Course Name: Basics of Crop Breeding and Plant Biotechnology

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Week: 06

Lecture-25: Introduction to Reverse Breeding

Hello everybody. Welcome to the SWAYAM NPTEL course on Basics of Crop Breeding and Plant Biotechnology. This is the lecture that is on reverse breeding. So, these are the concepts which will be covered in this particular part. First one, what is reverse breeding? We have heard about different conventional breeding strategies earlier, right? Now what is reverse breeding? We would like to know over here.

Then what is the process of conducting reverse breeding we will be discussing. Then the difference between the conventional and reverse breeding will be discussed. Then why reverse breeding is needed? What is the importance of reverse breeding? Those things will be discussed. Then gradually we will move into the different steps of reverse breeding procedure means, at least three different steps are involved there we will be discussing those things.

Then regarding the applications the crossing of desired double haploid lines, to develop superior hybrids will be discussed through reverse breeding approach. Then we will discuss about different applications of reverse breeding. What are the limitations of reverse breeding? It may not be applicable for all the crops; certain things are required. So, we will discuss about the limitations, and what are the future prospects. So, those things will be covered in this particular topic.. under this particular topic.

So, let us start reverse breeding. First of all, it is a novel plant breeding technique which

is designed to produce parental lines directly from any heterozygous plants. If we think about the conventional plant breeding, in conventional plant breeding, in conventional plant breeding, let us assume, that we have a plant P_1 and we have another plant P_2. So, P_1 is having genotype capital A capital A small b small b and P_2 is having genotype small a small a capital B capital B. So, we would get F_1 having genotype capital A small a and capital B small b, capital A and small b will come from this, and small a and capital B will come from this, after fusion of this male and female gametes will be having this F_1.

Now, if we go to in F_2 generation what we will see? In F_2 generation different combinations will be produced some of the lines will be having capital A capital A small b small b. Someone will be having small a small a capital B capital B, someone will be having capital A capital A capital B capital B, someone will be having capital A small a capital B capital B and so on. In this way, different plants will be available in F_2 generation, or F_3 generation. These are known as F_2, F_3 these are known as segregating generation, right, because from here the segregation starts within the different genes available on the chromosome. So, due to this segregation different genetic constitutions could be available in F_2 onwards.

So, through reverse breeding approach from any heterozygous plants, it may, it may be in the F_1 generation or it may be in the segregating generation. There from we have to start our procedure to get back the desirable parents. Ok! So, now Driks et al in 2009, he basically proposed the term reverse breeding, and it has not been commercialized yet. So, a lot of labs, as well as companies are working on it has been optimized in some of the crops later on, we will discuss, but it has not been commercialized. Now this is the process outline, the brief process through which the reverse breeding is done.

First, as I was mentioning this is the heterozygous heterogeneous population. Let us assume, we are dealing with a cross-pollinated crop. We have a maize field, in this maize fields several plants are available and open pollination is practiced. So, different plants are available, over there open pollination is practiced. So, if we identify some suitable plants, its genetic constitution might be unique compared to others, because open

pollination is taken place.

In cross-pollinated species the heterozygosity is maintained and this population will be heterogeneous, also not all the plants will be similar in nature. Ok! Due to different combinations of different genes, different alleles we may see the heterogeneous population in cross-pollinated species. So, from that heterogeneous heterozygous individual we can identify a suitable plant that is a suitable hybrid or suitable heterozygous one. So therefrom, we can start our breeding, this reverse breeding. So, first superior heterozygous plant of unknown parentage, because we do not know that this plant has been generated by mixing of male and female gametes of which plant in the earlier generation, because the open pollination was practiced over there right.

So therefrom, we are initially identifying the final product, that this is the best heterozygous individual in this heterogeneous population. Then we will apply the reverse breeding procedure on to this plant and finally, we can develop parental lines, we can develop two different parents. So, if we cross those two parents again, we can get this particular heterozygous superior heterozygous plant. So, if we assume, this as our final end product, ok, we are trying to make two suitable parents for this particular end products means, if these two parents are crossed anytime we can get such superior heterozygous lines in future. Now, this slide shows the difference between reverse breeding and traditional breeding that is conventional breeding.

So, in conventional breeding or traditional breeding what is practiced here, first we select the lines, as I was mentioning first, we select the lines, therefrom we start our experiment. If we think about in self-pollinated crop, in cross-pollinated crop first we need to select the parents then ultimately, after crossing it we can produce the hybrid seeds, or we can go to a couple of generations to get a suitable individual we may identify the transgressive segregants also. Ok! So, we start from the suitable parents ok, while, in case of reverse breeding we start from the heterozygous plants the selection of heterozygous plant is done from the field. Then gradually through reverse breeding approach we will try to identify the different types of parents, which will be crossed to make this parent

will be developed basically from the heterozygous individual. Ok! So, production of parental lines is done from the heterozygous individuals through reverse breeding.

