Course Name: Basics of Crop Breeding and Plant Biotechnology

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Lecture-52: Molecular Analysis of Transgenic Plants II

Welcome back. So, we will continue again. Gradually we will move into the copy number determination. Ok! In copy number determination, I will try to use the same construct, but the plant numbers, three putative transform plants will be used over here. Ok! Suppose we have used the same construct promoter, then the gene of interest and the terminator, it is available in the vector here we have the left border and the right border region. Ok! Over here what were the restriction sites? *Eco*RI, *Bam*HI, *Sac*I and *Hin*dIII here we had *Eco*RI, here we had *Bam*HI, here we had *Sac*I and here we had *Hin*dIII. Ok!

This restriction enzymes, have been used and suppose three different plants are being used over here. We had plant 1, we had plant 2, and we had sorry we had plant 3. Suppose for your understanding I am just making two chromosomes over here. Ok! In each of the plant two chromosomes are available.

A particular rice variety has been used for plant transformation, and in the same variety in different plant lines our transgene has been integrated at different position. Ok! So, suppose over here we are planning to use again *Bam*HI restriction enzyme. Ok! Because, this is at one side of our gene of interest. Ok! So, over here only one *Bam*HI site is available and over here two *Bam*HI sites are available. This one was 1.5 kb, this one was 3.5 kb, this is 2.5, 3 and 1 kb. Ok! Suppose the scenario was same over here 1.5, 3.5, then 2.5, 3 and 1. Over here the scenario was also same, let us assume 1.5, 3.5, then we will be having 2.5, 3 and 1.

Now suppose in plant number 1, transgene has not been integrated, just we are assuming we have to confirm it based on the autoradiogram, we have to tell it based on autoradiogram. For your understanding I am starting from here. In plant 2 suppose the transgene has been integrated over here, as I mentioned earlier almost similar to our earlier scenario. Ok! The transgene has been integrated over here while in plant 3, 2 transgenes has been integrated. In plant 3, one transgene has been integrated in chromosome 1, another one transgene has been integrated in chromosome 2.

So, should we see any difference in autoradiogram? Let us see. So, here also I am telling it that the gene of interest is from a different organism we are using. Suppose we are using a Bt gene, a gene from *Bacillus thuringiensis* we are trying to over express in rice system. Ok! So, that particular gene of interest will not be available in normal rice genome, until and unless our transgene will be delivered, we should not see anything in the autoradiogram. Ok! And over here we must have to have some sizes, the size of the promoter is 1 kb, size of the gene of interest is 2 kb and this part is 0.3 kb. Let us assume this is 1 kb, this is 2 kb and this is 0.3 kb. So, in all of this cases our promoter, our gene of interest and our terminator has been integrated, has been integrated within the genome. Now let us try to make the banding pattern. I am telling it once again this type of banding pattern will not be detected in actual southern hybridization process, because once we will digest the genomic DNA, we will see smear thousands of bands will be there.

In the autoradiogram only we can see where our specific probe is binding. This thing I am drawing for your understanding. So, in plant 1, in plant 2 and in plant 3 what will be the scenario? In plant 1 how many bands will get? 1, 2, 3, 4 and 5 bands will be obtained right. 1 the smallest one is 1 kb, then thereafter will be having 1.5 kb, then will be having 2.5 kb, then will be having 3 kb and 3.5 kb. This is we are talking about the gel. In case of plant 2 how many bands we should get? One band, second band, then from chromosome number 2 will get band number 1, from here will get 2 bands, right? Because, in between the promoter and gene of interest a *Bam*HI site is available in our construct. So, we will get this band.

So, here also we have to think where the integration has been taken place. Suppose out of this 3 kb region this size is 0.5 and this size is 2.5 initially it was 3 right initially this whole part was 3.

So, now after 2.5 kb from here our transgene has been integrated. So, we will get a particular band of this it is size will be 2.5 + 1 that is coming from the promoter, that will be 3.5 kb while, another band will get from here, where our gene of interest, terminator and this 0.5 kb will be available right means 2 + 0.3 + 0.5 = 2.8 kb will be getting from here, then another 1 kb that is available. So, we will be getting 1, 2, 3, 4, 5 and 6 fragments, right? So, let us draw it we will be getting a band at around 1.5 in plant 2, we will be getting 3.5 kb band in plant 2, those are coming from here, then we will be getting 2.5 kb band in plant 2, then we will be getting another 3.5 kb band that is this one a 2.8 kb band 2.8 kb band and a 1 kb band. Ok! So, over here we had 1, 2, 3, 4, 5 bands, but over here we are having 6 bands. Ok!

