Course Name: Basics of Crop Breeding and Plant Biotechnology

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Lecture-26: Applications of Reverse Breeding

Welcome back. So, we will continue again. Now, third things what is needed for doing this type of reverse breeding, one was selection of genes. Those genes could be selected and through RNAi strategy we can silence the function of those genes. We can inhibit their protein production mechanism. Next one, we have discussed about double haploid production.

And third one, we need to identify the suitable plants based on marker assisted selection from the double haploid progeny. Ok! So, let us assume this is a particular F_1 plants and different markers. Suppose, we are talking about a particular SSR markers, once we will be covering the molecular marker parts, this thing will be clearer. Suppose, we are talking about a simple sequence repeat markers in this particular F_1.

We have identified a plant from the field, it was found to produce in SSR, suppose, different SSR markers are used 556, 759, then 1391 these are different SSR markers assume. This 556 marker, is supposed to produce 200 base pair amplicon in this F_1. This 759 marker is supposed to produce 350 base pair amplicon in this marker, in this F_1 plant, in this F_1 line. Ok! 1391 this SSR marker, maybe, it is producing 100 base pair amplicon in this F_1 line. Ok! These things, first, we need to identify means what F_1 or what heterozygote plant we are selecting at the initiation process.

Then, we need to switch off the gene expression mechanism through post transcriptional gene silencing by RNAi construct. Then, we need to develop the double haploids, later

on, we will describe the steps once again and suppose these are the different double haploid lines we have generated. And those double haploid lines will be screened using these SSR markers once again because, our target is to identify complementary lines. We need to identify two lines which are complementary to produce this particular F_1 or hybrid or heterozygous individual. So, if you see over here, among these two lines in first one for SSR marker 556 what we were using here, this band is available of 200 base pair.

Let us assume this is the double haploid line 7, and this is the double haploid line 6. In double haploid line 7, the 350 base pair band is not available, maybe, some different band is there. Well, if you consider the double haploid line 6 here, that band is available in case of SSR 759 we can get the 350 base pair amplicon. It means, this two can complement each other, this one is not available here, it is available here, while, the SSR 556 is available in DH 7, it was not available in DH 6. In this way, the double haploid 6 and 7, they are showing perfect complementarity.

So, these two plants will be chosen, these two double haploids will be chosen as a parents through this reverse breeding approaches. Now, we will be discussing about the process of reverse breeding that is the crossing of desired double haploid lines to develop superior hybrids. Ok! So, to describe this process, first, we need to start from our parents for your normal standing. Suppose, we had two different parents P_1 and P_2, this is the one homologous chromosome and this is another homologous chromosome. Ok! Suppose in P_1 parent this is chromosome number 1 and 1′ and this is chromosome number 2 and 2′. Ok!

While in plant 2, I am just drawing over here, only one set of chromatids for easy understanding. In plant 2, we are assuming that both the parents are diploid and diploid parents will work properly in reverse breeding approaches. This is the smaller chromosome this is 1, 1′ and this is 2, 2 ′. Suppose in this individual, our chromosome was shaded. Shaded means suppose over here, capital A capital B, these genes are available while over, here small a small b these genes are available.

This is the second pair of chromosome in parent 2. So, in this way, suppose, initially we had two different parents. So, in conventional breeding, we are we have identified parents, we are getting F_1. So, what will be our F_1? One set of chromosomes will be coming from here and one set of chromosomes will be coming from here in naturally. So, F_1, F_1 will be like this, means 1, 1′, 2, 2′.

So, one set is coming from here and one set is coming from there. So, the chromosome structure will be like this. So, suppose here, capital A capital B capital C these genes were there and here, small a small b small c these genes were there. While over here, suppose, capital D capital E was coming from here, small d and small e is coming from here, in this way, from two parents, our chromosome have come in the F_1 hybrids. Now, once we are doing selfing of this F_1.

So, once we are getting the recombinants, different combinations will be taken place conventionally. Still now, we have not started the reverse breeding approaches, this is the conventional breeding process we are talking about. Ok! So, over here, in conventional breeding, no reverse breeding approaches has been taken. So, crossing over will be taken place right! Crossing over will be taken place between the homologous chromosomes. So, finally, different types of recombinants will be obtained.

