Plant Developmental Biology
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Lecture - 04
Molecular Genetics of Plant Development - I

Welcome to today's class of Plant Developmental Biology, where we are going to learn how to study plant developmental biology.

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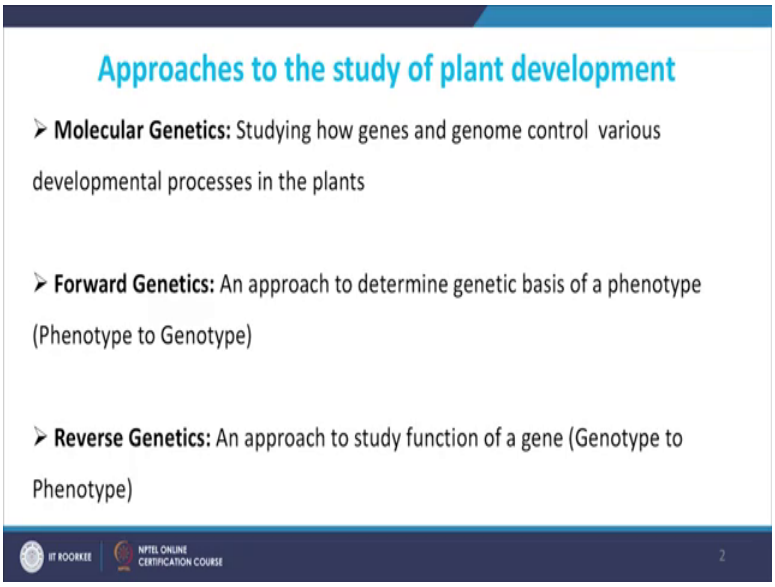

A prominent approach which we take to study plant developmental biology is Molecular Genetics based approach. Molecular genetics is, to study how a particular gene in a genome regulates a particular developmental process in the plants. So, there are two major approaches in the molecular genetics, which we usually take to study plant developmental biology: forward genetics based approach and the reverse genetics based approach.

So, in forward genetics we begin with a mutant or with the phenotype, and then try to identify what is a gene associated with that phenotype. Whereas, in case of reverse genetics first we define our gene of interest, and then we try to understand or we design experiment to pinpoint what is a specific developmental function of that gene.

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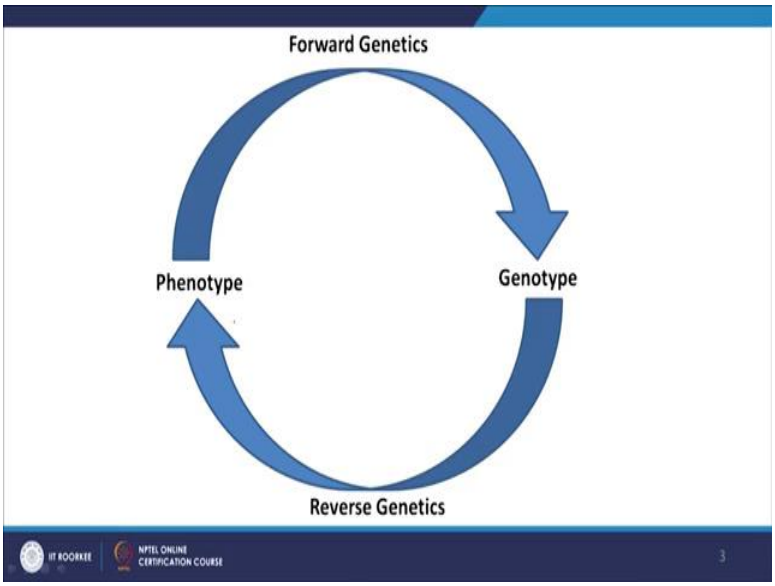

So, you can look at the schematic diagram. In forward genetics, we start with the phenotype and then we map the associated gene or the genetic loci which is associated with this phenotype. Whereas in case of reverse genetics, we have gene, we identify the gene first and then we try to understand, does this gene has any role to play in the plant development, and if it is so, what is its specific function in the plant growth and development?

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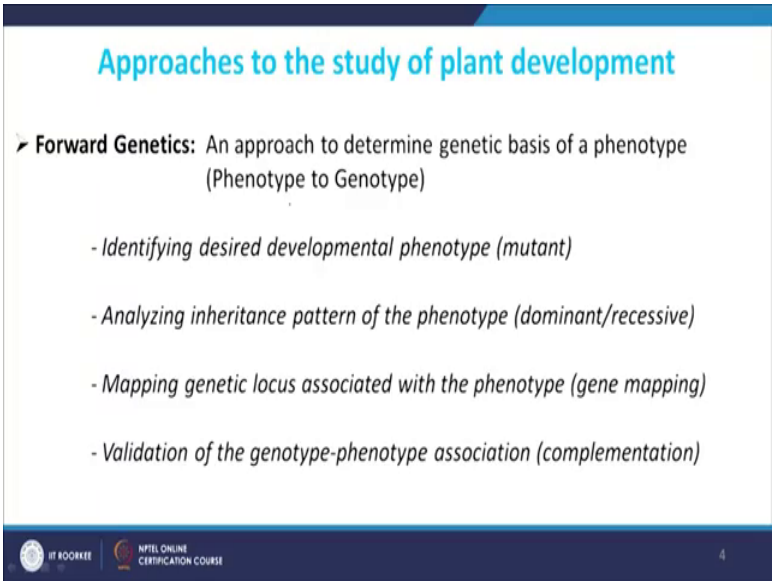

First we will discuss about the forward genetics based approaches. First step of the forward genetics is that you should have a mutant or any variant. Mutant means a particular defect in the development. So, defect is important because if you do not have abnormality, it is difficult to trace what is the normality or what is the mechanism which is regulating the normal development.

So, typically what are different steps which we follow during forward genetics based approach? The first approach is identifying a desired developmental phenotype or a mutant with the phenotype. Then once we identify this mutant, we are going to analyse genetics or inheritance pattern of this mutant whether it is dominant, recessive, semi dominant.

