

Course Name: Basics of Crop Breeding and Plant Biotechnology

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Week: 02

### Lecture-08: Backcross Breeding

Hello everybody. So, today we will continue the module 2 i.e. on Principles of Conventional Plant Breeding and this is the second lecture on the particular module. Today we will discuss about backcross breeding. So, these are the things which will be covered in today's lecture. First of all, we will discuss how selfing increases homozygosity. We have heard that during pureline selection, during pedigree breeding method.... we used to do selfing.

So, how homozygosity is increased through selfing? First, we will discuss it. If you recall the findings of Johanssen who gave the pureline; pureline selection method, there also he mentioned that if selfing is done, then homozygosity is increased. So, first we will discuss that part. Then gradually we will move into the backcross.

What is backcross? What are the different requirements of backcross breeding? Then gradually we will discuss two different types of backcross breeding mostly which are used in conventional plant breeding approach. First one is dominant gene transfer, next one is recessive gene transfer. Then gradually we will move into the different applications of backcross breeding and finally, we will discuss the merits and demerits of backcross breeding and how backcrossing improve homozygosity, will be discussed later on. Initially, we will discuss the improvement of homozygosity through selfing, and later on we will discuss about the improvement of homozygosity through backcrossing, whether there is any difference or not, will be discussed. So, first let us discuss that how

selfing

increases

homozygosity.

So, suppose we have one plant having genotype capital A capital A, let us assume this is  $P_1$  plant, while we have another plant  $P_2$  its genotype is small a small a. We are discussing these things by considering the population as a homozygous in self-pollinated crops. So, the  $P_1$  is homozygous dominant while  $P_2$  is homozygous recessive in nature. So, we are crossing it, here from only one types of gametes will be produced and here from also only one types of gametes will be produced. If crossing is made between these two will be having capital A small a genotype in  $F_1$  generation.

In  $F_1$  generation will be having capital A small a genotype that, is it is 100% heterozygous. If we think about the homozygosity; 0% homozygosity is there. Now we are starting selfing here just we have done the cross then once we will do selfing of all the plants in this generation two types of gametes will be produced capital A and small a male gamete will be these two female gametes will be also capital A and small a. So, if we make the checker board will be having capital A capital A, will be having capital A small a, will be having capital A small a and will be having small a small a in  $F_2$  generation, isn't it? If you recall the segregation of a single gene the monohybrid crosses in Mendel's theory, they are also in  $F_2$  you will get similar types of things.

So, in  $F_2$  will be having capital A capital A, will be having capital A small a, and small a small a. What will be the percentage of capital A capital A individuals? Here from one-fourth is capital A capital A. It means 25% will be capital A capital A. Then if you see the checker board, the capital A small a; it will be half of the population means will be getting 50% of individual in  $F_2$  generation where genotype will be capital A small a and small a small a will be remaining 25%. So, over here if I write in  $F_2$  generation what is the heterozygosity percentage? Heterozygosity percentage is 50%. In  $F_1$  generation 100% heterozygosity was there, here heterozygosity has been reduced and homozygosity: it will be 25+25 means it is also 50%.

Means, 50% homozygosity you are observing in first generation of selfing this is the

first generation of selfing. Let us take all the plants and we are selfing it once again. In the self-pollinated crop selfing is pretty common. So, let us think that we are selfing all the plants and we are getting the seeds for next generation. What will be our next generation i.e. F<sub>3</sub>.

In F<sub>3</sub> generation, from selfing of this particular plant capital A capital A all the progeny will be having this genotype capital A capital A, from this one capital A small a will be having 3 types of genotypes upon selfing. We will get capital A capital A, we will get capital A small a, as well as small a small a while from here in next generation upon selfing all the individuals will be small a small a, isn't it? So, now this 25% will come over here if you consider the total population in F<sub>3</sub> where, 25% is directly coming from there, then this 50% will be divided into 3 parts. One fourth of this 50% will be capital A capital A one fourth of 50% means 12.5%. Ok!

It will be capital A capital A, will be capital A small a; the half of this individual will be capital A small a heterozygous in nature. So, it will be 25% means half of this 50% while the small a small a individual will be 12.5%. From these particular types of parents, we will get these 3 different things while, from the small a small a plant in F<sub>3</sub> generation all the progeny will be small a small a. It will be 25%. So, if we calculate the homozygosity percentage in F<sub>3</sub> generation, capital A capital A types of plants will be 25% and 12.5%, right?

