Course Name: Basics of Crop Breeding and Plant Biotechnology

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Lecture-47: PCR Screening

Hello everybody. Welcome to SWAYAM NPTEL online course on Basics of Crop Breeding and Plant Biotechnology. Today we will start that is on the Selection and Screening of Transgenic Plants and this is lecture 1. Here, we will be analyzing the Analysis of Transgenic Plants. So, these are the concepts which will be covered under this particular topic. First of all, we will be discussing the screening of transformants, then, we will discuss PCR screening method, how we have to design the primers for screening of the putative transformants, then gradually, we will move into the molecular screening of transformants, then how can we plan to screen the over expressed plants and how can we screen the silenced plants those things will be discussed and finally, screening of promoter-reporter constructs will be discussed.

So, let us start the screening of transformants. So, once we have to screen transformants, first we need to plan a particular experiment. Ok! So, let us think that we have developed a particular construct, where we are having a particular promoter, then under this promoter, suppose, we are trying to over-express a particular gene and we have a terminator. So, this whole thing suppose we have cloned in a binary vector.

Suppose, as a binary vector we are using pCAMBIA1301. So, in pCAMBIA1301, what is available? In this vector, left border is available, the right border region is available and in addition to that the origin of replication and those things will be there, the selectable marker gene for bacterial selection will be here. Let us assume, what is available in between the left border and right border region? Ok, the origin will be there, the bacterial

selectable marker gene might be here. Ok! So, within the left border and right border region, what is supposed to be available in pCAMBIA1301 vector? Over here, that is *hptII*, hygromycin selectable marker gene, is available.

Let us assume, this is the hygromycin selectable marker gene and for expression of this particular gene, definitely a promoter is needed and it is being expressed under CaMV35S promoter and thereafter a terminator is also available. This is the terminator region. So, this hygromycin construct is available within the pCAMBIA1301 vector. Thereafter within the MCS region, we have cloned our desirable gene, over here, the multiple cloning site was available. In this region, we have cloned our over-expression construct and thereafter in pCAMBIA1301 vector, another *GUS* gene is also available. Ok!

You know that GUS is a reporter gene. So, a *GUS* gene is also available under a particular promoter and a terminator is also there. So, this all things are available within the left border and right border region. So, if we use this particular construct for plant transformation through *Agrobacterium* mediated plant transformation, then what *Agrobacterium* will do? It will transfer whatever things available in between the left border and right border region, this whole part will be transferred into the plant genome. Ok! So, we have already discussed different plant transformation methods of rice calli or tobacco leaves either through *Agrobacterium* mediated transformation or through other different means direct gene transfer method.

So, suppose, we have started our experiment on tobacco, tobacco leaf disc was infected with *Agrobacterium* suspension culture and within that *Agrobacterium* strain this particular plasmid was available. Hence, the recombinant *Agrobacterium* cell was used to infect this leaf disc, tobacco leaf disc. And thereafter, within 7 to 14 days, we can see a lot of, a lot of shoot buds will be coming from the different parts of the leaf. Some

shoot buds may come from the intermediate position and from the surface area, most of the shoot buds will be coming out. So, at that time, we have to put something in the media so that we can screen the transformants, because, we are discussing the screening of transformants.

If we think about each leaf disc, suppose it is a leaf disc, 1000 cells are there, within this 1000 cells, the *Agrobacterium* will not deliver this particular LB to RB region in all the cells, maybe out of them, 10% cells will be transformed. So, suppose this cell, this cell and this cell has been transformed while some other cells are there, I am drawing it with red color that are non- transformed. So, until and unless we select it properly in normal media, the seedlings may come out from all of the cells. So, some selection is needed. We have already discussed about some positive selectable marker and negative selectable marker.

In positive selection, we have discussed about main A gene or other different genes, in negative selection, we can do through hygromycin, kanamycin those different antibiotics or using different herbicides etc. So, if we have to start our experiment using pCAMBIA1301 vector, we know that hygromycin resistant gene is there. So, in the media we have to add hygromycin. So, if we add hygromycin in the media, then those cells which are red in color, which may form the shoot-lets, which may form the shoots, their growth will be hampered and basically, they will be killed. While those cells where our transgene has been integrated, the region between left border to right border has been integrated those things will be grown in the media.

