Course Name: Basics of Crop Breeding and Plant Biotechnology

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Lecture-45: Gene Transfer Methods

Hello everybody. Welcome to NPTEL online course on Basics of Crop Breeding and Plant Biotechnology once again. So, today we will start discussing that is on plant genetic transformation, discussing gene transfer methods. Overall, we will discuss about the gene transfer methods in plants, thereafter we will discuss some of the examples of plant transformation. These are the concepts which will be covered under this topic. First of all, we will be discussing the methods of gene transfer in plants, then *Agrobacterium* mediated gene transfer will be discussed in detail, then the Ti plasmid and T-DNA part which is involved in *Agrobacterium* mediated gene transfer method that will be discussed.

Then we will give some examples of transformation process in tobacco and transformation process in rice. How these two plants are transformed, we will be discussing. So, let us start the methods of gene transfer in plants. Different methods are there, these methods comes under direct gene transfer method.

No *Agrobacterium* or other means are used over there, directly naked plasmid or digested plasmid could be used in this type of direct gene transfer method. This has also two categories, first one is physical method. In physical method, different options are there, means through electroporation we can transfer a particular gene within the plant system. Basically, through electroporation within the suspension cell culture, we can transfer our gene which will be available in a plasmid. Ok! In most of the cases different tobacco suspension cell cultures are available.

So, using those cell suspension cultures using electroporation method by providing electric pulse we can transfer our plasmid into those genes. By those type of electroporation method, we can evaluate the GUS expression, GFP expression or luciferase expression in the transient through the transient method in the cell lines. Then coming to another method that is particle bombardment. Through particle bombardment method, a gene gun or a ballistic gun is used. Using that particular gun, we can bombard the DNA in the different tissues.

So, over here, the callus could be utilized, over here, the leaf part could be utilized, embryonic callus could be utilized. So, different plant tissues could be utilized in particle bombardment method. Initially, this particle bombardment method was one of the important method of gene transfer in plants. Later on, *Agrobacterium* mediated gene transfer became more popular. So, next one is micro-injection, here using a minute needle we can insert our target gene within the desired cell.

Then the liposome mediated gene delivery is another physical methods and silicon carbide, the small nano-particle like structures, it could be utilized also to transfer, to deliver the target gene, naked target gene into a particular cell. Now in the chemical methods, two methods are mostly used. One is PEG mediated, i.e., polyethylene glycol mediated gene transfer method. Another one is DEAE dextran mediated methods. So, our major discussion will be starting from here *Agrobacterium* mediated gene transfer.

So, basically two types of *Agrobacterium* strains are used for *Agrobacterium* mediated gene transfer. One is *Agrobacterium tumefaciens* that is harbouring Ti-plasmid. Ti stands for tumor inducing plasmid and using *Agrobacterium tumefaciens* strain, we can do the gene transfer. Another *Agrobacterium* strain which contains Ri-plasmid that is root inducing plasmid. So, using those particular *Agrobacterium* strain also we can transfer our gene within the particular plant tissues.

So, most of our discussion will be associated with the Ti-plasmid mediated gene transfer

methods. That is, we will be discussing the *Agrobacterium tumefaciens* and its mode of action little bit in detail. So, vector mediated gene transfer is either carried out by *Agrobacterium* mediated transformation, or by use of plant viral vectors. Different plant viral vectors are also used, but this part will not be covered over here. The virus mediated gene transfer method will be a part of will be important part of virus induced gene silencing.

It might be discussed in some other classes. Ok! So, here, we will be focusing on *Agrobacterium* mediated gene transformation method. In *Agrobacterium* mediated gene transfer, *Agrobacterium tumefaciens*, these particular bacteria which is available in the soil and it is a gram-negative bacterium. Ok! It belongs to the bacteria family Rhizobiaceae. Two strains are used, I have mentioned earlier one is *Agrobacterium tumefaciens* another one is *Agrobacterium rhizogenes*.

The tumefaciens cause crown gall disease, a tumor like structure occurs within the plant system by the involvement of this particular bacterial infection. While *Agrobacterium rhizogenes*, it can induce hairy root formation. Let us discuss the history of *Agrobacterium*. So, in the last 100 years, the study on *Agrobacterium* has remarkably revolutionized the plant molecular genetics or the genetic engineering of plants. Initially, I was telling that once the plant transformation process initiated, at the time, the biolistic gene gun was very famous, but some problems are associated in biolistic gene gun.

