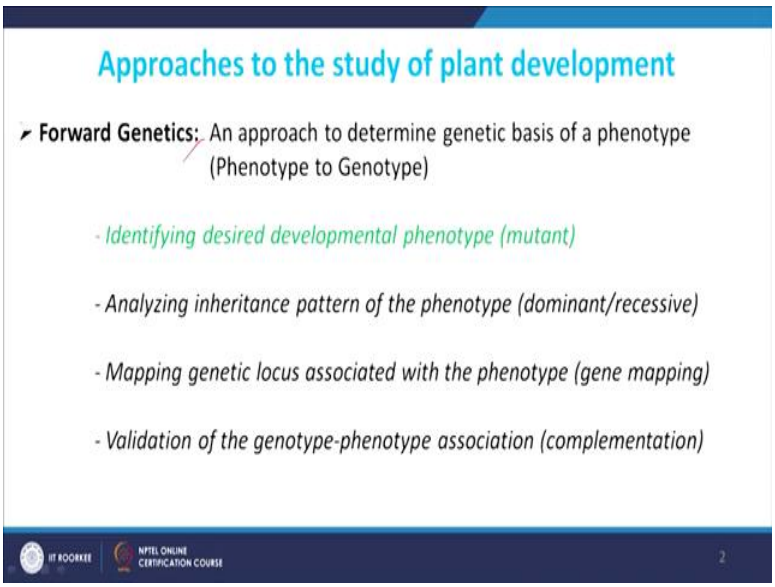
Plant Developmental Biology
Prof. Shri Ram Yadav
Department of Biotechnology
Indian Institute of Technology, Roorkee

Lecture - 05
Molecular Genetics of Plant Development - II

(Refer Slide Time: 00:32)

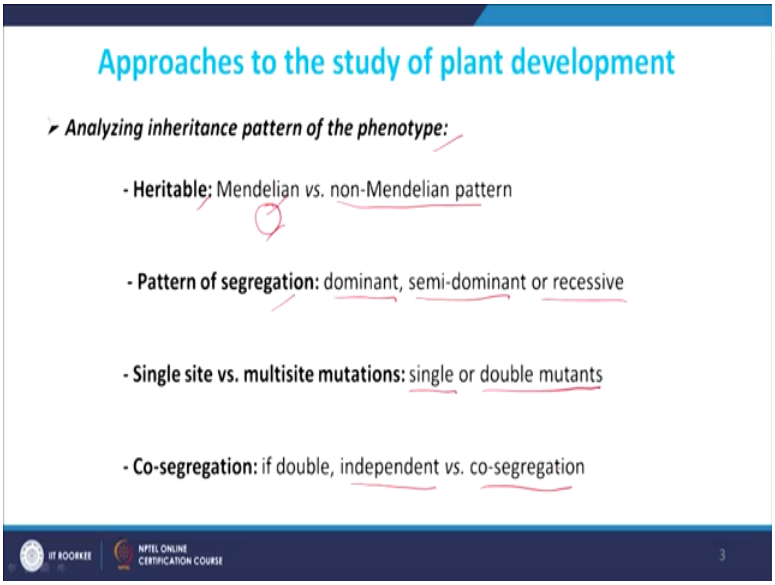


Welcome to this class of Plant Developmental Biology. So, in the previous class we started studying forward genetics based approaches to study plant development and where there we have discussed that what are the different ways how to generate or how to identify a particular developmental mutants with a defect or with a phenotype. So, let us assume that we have identified or we have isolated a particular mutant which has some developmental defect or some phenotype which is of our interest then how to move ahead.

So, the next step what we do or what we do is that we first analyze the nature of mutants. What kind of mutants it is, what is its inheritance pattern? Because we are using genetics based approach. So, it is very important for us to know whether the mutation is heritable in nature or not that is the first question. There are some mutations they if they are in the germ line they can be inherited to the next generation, but if it is in the somatic tissues if they are the somatic mutations they will disappear in the next generation. But for us to

understand more in the detail to establish the proper mutants it is important to have heritable mutants.

(Refer Slide Time: 01:52)



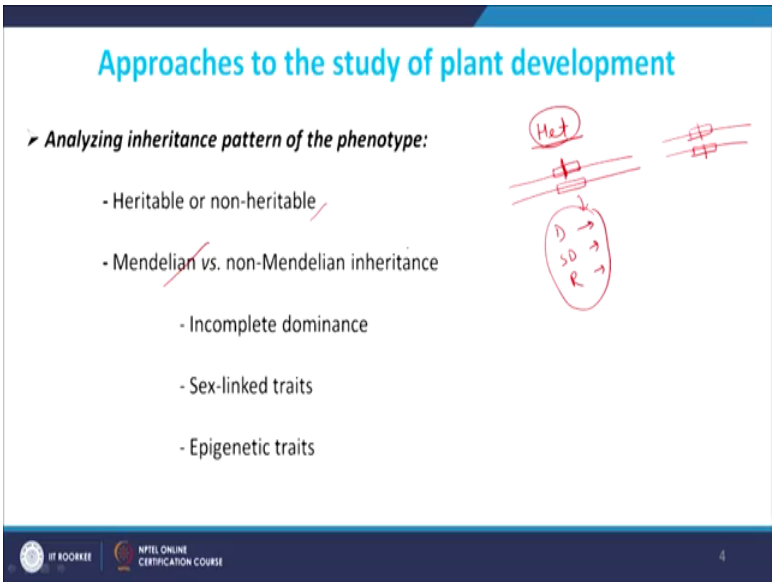
The first thing and then second thing what we do? We try to identify, if it is heritable what is its pattern of inheritance, does it follow Mendelians law? So, you would have studied in your basic course of genetics that Gregor Johann Mendel has given three laws of inheritance how a particular genes or genetic mutants are getting inherited in the next generation.

So, if the mutants are heritable in a fashion which can be justified or which can be explained by the process of Mendel's or by the Mendelian law or if they are not following then that kind of inheritance is non Mendelian inheritance. We will be interested in having Mendelian way of inheritance which makes our life easy to study the plant developmental biology. But if it is heritable and let us assume that it is following the pattern of Mendel's heritance, then we look and we analyze what is the pattern of segregation or what is the pattern of heritance, is the mutant dominant in nature semi dominant in nature or recessive in nature; first.

Next things: if you remember the last class or if you recall the last class, what we have found that in this technique there is a possibility to have multisite mutation. So, we will also like to know whether the mutation or the phenotype is because of a single mutation or it is because of a higher order mutation let us assume the double mutation.

Double mutation means there is a possibility that there are two mutants in two independent genes or and the result of these two mutants might give the phenotype. And if this is the case then what happens, how they segregate, do they segregate independently? So, there is a first possibility where there is a two mutation but both the mutant or both the locus; both the loci are segregating independently or they segregate together; co-segregation.

(Refer Slide Time: 04:15)



So, first thing we would like to rule out all this possibility with our interest or mutant of interest. So, for first thing what we do as I said that heritable or non heritable. The mutants if mutant is heritable, then if you collect the seeds from the mutants and grow to the next generation, in next generation you will get the phenotype, but if your phenotype is disappearing from the next generation then your mutation are is non heritable.

