Course Name: Basics of Crop Breeding and Plant Biotechnology

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Lecture-35: Vectors and Plasmids used in Molecular Biology

Welcome back. So, we will continue again. Now, let us discuss about different types of vectors. First of all, we will be discussing about plasmid vectors, because plasmid vectors are mostly used in molecular biology. So, plasmids are small circular DNA molecules, distinct from a bacterium's chromosomal DNA. If you think about a particular bacterium in the bacterial cell, the chromosomal DNA is available, along with some double-stranded close circular DNA molecules might be available. Ok!

Those DNA molecules is a plasmid, and those plasmids could be used as vectors. So, this type of plasmid vector could be modified through biotechnology to transport foreign genes. Once introduced in bacterial cells, these plasmids facilitate replication due to the availability of certain regions, later on, we will be discussing resulting in the production of copies of the foreign gene alongside the bacterium's native DNA. It means, that if the bacteria is replicated from 1 bacterial cell 2 bacterial cells will be produced, and from 2, 4 bacterial cells will be produced, and the chromosomal DNA will be replicated, in its own way in the new progeny. Ok!

But in these plasmids, some sequences should be there with the help of those plasmids will be replicated also. So, that it could be available in the progeny. So, now coming to another type of vector which is the viral vector. Viruses can be adapted as carriers for gene delivery by replacing their original genetic material with the desired gene. If you think about a normal bacteriophage virus!

So, in normal bacteriophage viruses, this type of structure is there most of them are made up of protein, and within that head within the head part the genetic material is available, and the genetic material it might be DNA or RNA whatever. So, once we are using viral vector ok, we can replace the original genetic material that is available within the virus. Because, once if this type of virus, let's assume the bacteriophage once bacteriophage infects a bacterium the protein part remains outside, but the genetic material goes inside and can cause damage. So, within that genetic material, different genes are available some of them are responsible for the occurrence of the disease, some of them are responsible for the multiplication of different viral parts and some of them will hijack the bacterial system they will hijack the transcriptional machinery. So, different sets of genes are there.

So, once we have to develop viral vectors we have to remove some sets of genes, especially those genes that are responsible for disease occurrence we can remove those parts and within that part, we can put our target gene. So, that once this DNA, will be entered into a bacterial cell it will amplify a large number of viral molecules or a large number of viral vectors will be produced, but that cannot cause infection. Ok! So, in this way basically, viral vectors are used. So, in this scenario, the altered virus is employed to infect specific target cells facilitating the transfer of genetic cargo into the host organism. The genetic material could be transferred, but it cannot cause any infection in the bacteria or it cannot cause any infection in the targeted host.

Now BAC or bacterial artificial chromosomes and YAC or yeast artificial chromosomes those type of vectors are also used. Bacterial artificial chromosomes and yeast artificial chromosomes are specialized carriers, crafted to transport substantial DNA fragments. If the DNA fragment that we are trying to transfer from one organism to another organism is huge if the size is more. Ok! Even the complete sets of genes or even the huge genomic region, if we have to deliver then we have to use these things the BAC or YAC. Through plasmid generally, we can transfer up to 10 kb up to 15 kb through viral vectors we can transfer a little bit more, but using bacterial artificial chromosomes or yeast

artificial chromosomes, large-size DNA could be transferred.

And, typically they are utilized in biotechnology and these vectors play a key role in cloning sizable DNA segments for in-depth genomic investigations. Now coming to a hybrid vector, that is a cosmid vector. So, cosmid vectors are hybrid they combine the elements of plasmids and bacteriophage, a type of virus that infects bacteria based on that the viral vectors could be generated and the cosmid vectors can carry larger DNA fragments than typical plasmid vectors. This is another type of vector expression vector. So, these vectors are tailored for protein expression, they are specifically crafted for this purpose, for the expression of a particular gene, we need certain elements.