So, basically the selection of end product at the start of the breeding cycle is done, in case of reverse breeding because our end product will be this, that was found to be highly suitable in the field. Ok! So, we are starting from this end product, in case of reverse breeding. Now, why reverse breeding is required? First of all, since maintaining hybrid stability is the difficult task, we can go for reverse breeding. If we consider about maize ok, in maize or in most of the cross-pollinated crop, the crossing is taken place between different plants. So, each plant is heterozygous in nature, suppose different plants are there its genetic constitution will be different from this one, will be different from this one.

So, if open pollination is taken place, pollen grains can come from here, as well as here in this plant. So, different types of seeds will be produced from this plant, right. So, next generation its progeny may show some difference. So, in this way, suppose, a hybrid variety has been developed in cross-pollinated species. So, in next generation if we have to get the similar type of performance, those parental combinations should be used otherwise, the stability of the hybrid will be diluted, its quality will be reduced eventually, due to mixing of different gametes, right?

So, we can develop the proper parents of this one through reverse breeding. Next one, the parental lines need improvement first, so as to improve hybrid performance. Suppose in cross-pollinated species we are mixing two populations, right, two populations are basically crossed. Here, different plants are there not a single plant, sometimes single plants are used sometimes, inbred lines are used for hybrid development, but in most of the cases, one inbred line is crossed with an open-pollinated variety like that. Ok! Based on GCA, SCA general combining ability, specific combining ability we have discussed it initially.

So, based on that we can attempt the cross. So, we are not sure that which cross

combination will give the maximum potential, will give the maximum yield or maximum suitable performance. Ok! So, there is a need to improve the parental lines also. So, if we start from our end product, and if we identify its parents, if we can develop its parents that would be best option. Ok! Because if we cross these two parents, every time we will be getting this superior individual.

Third one it is difficult to establish breeding lines for uncharacterized heterozygotes. As I was discussing in cross-pollinated crops here, different heterozygotes are produced each seed are heterozygotes, heterozygotic in nature. So, next generation once we will grow it then, only we can see its performance. So, those are in uncharacterized state. Ok! So, the breeding lines for these uncharacterized heterozygotes means, which breeding lines, which combination of breeding lines will be suitable.

This identification will be difficult, but through reverse breeding we can easily do it. Next one parental genotype is preserved by clonal propagation or apomixis. Sometimes the parental genotypes could be maintained by clonal propagation means, by vegetative propagation or apomixis, but further improvement is hindered by the adaptation of the parental lines. If we maintain it through apomixis or clonal propagation we may face some problem in its adaptation because, in each and every year the climatic scenario is changed. So, its adaptability will be changed also, maybe its progeny which was suitable in 2001 it may not be suitable in 2005.

So, suppose we are maintaining the parents through clonal propagation in 2005, its progeny performance may not be up to the mark. Ok! So, the new traits development will be prevented, or the further enhancement of the traits will be prevented, though, if we go through this approach, if we do the clonal propagation or apomixis of the parental lines. So, if we start from the end product, and if we can identify the suitable parents, it would be better. These are the different steps of reverse breeding. So, first step is suppression of meiotic recombination. Ok!

One thing is mentioned here meiotic recombination. If you recall the mitosis and

meiosis, which was discussed in Mendelian genetics class during meiosis, what happens 2 steps are there meiosis I and meiosis II. In meiosis I, what happens, the homologous chromosomes are separated in meiosis I, the homologous chromosomes are separated. Ok! Suppose one pair of homologous chromosomes is being shown here. So, this 2 will be separated, this will go in a particular pole, it will go in another pole in meiosis I.

While in meiosis II the chromatids of each chromosome will be separated the chromatids of each chromosome will be separated. Now in meiosis I, different steps were there in meiosis I in prophase; leptotene, zygotene, pachytene, diplotene, diakinesis, different stages were there. So, in pachytene stage the crossing over is taken place, between the non-sister chromatids of homologous chromosome, the crossing-over is taken place. Due to this crossing over, whatever the alleles available over here in this chromosome, and this chromosome, they may be combined means, different combinations could be generated due to this crossing over. So, if we have to undergo reverse breeding, if you have to do reverse breeding, our first target will be suppression of meiotic recombination, this recombination process should be suppressed later on we will discuss it once again.