And then once we know the pattern of the mutant or the inheritance pattern of the mutant, then we go and we try to map the genetic loci. So, we try to understand what is the genetic loci which is actually responsible for this developmental defects? And then final step in forward genetics is, we then validate the genotype and phenotype association.

So, how do proceed? The first step normally we take a mutant or any variants. Natural variants are also available to study, and then we finally, do the complementation to validate. So, the first important thing in any study of plant developmental biology is to fix your biological question.

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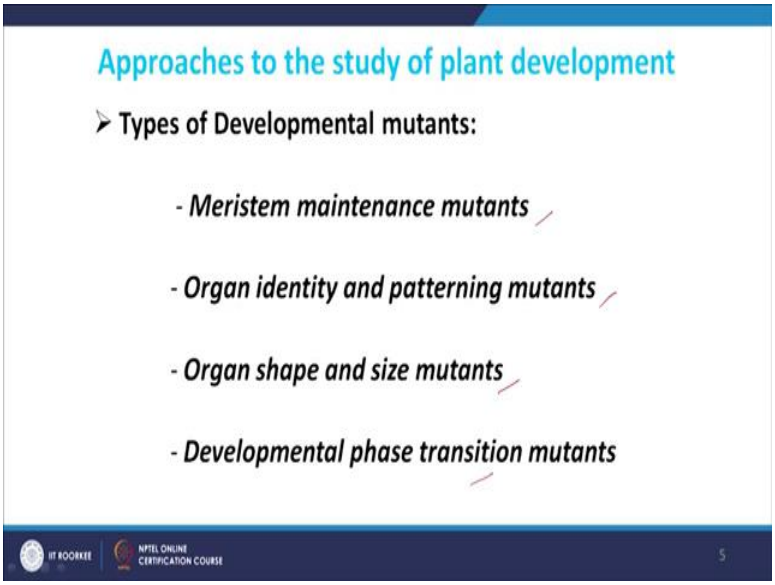

So, if you recall our previous classes, we have discussed about different developmental events which takes place during the process of plant growth and development. So, depending on that you can first choose your questions. So, you can define that which aspects of development you want to study.

For example, are you interested in studying what are the genetic loci which is responsible for maintaining the meristem, organ identity or patterning. So, you want to understand that what are the genes or what are the, genetic element which is responsible to define a particular identity of an organ or a tissue, or you can identify what is the genes or what are the genetic elements which is responsible for patterning, this is important in the process of a plant architecture maintenance.

Then you can identify mutants which shows abnormality in shape or size of a particular organ. You can study what are the developmental mutants which affects phase transition. During growth and development, a plant passes through different phases starting from the embryogenesis phase. Then in the post embryogenesis first it undergo the process of juvenile development, then there is a transition from juvenile to the adult vegetative development. And then from adult vegetative development, there is another major transition from vegetative to reproductive development. So, these phases are regulated in time. So, what we can do? We can identify a variants or a mutants where this stage or this transition are defective.

Another important thing if you recall previous class, we have talked about the activation of branching or activation of secondary growth. So, both this path processes are regulated and it occurs only at a certain time point. So, it is also important that they are also regulated by genetic element. So, we can also identify or screen a mutant where these processes are defective. So, now we will go and look what are the different ways to identify the mutant.

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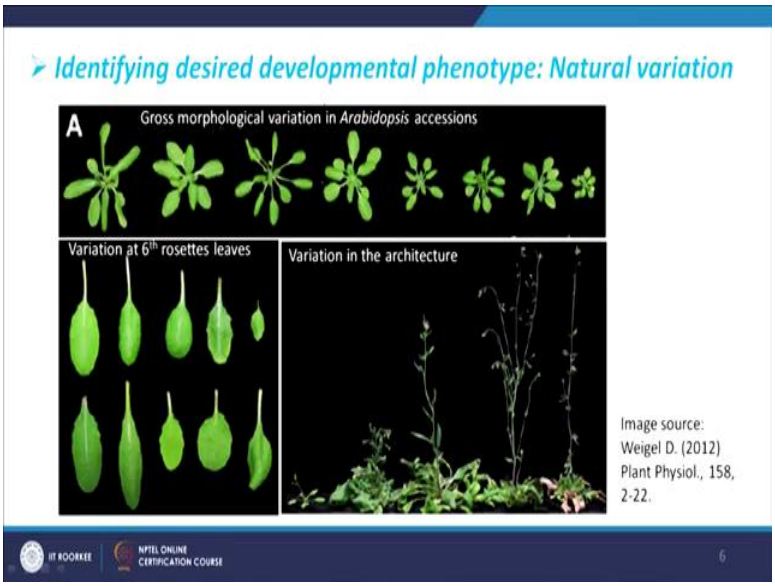

So, the first step of forward genetics we are taking here to identify a desired developmental phenotype or desired mutant. The first and maybe more important with respect to the breeding approaches was to look the natural variation. In this nature, there are lot of variations in the developmental stages, organs, structure and function. So, if you look for example, this upper panel, here are different accession of *Arabidopsis thaliana*. And there are morphological variations. If you look their leaves, the number of leaves, shape of leaves, size of leaves, there are huge amount of variation even within the same species.

We can choose any one of these two contrasting accession with the contrasting or with the different trait, and then you can try you can try to identify the associated loci. If you look here, variations at the 6th rosettes leaves, it is across different accession. You can see that even not only the overall morphology, but shape and size of a specific organ also shows a large amount of variation across these species or across the accessions.

Here is the variation in the architecture. So, you can look these all plants are compared at the time of after transition floral transition has occurred. And you can see that across these different variants or different accessions, there is a large amount of variation in their overall or final plant architecture. So, this could be one source where you can identify a particular trait of interest, and you can you want to study the associated gene.