Later on, we will discuss, how they commonly include regulatory components, like promoters and enhancers, which stimulate the activation of the inserted gene within the host organism facilitating protein production. Now, mostly we will discuss, about the different types of plasmids, and what type of plasmids will be suitable for molecular biology those things. So, the plasmid is a small circular double-stranded DNA molecule found in bacteria, and certain other types of cells like yeast, it exists independently from the chromosomal DNA earlier we have mentioned, and it replicates autonomously within the host cell. So, plasmids can be classified into various types, based on their function, sizes, genetic contents, and how they interact with the host cells. So, based on these things different types of plasmids are there.

So, these are the examples of different types of plasmids, fertility plasmids are there conjugative plasmids are there, degradative plasmids are there, col plasmids, virulence plasmids are there, and resistance plasmids are there. So, what is fertility plasmid? The fertility plasmids, they carry genes responsible for the formation of sex pili, within 2 bacterial cells, the sex pili could be formed if the fertility plasmid is available in any one of them, and it facilitates the transfer of genetic material between the bacterial cells through conjugation. Ok! Those bacteria that will have F plasmids, they will form this type of sex pili and with the help of these plasmids they can transfer the genetic material through conjugation process through bacterial conjugation. Then, resistance plasmids that

are R plasmids, they contain genes that confer resistance to antibiotics or other antimicrobial agents. Some bacteria that possess this type of plasmids, they will show tolerance to those specific antibiotics.

Maybe some of you have heard about the antibiotic resistance, antibiotic resistance generation in human beings, or in different crops. Ok! So, if an individual is to take a particular antibiotic indiscriminately, means no proper dose is maintained suppose anyone is having a fever, he has taken a couple of pills after 1 week or so, again 2 pills have been taken 2 antibiotics have been taken. So, in this way some microbes, which are available in our system in our gut or whatever. So, some of them might be converted into resistant bacteria. So, this type of resistant plasmid might be generated over there, and once means the bad effect is found once, that type of resistant bacteria is generated within the system.

At that time, if a proper antibiotic is given, for 5 days, or 7 days still the bacteria could not be controlled, because at that time the bacterial population having that type of resistant plasmid might be more in the system. So, in this way, the bacterial, resistance is generated in most of the systems. Ok! So, they often carry genes including enzymes that can inactivate antibiotics. So, if our plasmids are available within bacteria they possess some genes that code, for different enzymes that can inactivate the antibiotics, or the efflux pumps means, due to the availability of some genes, this efflux system is available within that bacteria. So, if they can expel the drugs from the cell, if the drugs or antibiotic comes into their system, they can expel it due to the availability of efflux pumps.

Now, coming to virulence plasmids, these plasmids harbor genes that enhance the pathogenicity of bacteria, enabling them to cause diseases. Later on, we will be discussing about, *Agrobacterium* a particular gram-negative bacterium that can cause crown gold disease in plants. So, it has a particular plasmid in the native *Agrobacterium* strains and some virulence genes are available, there, due to the availability of the virulence gene, it can cause the infection in the plant. So, the virulence plasmid they

contain genes including toxins, adhesion factors, or other virulence determinants with the help of that they can cause disease in the target organism. Then coming to col plasmids, col plasmids produce colicins which are bactericins, that kill closely related bacterial strains providing a competitive advantage to the host bacteria.

Suppose, three types of bacteria are available belonging to the same group among them one produces colicins, and one has col plasmid. Then, it will produce that particular compound, and the rest of the bacteria will be killed. So, that its growth will be favored the competitive advantage will be obtained by these particular bacteria. Then we will be discussing about degradative plasmids. These plasmids contain genes including enzymes, that enable bacteria to degrade and utilize specific compounds like hydrocarbons, pesticides, aromatic compounds that are not usually used as nutrients.

So, which are not commonly used as nutrients those things could be utilized properly by these degradative plasmids. Then conjugative plasmids, this plasmid possess the machinery necessary for their transfer between bacterial cells via conjugation. So, basically, F plasmids, are a kind of conjugative plasmid that also then comes to shuttle plasmids or shuttle vectors. So, these plasmids can replicate in multiple host species, making them valuable tools in genetic studies across different organisms. They can be grown in two different organisms.