Through biolistic gene gun, first of all, although we have to use a rupture disc. So, that not too much pressure, not too much force will be applied on the tissue on the tender tissue, but still most of the tissue become damaged. Ok! Although the gene delivery could be observed over there, but once the tissue is damaged, then we cannot make a successful transgenic plant from there, that type of problem was faced by different scientist. So, thereafter, gradually they shifted their research to *Agrobacterium* mediated gene transformation process. So, in 1907, Smith and Townsend, they first identified this particular bacterium, at the time they did not identify this bacterium, they basically identified this particular disease-causing bacterium for crown gall in ornamental plants

and fruit trees.

So, crown gall formation, the crown gall formation was taken place in ornamental plants and fruit trees, means, in some of the trees such type of gall-like structures are formed. So, due to the effect of the *Agrobacterium tumefaciens* infection, ok, this type of gall-like structures are observed as tumorous growth-like structures are observed. And Smith and Townsend first time in 1907, they reported that some bacteria is causing this particular disease. After 30 years, Armin Brown, he studied this unusual plant disease also and later on, gradually, they identified the particular bacteria associated with this crown gall formation and they came to know that in this bacteria an unusually large size plasmid is available that is responsible for this crown gall formation, a large size plasmid. So, what is plasmid, we have discussed already those are extra chromosomal DNA ok, those are circular double-stranded DNA structure, other than chromosome, it is available in different bacterial system.

So, the part of the plasmid from this large plasmid, this large plasmid which is available in *Agrobacterium tumefaciens*, the part of this plasmid is transferred and integrated randomly in the plant tissue. So, few things are important, part of the plasmid is transferred, not the full plasmid is transferred ok, its part is transferred. Next, it is integrated randomly in the plant tissue means it has been found that it is integrated within the plant genome, not plant tissue within the plant genome basically it is integrated through illegitimate recombination process. Now let us know little bit more about the crown gall disease and Ti plasmids gradually. So, *Agrobacterium tumefaciens* it infects the damaged and wounded plant tissues and induced the formation of plant tumor.

So, earlier I have told that, this bacteria is available in the soil it is a gram-negative bacteria, it is available in the soil. So, in the soil, once a plant root used to grow due to some infection some damages may be occurred, some cut surface may be occurred. Ok! So, from that damage or wounded part, basically this *Agrobacterium* enters into the plant system, and thereafter different steps are there, finally, they can induce the formation of plant tumor. Then the release of phenolic compounds like acetosyringone, a very

important compound that is responsible for *Agrobacterium* mediated infection and hydroxy-acetosyringone. So, these phenolic compounds are produced at the site of damaged plant tissue.

Once a plant tissue is damaged, once a root tissue is damaged, this type of acetosyringone and hydroxy-acetosyringone, these compounds are produced in the damaged part. And basically, it works as a signaling compound once these things are secreted, then *Agrobacterium* strains come closer to the infected region or the damaged part of the root ok, and it helps in entry of the bacteria in the plant tissue. Then, the bacteria release its tumor inducing plasmid, the tumor inducing plasmid, its name abbreviated as Ti-plasmid. So, it releases it in the plant cytoplasm which cause crown gall formation and eventually, it is integrated within the chromosomal part within the genomic region. The segment of Ti-plasmid, is transferred to back is transferred from bacteria to the plant is called T-DNA.

It means the full Ti plasmid is not transferred, the part which is transferred that is known as T-DNA, transfer DNA Ok! So, basically, this T-DNA part is transferred which integrates in the plant genome. The T-DNA carries gene encoding sequences which codes protein involved in biosynthesis of growth hormones, different growth promoting hormones are there, later on, I will tell it once again along with some plant metabolites such as amino acid derivatives, opines and sugar derivatives that is agropines. So, this is the structure of a Ti-plasmid the basic structure Ok! Here, two things are available, one is LB i.e., left border region another one is right border region.

Then, in between this left border and right border region, you can see few genes are there. The auxin, a phytohormone producing gene, cytokinin, another phytohormone producing gene is available over there. Then opine is another gene that is involved in bacterial growth and development, these things are available within this part. In addition to that, in this Ti plasmid, some virulence region is available '*Ori*' i.e., origin of replication region is available and opine catabolism genes are there also. So, let us see what are their functions?