Means 37.5% will be capital A capital A, while small a small a percentage will be 25% and 12.5% i.e. 37.5%, and heterozygous individuals will be there only 25%, capital A small a. So, homozygosity percentage will be there  $37.5\% + 37.5\% = 75\%$  while, heterozygosity will be 25% only. So, in this way if you see upon first generation of selfing our homozygosity has become 50% in earlier generation, homozygosity was 0%. If we go to second generation of selfing, i.e. in F<sub>3</sub> population here homozygosity will become 75%. So, the homozygosity is increasing after each type of selfing. Now can we make any formula for that? So, let us try to make some formula.

So, so far whatever we got in F<sub>1</sub> generation, homozygosity percentage was 0, heterozygosity was 100%. In F<sub>2</sub> generation homozygosity was 50%, and heterozygosity was 50% in F<sub>2</sub>. In F<sub>3</sub> generation, the homozygosity became 75%, and heterozygosity became 25%. Ok! So, this is the first selfing generation, this is the second selfing generation. Let us make a formula.

We can say that  $(1/2)^n$  = heterozygosity, the frequency of heterozygosity which is observed through selfing where n = number of selfing generation. In F<sub>2</sub> i.e. first generation of selfing if we use this formula  $(1/2^1)$ , first generation of selfing means half i.e. 50%. So, if you see in F<sub>2</sub> generation, the heterozygosity was 50%. If we go to F<sub>3</sub>, let us see  $(1/2)^2$  i.e. F<sub>3</sub> second generation of selfing. So,  $(1/2^2)$  i.e. 1/4 means 25% heterozygosity will be there. In this way using this particular formula, we can calculate the heterozygosity percentage which is observed through selfing.

Now what will be the homozygosity percentage? The homozygous population, will be  $[1 - (1/2)^n]$  right! So, it is supposed to be the homozygosity percentage where n is same, n = number of selfing generation. So, let us use this formula in F<sub>2</sub>, what we will observe in F<sub>2</sub> is the first selfing generation right? Here homozygosity will be  $[1 - (1/2^1)]$  means  $(1 - 1/2)$ , i.e. 1/2 means 50%. Over here homozygosity was 50% in F<sub>2</sub> generation.

If you go to F<sub>3</sub> i.e. second selfing generation i.e. n = 2, there homozygosity will be  $[1 - (1/2^2)]$  i.e.  $[1 - (1/4)]$ ; i.e. 3/4, it means 75%, isn't it? So, this is the homozygosity percentage in F<sub>3</sub>. So, in this way using this particular formula we can calculate the homozygosity percentage through selfing where n = number of selfing generation. If you have to calculate the frequency of homozygous dominant or homozygous recessive; we know that through selfing homozygous dominant as well as homozygous recessive will increase in the same rate. So, the frequency of homozygous dominant through selfing will be half of this things  $1/2[1 - (1/2)^n]$ . Ok!

So, we can simplify this formula means  $1/2 [(2^n - 1)/2^n] = [(2^n - 1)/2^{n+1}]$ . So, the formula will be  $[(2^n - 1)/2^{n+1}]$ . Using this formula, we can calculate the frequency of homozygous

dominant or the frequency of homozygous recessive through selfing. Ok! You guys can try this formula whether in  $F_2$  or  $F_3$  we are getting similar types of frequency or not. Ok! So, now gradually we will move into the next part, backcross.

So, I think you guys can recall the test cross method, Mendel discussed about test cross. In test cross basically, either the unknown genotype or the  $F_1$  genotype was crossed with the homozygous recessive parent. Ok! To check whether it is true  $F_1$  or not? If it is a true  $F_1$  then, through test cross we could get 1: 1 ratio. Now in backcross. In backcross it is simply described as a cross between, the hybrid with any one of its parents, the hybrid  $F_1$  is crossed with any one of its parents. Now, for backcross breeding, the hybrids and its progeny, the hybrids i.e.  $F_1$  and its progeny means after crossing of  $F_1$  with any of this parent will get the progeny, those progenies will be repeatedly crossed and generally 6 to 8 generation of crossing is done over there.