So, from one organism it can be transferred into another one also. Now we will be discussing about plasmids used in molecular biology. What type of plasmid, we should get or whatever plasmid is required for proper functioning, or for doing proper work in molecular biology. Ok? First, we will be discussing about cloning plasmid. So, what is plasmid or we have discussed is a closed circular double-stranded DNA molecule.

Let us assume this is the closed circular double-stranded DNA molecule. So, for proper

utilization in molecular biology a plasmid must be having three parts. First one we need an origin of replication. So, it is a sequence that should be available in the plasmid if that sequence is there then this plasmid could enter within a bacteria and it can replicate over there. Ok! For suppose somehow we have inserted this plasmid in a bacterial cell.

So, once the bacteria will grow, from one bacterial cell, two bacteria will be produced from two cell, four cells will be produced, the bacteria will be multiplied. Its chromosomal DNA, will be multiplied eventually, but in plasmid until and unless the origin is available it cannot be multiplied, it cannot be grown properly. So, in molecular biology what is our target? In molecular biology, our target is to clone a particular gene, within this plasmid and through this cloning process, we are trying to make more number of copies of that particular gene, so that, that particular gene will be amplified, or more copies will be produced and all the copies should be same, should be a like the sequence. So, for this purpose, we have to clone a particular gene in plasmid and until and unless the plasmid is replicated our target gene will not be amplified within the bacterial system. So, this is the first thing we need.

Next one, for utilization of a particular plasmid in molecular biology we need a selectable marker gene. We need a selectable marker gene. Let us assume a selectable marker gene is there. So, what type of selectable marker genes are used in plasmid mostly? They could be kanamycin resistant gene, they might be ampicillin resistant gene, they might be tetracycline resistant gene. So, these antibiotic resistant genes are used as selectable marker gene.

So, this resistant gene are placed in plasmid, and those genes should be placed under a particular promoter and a terminator also. So, P stands for the promoter and T stands for the terminator. So, what will be the function of this promoter and terminator? Once this plasmid will enter into the bacterial cell then within this promoter the RNA polymerase

will bind and thereafter from this selectable marker gene, the mRNA will be produced. And the mRNA production will be stopped in the terminator part, and from that mRNA finally, the protein will be produced from that selectable marker gene and that protein will help these particular bacteria to survive on an environment where the toxic antibiotic is given. Ok!

So, let me tell it once again. Suppose, we have done a bacterial transformation in a particular vial. Suppose, 3 bacterial cells are available, we have done transformation using this particular plasmid, this plasmid has entered into a particular bacteria while in rest of this bacteria the plasmid has not entered. Now, after transformation this bacteria this bacterial population will be grown on Petri plates. On those Petri plates will use some sort of media, mostly LB or some other media will be used and within that media will use a specific antibiotic, which antibiotic will be using based on the selectable marker gene available on the plasmid. If kanamycin resistant gene is there, we have to use kanamycin antibiotic, kanamycin 50 antibiotic will be added in the media.

So, once we will spread this bacterial culture in the media, then only this bacteria or its progeny can sustain it can form colony. Rest of the bacteria, cannot grow because the antibiotic resistant gene has not been inserted over there. So, in this way we will get the colony from those bacteria, where our desired plasmid has been entered. So, this is the purpose of having the selectable marker gene, based on that we will identify the recombinant bacteria. Those bacteria where our plasmid has been entered. Ok!

Third thing, we need is that multiple cloning site. So, basically these three things must be there in a particular plasmid, which should be used in molecular biology. One is origin of replication, second is selectable marker gene and third one is multiple cloning site. In multiple cloning site what is available? Earlier we have discussed about different restriction enzymes right. In this multiple cloning sites, different restriction enzyme sites

are available. Ok!

So, let us assume over here *Eco*RI sites is available. Thereafter *Bam*HI site is available. Thereafter *Sac*I site is available. Thereafter *Sma*I site is available. Thereafter *Hin*dIII restriction sites are available.