Another important thing if your mutation is inherited in Mendelian fashion, then what are you expecting? You are expecting a result depending on the fact what is your initial mutation, if your initial mutation is let us assume heterozygous. Heterozygous means for every chromosome we have a pair of chromosome pair of homologous chromosome. So, if let us assume this is a gene where there is a mutation here.

So, if mutation is in only one allele or one loci or in the one homologous chromosome and mutation is not present in the corresponding locus of another homologous chromosome then this condition is called heterozygous. In homozygous condition what

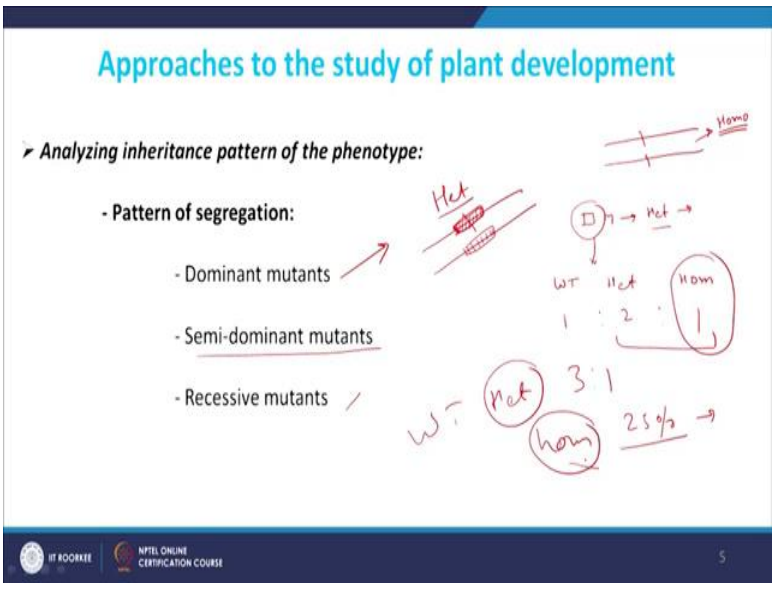
happens that you can expect that mutation is present at both the allele, here as well as here, here there is no mutation.

So, if your mutation is heterozygous, then you can go to the next generation and look the segregation pattern and it depends on what is the nature of your mutation, if it is a dominant then you expect a particular ratio, if it is semi dominant then you expect another genotypic and phenotypic ratio and if it is recessive then you expect a different pattern of ratio, what would be those ratio we will see to the next slide.

But another possibility is that if it does not follow the Mendelian pattern of inheritance, what is the pattern of inheritance? There are a lot of cases which has been found naturally as well as induced way which are not following the Mendel's pattern of inheritance.

For example, if the dominance is not complete or incomplete dominance or if there is a epigenetic mutations which is not getting transmitted; which is not inherited to the next generation or the loci which are sex linked traits because sex linked there is a difference of the homozygosity is not fully maintained because one chromosome is x and other chromosome is y ok.

(Refer Slide Time: 06:49)



So, let us assume that the mutant or we will be more interested or we will be more happy to have a mutant which is following the Mendelian pattern of inheritance. If it is

following the Mendelian pattern of inheritance, then what could be the nature of mutants?

So, first thing is that if your mutant is dominant and if you have a heterozygous loci for the mutants you can expect that you can expect a phenotype even in the heterozygous condition, but if your mutation is recessive in nature then the just it disruption of one loci is not sufficient to give the phenotype.

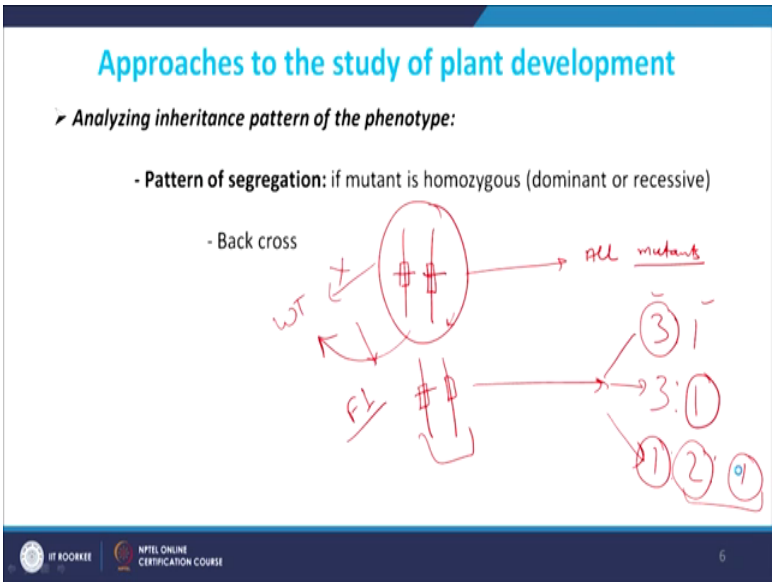
In this case you have to have mutation in both the genetic loci and if this is the case then you will see the phenotype. So, your phenotype will be only if you have mutation in both the homologous chromosome and this is the condition called homozygous condition. So, what happens? So, how to study this? So, you take this plant mutant plant and go to the next generation.

In next generation let us assume your mutant plant was heterozygous. And if it is heterozygous if your mute if mutation is dominant it will already have a phenotype, but if it is recessive then your mutant plant will not have phenotype, but if you go to the next generation you are going to get wild type heterozygous and homozygous population in the ratio of 1 : 2 : 1 and if as I said if it is dominant then you expect these 3 to be the mutant.

So, your phenotypic ratio is going to be 3 mutants and 1 wild type, but if your mutation is recessive in nature then only this homozygous which is like 25 percent of the total population is going to have the mutant phenotype. So, if you look the segregation pattern and if you quantify the number of mutants in the next generation you can tell whether your mutant is dominant or recessive in nature. Another condition what happens is the semi dominant.

So, in semi dominant mutant condition what happens that you have wild type, heterozygous and homozygous. Now, the heterozygous and homozygous in case of dominant mutant purely dominant mutant they have a same phenotype. In case of recessive mutants only homozygous will have a phenotype, but in case of semi dominant mutants the heterozygous and homozygous both will have a phenotype, but the severity of the phenotype will be more in case a homozygous than the heterozygous. So, you have a range of phenotype in this case.

(Refer Slide Time: 09:49)

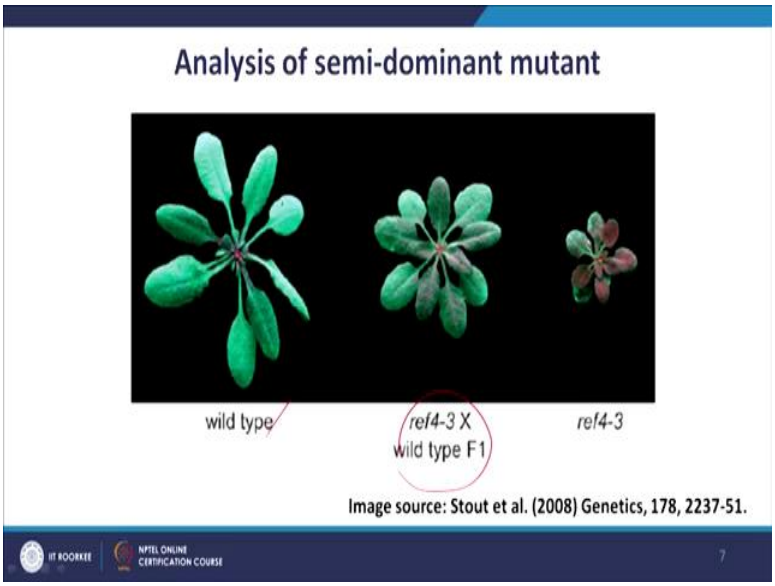


Another case how to rule out: let us assume that your original plant or the mutant plant is homozygous. If it is homozygous which means that both the loci is mutated. If both the loci are mutated, then next generation if you go to the next generation all the plants are going to give the phenotype; all mutants. Here it is very difficult to tell what is the nature of your mutants, whether it is dominant or recessive it does not matter because this both the loci are mutated, so they will not segregate.