Suppose, this is the recombinants of a particular one, we should draw at least two pairs of chromosomes, right? Suppose, we are talking about three different lines where recombination has been taken place and, in each line, we are drawing the chromosome number 1, 1′ and 2, 2′. In this way, different combinations will be there. So, due to recombination suppose over here, this type of combination may be generated in one, in another one due to crossing over, this type of things may be available over here. Suppose, this type of combination has been taken place over here, suppose, this type of combination has been taken place.

Suppose, in this way, various combinations have been created in different recombinants

right! Since we know that if the condition was in F_1, A a B b C c; then different number of combinations could be generated, for each and every gene, at least three combinations will be created for A gene: A A, A a, a a. For B gene: B B, B b, b b in this way, different combinations will be created and they will be permutated together, permutation-combinations will be taken place among these different genes. So, eventually, we can get a different type of recombinants. Ok! So, suppose out of these recombinants, if we think about the gene over here, our gene was capital A capital B capital C right?

So, in this one capital A capital B capital C was there, while here, capital A small b was coming right, suppose capital C was there. In this way, different genetic constitutions will be created, over here it will be capital A capital B capital C, but here it will be small a capital B capital C; in this way, different combinations could be created right. So, now suppose, we are thinking that this one is performing best in the field. Suppose, we have identified these particular recombinants that is showing best performance in the field. So, this is our initial thing, this is our initial combination in the reverse breeding approach.

So, till now, we have discussed about the conventional breeding strategies, two parents were there, we have crossed it, we have got F_1 in next generation or in next-to-next generation due to selfing and recombination we have got different recombinants. And out of these recombinants, based on its field performance or based on its some quality issues, quality parameters, we have chosen a particular individual, its genetic constitution is like this. So, we should start our reverse breeding from here to identify the parents in such a way, so that, easily we can get this plant again and again, that is our motto, that is our objective. So, let us draw this particular recombinant once again. So, we had the combination like this for chromosome 1 and like this for chromosome 2 means 1, 1′; 2, 2′.

This combination we got, it was found to be best in the recombinants. So, we have identified these combinations, we can identify these combinations through molecular markers also. Through molecular marker, we can tell which allele is available over here and which allele is available over here, means, whether for this particular allele it is

heterozygous or not. For this particular allele available over here, it is heterozygous or not, we can identify from here, those recombinants will be identified through molecular markers first. Then, we need to transfer the T-DNA or we need to transfer the transfer DNA.

We know about the transfer DNA available in *Agrobacterium*, basically, it is a material, it is a genetic region available within the Ti plasmid of *Agrobacterium*, *Agrobacterium tumefaciens*. In *Agrobacterium tumefaciens*, the Ti plasmids are available that is tumor inducing plasmid. So, once we will discuss the *Agrobacterium* mediated transformation process, we will discuss it in more detail. So, for the time being, we are just telling that by modifying the original Ti plasmid, different vectors are produced nowadays. Those vectors are used for plant transformation, different binary vectors are used.

So, in those binary vectors, some T-DNA region is available means left border and right border region is available. So, whatever we put between left border and right border, basically, that part, that T-DNA or transfer DNA part is transferred in our targeted plant. So, within this left border and right border region, within this T-DNA we can put the gene silencing construct what we have used earlier. Earlier, we have made an RNAi construct of RecA gene or different genes. So, in this way, we can put our RNAi construct over here.

So that, through the *Agrobacterium* mediated transformation, this T-DNA could be delivered within the plant genome or using the non- *Agrobacterium* mediated transformation also we can deliver this T-DNA region into the plant genome. So, now assume, this one was our suitable recombinants, we have delivered the RNAi construct through T-DNA within this plant. Ok! Its genetic constitution initially was known to us like this. And now suppose, our transgene has been integrated over here in the chromosome number 1′ our transgene has been integrated over here in chromosome number 1′ in a particular plant. So, we got a particular transgenic line where our transgene has been integrated over here.