How 6 bands? Because this is 1 kb, this is 1.5 kb, this is 2.5 kb, now we got 2.8 kb a new band ok, then two 3.5 kb bands we are getting one 3.5 kb is coming from here and another 3.5 kb is coming from here, because within this 3 kb region our T-DNA has been integrated right? So, basically double bands are available at this position. Now let us see what will be the scenario in plant 3, once we can understand this, then we will talk about the hybridization part once again. In plant 3, suppose the integration has been taken place at 3 kb away from here, let us assume this is 3 kb and this part is 0.5 kb. Ok! So, in this way we have to make the fragments from there one will be 1.5 kb, then over here will be having 3 kb + 1 kb means a 4 kb bands will be obtained over here. Ok! And since I am telling that, in this part, in this part of the chromosome 1. Ok! In plant 3 the integration has been taken place and, the integration has been taken place that place is 3 kb away from this position the 3 kb away from this position. Ok! So, it is after digestion with *Bam*HI, *Bam*HI will digest over here and this fragment will be 3 + 1 and this fragment will be 2 + 0.3 will be 2 + 0.3 that is coming from the construct plus 0.5, 0.5 is coming from the chromosomal part. Ok!

So, it means 2.8 kb. So, 2.8 kb will be coming from here and 4 kb is coming from there ok, chromosome number 1 is done. In chromosome number 2 will be having 2.5 kb bands that is coming from here will be having similar things which has been observed over here these 4 and 5 bands, that is 3.5 kb and 2.8 kb bands. Another 2.8 kb bands will be there will be getting a 3.5 kb bands. Ok! And finally, will be having a 1 kb band these things will be available in plant 3.

Now we are using gene of interest specific probe in this particular hybridization. Once we will use gene of interest specific probe in plant 1 no integration is there. So, the probe will not bind. In plant 2 once as we are using gene of interest specific probe which particular band will be highlighted means 3.5 or 2.8 definitely it will be the 2.8 because our gene of interest is this one the green one over here. So, the 2.8 kb band will be highlighted in plant number 2. This is not the 3 kb band, because within the 3 kb region that integration has been taken place 2.8 will migrate close to 3 kb. While in case of plant 3 what will be the scenario, as we are using gene of interest specific probe. Our probe will bind at 2 positions, 2 different locations one will get the same 2.8 kb. And another one will be also 2.8 kb. So, if two 2.8 kb bands are there, they may not be visualized properly they may not be separated properly. So, using this gene of interest specific probe we cannot distinguish it whether in plant number 2 and 3 same integrations has been taken place or not same number of integrations has been taken place or not could not be observed from here, but if we use probe 2. So, our autoradiogram will look like this in P_1 will not see anything in P_2 will see a signal at around 2.8 kb and in P_3, will see a relatively strong signal at around 2.8 kb because from here 2.8 is coming and from here also 2.8 will be getting which will be similar to this one. Now I am just using a different probe to analyze these 3 plants the P_1, P_2 and P_3 suppose the same digestion, same everything has been done we are using promoter specific probe. Once we will use promoter specific probe in plant 2 this part will be highlighted right? Because promoter is in this side this part will be highlighted. So, basically over here promoter specific probe will bind this part while in case of plant 3 promoter specific probe will bind over here its size is 4 kb and, another promoter specific probe will bind over here this size will be

same to 3.5 kb it will be similar to this one. So, once we will, here we have used gene of interest specific probe and here we are using promoter specific probe. Here in plant 2 will get a band at around 3.5 kb while in plant 3 will get 2 bands at around 4 and 3.5 kb. So, using 2 different probes we can confirm whether the single integration has been taken place in the transgenic lines or whether double integration has been taken place in our transgenic lines.

So, in this way the copy number can be determined through Southern blot analysis. In our next class again, we will discuss how different southern analysis could be done in detail. Thank you.