So, to rule out the possibility you will have to make a back cross to generate heterozygous. So, you will take this homozygous mutant plant cross with the wild type plant and generate a F1 which will have only one locus mutated another locus as a wild type. This is heterozygous plant and then take this heterozygous plant for the next generation and look the phenotype.

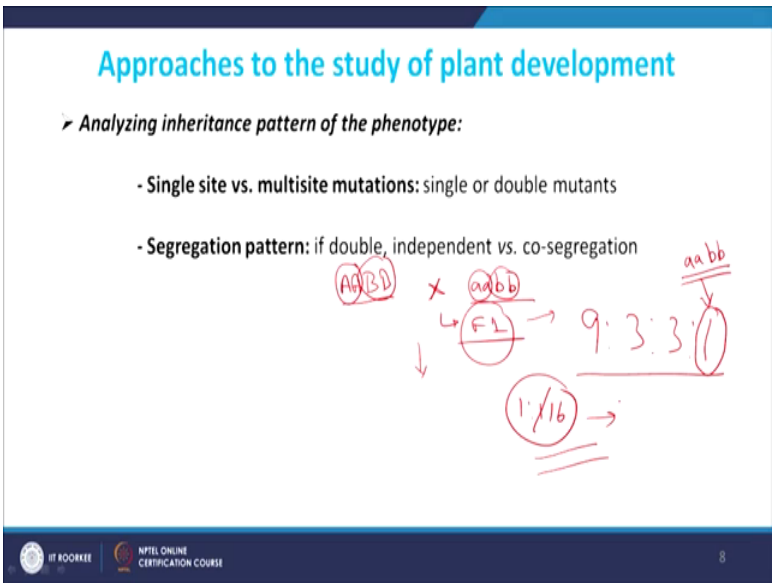
If it is dominant, if your mutation is dominant you expect 3 : 1, 3 mutant 1 wild type, if it is recessive then you expect same ratio 3 : 1, but here mutant will be 1 and dominant mutant will be 3, but if it is a semi dominant case you are expecting 1 : 2 : 1. So, 1 will be pure wild type absolutely normal, 2 will have some phenotype, but 1 fraction this is going to be more severe than this one. So, essentially this both population will have the phenotype, but severity will be different.

(Refer Slide Time: 11:44)



So, look here this is a case of semi dominant mutants. So, this is wild type and this is homozygous. If you compare wild type and homozygous look the phenotypic severity this plant is very small, the leaves are very small shape are very defect, but if you look the F1 population which is basically heterozygous; heterozygous is the severity of phenotype is intermediate. So, it is less than the wild type, the growth is less than the wild type, but more than the mutant. So, this is a kind of semi dominant mutant phenotype.

(Refer Slide Time: 12:32)

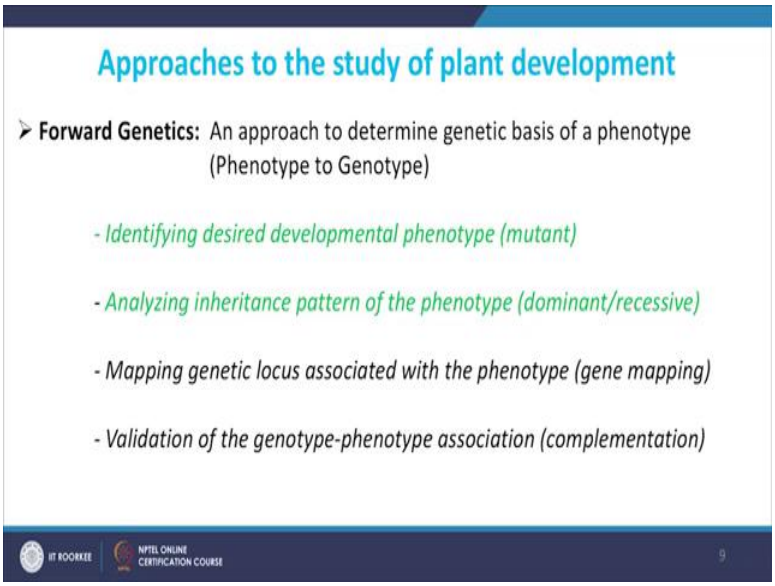


So, next thing what we would analyze with this mutant is whether mutation is single site mutation or multi site mutation. How we can do that? We can do this by simple doing the Mendelian cross. So, let us assume that you have a two mutations; one is a and another mutants is b in gene b and you will have wild type and then you have mutant. Let us assume the phenotype is because of the double mutant phenotype and you if you make this kind of cross go to the F1 generation and look the phenotype. So, this is wild type this is a mutant one and go and look the F1 plants, this will tell an idea whether your mutant is dominant or recessive.

If your F1 plants they already have the phenotype which means that your mutants are dominant, if your F1 plant do not display any phenotype which means your mutants are recessive. And then you go and make a cross go to the F2 generation and now it depends on what is the segregation factor pattern of both the loci. If the segregation of both the loci are totally independent and if mutation is because of the disruption of both the loci because of the mutation in the both the loci, then you are expecting a typical ratio 3; 9 : 3 : 3 : 1, the phenotypic ratio which we always get when you make a dihybrid cross; Mendelian dihybrid cross.

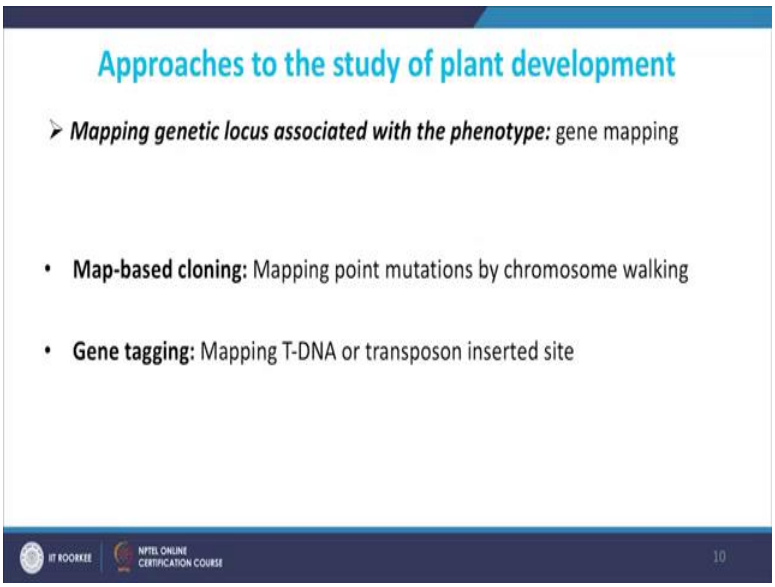
And in this case this population the 1 this is the population which will be homo double homozygous; double homozygous for the mutant. So, this will be a small a small; a small b; small b. So, if we get 1 out of 16 as a mutant in F2 generation, this tells that your this is a result of double mutant both the mutants are recessive in nature and they are not co segregating they are basically segregating independently that is why we have got this particular ratio. But if they are genetically linked, if they are co segregating the ratio will be totally changed depending on the strength of linkage ok.

