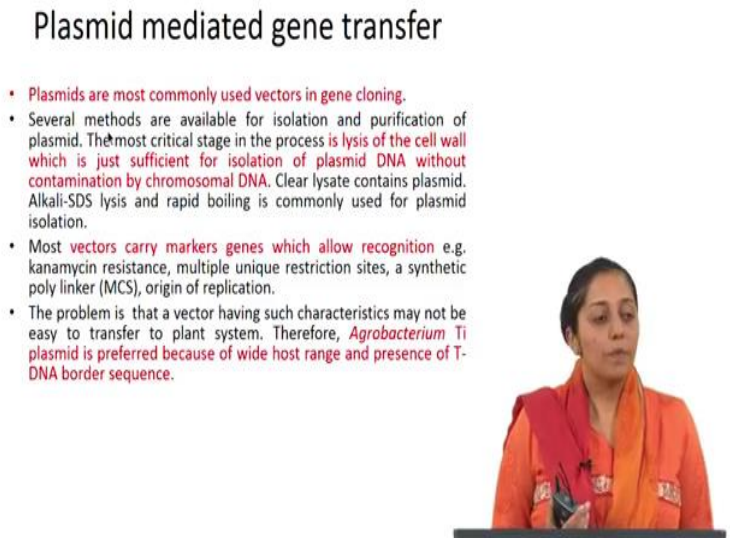

So, plasmid mediated gene transfer. Plasmids are most commonly used vectors in gene cloning. What are the different types of plasmids used? Different types of plasmid which are used are, Ti plasmids or Ri plasmids. Ti plasmid is tumor inducing plasmids and Ri is root inducing plasmids. Now I will come to certain diseases in nature or because of bacteria which is *Agrobacterium* which contain these plasmids.

Now, these plasmids will have a set number of genes. Why do you think a bacteria infects the plant? It contains certain genes which cannot be expressed when the plasmid is present in the bacteria, but as it gets integrated into the plant they will express these genes and lead to opine production. These opines are carbon and nitrogen sources for the bacteria.

So, now in this course, the portion which gets integrated will have certain oncogenic genes. Now the plant is allowing this infection to happen because it has certain auxin and cytokinin genes as well which get integrated and cause some biochemical changes leading to neoplastic growth at the point of infection. Now morphogenetic events can either lead to rapid multiplication of cells causing crown gall formation or organogenic/adventitious root formations which are called as hairy roots.

Therefore, these *Agrobacterium* and the plasmid in it will be modified or disarmed; Disarmed means removing these oncogenic genes - the auxins and cytokinins genes. In that place multiple cloning sites (MCS) is put there with different restriction sites so that the desired gene can be

integrated. Now this whole cassette is included in the T-DNA region. The portion of the construct which gets integrated into the plant chromosome, is called T-DNA.

Now, this portion contains a right border and a left border. These are direct repeat sequences - around twenty five base pairs long. Even outside this T-DNA there are different regions of the Ti plasmid or the Ri plasmid. There are some genes which are responsible for the breaking and the multiplication of this T-DNA, processing of this T-DNA, transfer of this T-DNA into the plant cell and protecting it till it reaches the chromosome of the plant cells and gets integrated.

These can be virulence genes and other genes which are required in the entire packaging of the T-DNA. So, it is not T-DNA alone, but it is T-DNA with other proteins which are involved in the process of movement till the plant chromosome and even its interaction with the plant proteins thereby facilitating the integration in the plant chromosome.