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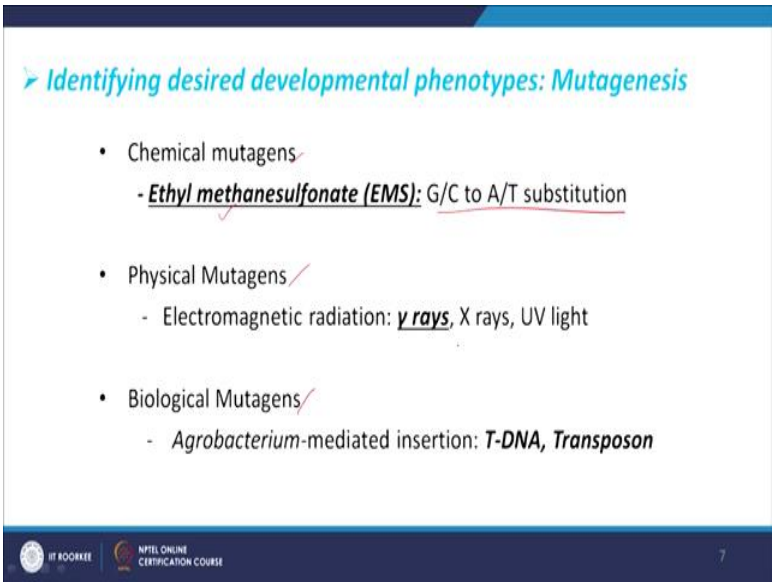

Second way of moving is you can induce the mutations. So, you can basically create a mutant plants where you can look for a special phenotype. There are many way to do that, take few example of very widely used way to study or to generate the development of mutants. There are three major category: chemical mutagens, physical mutagens and biological mutagens. In chemical mutagens the most frequently used chemical is EMS; EMS generates a point mutation. It creates a change of the nucleotide in a way that GC base pair getting converted into AT base pair. This is a kind of point mutation point mutation has a lot of benefit.

Second mutagen is physical mutagens where you can use different electromagnetic radiations gamma rays, X rays, UV light and they can induce random mutations in the genome. And we will take an example of a gamma rays radiation. Third way of generating mutants are biological mutagens, where we are using a bacteria which is called *Agrobacterium*. Using this *Agrobacterium* mediated transformation system, where we can use either T-DNA or transposons based system, and we want to create a kind of gene knock out by insertional inactivation.

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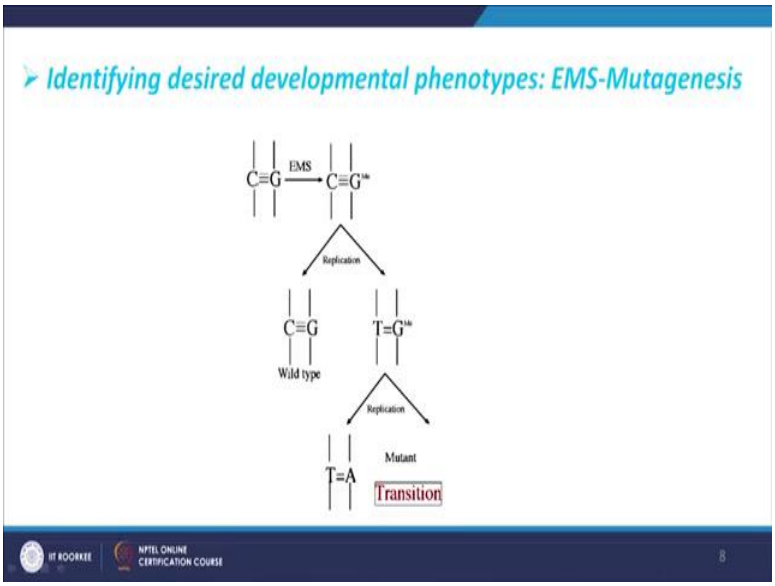

How EMS works? let us assume here is a GC base pairing. And if you treat with the EMS, what it does; It changes or modify this nucleotides the G and adds to it methylene. And once this modification has occurred, in the next round of replication this methylated G cannot pair with the C, instead it can pair with the T. So, now it has added T in place of C in one strand and once this DNA will undergo the second round of replication at the place of T, naturally A will be added. So, if we look from here to here there is a transition of CG base pair to TA base pair. This is creating a point mutation.

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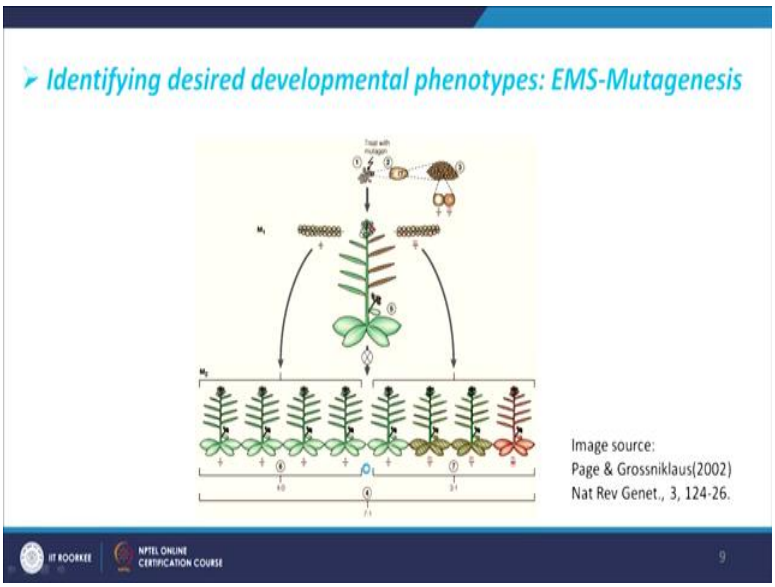

And this is one of the very powerful chemical mutagen which we are using or people who are working in the field of developmental biology is extensively using to generate point mutations. What you do with this one? So, let's take typical example of *Arabidopsis thaliana*. So, you take a large number of seeds, and expose the seeds with the chemical. And what you are expecting? You are expecting a random mutations in some of the seeds, anywhere in the genome.