The T-DNA region flanked by left and right border means, left border and right border, this region is known as T-DNA region. The T-DNA region flanked by left border and right border along with the genes for biosynthesis of auxin, cytokinin and opine that is basically transferred from this Ti-plasmid into the plant cell from this *Agrobacterium* into the plant cell. Now, what is the function of this virulence region? The virulence region is also known as *vir*. Ok! Several genes are there several *vir* genes are available over there, that is responsible for transfer of T-DNA and is located outside the T-DNA region. So, within the T-DNA region, it is not available, but it assists in the delivery of this T-DNA from this bacterial system into the plant system.

So, some genes are there some *vir* genes are there, those are involved in phosphorylation and dephosphorylation process. Some genes are there, they make this T-DNA into single stranded structure, some genes are there, they coat for some proteins that bind with this single stranded T-DNA and they helps in transfer of this T-DNA into the plant cell. So, in this way different *vir* genes are available and they basically help this transfer mechanism. Now coming to the opine catabolism region. So, opine catabolism region is responsible for coding the protein for the metabolism and uptake of opine. Ok!

Means catabolism, means the opines will be degraded, the metabolism will be done and it will be uptaken by the bacteria. So, you know why the ori is available for replication of this particular plasmid within the bacterial system within the bacterial cell. So, this is the basic structure of a Ti-plasmid which is available in *Agrobacterium tumefaciens*. So, once scientist identified the whole thing, then they try to modify this Ti-plasmid for genetic engineering. Because by this Ti plasmid, with the help of this T-DNA, *Agrobacterium tumefaciens* can naturally do genetic engineering in plants.

They can cause crown gall formation, means, they can deliver this transfer DNA. A foreign DNA could be transferred from bacterial system into plant system. So, using this concept, the scientists have modified this Ti-plasmid. How did they modify? They basically removed this gene from this Ti plasmid. So, first of all, the size of Ti plasmid is

close to 200 kb, it is a huge size.

The whole thing is not transferred, only region available between left border and right border is transferred. So, in between this part, if we can put our target gene, then easily we can deliver it into the plant system. So, scientist initially identified these two regions which are responsible for transfer mechanism or transferring any gene which would be available between these two. Then scientist worked on this virulence gene. So, using these two things scientist have developed binary vectors, Ok!

They have developed binary vectors. In binary vectors, binary means two vectors are there. In one vector they used different *vir* genes. While in another vector they used this left border and right border region and in between that, they have put their desirable target genes. As once these two things were delivered into *Agrobacterium* strain, then they found that with the help of the enzymes produced by this *vir* genes from this particular plasmid, finally, the T-DNA available in another plasmid could be delivered into the target plants. So, in this way, the scientific communities have modified the basic Ti plasmid as per their required, as per their requirement.

Now, let us discuss the T-DNA transfer and integration process once again. First of all, the signal transduction to *Agrobacterium* species. How the signal transduction is initiated? Earlier we have mentioned that once a root tissue is damaged or wounded, therefrom some compounds are released, acetosyringone related compounds are released and once it is released, then that signal help *Agrobacterium* species to come over there. Then attachment of *Agrobacterium* species to the plant cells, at that cell, the *Agrobacterium* binds over there, different bacterial molecules come close to that particular cut tissue. Then production of virulence protein, different *vir* gene become active and virulence proteins are produced.

Thereafter, production of T-DNA strand, mean single stranded T-DNA is transferred then, the transfer of T-DNA out of the *Agrobacterium* species and transfer of the T-DNA in plant cell and integration. So, in this way, basically T-DNA transfer and integration

process is taken place. I am not going into too much detail, because, in this topic, mostly we will be discussing about the plant transformation methods. Now, these are different *Agrobacterium* strains which are mostly used for plant transformation. The GV strain means GV3101 and GV3850, those strains are very popular, they can be used for transformation of tobacco and other different crops *Arabidopsis* and other different crops.

In case of rice mostly EHA105 and EHA101 these two strains are mostly used for rice transformation, while for *Brassica* transformation, for tobacco transformation LBA4404 strains are mostly used.