

Course Name: Basics of Crop Breeding and Plant Biotechnology

Professor Name: Dr. Joydeep Banerjee

Department Name: Agricultural and Food Engineering

Institute Name: Indian Institute of Technology Kharagpur

Week: 08

Lecture-33: Backcross breeding through molecular marker (part-II)

Welcome back. So, we will continue again. Now let us discuss about, Back cross-breeding using Molecular Markers. So, the discussion we have already started. So, suppose we have got the F_1 , if we see our previous slide this is our F_1 plant here capital A capital B small r and capital D. This one was coming from one parent and small a small b capital R and small d it is coming from another parent.

So, let me shade this and our resistant gene is here. So, now in back cross breeding F_1 should be crossed with the recurrent parent right. So, let us cross it with parent 1 over here, parent 1 was the recurrent parent. Here we had capital A, capital B, small r and capital D.

If you think about gamete formation, what types of gametes will be produced from here? Over here I have drawn only one chromatid for each chromosome. So, for each chromosome at least two chromatids will be there, the scenario will be like this ok, different shades will be available over here. Now what will happen in between the non-sister chromatids of the homologous chromosome, crossing over will take place during gamete formation right? If you recall the meiosis process in pachytene this crossing over will be taken place and once the crossing over will be taken place, then different types of gametic combinations could be produced. In some of the gametes, this part may come from this chromosome, rest of the part may come from this chromosome.

In some gametes, these two parts has come from the P_2 plant, and rest of the part may

come from the P₁ plant. In some of them, this type of scenario may be there also. . Ok!
In this way, different genetic combinations could be created during gamete formation because over here the chromosome constitution two chromosomes are different right. Genes are the same, the same sets of genes are available, but most of them are in heterozygote condition. So, segregation will take place and if you think about the R gene capital R or small r over here the capital R will be available over here the capital R will be available while in this case, the small r will be available. Ok!

Let me tell you about the names of different alleles over here. So, it will be more good suppose capital A small b capital R and small d are available in this allele.. in this gamete. In this gamete over here, the small a capital B small r and small d are there. If you see this one here; small a, i.e., coming from parent 2 you can recall capital B, capital R, and capital D. Ok! This type of different combination may be there, while if you think about the gamete being produced from this recurrent parent only, one type of gamete will be there because they are these homologous chromosomes, they are homozygous in nature for all the genes.

So, only one type of gamete, will be produced from this particular one right? So, once they will fuse then we will get different sets of BC₁ plants, because BC₁ generation is being created F₁ is crossed with one recurrent parent. Let us assume this one is coming and fusing with this one what will be the scenario? The scenario will be so, here small a capital B capital R and capital D, while from here capital A capital B small r and capital D is coming. So, this could be the genotype of a particular BC₁ plant we can screen it through the molecular marker, if we recall the foreground selection, first we need to select it whether our capital R allele is there or not because, we have already developed a molecular marker to detect capital R i.e., responsible for the resistant trait. Then using the along with the forward selection if we think about the other selection process for rest of the genes ok then using molecular marker we can tell that ok capital B is there while for A gene some heterozygote condition is available, but for capital B gene and for capital D gene it is almost close to our recipient parent recurrent parent means i.e., our target.

So, in each and every generation of back cross first we need to screen for the foreground selection then we have to do for the background selection. Ok! So, now through some models let me discuss this particular process once again, i.e., the objective of doing backcross breeding using molecular markers. Suppose this is an elite rice plant, as I was mentioning earlier it can produce a good amount of yield, but it is susceptible to a particular disease, while we have another rice line suppose this one was our P_1 and this one was our P_2 and we have another rice line its yield is very poor, but it is tolerant to a particular disease the disease resistance gene is available over here. Now what will be our target in backcross breeding, our target is to transfer this particular resistant gene in the elite rice plant background. So, that the disease-resistant elite rice plant could be generated.

So, i.e., our target. So, using that what I have mentioned earlier mentioning different genes A, B, R and D here we will be discussing about the different colors. So, your understanding will be a little bit clearer. So, we have a particular plant parent 1 i.e., being used as a recurrent parent, while we have another donor parent i.e., containing the resistant gene, but for the rest of the gene, it is inferior in nature. If you cross them will get the F_1 one chromosome, will come from each of the parents and these are the different gametic combinations which will be produced from this particular F_1 plants, ok because crossing over will be taken place in between the non-sister chromatids of the homologous chromosome.