So, in this way, initially we have to screen the transformants so that we can reduce the plant population and those plants will be survived on the media where in 90% cases our transgene has been delivered. So, maybe if it is a leaf tissue, maybe one or two shoots might be coming from there at final stage, rest of them will be killed. Ok! We can see some black spot over there in tobacco, the calluses also formed the plantlets or shoots are also emerged in the media at the same time, but we have to continue the selection using hygromycin all the time. So, in this way, once we will get some putative transformants

means some putative plants which are being selected in hygromycin containing media, those will be further grown into rooting media. In rooting media, it will be grown, then ultimately the root formation is taken place. Ok!

So, in this way, initially we have to do screening based on the particular vector what we are using for plant transformation, based on that vector, we have to design that which particular antibiotic or selectable marker we are going to use, which type of selection we have to do, then ultimately, we will get some putative transformants. So, once we will get the putative transformants, next our subsequent screening through PCR will be started. So, before starting the PCR, once again, I am drawing this particular gene drawing this particular gene construct, we had a promoter under that promoter we had a particular gene and thereafter, we had a terminator. So, this thing was available in pCAMBIA1301 vector. In pCAMBIA vector, the left border and right border is there, over here, we have *hptII* gene, its promoter and its terminator and we have a *GUS* gene also over here with its promoter and terminator.

Now, we have already got the putative transformants means the transformants which are putative in nature, means, we are thinking that our transgene has been delivered over there. Now, we have to screen those plants through PCR initial screening. So, for doing PCR, first we need to isolate the genomic DNA, gDNA isolation then we have to design the primers then we have to analyze it through PCR. So, if such type of over-expression construct is used for selection purpose such type of construct is used for plant transformation. Suppose the gene what we have used that one is a rice gene suppose *OsGLP11* this particular gene has been used, rice germin like protein 11 gene has been used for plant transformation.

So, this gene has been taken from rice and we have done transformation in tobacco. In tobacco, this gene sequence is not at all available. In that case, we have to design the gene specific primer. Suppose, it is a control, tobacco leaf in control means it is untransformed plant. This is the untransformed tobacco leaf ok, while we are having one leaf from two putative transformants.

Let us assume take it as P_1 and P_2 those are putative transformants. We are thinking that over here our transgene has been delivered. For screening it, definitely we need at least a control plant, the untransformed control plant. So, thereafter we have to design the primer, we are talking about gene specific primer. We have used a rice gene for tobacco transformation.

So, that gene is not available in tobacco. Then if we use *OsGLP11* specific primer, suppose, we have designed the primer and it is supposed to produce an amplicon of around 500 base pair. Suppose, it is about to produce an amplicon of 500 base pair. Then, what are the things we need to screen through PCR? Here, we need to have a positive sample, we need to have a negative sample and other putative transformants 1, 2, 3 how many plants we are going to screen will use their genomic DNA also. So, in our PCR screening, we need to have a positive sample, we need to have a negative sample and different putative transformants. Ok!

Now, what will be used as a positive sample? For positive sample, we have to use this particular plasmid where our *OsGLP11* has been cloned. Ok! Basically, the plasmid is used, plasmid DNA is used as a positive control where our target gene is available and in PCR, we must get band from there. Within the negative sample, 2 types of negative samples are used, in such type of screening, one is the untransformed tobacco leaf sample where tobacco DNA will be there, but our gene should not be available, it has not been transformed and another one will be only water. In that sample, water will be added along with primers to check whether any artifacts are coming in the PCR reaction or not. So, thereafter, if we screen in this way, in positive we are supposed to get a band at around 500 base pair, out of these 3 plants, suppose, in one plant we are getting bands, in other 2, we cannot see anything and in negative plants we should not expect anything because *OsGLP11* is not available in the control tobacco plants. So, in this way, we have to do the PCR screening using the gene specific primer.