(Refer Slide Time: 14:50)



So, this is the preliminary genetic analysis one can do just after identify the mutants. Then the next step will be that you have identified the mutant, you know what is the pattern of its segregation, now the next important thing is to identify what are the genetic loci associated with this mutants which means that mapping the mutations mapping the gene. How to do that?

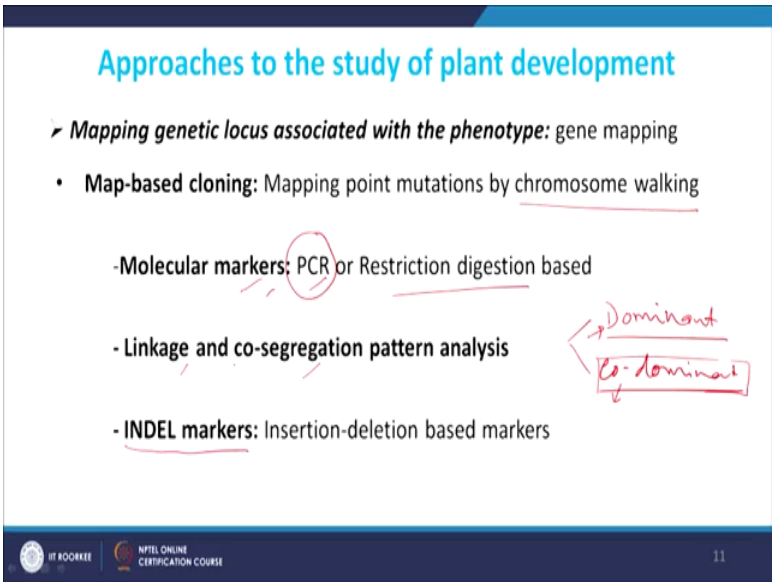
(Refer Slide Time: 15:16)



So, there are two approach depending on what is that what type of mutants you have generated, if you have generated point mutation for example, by EMS then we normally

take map based cloning based approaches and if you have generated mutation through the insertional mutation using T-DNA or transposon, then you can do gene mapping by gene tagging. So, now we will take the first approach map based cloning. So, this is done by some molecular marker.

(Refer Slide Time: 15:53)



So, this is very important for you to understand and what happens here we do by chromosome walking. So, there are certain things, so there are molecular markers already reported or people have identified molecular markers, what are molecular markers may be we will see to the next slide, but this can be either PCR based marker or restriction digestion based markers. For quick analysis we prefer PCR based marker because this is very fast and then what we do, we try to analyze linkage and segregation pattern between these marker, molecular markers and the phenotype ok.

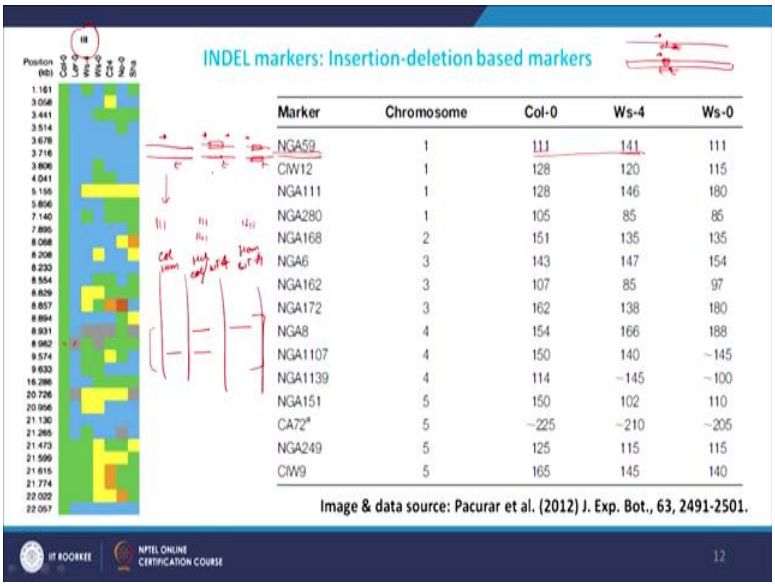
One very important marker which is preferably being used are INDEL markers. These markers are produced at the site of insertion and deletion. So, basically in markers you can have two kinds of marker; one is called again dominant markers and another are co-dominant markers. So, co-dominant marker; so in case of dominant marker what happens that this marker will give a pattern in one particular genetic background and it will not give a band in another genetic background.

For example, if you are using PCR based, but in co dominant in both the condition or both the genetic background, it will give a band, but the size of bands will be different.

So, for our use or for most important thing co dominant markers are very good markers because in case of dominant marker when you see the band you are sure that this is present, but if you do not see the band you are not 100 percent sure that absence of band is actually because this is a marker, it could be simply a technical problem of the PCR.

So, this that is why in co dominant markers you see both the bands in both the condition, but based on this different size you can clearly tell this is this genetic background, this is that genetic background ok. So, for example, in most of the plants these markers has been well identified, well established and now people know if you take some example of *Arabidopsis thaliana* model plants.

(Refer Slide Time: 18:10)



Marker	Chromosome	Col-0	Ws-4	Ws-0
NGA59	1	111	141	111
CIW12	1	128	120	115
NGA111	1	128	146	180
NGA280	1	105	85	85
NGA168	2	151	135	135
NGA6	3	143	147	154
NGA162	3	107	85	97
NGA172	3	162	138	180
NGA8	4	154	166	188
NGA1107	4	150	140	~145
NGA1139	4	114	~145	~100
NGA151	5	150	102	110
CA72*	5	~225	~210	~205
NGA249	5	125	115	115
CIW9	5	165	145	140

So, this is chromosome number III of *Arabidopsis thaliana* these are different varieties of *Arabidopsis* Columbia, Landsberg all this kind of C 24. So, different variety and what people have tried to identify, what are the markers? Markers basically are the genetic elements which is different in two variety. So, for example, if you look this is Columbia, so Columbia has been taken as a reference background and if you look that Ler, so, here if you look this is, so there is a difference here.

So, Columbia has a different difference in the genetic loci with the Ler and you can choose this region and if you design a specific primer for this region, then that primer can really distinguish whether it is Columbia background or it is Ler background and that kind of markers you could use to identify whether a particular region of a chromosome is

from the Columbia background or from the Ler background. These are some names of these markers. For example, if you look because of some insertion some deletions there is a difference, so this is NGA59. So, this is the name of one marker in this case you have identified a region where there is a difference.