So, why it is done later on we will discuss, with one of its parents. So, generally one parent is used again and again in backcross breeding. The parent which is used again and again i.e. known as recurrent parent, while another parent i.e. used mostly for one time i.e. used as donor parent i.e. known as donor parent. So, we will discuss these things once again later on. So, what are the objective of backcross breeding method? So, suppose we have a mega rice variety Swarna.

Swarna is a mega rice variety i.e. grown all across India, it has a good amount of yield, the farmers accept this variety easily. The variety is almost adaptable to different agro-climatic conditions. The consumers prefer the seed of this particular variety. So, there is no problem in acceptance, but this variety is susceptible to a particular fungal disease. Let us assume we are talking about sheath blight of rice.

It is a fungal disease, and the Swarna variety is susceptible to this particular fungal disease. So, this variety is mostly good. It has one drawback. If somehow, we can improve this drawback so, we could get more yield, the farmers will be more benefited. So, to improve this minor defect of a particular desirable variety we can go to backcross

breeding

method.

So, what are the requirements for conducting backcross breeding method? First of all, we need a suitable recurrent parent. If I continue our previous discussion let us have, we have the Swarna rice variety. It is highly acceptable by the farmers by the consumers. So overall, this variety is highly adaptable, but it has some minor defects, i.e. its susceptibility to sheath blight of rice. So, we are trying to improve this variety through backcross breeding.

So, this variety will be used as a recurrent parent. Recurrent parent means, this parent will be used again and again in the backcross breeding method, and it is lacking any one or two specific traits. Next one we need to have a suitable donor plant also. As we are trying to introduce the sheath blight of rice resistant gene from some other genotype, we need that variety also where from we will take the resistant gene. Let us assume that Tetap it is another rice variety i.e. not too much familiar in Indian agro-climatic condition.

Most of the rice genotypes are susceptible to sheath blight of rice, but from the earlier literature it is understood that this, Tetap rice variety is showing resistant to sheath blight of rice. So, we can transfer this resistant gene from Tetap into Swarna. So, Tetap will be used as a donor parent. Ok! Rest of the characters are not desirable, but this resistant trait is available in the Tetap variety in the donor parent. And another thing we have to consider, we must have to consider is that, the donor parent should have that particular trait what we are interested to transfer in very intense form.

It should not be semi resistant, or it should not be semi tolerant, it should be highly resistant in nature. So, that the robust trait which is available in Tetap could be transferred into Swarna through backcross breeding. Third one we should target those characters here; we are trying to target the disease resistant trait. Ok! For sheath blight of rice disease. So, in backcross breeding we should target those characters which will be highly heritable in nature, means those are controlled by genes 1 or 2 genes. Ok! And

environment will have least impact on that.

So, we should target those traits. Next one, sufficient number of backcrosses is done for full recovery of the recurrent parents, because our target is to get this variety along with the resistant gene from the Tetap, isn't it? So, sufficient number of backcrossing should be done generally 6 to 7 generation of ... backcrossing is generally done. So, let us think that, this is the Swarna genomic DNA just I am not talking about different chromosomes available in Swarna, just assume it is the genomic region of Swarna while, this is the genomic region of Tetap. Ok! Since Swarna some genes are there, i.e. responsible for plant height i.e. preferred by the farmers and consumers. Some genes are there i.e. responsible for rice seed type, whether it is sticky or non-sticky, it is also preferred by the farmers. Ok!

But it has one gene i.e. making it sensitive to seed blight of rice while, it has another gene i.e. responsible for its maturity i.e. also preferred by the farmers. So, in this way most of the genes are good in Swarna variety while, if you consider Tetap is not at all adaptable in our agro-climatic zone, its seed type is not accepted by the consumers or farmers available in India. But it has the gene which is responsible for sheath blight of rice, that gene is available, that resistant gene is there while, it has for another trait, the maturity is also, maybe it may be longer duration in nature. Ok! So, farmers will not accept it. So, our target is to take only this part over here.

So, that ultimately, we can get a Swarna like things where this particular resistant allele will be coming from Tetap, is not it? So, we need to get full recovery of the genome of the recurrent parent, by repeated backcrossing by 6 to 7 generation of backcrossing. Now, before discussing two backcross breeding methods conventionally used i.e. the backcrossing for dominant gene transfer, and backcross breeding for recessive gene transfer. First, we have to know that the trait with which we are trying to plan our experiment? Which we are planning in our experiment? Whether the trait is controlled by the dominant allele or the recessive allele, first we need to know that. Ok! So, suppose we have a variety X and we have another variety Y.