And then you generate a population where you will have some non-mutant, some mutant. And whenever this mutation will occur in the germ line and you take this plants grow them, there is a possibility, if your plant has come from the non mutated seeds, it will create a normal pattern, but there is a possibility that you screen a plant which is coming where the genome has been already mutated. So, you have generated some kind of point mutations and as I said that this way of generating mutation is totally random. So, you have to screen a very large population of mutant to find your desire mutant.

Then you screen this population and look for your phenotype, fix your phenotype. So, first thing let us assume that if you want to study the root development, then you look a mutant where root is defective. If you want to study the shoot development, find or screen for mutant, where shoot is defective, flower is defective, whatever your interest is, you can fix it and then try to identify a mutant phenotype. And we know from the genetics this mutant can be either dominant, recessive, it can be either in homozygous condition or heterozygous condition; you can analyze, and you can understand that what is the pattern.

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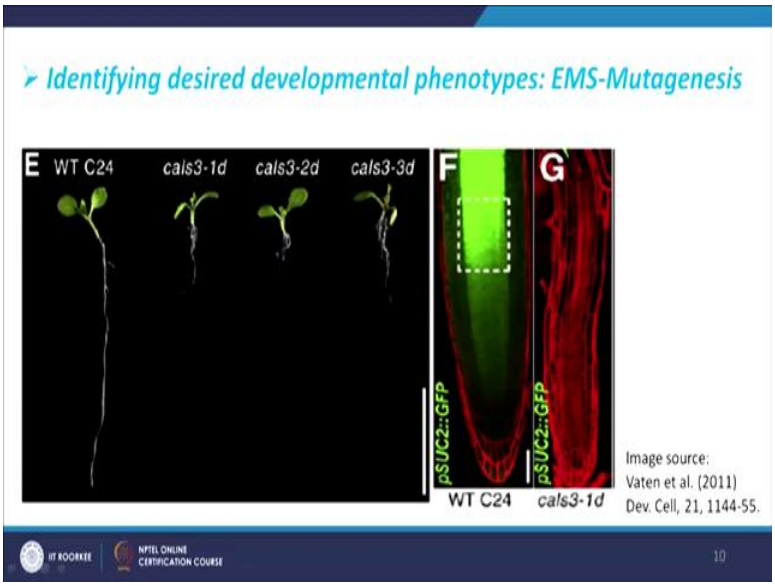

So, I will take some examples. So, if you look here, this is *Arabidopsis* root, this is a typical or very early stage *Arabidopsis* seedling. When this seedling was EMS mutagenesis and was looked for some kind of defects in the root, it was observeed that if the shoot developments look quite normal. But there were three independent mutants, which was identified from this population where root development was affected.

So, you can see they have very short root.. You can even make a very sophisticated genetic screen. For example, if you look these two pictures, so here we want to identify the tissue patterning in a very special tissue which is called phloem. If you look this signal, this is a protein; green fluorescent protein. And if you express or if you produce the green fluorescent protein in the phloem cells, it can move through the phloem cells, and it get distributed in the root tip.

Now, if someone want to identify what are the genetic locus or what are the gene which is responsible for giving a proper identity to the phloem cell or regulating a proper differentiation to the phloem cell, you can mutate this plants and look the plant where this property of phloem is affected. So, if you look the mutant, here you can see that we have the green fluorescent signal somewhere here, but somehow the fluorescent is not moving, the GFP is not moving.

So, this tells that in this mutant some aspect of either phloem developments are affected or phloem function which is transporting the molecules are affected. So, you can fix your

biological question here, and then you can go and trace back what is the genetic loci associated with this one ok.

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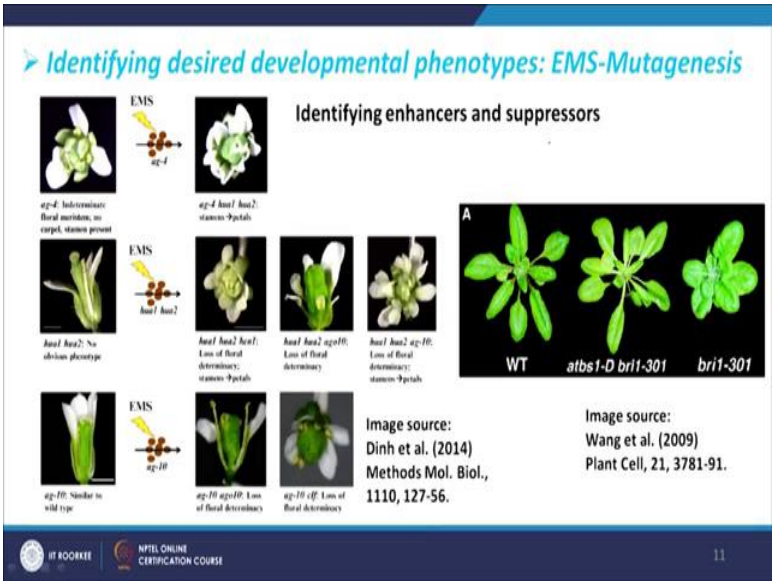

Similarly, this mutant approach you can take, let us assume that you already have a mutant, you know function of a particular gene through the forward genetics. You can further try to identify other genetic component of that entire pathway by mutagenizing your mutants and screening for second site mutations; identifying enhancer, identifying suppressor.

Take this examples here. This is a gene which is called *AG; AGAMOUS* gene. In single mutant, you can see some phenotype, a phenotype is indeterminate floral meristem no carpel, but stamen is fine. When this mutant was mutagenize and looked for another second site mutation, so there was another mutant which was identified *hua 1*, *hua 2*; and these mutants along with the *agamous 4* mutants, double mutants. If you look all other the mutant phenotype is still there, but even stamens are getting converted into petal. So, second site mutation can enhance the first mutation.

Similar case, here *hua 1*, *hua 2*, alone they do not have any phenotypes. So, if you take a single mutant, there is no obvious phenotype, but when you screen for another higher order mutants, so *hua 1* and 2 with *hen 1*, there is a loss of floral determinacy there is homeotic conversion of stamen into petal. Similarly, *hua 1*, and *hua 2* with *ago 10* gene

there is loss of floral determinacy, with *agamous*, there is loss of floral determinacy and conversion.