So, for example, in this region if you compare this and this is base pair. So, this is 110, this is 141, so difference is 30 nucleotide which means that in Columbia either there is a deletion of 30 nucleotides in this particular region or in Ws4 there is this insertion of 30 nucleotide. So, if you look here, so what happen? If you take Columbia genome so, there is a region here and if you take corresponding Ws4 genome here is a region, but there is a small insertion.

So, if you design a primer here, if you design a primer here and if you say primer here and here, what you are going to get from Columbia background? You are going to get this band size which is 111 and from this background you are going to get this. So, this is 111 plus this 30 nucleotide insertion which is 141. So, if you take these and if you run the gel, what you can expect? Another important thing you can expect here. So, let us assume if a genetic loci is only Columbia, then if use this marker you are going to get 111 base pair band.

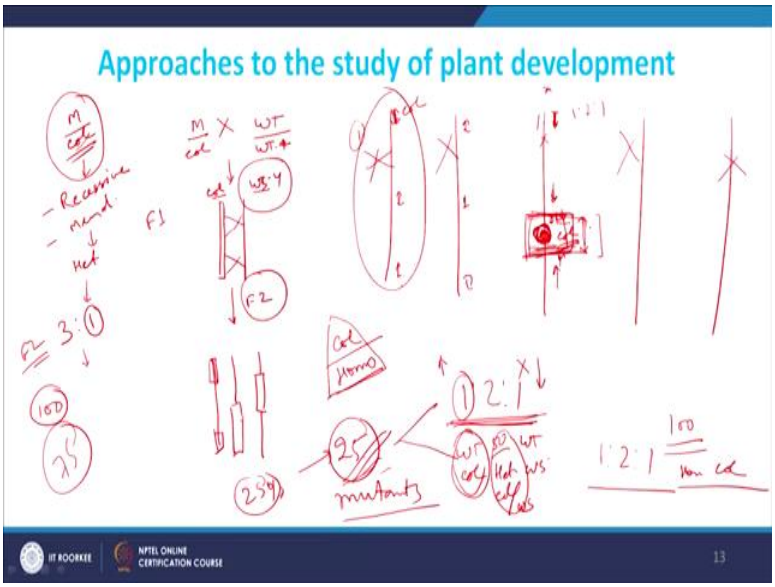
If this loci is heterozygous one loci is Columbia and one loci is Ws and if you use here then what are you going from the Columbia loci you are going to get a band of 111 from this Ws4 loci you are going to get a band of 41 and if you have a genetic loci which is for this region which is homozygous for Ws4 and if use the same primer you are only going to get a band of 141 nucleotide.

So, if you run on the gell you can clearly see this kind of pattern, if it is homozygous for Columbia then you are going to get a band of this size, if it is heterozygous for Columbia and Ws4 you are going to get a band of this size and a band of this size. So, this is coming from one locus, this is coming from another locus, but if it is homozygous for Ws4 then you are going to get only one band of 141 base pair.

So, just looking this banding pattern and looking this set of primers you can clearly distinguish what is the genetic loci on the chromosome number 3 or somewhere here wherever this marker is located. So, this marker for example, is located on the chromosome. So, this way the entire genetic map has been prepared for all the

chromosomes and large number of molecular markers has been identified. If we have to map a gene, then what you can do? You can simply choose these genes and show association and look their segregation pattern with your mutant.

(Refer Slide Time: 22:48)



So, what you do basically? Let us assume I have a mutant plant which is in Columbia background and I have I know that this plant this mutant is recessive in nature, it follows Mendelian pattern of segregation which means that if I take heterozygous of this and go to the next generation in F2 generation I am expecting that 3 : 1 ratio and 1 this ratio 25 percents plants are going to be mutant. So, if I take this plant and mutant plant and cross with wild type, mutant is Columbia background if I take Ws4 background.

And if I cross them what I am going to expect? In F1 generation I am generating heterozygous. Heterozygous means half set of genomes are coming from Columbia background another half set of genomes are coming from Ws4 background this is heterozygous. And then if I allow them for selfing and go to the next generation there will be a lot of crossing over in some chromosome 1, some chromosome 2 and basically what it is doing that there is merging of the genome.

So, if you go to the next generation and go to the F2 generation the genomes of Columbia and Ws4 will be totally merge and you may find a region different kind of possibility you can find a chromosome like this, you can find the chromosome like this. So, any possibility whatever you can imagine you can find here in the F2 generation, but

what one thing we are doing that, then we take this plant. So, let us assume I have taken 100 plants from here from F2 population and here as well; so from this F2 population.

So, the 25 percent of the plant which will be like let us assume approximately 25 plants they will be the mutant, they will show the phenotype. So, what it means that for this mutant since my mutation is coming from the Columbia background and this mutation is totally absent in the Ws4 background which means that in these 25 plants the locus where this mutation is it will be homozygous for Columbia. Why this will be homozygous for Columbia? Because it will if it will be homozygous then and only then it will have the phenotype. Since it has a phenotype it means that it is a homozygous; homozygous for the mutation.

And since mutation is only coming from the Columbia which means that region where there is a mutation will be only from the Columbia and there will be no region from the Ws in that one. Now, if you take all these 25 mutant plants with the mutant phenotype and check for their different chromosome, let us assume there are 5 chromosomes typically in *Arabidopsis* and if I measure a chromosome here a marker here; a marker here; a marker here I do use this PCR based molecular marker for different region.

And let us assume that my site of mutation is somewhere here then what I am expecting? If I take this 25 plant, so my mutants are fixed here. So, this locus is homozygous for Columbia in these 25 plants I am not talking about remaining 75 plants there might be something different, but in this 25 plants which clearly shows the phenotype this locus is homozygous for Columbia.

Now, if I look; so in these 25 plants this will be this region will be Columbia, but all other region can be anything as per the Mendelian pattern. So, if I look this region or if I check this region in this 25 plant, what I am going to expect? I am going to expect a pattern of this. So, out of these 25 plants 25 percent of this population will be wild type for Columbia; 25 percent of this could be wild type for Ws and 50 percent of these can be heterozygous for Columbia and Ws, this is a typical pattern Mendelian pattern here.

So, what I do? I check different regions of the chromosome and if I found on the chromosome let us assume on chromosome number 1 all three marker shows independent assortment with respect to the phenotype, if they are giving 1 : 2 : 1 ratio for these marker which means that my mutation is not present in this gene in this

chromosome. Similarly, you rule out this chromosome; you rule out this chromosome; you rule out this chromosome; you rule out this chromosome.

But once you will reach to this chromosome and if your mutation is here, if you look here a marker which is present here since this is far from the site of mutation there is still possibility that there is a crossing over events here. But once you start moving this is called chromosome walking, if you check for here you might still get 1 : 2 : 1 segregation for this marker in this 25 plants, but once you will come closer to the site of mutation either from this direction or from this direction, what you are expecting. The wild type Ws start decreasing, then heterozygous or homozygous Columbia will start increasing.

So, then there will be a deviation you cannot expect 1 : 2 : 1 and then if you come closer; closer; closer and let us assume if you look this marker this might be present very close or very near to the sight of mutation. In all these 25 percent there is a possibility that it might be either only heterozygous or only homozygous for the Columbia and then if you come down; come down there will be a point where all the plants will have only Columbia genome.