In variety X suppose this is the genetic condition constitution while, in variety Y the genetic constitution is like this. Out of these two varieties the variety Y is showing drought tolerance while the variety X is drought susceptible. So, if we attempt a cross between these two varieties, what will be getting in F<sub>1</sub>? In F<sub>1</sub>, capital A small a genotype will be there. Let us assume, we are checking it in the field, and in the field, we are observing that all the plants are drought tolerant. We are observing, all the F<sub>1</sub> plants are drought tolerant.

It means we can conclude that the capital A allele is responsible for drought tolerance isn't? It means as capital A is here, in F<sub>1</sub> generation we are getting drought tolerant phenotype. So, the capital A might be responsible for drought tolerant traits, and for this particular experiment, we have to go for dominant gene transfer because the dominant gene is controlling this character. Now, let us think another experiment where, we have a variety A and we have another variety B. The variety A is resistant to salt tolerance. While variety B is susceptible to salt tolerance, it is susceptible to salt stress.

We are making a cross, we are getting this genotype. Now if we go to next generation it is supposed to be, all should be supposed to be salt tolerant in nature. All the F<sub>1</sub> because the capital A is coming from here, the salt tolerant individual in F<sub>1</sub>. So, all the plants will be salt tolerant and here also we have to go for dominant gene transfer, but under certain condition.... under certain condition we should go for the recessive gene transfer. How can we determine it? Suppose we have another variety P and another variety Q. Here P is having genotype small p small p and Q is having genotype capital P capital P, where small p small p is responsible for suppose white colored flower and capital P is responsible for purple colored flower.

White colored flower is less toxic. Suppose we are discussing about lathyrus, we know that lathyrus is a pulse crop, there some toxic components ODAP is available. So if that toxic component is more, means the seeds are detrimental for the consumers. If the toxic components are less, seeds are seeds could be accepted by the consumers. So now let us

assume, that the white colored flower this trait is associated with the low ODAP content. Let us assume that the white colored flower is associated with the low ODAP content.

So we are making a cross between variety P and variety Q. We are getting  $F_1$  individuals, we are observing all the plants are purple. So, is it desirable to us? I.e. not desirable to us, because if purple flower is there it means the ODAP content will be more, ODAP content will be more and it will be toxic for the consumers. Let us go to the next generation in  $F_2$  generation. There we can see the segregation, capital P capital P will be there, capital P small p will be there and small p small p will be there. This small p small p genotype will show the white flower, means in  $F_2$  generation we are getting our desired type in 25% of the population.

So if a particular trait is transferring in this way, then we have to do the recessive gene transfer through backcross. So now gradually we will discuss about the dominant gene transfer method of backcross breeding. So let us take the example of Swarna rice. Let us assume, this is one of our parents and its genotype is small a small a while, we have another genotype Tetap i.e. plant 2, its genotype is capital A capital A. We are discussing the sheath blight of rice; we are discussing the sheath blight of rice resistance transfer.

Suppose we found that in the Tetap variety the dominant gene is available while, in the Swarna which is a desirable variety, which is accepted by the farmers and consumers, which has good seed quality, but it has some lacking; it is susceptible to sheath blight of rice. So, we are attempting the cross in  $F_1$  generation we will be getting capital A small a. So, if the homozygous dominant parent was Tetap i.e. responsible for sheath blight of rice resistance, then all of our  $F_1$  is supposed to be resistant in nature because capital A will be coming there in one copy. So over here we do not have to do any selection no selection is done, but all the plants we have to cross with the recurrent parent. I have mentioned about recurrent parent means the parent which is used again and again in the backcross breeding right.

Here, Swarna is our recurrent parent i.e. the desirable parent, only one trait is lacking

there. So, all the  $F_1$  plants are crossed with Swarna. After doing this cross, here from we will get only one types of gametes, here from we will get two types of gametes; capital A and small a. So, in next generation after fusion of these gametes we will get capital A small a and small a small a. This generation is known as backcross one generation i.e. first generation of backcross.