For confirming whether we are getting right result or not, we can use hygromycin specific primer also. If we use hygromycin specific primer, we will get similar type of result because in our vector *hptII* gene is available. In the putative transformed plants

which has been selected through hygromycin, there this hygromycin resistant gene might be integrated. So, it is showing the resistance mechanism and in the negative plants, we should not see such type of bands. Ok! So, using hygromycin may be at around 700 base pair we are getting the amplicon.

So, we can get it in these 2 samples. So, this type of PCR screening could be done for analysis of different over expression lines. Now, if we have to screen the gene silenced plants, how can you screen it, that is a very interesting question! So, now, if we have to screen the gene silenced plants, then the primer designing will be highly important. So, let us make a particular construct, suppose over here, our gene is available then we are having the linker region, the gene is available in another orientation and suppose we had our promoter over here and we had our terminator over here.

This whole thing is available within the pCAMBIA1301vector or something. Here, the hygromycin gene is also there, the *GUS* gene is also there, these things are available, this is the left border and right border region. So, once we have to screen the putative transformed lines which has been generated using gene silencing construct, how should we design the primers? Ok! If you carefully see these particular sequences, what sequence is available over here, the same sequence will be available over here, right? Similarly, the sequence which is available over here, that will be available over here also, because a particular sequence has been flipped in two different orientations to make this silencing construct, this is the hp-RNA part, right!

So, same sequence has been just placed in reverse and forward orientation or forward and reverse orientation. So, if we design the primer from here, then it will be highly tricky. So, for this screening, if we have to screen the putative transformed plants, we have to design a primer, we have to design a primer from the promoter region and we have to design another primer within the linker region. Ok! Because, this part will be available once within the transgene. So, to make it sure, we have to use a particular promoter that is not available in tobacco, right!

So, suppose, we are trying to silence a particular gene, that gene is available within tobacco. So, the control tobacco plants are there, means untransformed, we have tried to reduce the expression of that particular gene through gene silencing construct, right? Gene silencing is done to reduce the endogenous transcript. So, the endogenous transcript should be there, then only, it will be reduced. So, a tobacco gene will be used in this type of construct. So, in the putative construct, in the putative plant, putative transformants, suppose, we are playing with *NtSOS1* gene, *Nicotiana tabacum SOS1* gene, Salt Overly Sensitive gene. So, that gene was available within the untransformed plant and within the putative transformants also, this gene will be available. Just try to think about two different plants, this one was the initial untransformed plants, there this gene is supposed to be available and over here, the gene is fully functional, its mRNA will be produced, but as we have done RNA mediated gene silencing using this particular gene part in reverse and forward orientation, we are supposed to get reduced mRNA. We are supposed to get reduced mRNA in the putative transformants and this is the untransformed one. So, at the DNA level the gene is available in untransformed one as well as in the putative transformants one. So, as we are doing PCR screening at DNA level.

So, both the genes will be amplified, we may not see any difference, but if you think about this particular transgene, there the promoter is unique, the linker part has been taken from any unrelated species. So, these two primers will be unique, using this promoter and linker specific primer we can screen these two plants. Here also, we have to use the positive samples, using the plasmid DNA we have to screen the putative transform lines 1, 2, 3 and we have to screen the negative samples also. Ok! So, in negative sample, if we use such type of primer, promoter and linker specific primers, then we should not get any bands in the negative plant samples. While, in case of water samples, where only water will be used and primers will be used, there also should not get any bands, but if our transgene is successfully delivered, then in the transformed lines, we may get some band that will be migrating similarly in the positive sample also.

So, in this way, through PCR, we have to screen different transformed lines. In case of

over-expression construct, the primer designing is pretty easy, we can use gene-specific primer, we can use hygromycin specific primer. While in case of gene silencing construct, from the gene we should not design any primer for PCR screening, we have to design the primer from the promoter and linker part that will be unique. And over here, in the gene silencing construct also, we can use the hygromycin specific primer for base selection. Ok! So, in this way, the PCR screening is done.