This provides a very powerful way to perform a very special or targeted genetic screen and identify double mutant, triple mutant, and then try to understand the entire genetic pathway. You can trace out the genetic pathway what all genes are responsible to regulate a particular process of development.

Another important thing is screening a suppressor. So, let us assume that I have identified a mutant where leaf is defective. If you look here this is wild type there are so many leaves and shape and is different. But when you have a mutant where number of leaves and the shape and size of leaves are altered. Now, I have identified one gene or one genetic component which is responsible for this patterning, I want to identify what are the other gene or what are the other genetic components which are interacting with this gene to provide this particular shape and size.

You can take this mutant and now you can look for double mutant where even the previous mutants are getting suppressed. This is called suppressor mutants. In these cases, you are adding them and you are identifying more severe mutant phenotype. Here we are looking for a mutant where even the existing phenotype is disappearing or getting supressed. So, this technique you can use to identify both kind of genes.

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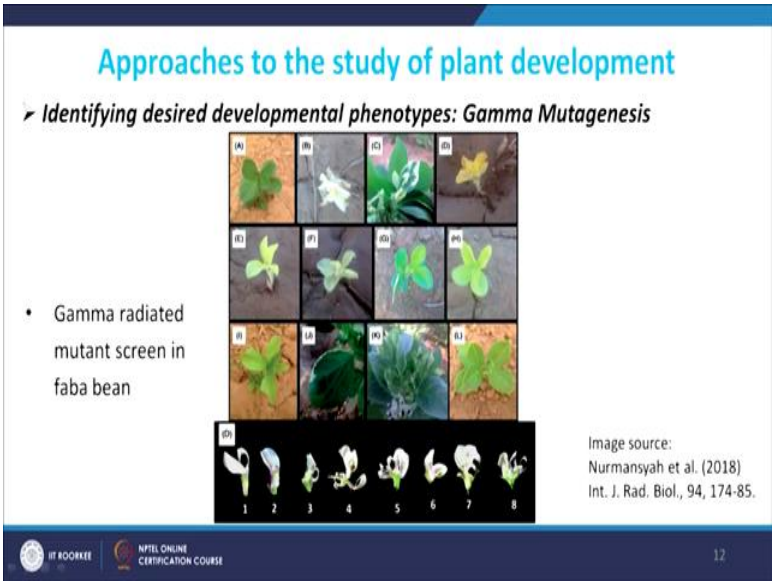

I will not go in detail, but you can look this picture here gamma mutagenesis, physical radiations being used to mutagenize the plants. And what you can see that there are lot different kinds of mutants with different phenotype, you can see chlorophyll phenotype, leaf phenotype, shoot phenotype, flower phenotype, all this kind of phenotype or mutants can be generated with this mutagenesis.

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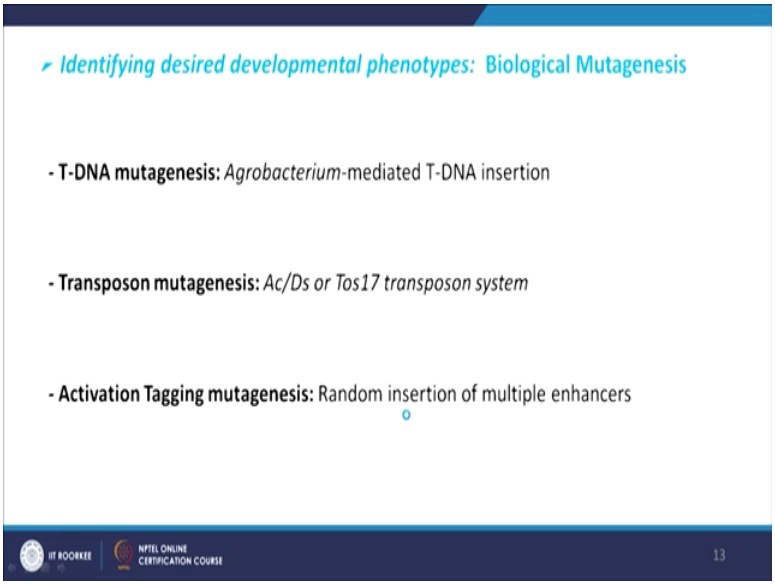

And third another important way to generate a desired developmental mutants is biological mutagens. Here there are three major approaches which is being taken place. First one is T-DNA mutagenesis which is done by *Agrobacterium* mediated, T-DNA insertion. Second is transposon based mutagenesis and third is activation tagging based mutagenesis. Transposons are the mobile genetic elements which is naturally present in the organism, and they can actually jump in the genome that is why they are all also called jumping genes. So, we can use them as a technique to basically disrupt a particular gene and identify the mutant phenotype.

Basically this process is called insertional inactivation or insertional mutagenesis. So, what happens? If you take T-DNA or transposon. You are allowing this molecule to randomly get inserted in the genome. And if it happens that it got inserted in the gene or the regulatory elements of the gene, it can affect the expression or function of the gene and that results in some mutant phenotype.

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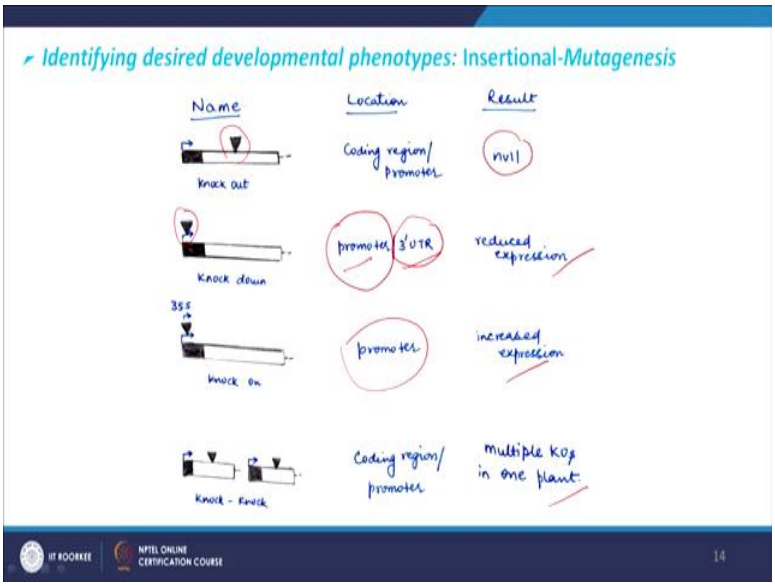

So, these are some possibility. Let us assume that if this got inserted in the coding region, it may disrupt the coding region and you might get a loss of function phenotype. So, where the gene is disrupted, no functional proteins are made and that is why you will have a phenotype because of the loss of the genes.