Next our target will be developing production of double haploids. Ok! So, what is haploid, what is double haploid, those things will be discussed means, anyway we have to develop double haploids. And third one is the selection of complementary lines or parents through marker assisted selection. So, these three steps are there, first we need to suppress the meiotic recombination, next one we need to produce the double haploid and then selection of the complementary lines through marker assisted selection or through marker assisted approaches. So, now we will be discussing about the suppression of meiotic recombination. Ok!

So, let us try to find out how the recombination is taken place, suppose one pair of chromosomes is there. This is the homologous chromosome, I am talking about a particular chromosome 1 and 1′ suppose, this is available in a particular plant. The 1′ chromosome has different sets of genes compared to 1. So, what happens during crossing over, in this way the chromosomal parts are exchanged, the parts of the chromosome in

the homologous chromosome it is exchanged. And these are non-sister chromatids, these two are sister chromatids of this chromosome these two are sister chromatids of chromosome number 1′.

Within the non-sister chromatid like this, and this of the homologous chromosome in this way the exchange is taken place. And once this crossing over taken place thereafter, what happens here some part of this chromosome could come while, over here some part of this chromosome could come. Let us assume, it was chromosome 1, it was chromosome number 1′ as I was talking about. Ok! After crossing over once in diakinesis those two homologous chromosomes are separated then in this way, some part of the chromosome from 1′ will be exchanged with chromosome 1 and vice versa. So, in this way this type of structure could be generated.

Now, if you think that here capital A allele was there, and here small a allele was there. Ok! While, over here capital B, and here small b allele was available initially. So, after this exchange, in this one along with small a capital B will be there while, in this arm in this non-sister chromatid constitution was capital A capital B like this one. Here, it was capital A capital B here it was small a small b. While, over here we can get capital A small b small a small b.

In this way the alleles available in the homologous chromosome could be exchanged, you can see over here. Here small a has come with capital B, here capital A has come with small b, this is the crossing over the effect of crossing over. So, this is normally occurred during gamete formation in heterozygote individuals. It is heterozygote means, capital A small a capital B small b this condition was there, capital A small a capital B small b this condition was there, in the heterozygote. So, during this gamete, its gamete formation these things will be arise means, this will be happening in meiosis I.

This homologous chromosome will be eventually, separated and in meiosis II basically 4 chromatids will be separated. So, one will be having capital A and capital B one will be having small a and capital B, one will be having capital A and small b, and one will be

having small a small b. In this way these things will go to different gametes. So, how the suppression of meiotic recombination is conducted basically? So, in this way the normally spores are produced in plants in the heterozygotes. Now the spore formation process is regulated by suppression of recombination during the reverse breeding approach. Ok!

So, in reverse breeding we need to suppress these things, we need to suppress this recombination, this crossing over, this recombination we need to suppress. So, that whatever is available over there, will go to its gamete and whatever is available in this, chromosome will go to its gamete these things we need to suppress. Now, how to suppress meiotic recombination means, what are the different approaches to suppress the meiotic recombination. First let us discuss, about some genes, mention about some genes which are responsible for meiotic recombination. It was found that some genes like *DMC1*, *RecA* and *Spo11*.

These 3 genes are involved in recombination process, different proteins are produced. So, that the non-sister chromatids could come closer, some genetic material could be exchanged. So, those proteins are produced by these genes, these genes play role in those recombination process. Now, if you have to stop the recombination process, we can do RNAi mediated knock down, or RNAi mediated silencing of this gene during spore formation. Ok! How RNAi approaches could be taken later on we will discuss.

So, basically through RNAi, that is RNA interference approach we can reduce the expression of this gene, we can reduce the expression of *DMC1*, *RecA* or *Spo11* gene. So, if their expression is reduced, if their transcript production is reduced then eventually, its protein production will be reduced also and finally, the recombination process will be hampered. Then other than these 3 genes, we can apply some exogenous chemical compounds also that cause inhibition of recombination, it is mirin, it is another compound we can exogenously apply. Ok! Basically, this compound causes arrest in G2 phase of cell cycle by inhibiting phosphorylation of ATM.

Now, let us discuss how RNA silencing works. Suppose, we are trying to silence the *RecA* gene, how we can take the RNA silencing approach. So, for gene silencing. Ok! So, let us assume we have a particular gene let us assume, this is the *RecA* gene. So, this is the gene sequence we are starting from ATG, different, this is the coding sequence of the gene for RNA silencing we need to target the coding sequence. Ok! Suppose, this is the region we are targeting, and here the stop codon is there.