So, then you might get a condition where 100 percent of this 25 plants will have homozygous Columbia and they do not have any genotype for Ws this is called your mapping window. So, now, we do from this direction we keep on mapping and we found that here is a marker which is 100 percent Columbia, here is the marker which is 100 percent Columbia, now this is my window.

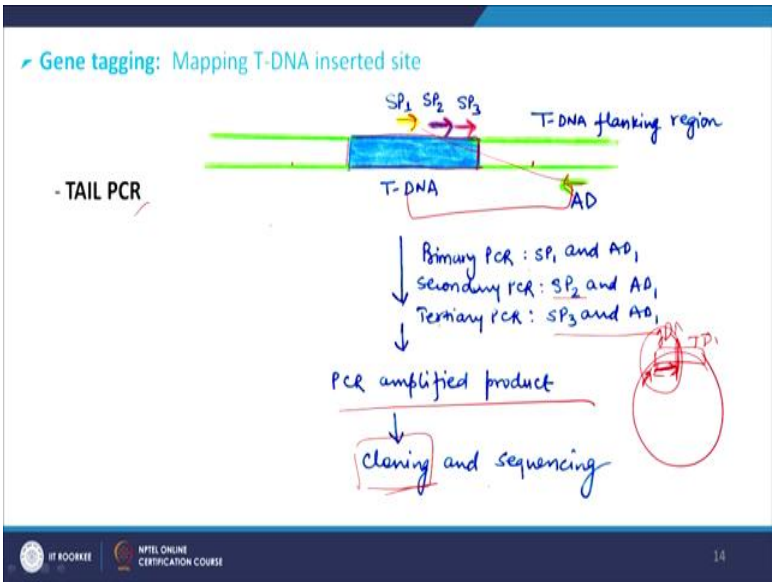
Now, I know that the mutation is somewhere in this window. If you want to further go and fine tune this mapping, then you will have to increase the number of mutant plants this is not possible with 25 plant, you can go 1000 plants more, more. And then you can do the fine mapping and you can do, but these days since we are in the post genomic era, most of the plant model plants their entire genome has been sequenced, now in the different varieties of those plants genomes has been given sequenced and now sequencing is no longer very costly or very time consuming process. So, we can take this mutant and go and sequence whole genome sequencing.

And then why we have done this is called rough mapping, why we have done this rough mapping because this entire plant was exposed with the EMS. So, there is a possibility

that there is a large number of point mutation in the genome, but we have to find out which mutation is actually responsible for the phenotype, all mutation might not be responsible for the phenotype.

And then if we have already defined this window, then we can only look what are the mutations in this window and then one by one you can rule out depending on your knowledge if something is in the inter in the intergenic region we can rule out. Similarly you can point out and you may find may be one point mutation or two point mutation in this gene which might have the relevant phenotype. Then we can take in the next step and validate that actually this mutant is responsible for the phenotype.

(Refer Slide Time: 31:49)



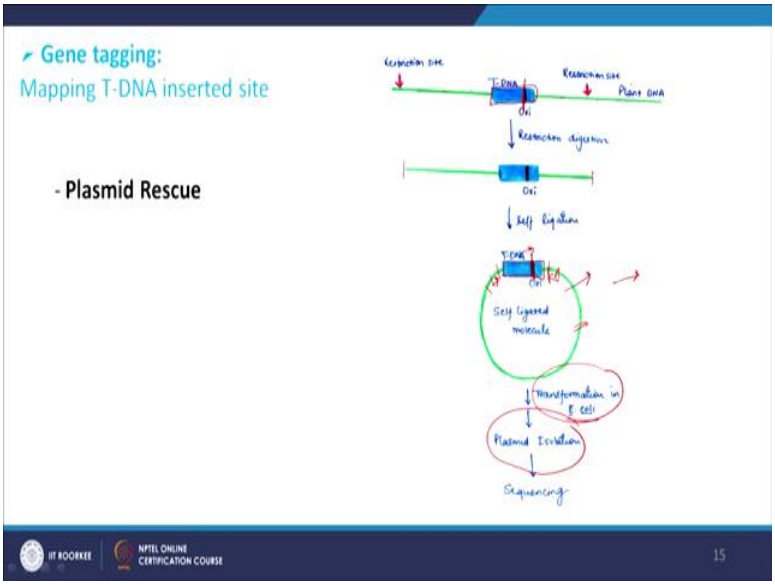
So, this is to map a point mutation whereas mapping T-DNA insertion site is relatively easy and it has been done with the several way. One way of doing it that this is called TAIL PCR. In TAIL PCR what you do? Since, this T-DNA region which you have engineered, so, you know the sequence, you can design some primers for this T-DNA. For example, SP 1 primer, SP 2 primer, SP 3 primer and let us assume this is integrated in the genome and this might be the plant genome and then you can design an arbitrary primers here.

So, this primer basically this is not very specific this is more kind of random primers. So, it can bind in a very or you have a kind of pool of primers. So, you might find a primer which might bind here and this primer if you run first primary PCR with SP 1 you can

enrich this amplification, then you use another primer keep on enriching and if you do a combination of primer you might amplify this region, the region from here to here. And then you take this PCR amplified products, clone in a vector how to do cloning you would have studied somewhere, take this PCR fragment and clone in a cloning vector and now if you have clone in a cloning vector this is your fragment, in fragment you have some region of plant genomic DNA and some region of T-DNA.

Now, you have some primer binding site here sequence from here and identify what nucleotide sequence is coming from the plant and then you can do, you can check this go to the website you just try out what is the sequence and where this is positioned in the plant this way you can map that what is the site of insertion.

(Refer Slide Time: 33:35)

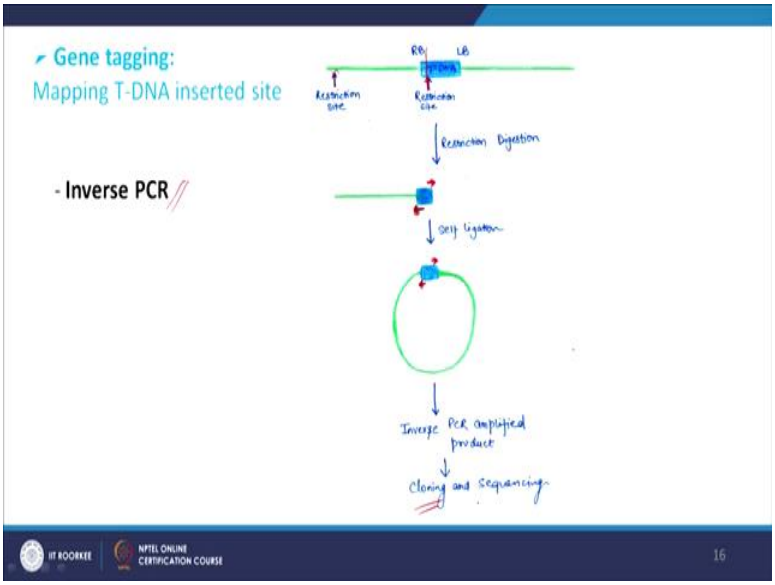


Another way of mapping is plasmid rescue. Here what you do? When you are engineering your T-DNA you put a origin of replication for bacteria. You know that if origin of replication is present, then this DNA if it is circular in nature it can propagate in the bacteria it can replicate in the bacteria. Take this let us assume that this T-DNA is integrated in the plant genome use some enzyme site. So, let us, but ensure that this enzyme site is not cutting in the T-DNA.