Second possibility is that if the insertion somehow happens in the promoter region or untranslated region. So, promoter regions you know this is very important to regulate expression and expression pattern of a gene. UTR regions - Untranslated Region, they directly are not involved in coding the protein, but lot of regulations occurs through the untranslated region. So, if there is insertion or disruption because of the T-DNA or transposons in this regions, what we will expect? We can expect either reduced expression or altered expression pattern. And in both the case, we might have the phenotype.

Another way is that the insertion happens in a gene or in the genetic elements and it can also enhance the expression. This is more relevant when we talk about the activation tagging, but it has been also seen that if insertion happen somewhere in the promoter or it happens in the element which is like silencer or repressor of a gene. And if this insertion suppress or kills the regulators, then you might have increased expression.

But again as I said that this processes are totally random. So, there is also possibility that there is a multiple insertion in the same genome at the multiple site and this may generate a multiple knockout or knock down whatever is the condition ok.

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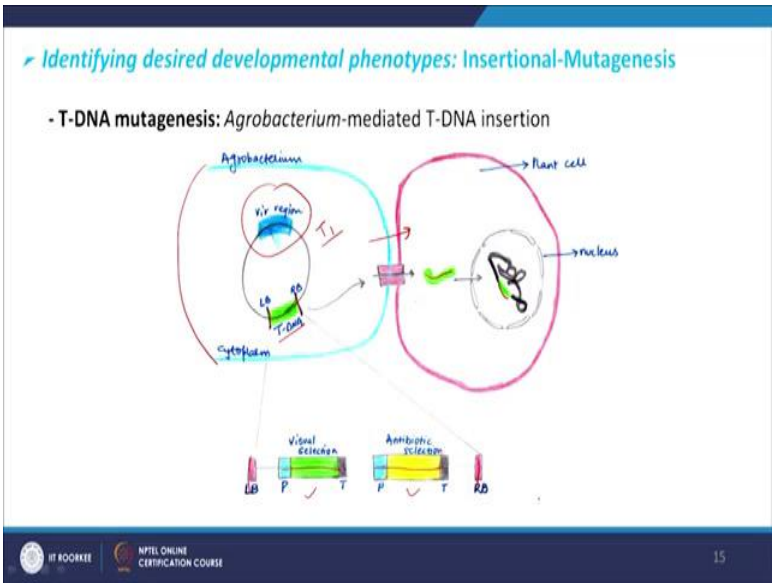

So, we will briefly discuss how T-DNA mutagenesis occur. So, *Agrobacterium* is a bacteria which basically is known to infect the plant cells. And it has a plasmid which is called Ti plasmid. Plasmid is extra chromosomal genetic material. When the bacteria infect a plant cell, a section a portion of this plasmid Ti plasmid which is called T-DNA is transferred in the plant cell. And then this T-DNA get integrated in the plant genome, but that integration is totally random. So, it can go and integrate anywhere.

If you want to use this technology, and if you want to create and there are certain elements which is may be more relevant in the making transgenic, we will discuss in future. And what happens here that if you can genetically engineer this T-DNA, you can basically use this technology to generate mutants. So, what you do, in this left border and right border you can put either a visible marker, this will help you to identify the plants where insertion has occurred or you can put a kind of selection marker. Selection marker, you can put an antibiotic resistance gene.

So, you can basically directly select the transformant using this antibiotic. And then use this and generate a large number of a transgenic plants. And then screen them either based on the visual selection marker or by selecting on the antibiotic. And this has been

very extensively used in model plant *Arabidopsis thaliana*. And in fact, of full fledged collections of this T-DNA insertion plants or mutants has been generate, and it has been stored or maintained as a firm of a big program where anyone across the globe can order these lines, they can use for then their own specific study.

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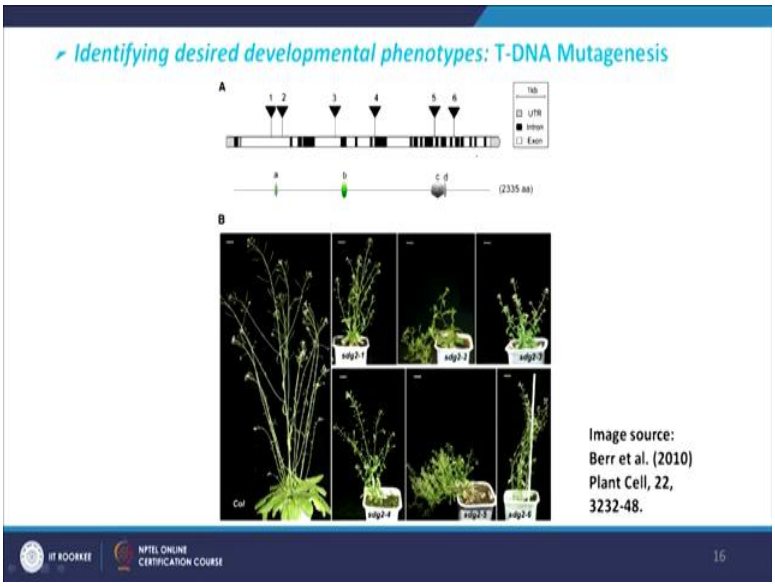

If you take one example this is one gene, and what it has been found that through the process of T-DNA insertion, multiple site insertion has been generated. So, in some mutants it was inserted here; here; here; here.