So, if our transgene is integrated over here, in this plant, this transgene will be expressed means from this particular transgene, the siRNA will be produced and those siRNA will eventually block the recombination process in this particular plant, isn't it? So, in this particular plant, the recombination process will be hampered. So, if recombination process is hampered. So, the recombination among these two chromosomes, sorry, the recombination among these two chromosomes will not be possible and let us see what types of spores will be produced from this plant. So, from this plant, 4 different spores will be produced, 4 different types of spores could be produced.

This is the larger chromosome, this is the smaller chromosome, this is the larger chromosome, this is the smaller chromosome, this is the larger chromosome and this is the smaller chromosome. So, now, over here, what type of combinations could be created? 1; chromosome number 1 can go with chromosome number 2; in chromosome number 1 we had shaded over here. It can grow with chromosome number 2 in a particular spore. Then chromosome number 1 can go with chromosome number 2′. Ok! So, 1 is going with chromosome number 2 here chromosome number 1 is going with chromosome number 2′.

Then the chromosome number 1′ can go with chromosome number 2, the chromosome number 1′ can go with chromosome number 2 and last one, the chromosome number 1′ can go with chromosome number 2′, right! In this way, 4 types of combinations could be produced 4 types of gametic combinations or spore combinations could be produced from this plant. Now, suppose, only one T-DNA integrated line will not get, maybe, we have to get a number of transgenic lines. Suppose, in another line, we got another transgenic line; here, chromosome the T-DNA integration has been taken place in chromosome number 2. Ok! Let us assume, here, the integration has been taken place in chromosome number 2.

This one was 1 and 1′; 1 and 1′ and this is 2 and 2′; in 2, this one was the structure, in 2′, this one was the structure. Now, let us assume that over here, our T-DNA has been integrated, this is our T-DNA. In this case, this one was our T-DNA. So, let us see what

type of gametes will be produced from here, herefrom also 4 types of gametes will be produced. Let us assume, this is the larger chromosome, this is the smaller chromosome, this is the larger chromosome and this is the smaller chromosome.

So, we will get 1 and chromosome 2 over here 1 and 2 we will get 1 and chromosome 2′, 1 and 2′, we will get 1′ and 2, in 1′ the situation was like this and 2 and we will get 1 ′ and 2′, in 1′ it is like this and in 2′ it is like this. So, this type of combinations will be available. Now, if we think about our T-DNA which has been integrated over here, that will be transferred in the next generation also that transgene. So, over here, wherever the 1′ is available try to see it carefully, wherever the 1′ is available, like over here, our T-DNA will be available, over here our T-DNA will be available. Ok! While, if we see the gametes produced from here, wherever the 2, chromosome number 2 is available, then our T-DNA will be available there and our T-DNA will be available over here.

Now, our next step, we have stopped the recombination process we have identified the spores, now, we have to make the double haploids. So, during double haploid production we can use our molecular markers and using molecular markers, we need to identify those plants after double haploid formation which will be complementary in nature. Right? So, for being complementary in nature, let us assume we have selected this one as our targeted double haploid. So, let us see what will be getting from here, if we make double haploid of this plant what will be getting? If we make double haploid of this plant, the upper part will be shaded and this lower part will be shaded, this is the chromosome number 1, 1′, 2, 2′and suppose over here, we have made the double haploid from this one. Ok! Herefrom, what will be the condition of this double haploid? This will be the first set of chromosomes, the larger one, this will be the smaller set of chromosomes, we are making double haploid lines, from this haploid it will be doubled, the chromosome number will be doubled.

So, now try to see it carefully, this is suppose, double haploid line X, this is the double haploid line Y. So, if we cross this two plant can we get this line? Can we complement both of them? Over here in chromosome number 1, the shaded region is available that

may come from here, over here in chromosome number 1′, the shaded region is available it may come from here, in chromosome number 2, the shaded region is available on top that is it is not available over here, it is not available over here also. So, these two double haploid lines will not be complementary based on molecular markers, we need to identify it. Now, let us assume we have attempted double haploid from here, let's see what will be getting this two are larger chromosomes and this two are smaller chromosomes right? The constitution will be like this and this in the double haploid.