So, if you use a restriction site it might come here; here; here; here anywhere in the plant genome and then when it will digest use this fragment and you can re-ligate it. So, re ligation will generate a this kind of molecule where you will have T-DNA as well as a

fraction of plant genomic DNA. Since, you have origin of replication this is a kind of your plasmid you transform to the bacteria *E.coli* bacteria and in bacteria it can propagate, extract the plasmid and sequence using T-DNA specific primer and then you can identify what are the sequences which are just flanking the T-DNA and you can basically position or you can identify the locus of integration.

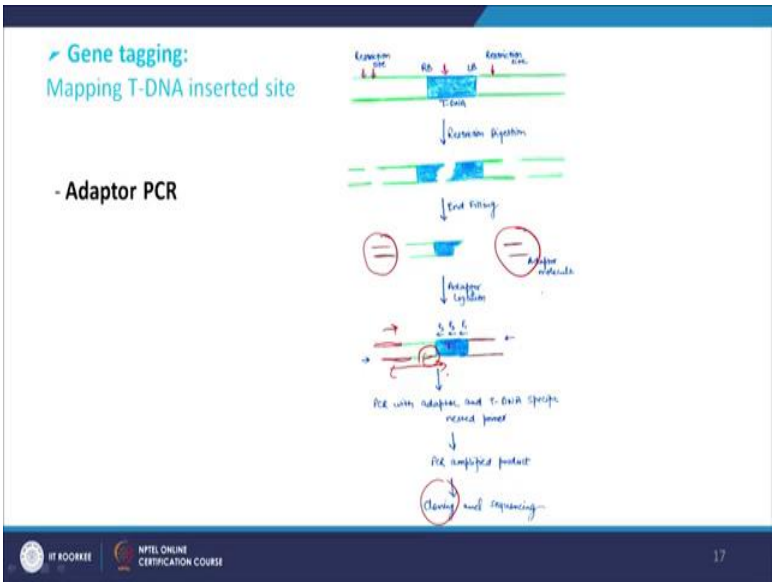
(Refer Slide Time: 34:56)



Third way of doing identifying the site of insertion is inverse PCR. In inverse PCR what you do? Basically you choose a site restriction site which cut at least once in the T-DNA and then somewhere in the genomic DNA.

When you will digest it you know the sequence where it cut, you have designed a primer which is specific to the T-DNA another primer which is specific to this region and then you re-ligate it; when you re-ligate it this will. So, you have a primer positioned here; you have a primer positioned here do the PCR. And this will amplify this region and clone this region in a particular cloning vector sequence them and identify what are the flanking sequence in the T-DNA.

(Refer Slide Time: 35:50)

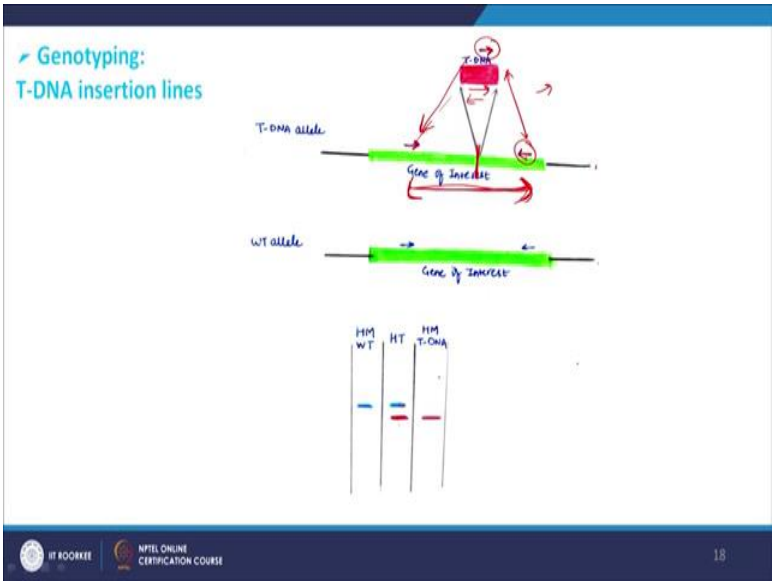


In another technique which we are using to map the site of an insertion is adaptor PCR. Adaptor PCR is quite similar, but what we do that you digest with the restriction enzyme and then in the end what you do fill this end depending on what kind of enzyme you are using are you using blunt end enzyme, sticky enzyme, but then additionally what you do? You add some adaptor molecules at the both the ends of this molecules.

And when you add these adaptor molecules since you know the adapter sequence you can have a primer specific to the adaptor sequence, you know the T-DNA sequence, you can design another set of primer and you can do the PCR amplification, take this fragment, clone and sequence. So, you can identify what sequence this is and depend based on this you can position it in the genome; in the plant genome ok. As I said that this has been very extensively used and lot of transgenic lines has been generated in *Arabidopsis* it is deposited in the database.

Now, simply based on your mutant phenotype or if you want to identify genes or if you know the genes you want to identify the phenotype you just simply order those mutants and if you order the mutants you know that there is a mutation and you know that there is a T-DNA insertion, you can simply go and do the genotyping and validate that actually there is insertion.

(Refer Slide Time: 37:14)



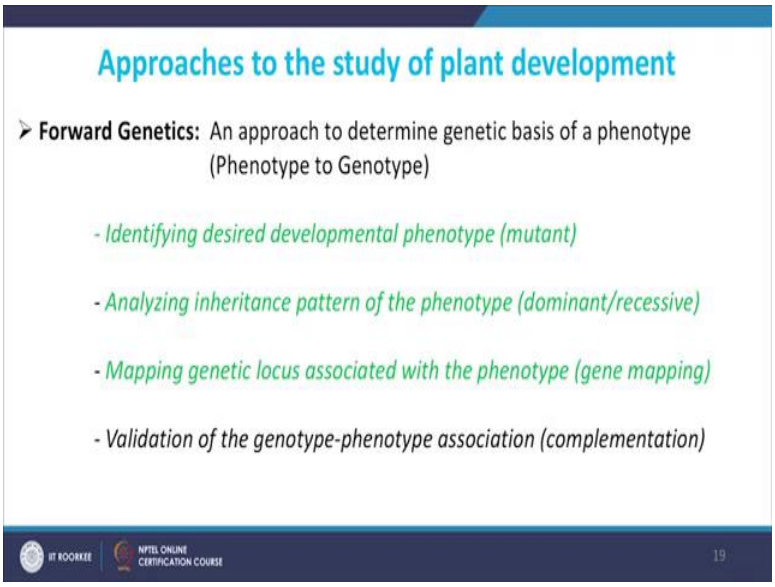
So, what you do for doing this? So, let us assume this is the gene of interest and you know the site of insertion because this is already established, sequenced, mapped, everything is done. Now, you design a primer in the gene, forward primer and reverse primer just outside the flanking region of the T-DNA and use another primer in the T-DNA and if you do the combination of PCR.