So, you can say that for this gene at least 6 allele has been generated through the T-DNA insertion and all the 6 mutants has phenotype. If you look this is a normal wild type plant, and the mutants this is 1, 2, 3, 4, 5, 6, depending on their site of insertion, there is a variations in the phenotype. So, all these mutants they can be simply generated by integrating the T-DNA.

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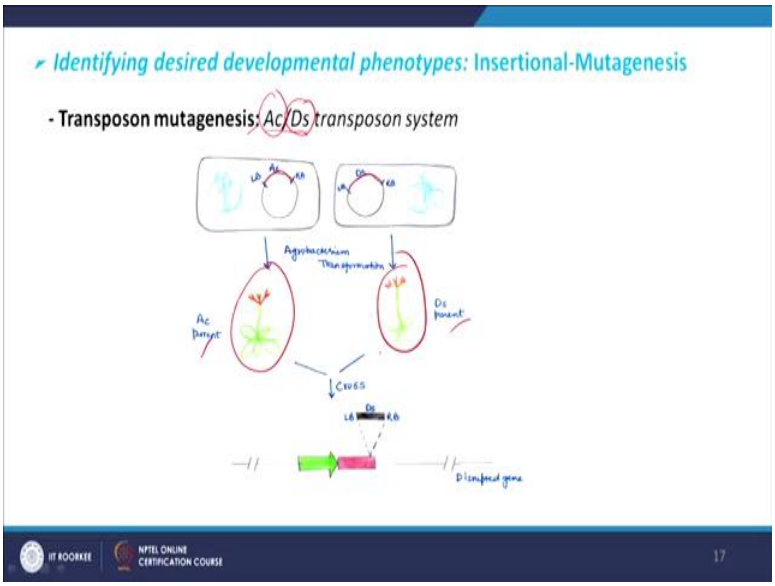

Second way of generating mutation is transposon based mutagenesis. One very commonly used transposon is Ac Ds based. Ds is the element of the transposon which actually jumps, which get excised from one place and get integrated at another place. And Ac is the part which helps in the process. So, what you can do you can generate a Ti plasmid you can engineer Ti plasmid and you can put Ac here in one Ti plasmid in *Agrobacterium*; you can put Ds in another Ti plasmid in the *Agrobacterium*. And what you do you can generate independent transgenic lines one for Ac elements one for Ds element.

The important thing is that Ds element cannot jump until and unless Ac element is present, but the good thing is that Ac element can work in trans, so they need not to be together. Even though if they are present in the same plant at different genetic loci, it is fine, they can still work.

And then what you do you take this both plant; and if you cross them you are basically bringing about Ac element as well as Ds element in the same transgenic plant, and once this Ac is available this Ds will start transposition. And during that this transposition it can go and it can inactivate or it get inserted or integrated at different genetic loci and which might create a developmental mutant.

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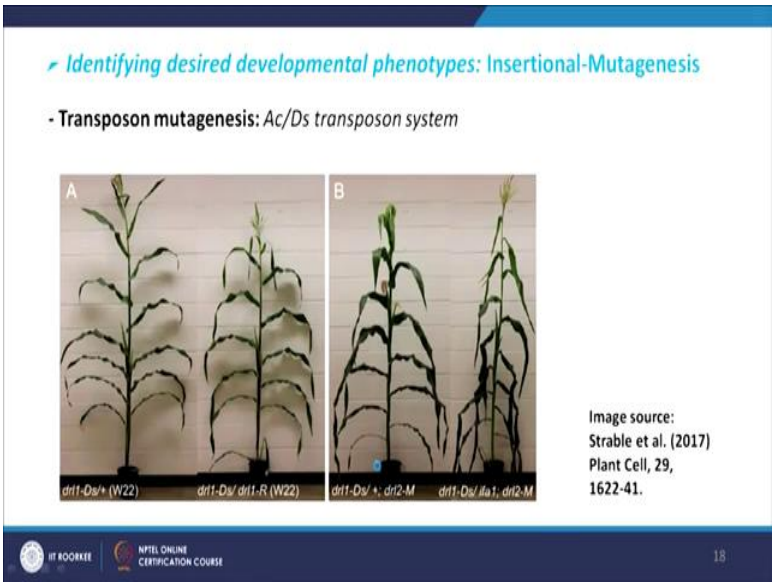

See the example here. This is maize plants, some mutants had been generated. If you look the wild type plant, these mutants, the leaves are not looking normal. This is more kind of drooping leaf they are falling off.

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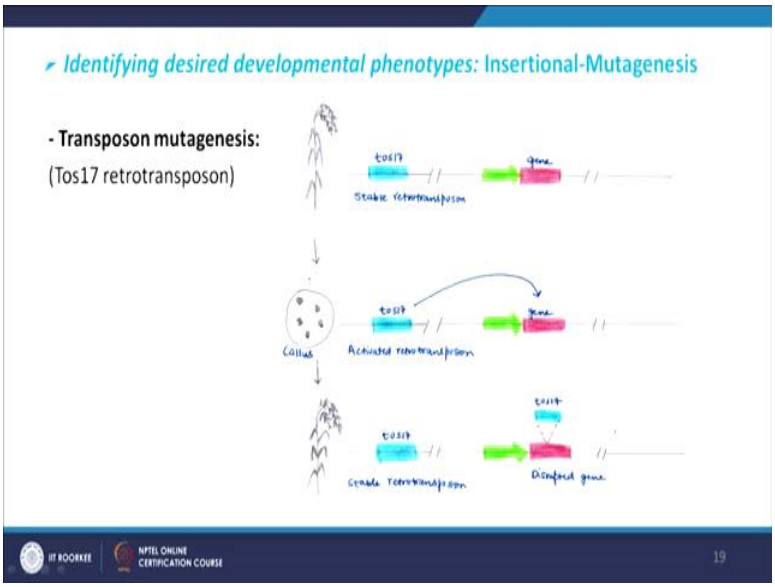

Another very important transposon which was used which is being used for generating mutation is Tos 17. Tos 17 is a retrotransposon and it is very important because this transposon is normally stable at the adult plant or at the later stage of development. So, the element is present, but it is not very actively undergoing the process of transposition.