So, basically in the MCS part within a plasmid in the multiple cloning site in this part different restriction enzyme sites are available. Ok! And those restriction enzyme sites are unique in nature. So, throughout this plasmid, those sites will not be available because using this site we can do different types of cloning. Using these sites, we can do cloning maybe, we can clone a particular promoter in between, *Eco*RI and *Bam*HI. We can clone a particular gene of interest in between *Bam*HI and *Sac*I. In this way those multiple cloning sites will be used.

Now let us discuss about the expression plasmid. So, it is a little bit different from the cloning plasmid. In cloning plasmid, we need to have origin of replication, selectable marker gene and multiple cloning sites. In expression plasmid, those things should be there the origin of replication, the selectable marker gene and multiple cloning site. But in addition to that this type of plasmid is mostly used for expressing a particular gene. Ok! So, here some sort of promoters are needed at the end of different multiple cloning sites, basically different promoters are used over there like, SP6 promoter or T7 promoter those type of promoters are used. Some ribosomal binding sites are available. So, that the once the mRNA is formed the protein synthesis could be initiated properly. Ok! So, those are the features of expression plasmid, the promoter should be there the ribosomal binding site should be there those features are available in expression plasmids.

Now will be discussed about *Agrobacterium tumefaciens*. So, it is a natural genetic engineer means, it is a type of gram-negative bacteria it is available in soil and it can infect more than 100 dicot species available on the earth, more than 200 species even it can infect. So, if you see the structure of different old plant, somewhere you can see such type of gall formation. Such type of gall formation is basically occurred by the action of

this particular bacteria *Agrobacterium tumefaciens*. It can cause the gall formation, and the name of that disease is crown gall disease. Ok!

So, what is available in this particular bacteria? Since, a long time different scientists have tried to characterize this particular bacteria, and they found that within this *Agrobacterium tumefaciens* a plasmid is available, a big size plasmid is available its name is Ti plasmid. The Ti name came from the tumor inducing plasmid. So, this type of plasmid can cause tumor in plants or crown gall disease in plants. Ok! So, in this type of plasmid in Ti plasmid, already we know it is a circular double-stranded region. So, it might be having some origin of replication it might be having some selectable marker genes.

So, in this Ti plasmid different virulence genes are available different virulence genes *vir* A, D, E different sets of virulence genes are available over there. In addition, to that in this Ti plasmid, another region is available that is known as T-DNA. T-DNA stands for transfer DNA. So, what does this bacteria do in a plant? So, once this *Agrobacterium tumefaciens*, which is available in the nature if they infect a dicot plant, they basically transfer this T-DNA region from this plasmid into the plant genome. So, it transfers this T-DNA region from bacteria into the plant genome.

So, how do they transfer? We will not go into the detail, but at the end of this T-DNA two regions are there, one is known as left border, one is known as right border region. These two regions are very crucial, if these two regions are available then it can transfer this whole region into the plant genome. So, after initial analysis the scientist found that in this T-DNA region, different genes are available means auxin producing genes, cytokinin producing genes, some opine producing genes are available over there. So, we know that auxin and cytokinin are different phytohormones. So, those are responsible for cell division for cell elongation those things.

So, once this thing is transferred into the plant genome then within the plant system the cell division becomes enormously fast, in certain part the cell becomes enlarged the cell

elongation is also taken place. And with the help of this opines, basically some sorts of sugars are metabolized and those metabolized sugars are eventually utilized by this *Agrobacterium*. So, if you think about these auxins and cytokinin genes, mostly those genes can cause the tumor formation or gall formation within different dicot species. Once the native *Agrobacterium tumefaciens* infect the dicot plants. So, in this way since long time the *Agrobacterium tumefaciens* have transferred this T-DNA into the dicot plant genome, and they have generated numerous transgenic plants naturally.

A few years before maybe during 2012 or 2014 a publication came in a good journal maybe in science or nature, that they reported that several sweet potato strains were characterized from different parts of the world. And, within those sweet potato genomes they identified that some parts have been transferred from this *Agrobacterium*, means some T-DNA part have been transferred. So, since the last 100 or 200 years this *Agrobacterium tumefaciens*, has engineered different plants naturally. Now, how can we utilize it for our purpose we will be discussing later on. Thank you.