So, we have taken this part of the gene, sometimes in RNA silencing we can take some part of the UTRs also, but mostly we can play with the coding region. So, now what we have to do, we have to place this gene in forward and reverse orientation. We have to place this gene in forward and reverse orientation flanking a linker. So, basically it is better if we put the gene in reverse and forward orientation means, reverse orientation could be kept in the first part. So, it would be better, later on we will discuss.. once we will be exclusively discussing the RNAi construct preparation, then we will discuss these things.

So, anyway for the time being you can understand, that the gene fragment, the coding sequence of the gene could be placed in this orientation, this is the start to stop that is if you think it is ATG …. different sequences are there, and at last TAA is available. While, in reverse orientation this is the 5′ to 3′ strand of the DNA, here this is the 5′ to 3′ in this way over here it will be ATG means, the gene is placed in reverse orientation ..... and at last it will be TAA. So, in this way we have to put our gene, it is complementary sequence will be here, let us draw the complementary sequence it is TAC . . .. ATT over here, it is complementary sequence was TAC ATT. So, during RNA silencing construct preparation, we have to put a particular gene, a target gene in forward and reverse orientation flanking a linker. Linker is the non-specific DNA means that DNA sequence should not be available in our target plant, where we are applying this RNAi construct and this whole things this is known as the hpRNA part hairpin RNA construct.

So, it will be expressed under a promoter, we know that promoter is needed for expression of any gene, because on this promoter RNA polymerase and other different

transcription factor binds, and it will start transcription in this way from here, and we have to put this hpRNA construct under a terminator also. So, that the transcription is stopped properly. Ok! So, promoter is needed for initiation of transcription, and terminator is needed for stopping the transcription process. So, in this way we have to make the RNAi construct, we have to put our gene in forward and reverse orientation, this is the forward and this is the reverse orientation flanking a linker, and we have to express it under a promoter and a terminator. So, if we deliver this constructing plant system what will be happening? Suppose, it has been integrated in the plant genome.

So, RNA polymerase will bind to the promoter region and finally, the mRNA will be produced from here. What will be the sequence of the mRNA? In this DNA the coding strand template, strand those two strands are there, this one strand is the template strand and the 5′ to 3′ strand is the coding strand you know. So, our mRNA will be AUG .... different sequences then UAA, then this is the linker region it will be transcribed also in our mRNA and thereafter, it will be UUA and finally, we can get CAU. So, this will be the structure of our mRNA because, mRNA will be complementary to the template strand right, it will be complementary to the template strand means, it will be similar to the coding strand just except the T, there will be uracil in RNA, no thiamine bases are there, they are uracil residues will be available. Ok! So, now in the plant system this type of mRNA will be produced from this particular construct.

Now, you know that RNA is mostly single stranded in nature, but if it gets some complementary sequences, it may form double stranded structure. So, now in nature if this type of sequences are available, it will try to form this type of structure while, this is the 5′ end, here we had AUG sequence, then we had UAA sequence, then this is the linker mRNA sequence, the mRNA which is produced from the linker part, then we will get gradually UU and A and at last we will get CA and U at the 3′ end, right? So, in this way a structure could be formed where A can pair with U, U can pair with A, G pairs with C, in this way a double stranded RNA structure could be formed and the linker region will form the loop like structure. So, in most of the eukaryotes a particular protein is available that is Dicer or DCL. So, this group of proteins, what they do, their structure

is almost like this basically using this structure they binds on the double stranded RNA.

If double stranded RNA is available in any eukaryotes Dicer will attack, those things, those double stranded RNA, and attacking those double stranded RNA it will make small double stranded RNA, small double stranded RNA will be produced. Basically, Dicer will chop the double stranded RNA available in most of the eukaryotes. Why it is available? It is available for some protection purpose because if some virus attacks those organisms which is having the double stranded RNA as the genetic material, those viruses genetic material could be easily cleaved. So, it is a defensive mechanism. So, if artificially we put our gene in this way, therefrom also such type of double stranded RNA could be formed and by the action of Dicer, the small double stranded RNA, that is known as siRNA or small interfering RNA it is produced from this double stranded RNA by the action of Dicer.

So, thereafter this small interfering RNA, it has 2 strands you can see. So, these 2 strands will be separated, this is 1 strand and this is another strand, these 2 strands will be separated, and 1 strand will be eventually degraded in the system while another strand will target our desirable gene. Now, I am coming back to our previous slide, in previous slide we are talking about few genes that are responsible for recombination right, among them one was *RecA*. Suppose in rice plant, we are trying to prevent the recombination process the *RecA* normal recombinants A gene is there, in the cell normal *RecA* gene is transcribed, its mRNA is produced, normal *RecA* gene its mRNA is produced in the system, in normal cell they are from finally, the protein is also produced suppose, this is the protein produced from the *RecA* gene. Now, if we introduce this construct, this RNAi construct in this particular rice plant, this is the endogenous *RecA* gene, this is the endogenous RecA transcript production, and this is the transgene we are delivering or exogenously we are delivering this in the plant system.