But if you generate a callus from these plants, at callus stage this transposons get activated and it starts jumping. So, particularly for those plants where we can generate a transgenic plant using callus, it is very good way to generate a lot of insertional mutation of for this one. Rice is one very good example of it.

So, what you can do, you can make a callus; in the callus the transposition will occur, different genes or different loci will be disrupted. And then use this callus to just simply regenerate through the process of tissue culture and make transgenic plant. In these transgenic plant, you can identify a developmental defect, developmental mutant and you can generate collection of such kind of mutants.

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Here is few examples in rice which using this Tos 17 retrotransposon lot of mutants had been generated. This I am showing only few of them. You can look here this is thick culm. Then you have a kind of abnormal flower, you have endless tiller mutants, you have a very dense panicle, panicle is basically inflorescence.

So, dense panicle means you have more panicle branches, more number of flowers, more number of seeds. So, this all mutants they have been generated by using Tos 17 based retro transposition.

Both these approaches the Ac, Ds based approaches and T-DNA based approaches usually they generates loss of function mutants, because they might kill the genes, they are more prone to inactivate a gene.

Another way to a study a gene function is that if you can enhance the expression of a particular gene, and how can you enhance? So, here this is called gain of function. One way of enhancing is that you do not change their domain of expression. They still express in the same tissue, where they normally express, but their expression level goes up.

Second way is that if you change their domain of expression or you can make them to express in a cells or in the tissue, where they were not expressed earlier. And if this kind of genetic regulators are sufficient to give a proper identity or proper developmental events, if they are sufficient to initiate that kind of program, they will give a different phenotype.

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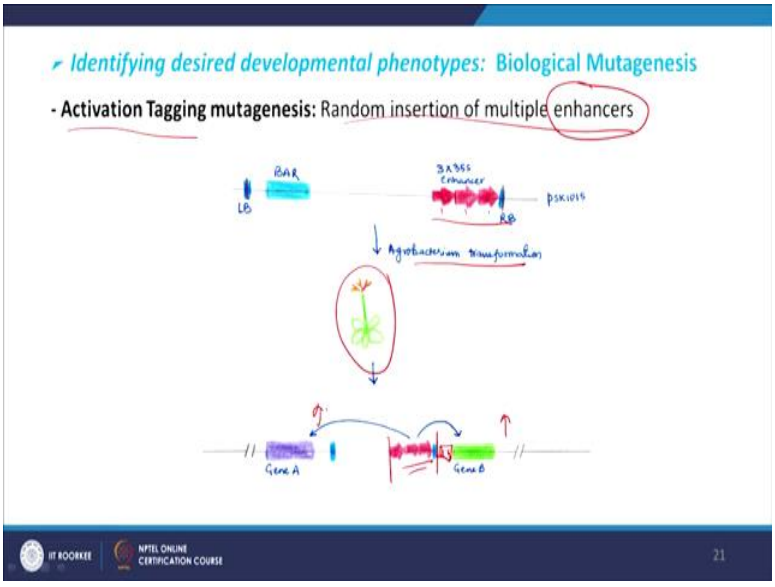

And this is being used through a process which is called activation tagging. What happens in the activation tagging? Random insertion of multiple enhancer. So, enhancers are the genetic elements, which can increase the expression of a gene. What people are doing? They are using multiple copy of enhancer and they are genetically engineering T-DNA or a Ti plasmid and they are using this enhancer through *Agrobacterium* mediated transformation and they are generating a large number of plants.

If there is a plant where there is a phenotype, most likely this phenotype could be because of the activation of a gene. So, if this enhancer elements, gets inserted somewhere here very close to promoter of a particular gene or the regulatory elements of this particular gene, there is a possibility that because of its enhancer activity, it will enhance the expression of this gene or this gene. And you might have a developmental phenotype in this case.

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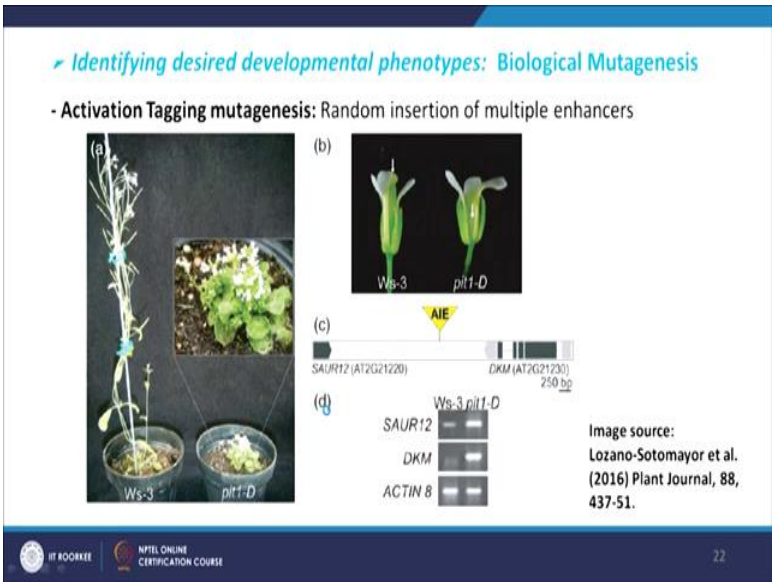

This is one example where activation tagging has been used to generate mutations. As you can see this is the site of mutations and this mutations can actually increase the expression. If you look this is a wild type RT-PCR, we will discuss some time in another class, how we do exactly. This is to measure to quantify the messenger RNA level of a particular gene.

So, if you look this gene and this gene, and if you take the expression label in the wild type, these genes are expressed at a low level, but when there is a activation tagging or insertion of the enhancer element in between you can see the expression level of these genes are significantly increased in these lines ok. So, we will stop here this class, in the next class, we will take this further and we will discuss if we have a mutant how can we go further and try to identify the genetic loci associated with it.

Thank you very much.