So, one PCR you do using gene specific forward and reverse primer and another PCR you do using T-DNA specific primer. Either with this depending on the orientation or this with the, it depends on what orientation this T-DNA got inserted. If T-DNA is inserted in this orientation then you would like to use this primer with this to have amplification, if it is in opposite orientation in this orientation then your primer position will change and then you would like to check with this, but what is important here.

Thus this band you are going to get only if your genomic copy is wild type and there is no insertion and this band you are going to get only if there is a T-DNA insertion you will not get on the wild type genome and thus we design primer in a way that there is a size difference between this amplicon size and this amplicon size. So, when we do the PCR if your both the locus are wild type, then you are expecting only one band of this size, but if your if one locus is insert having insertion another locus is not having insertion, then you are expecting a band size of this as well as a band size of this or this here.

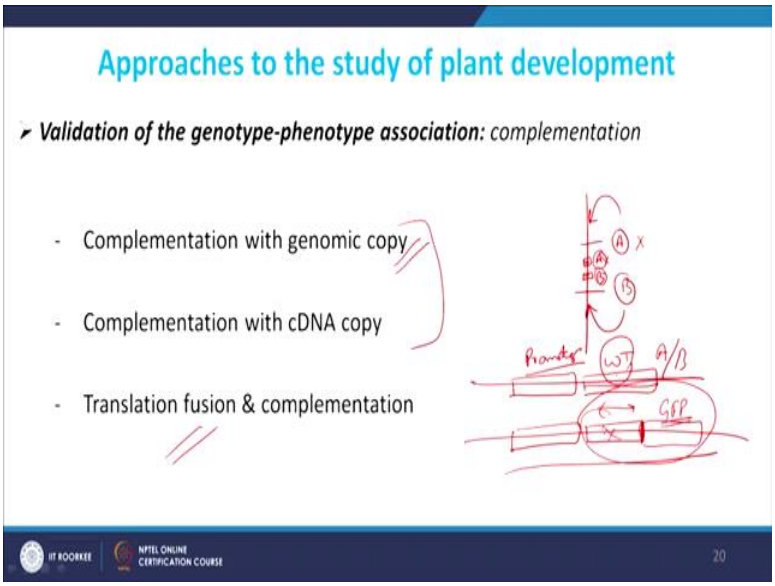
So, in heterozygous condition you will expect both the band, but if it if both the locus are disrupted and you have insertion in both the locus, then this primer will not give amplification only this primer will give the amplification and then you are expecting only one band of this smaller size. So, this is a way to genotype a particular mutant and to show that actually your mutation is homozygous, wild type or heterozygous for your gene of insertion ok.

(Refer Slide Time: 39:29)



So, now you have identified a mutant, you have analyzed the inheritance pattern, you have mapped the genetic loci and you have identified what is the site of insertion or point mutation. Now, next thing is to validate the genotypic phenotypic association.

(Refer Slide Time: 39:45)



So, how we do? Basically this is also called complementation analysis. So, let us assume that we have performed a forward genetic analysis, we have mapped a point mutation in a particular developmental or mapping domain or mapping window, let us assume a case where I have found two point mutation. So, if this is my mapping window if I have found two point mutation very close, but in two different gene.

Now, I have to rule out the possibility whether the phenotype is because of the gene A or because of the gene B. How can I do that? Then I take this mutant background and I compliment with gene A first and look if this can rescue the phenotype. In second experiment I complement the same mutant with B, then I look whether B can mut compliment the phenotype.

So, whichever gene will complement the phenotype or rescue the phenotype we will say that the mutation in genes A or gene B was actually responsible for the phenotype and this is the way to validate your phenotype. Yes, the complementation you can do either using the genomic copy you know there is a difference between genomic DNA and cDNA. In genomic DNA you have both exon as well as intron; in case of cDNA the introns are already spliced because you have made a DNA from fully processed messenger RNA there introns are not there and what we can do.

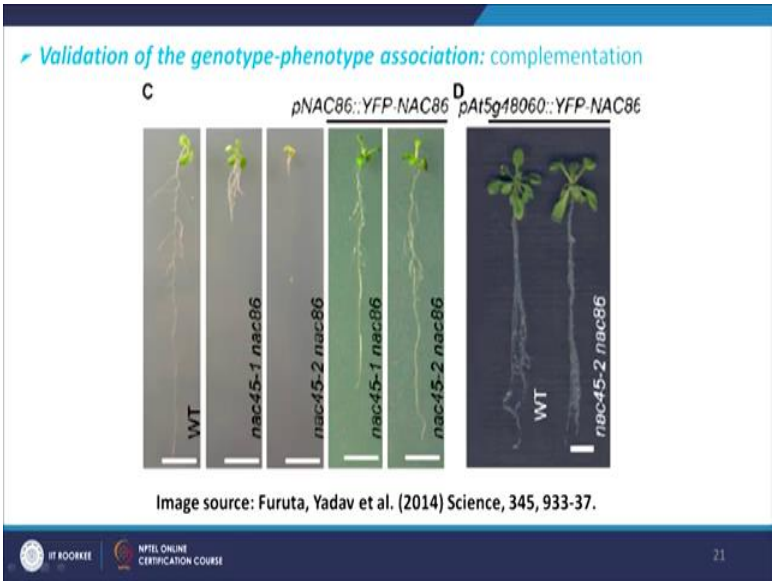
But if you want to complement the best way of doing the complementation use the promoter of the same gene. So, you are not changing the domain of expression, but you

are using now wild type copy of gene either it could be A or B you take that and put back in the mutant and what you are doing in the same expression domain where now mutant proteins was made you have started making wild type protein and you look whether it is complimenting the phenotype.

The only difference in these two way is that here either you can take a genomic DNA copy with exon and intron or you can take just cDNA copy without intron ok. Another way of doing complementation is what you can do? You can basically take the promoter of the same gene and you take the gene wild type copy of the gene and just mutate the last stop codon of the open reading frame and fused with some marker which is called GFP; Green Fluorescent marker or anything.

So, this will allow you to do two things you can look for the complementation as well as you can track the protein localization. So, you can also analyze where this protein is being localized, subcellular localization.

(Refer Slide Time: 42:43)



So, this is one example which I want to show you. So, this is wild type *Arabidopsis* plants growing very well, but if you look these are the two double mutants in both the double mutants the root growth is highly compromised they are very small rooted plant, but in this double mutant where both the mutants are lacking or both the genes are disrupted.

If you put back one of this genes, so, for example, *nac45*, *nac86* this is double mutant, but when we are putting *NAC86* in the *NAC86* promoter fused with the YFP; Yellow Fluorescent Protein, you can see this can complete totally complement the phenotype. Similar case here also you can see the mutants are getting complimented with this phenotype. So, now, this is looking very similar these plants are looking very similar to the wild type.

So, this tells that if you are bringing just one gene back the phenotype is disappearing which means that a single mutant might not have the sufficient phenotype. So, you have to have double mutant to give this phenotype and if any one of them you will bring back the phenotype is disappearing.

Now, we will stop this class here and in next class we will discuss the reverse genetics based approaches.

Thank you.