Now, if you see carefully this one, like suppose, double haploid new this one will be complementary with this one, means if we cross between this 2, if a cross is made then chromosome number 1 and 1′, 2 and 2′ it will be perfectly complementary like our suitable recombinants. So, this is the process in nutshell through which we can do the reverse breeding. Now, what are the end product of reverse breeding? The end product of reverse breeding is similar to the parental lines, we are trying to identify the parental lines. So that, whenever we need, we can make our targeted heterozygotes we can produce our desirable heterozygotes. Then, there will be no change in the genetic composition of the reverse bred plants, since RNA silencing is only restricted to meiotic crossing over.

If you see the earlier slide once again, whatever the lines we have generated, the double haploid lines we have generated complementary double haploid lines, the RNAi or T-DNA portion is not at all available over there right! The T-DNA portion is not at all available over there, means, T-DNA has been used for recombination process to block the recombination process only. Thereafter, we took those pores where our transgene is not at all available. And next one, the resulting offspring through reverse breeding is non genetically modified, our transgene will not be delivered. Now, what are the applications? First of all, reconstruction of heterozygote germplasm, what we have told just now, how the heterozygote germplasm could be reconstituted, how we can develop parents for that and we can reconstitute it, that is one major application.

Next one, breeding on single chromosome level. Ok! So, when reverse breeding is

applied to F_1 hybrid of known parents, chromosome substitution lines can be obtained. So, that is another important application of reverse breeding that is developing chromosome substitution lines. Ok! The homozygous chromosome substitution lines serve to be the novel tool for study of gene interaction. Ok! Later on, once we will discuss about the chromosome substitution line development, then we will discuss this part once again, the homozygous chromosome substitution line, how it may work, those things. Then it is possible to produce hybrids heterozygous for one chromosome.

We can produce the hybrids which will be heterozygous for only one chromosome. The offspring of such plants will show segregation for traits present on that chromosome only. The offspring of such plants will show segregation for traits present on that chromosome because the hybrid we are developing that will be heterozygous for one chromosome only. For rest of the chromosome, it will be same no variation is there, only one chromosome is differing between the parents. So, whatever the genes available on that chromosome, that will show segregation for rest of the gene will not see any segregation and improve breeding lines carrying introgressed traits can be produced. Ok!

So, let us see the how breeding on single chromosome level could be done. So, let's think that we have a particular hybrid. Sorry. So, before preparation of the hybrid let us think that this is our parents. So, suppose two sets of chromosomes are there, this is our parent 1 and it is being crossed, this is being crossed with parent 2.

In parent 2, different sets of genes are available as we are considering earlier also, this is the larger chromosome, this is the smaller chromosome, this is the parent 2. We have made a cross among them and we got a F_1 we got a F_1 like this. Ok! So, this is chromosome 1, 1′, 2, 2′; this is 1, 1′, 2, 2′. Ok! So, one set of chromosome has come from this parent, one set has come from this parent and after fusion we will get this F_1. Now, what types of gametes will be produced from there ok? Suppose, we found that this F_1 is desirable and in this F_1 we have attempted our reverse breeding approach in F_1 we have started our reverse breeding approach.

So, from here, what types of gametes will be produced? This will be one gamete, one gamete will be like this, one gamete will be like this and another gamete will be like this. So, these 4 types of gametes will be there, suppose, if this is 1, 1′and 2, 2′. So, in one case, 1 and 2 will be coming, one case 1 and 2′ will be coming, here 1′ and 2 and 1′ and 2′; these combinations could be developed. Now, we can do the double haploid production. So, let's see what will be the double haploids producing from here, this will be double haploids produce from here, herefrom this will be double haploids produced, from here we are producing double haploids, right!

This will be the double haploid from this particular gamete, and over here, this will be the double haploid produced from this particular gamete, right! In this way, double haploid will be produced. Now, if we start back crossing of this double haploid individuals with any one of this parents what will be the scenario? Our target is to develop some chromosomal substitution lines. If you see over here, if you see over here, here, one chromosome has come from this parent fully in this double haploid and one chromosome has come from parent 2 fully, right! Suppose, in spite of having two chromosomes, more number of chromosomes are there, this is just for your understanding just take this plant and if we do back crossing with our parent 2.