So, from this byproduct will be available, different small RNA will be available, that will be coming from our transgene, right? So, those small RNA sequence will be specific to the mRNA, because we have taken the coding DNA sequence over here in forward and

reverse orientation. So, definitely our mRNA will be having these sequences, and this sequence can bind the mRNA, this small RNAs can bind the mRNA, based on the sequence complementarity. So, if this binding is taken place, then again Dicer will work because, the double stranded RNA formation will be taken place. So, it will be attacked by Dicer again, and this mRNA will be chopped, this mRNA will be chopped.

So, in this way our endogenous transcript level will be reduced by RNA silencing mechanism, and if the endogenous transcript is reduced, if the mRNA becomes less, then the protein production will be lesser also and finally, the recombination process could be targeted could be hampered. So, in this way by targeting those genes we have mentioned earlier, we can reduce the recombination process, the efficiency of recombination will be eliminated means, non-recombinants will be produced. Now, our next step was production of double haploid lines, right? So, for producing double haploid lines, first we need to know, what is haploid? Ok! Haploid is the chromosome number available in the gametes of an individual, it is the gametic chromosome number. Ok!

Suppose, we have an individual where 2n = 4 means, it is an diploid organism, 2 chromosomes are available 1 1′ and 2 2′, 2 chromosomes are available in 2 copies, it is an diploid organisms. So, once its meiosis I will be taken place, what will be happening, 1 and 2 may go to a particular pole and 1′ and 2′ may go to a different pole, and during meiosis I and during meiosis II the chromatids will be separated. The chromatids will be separated. So, once these type of things, after meiosis II ultimately, the tetrad formation will be taken place, and in pollen grains, in egg cells these gametes will be produced, because this is the process of meiosis, this is taken place in the reproductive tissue during gamete formation.

So, the gametic chromosome number that is the haploid. Ok! Now, if we consider another plant where 2n = 6 means, it is polyploid in nature. Here basically, suppose, this set of chromosome has come from a particular species, this set of chromosome has come from a different species, and this set of chromosome has come from a another species, from another species. So, there also if each chromosome are available in pair, it will be

separated, and in next generation also in this way the haploids will be formed. So, just the chromosome number will be half, from the body cells in case of haploid, the chromosome number will be half, here n will be 3, here n will be 2, this is the haploid. So, now how the doubled haploids could be produced, how the doubled haploid could be produced?

So, we know that the haploids are produced during gamete formation, this is the gametic chromosome number. Now, we can do the tissue culture of immature pollen grains means, before the formation of tetrads, once the tetrads are formed, once they are matured then finally, the pollen grains are formed in the plants. So, we can do the culture of immature pollen grains. We can do the anther culture, and isolate microspore culture, to produce the colonies of cells from immature pollen grains also. So, in this way just before the final division, before the pollen grain formation we can do the tissue culture process.

So, that we can take those cells, where, the haploid chromosomes number are available, and they should be in a proper stage to regenerate to produce callus those things also. Ok! Then we need to transfer the cell colonies means, if we get the cell colonies from those microspore culture or from anther cultures or from immature pollen grains. Then once we will get some colonies, or some callus like structures then, we will transfer it to suitable growth medium. Once we will discuss about the plant tissue culture, then these things will be covered little bit, in more detail. So, through plant tissue culture approach basically, those callus or colonies of cells could be given different phytohormones, or different plant growth regulators, as well as sugars to induce shoot growth and subsequently root growth.

So, in this way, suppose this is the unopened flower bud. Ok! If it is in unopened condition definitely, the anthers will not be mature. So, the immature anthers are therefrom, we can do this type of culture. We can take the mature anther also at the uni-nucleate pollen stage, then we can form the callus. Once the callus will be observed, then we can put different combinations of phytohormones NAA, BAP different

phytohormones are used and eventually, we can develop the shoots then we can initiate the rooting process.

So, in this way we can develop the plants from this particular anther culture. And during this process, during this process sometimes the chromosome number is doubled, or artificially we can double the chromosome number by applying colchicine, another chemicals which is used conventionally in this type of double haploid production. So, using colchicine we can multiply the chromosome number. Ok! So, from one set of chromosomes, because in haploid you know the chromosome number was n. So, if we multiply the chromosome number, it will become 2n during this step and finally, we can get the plants which will be having each chromosomes in 2 copies.

So, in this way the double haploids are produced. During tissue culture we will be discussing this part once again. Thank you.