What is backcross? The crossing of  $F_1$ , the crossing of the hybrid with the recurrent parent; with any one of the parents... here we are using the recurrent parent. So, this is the  $BC_1$  generation. In  $BC_1$  generation we have to do selection for the sheath blight of rice resistance. If we screen this plant for sheath blight of rice resistance, these plants will be killed, while this plant will be survived because the capital A allele is available there. So here, selection for sheath blight of rice resistance will be done.

Whatever plants will survive, that will be selected based on the similarity to the Swarna, because some of the plants will show phenotypically similarity to the Swarna variety, those plants will be selected. So, we need to select those plants which are similar to recurrent parent and those plants will be crossed with the recurrent parent again. Whatever is survived from there we have to identify some plants which is showing similarity to the recurrent parent and i.e. crossed again with the recurrent parent. In next generation again we will get this type of plants i.e. known as second backcross generation  $BC_2$ . In  $BC_2$  here also we have to do selection for sheath blight of rice resistance, and we have to do selection of plants which are showing similarity to the recurrent parent and this one will be killed, this one will be survived and whatever the plants are selected it will be crossed with the recurrent parent once again.

So then we will get  $BC_3$  generation here also combination will be this and this. So, in this way we have to do the crossing of the selected individuals of each and every generation up to  $BC_6$  generation, 6<sup>th</sup> generation of backcrossing is done. So, after 6<sup>th</sup> generation of backcrossing again the population will be like this, once the population will be like this, here also we have to do selection for sheath blight of rice resistance and we need to identify those plants which are showing similarity to the Swarna, i.e. the recurrent

parent. So it will be crossed, it will be killed in the field and thereafter we do not have to do cross once again, we have to do selfing of the identified plants. So, if we do selfing, we know that from  $F_1$  upon selfing we can get  $F_2$  generation, here overall also once we do selfing then thereafter in next generation will be  $BC_6 F_2$  generation.

So here the plants are selfed and in  $BC_6 F_2$  generation, basically we have to grow plant to progeny row, here in this generation we have to grow plant to progeny row. If you think about the genotypic constitution over here, it should be capital A small a, means it should be heterozygous in nature. So, if we do selfing over here in this  $BC_6 F_2$  generation, some lines will be capital A capital A, some lines will be of capital A small a and some lines will be of small a small a, because we are growing plant to progeny row. All the plants will be screened again in this generation for sheath blight of rice resistance, the selection is done and then we will identify some plants, because these plants will be sensitive to sheath blight of rice, susceptible. It will not be survived. Next, we will grow to  $BC_6 F_3$  generation, in  $BC_6 F_3$  generation whatever the plants we are selecting from here its progeny again will be grown as plant to progeny row.

Now the plants which one is over here, means its genotype was, let us assume capital A capital A. So, its progeny all plants will be capital A capital A, no segregation will be observed in this line. While, in those plants where genotype was capital A small a, in its progeny some plant will be capital A capital A some plants will be capital A small some plants will be small a small a. So, in this generation  $BC_6 F_3$  generation again we have to do selection, selection for sheath blight of rice. After selection on those lines where we are getting similar type of phenotype and the plants are showing similarity to Swarna variety, all the plants are disease resistant, and it is showing similarity to the Swarna variety will be collected in bulk. Their seeds will be collected in bulk.

While, in those lines where we are getting segregation because we are using the inoculum of sheath blight of rice, and we can see some plants will be killed, some plants will be survived means, in a single line different types of plants are available, those plants or those lines will not be accepted. So, next we will go to  $BC_6 F_4$  generation. So, here we

can pull the seeds of those plants, which are showing resistant to the disease and which are showing similarity to the recurrent parent their seeds are pulled. In  $BC_6 F_4$  generation we can go to replicated yield trial. Earlier we have discussed about replicated yield trial, means our lines, our seeds will be grown in this way maybe in multiple locations suppose this is line 1, this is line 2, this is line 2, this is line 1, line 1, line 2 in different plots our variety our identified genotypes will be grown along with the standard check variety.

The check variety will show the resistant criteria of sheath blight, in this way for each and every location different check varieties are available, that can provide maximum yield, and for different disease also check varieties are there. Those varieties can show tolerance to the disease. So, in this way we have to use a couple of check varieties also in this replicated yield trial. Thereafter we will go to the next generation.

In next generation ultimately, we can prepare for seed multiplication. Then finally we can release the variety. So, this generation will be  $BC_6 F_5$ . So, in this way we can conduct the backcross for dominant gene transfer.