Suppose, we are taking parent 2, this is our parent 2 this one was and in back crossing what is done? F_1 is generally crossed with any one of the parents, right! Suppose, we are back crossing this F_1 or this double haploid with parent 2, we are trying to cross it with parent 2 again and again repeatedly. Then, what will be happening? For most of the genes, most of the chromosomes, it will be similar to parent 2 and in this way, we can develop a line where only one chromosome will be coming from a particular parent and for rest of the chromosomes, for rest of the chromosomes it will be like the parent 2. Ok! In this way, we can consider the breeding on single chromosome level. Ok! So let us discuss about the applications once again, the reverse breeding generates offspring complementing the desired parental traits.

The process involves using molecular markers for rapid trait identification, the process

involves the application of molecular markers for rapid trait identification. High throughput genotyping accelerates the identification of the complementing parents in population of double haploid lines, otherwise, it would be very difficult until and unless we do proper genotyping through molecular markers or something else, it could be difficult to identify good complementing parents from the double haploid individuals. Then, aids in studying gene interaction in heterozygous inbred lines. Ok! The quick identification of QTLs is possible by screening of population that segregate for traits present on a single chromosome. So, if we can make the chromosome substitution line, the traits available on a single chromosome, for that we can identify the QTLs also.

Then, chromosome specific linkage maps can be generated, it assists in detailed mapping of genes and alleles, the detailed mapping of genes and alleles could be done and it contributes to understanding the nature of heterotic effects in breeding, means, suppose five sets of chromosomes are available. Four chromosomes we are taking from a particular parent, only one chromosome is we are taking from two different parent, means, chromosome number 1 and 1′. Then we can understand the heterotic effect, whether we are getting heterotic effects for those genes available on chromosome number 1 or not those things could be identified. So, what are the limitations? First of all, the application of reverse breeding is limited to those crops where double haploid productions are commonly practiced because double haploid production is needed, we are stopping the recombination. So, we are getting the gametes after that and we have to make the double haploids that is needed and it is done in case of cucumber, onion, broccoli etc.

Then double haploid plants are rare occurrence or not formed at all, in some plants, in some plants like cotton, soybean, lettuce, tomato the double haploid production is very difficult. So, in those crops, this reverse breeding may not be applicable. In crops with haploid chromosome number of 12 or less, this technique can be easily applied if the haploid chromosome number is less or equals to 12, then we can try it. If the number of chromosomes is more, then it could be difficult, the process could be highly difficult. And the reverse breeding method can be applied to those plants where spores can be

generated into double haploids, means the haploid culture or anther culture should be applicable in those crops.

Otherwise from haploids, pollen grains we cannot develop the plants or we cannot make the double haploids. So, what are the conclusions of reverse breeding? First, reverse breeding introduces a novel breeding approach. It accelerates the breeding process by starting with superior hybrid selection, we start our process by selecting superior hybrids. Next, this method increases available genetic combinations and diversity. This particular method increases the available genetic combinations and diversity, means, if we can identify some important heterozygous plant, its parental lines could be generated.

Maybe in a couple of years after, we are identifying another one suitable line that is more adaptive under that particular scenario under the environmental conditions at that region. Ok! Then based on that, we can identify or we can make its parents once again. Ok! So, the available genetic combinations and diversity could be increased. Then large number of plants are generated, screened and generated without prior knowledge of their genetic constitutions. So, that is the things, means initially, large number of plants could be generated in the field, it should be screened without their prior knowledge, but once, we will get the suitable one then we can apply the reverse breeding.

The approach facilitates the identification of superior plant hybrids and the reverse breeding, reverses the conventional breeding sequence by first selecting hybrids or the recombinant one, and then recovering their parental lines. So, first, we need to select the hybrids or recombinants then we need to recover the parental lines. So, these are the future prospects, first of all the RNAi mediated reverse breeding is a juvenile method which needs vigorous study to overcome technical problems. The efficiency of double haploid production needs to be improved the crops like cauliflower, cucumber, onion, broccoli, where seed production is very difficult in those crops. So, in those crops, reverse breeding can be applied for the production of hybrid plants.

So, these are the references, you guys can go through this particular manuscripts if you have any doubts throughout this course. Thank you.