So, these different combinations, earlier I mentioned about 4 genes only, but in a chromosome 100 genes are there. So, a lot of combinations will be generated and these gametic combinations, basically will be produced from this F_1 plant, and suppose we have taken a particular gamete a particular gamete of F_1 that has been fused from a particular gamete coming from the recurrent parent, because one gamete will be produced from F_1 and one gamete will come from the recurrent parent after fusion will get the BC_1 plant, right? The first back cross-generation. So, in the BC_1 plant also different two chromosomes will be there in BC_1 in one chromosome all the genes are available from

parent 2, while in another chromosome in the BC_1 plant, one chromosome will be having i.e., similar to parent 1 while another chromosome is there and that is mixed like this. So, here again, recombination will take place during gamete formation, and suppose from that recombination, we have got one gamete like this and it has been fused with the gamete coming, from the recurrent parent and we got the BC_2 plant. So, if you think about this particular process as we are progressing from BC_1 to BC_2 . Ok! Our genetic constitution of the recurrent parent, is being more in the further generation in the subsequent generation. Ok!

Since, if you see over here the blue color is being more in the subsequent generation because, we are crossing it with this particular one again and again. So, maximum genome recovery of this one is being taken place. So, in this way from the BC_2 plant after recombination, this type of different types of gametes will be produced. Once the gametes will be produced crossing will be done, and we will go to BC_3 generation. In BC_3 generation, first we need to screen the plants whether that particular resistant gene or gene has been transferred or not i.e., foreground selection.

Then, we have to do the background selection for the rest of the genes, which means the total chromosome will be close to the blue-colored one right other than the red-colored one the resistant gene. So, ultimately after several generations of back-crossing followed by selfing ultimately this, type of elite disease-resistant plant will be obtained. And this is the way through which we can do the molecular marker-assisted breeding in the back cross. So, this is the process once again in a nutshell first we need a donor and a recipient plant so, for the donor we had earlier one plant 2 for the recipient we are using plant 1. So, we will get F_1 first test for true hybrid using a DNA marker, we have to make sure that whether our F_1 is a true hybrid or not or if it is the self-product of the female plant.

Next, the F_1 will be crossed with the recipient one or recurrent parent then we will come to the BC_1 , in a back cross one generation is what we have to do, we have to do foreground selection and recombination selection from one side. The recombination selection, I have mentioned earlier suppose this is our target gene, we have identified a

marker over here we have identified a marker over here. Suppose recombination selection will be done from one side along with our desirable gene. Next, we can go to back cross 2 generations; by crossing the back cross one plants which plants are suitable by crossing it with the recurrent parent we will go to the BC₂ plant. And over there also we have to do the foreground selection and recombination selection from another side, over here if we use M₁ marker, here we have to screen with M₂ marker. Ok!

Then, in the third case once we will go to the BC₃ generation, ok, then foreground and recombination should be confirmed using both sides of marker the marker available on the both sides, of the gene should be used along with the foreground selection. Ok! Then the background selection starts from here, means if you recall the homozygous how the homozygous percentage is increased. Ok! In selfing, we know that from F₂ generation in each and every generation of selfing 50 percent homozygous, it is increased. Similarly in the backcross also the homozygosity will be increased in the same rate and in BC₃ generation, means three generation of back cross has been done. So, almost close to 87 % homozygosity is there.

So, here from we have to start the background selection in the field and once the background selection is done then those plants will be grown or taken to the further generation. Ok! And, background selection on some best lines will be done using molecular markers through the field we have to see them we have to check them, whether phenotypically they are showing similarity to the recurrent parent or not based on that, we can screen it then we have to confirm it by background selection then we will go to the next generation. And at the end 2 to 4 improved lines in multiple location field trials, will be sent once we can identify some lines after BC₄ or BC₂, if BC₃ F₃ generation. Ok! Then if through background selection we can tell that, more than 99 % genome has been recovered from the recipient parent along with our target gene, which is which has to be confirmed through foreground selection, is there a few improved lines that should be sent for the multi-location trial for varietal release and those things. So, these are the references you guys can go through it, if you have any doubts, I think your concepts will be more clear. Thank you.