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: 02

## Lecture-07: Pedigree Method

Welcome back. So, we will continue again and now gradually we will be moving into the Pedigree Method. So, before moving to the pedigree method first I would like to discuss about the different features of pedigree method. In pureline if you recall the name, it was pureline selection, it is a selection process. Ok! But in case of pedigree, it is a pedigree method it is a breeding method. So, first of all the pedigree method deals with the segregating population. Segregating population means the population that is segregating. Suppose this is the genotype of a particular plant. Ok! In its progeny we can get capital A capital B capital B, we can get capital A small a capital B capital B as well as we can get small a small a capital B capital B. Ok!

In its progeny we can get these three different things means for A gene as it is in heterozygote condition, over here we can see segregation. While B gene is homozygote condition no segregation is there. Ok! But as it was heterozygote we can see segregation. So, if this one is recognized as  $F_1$ , then  $F_2$  upon selfing of the  $F_1$  first filial generation we can get these segregating individuals. So, in these segregating populations like  $F_2$ ,  $F_3$  etc. there, this pedigree method could be applied.

So, techniques like pureline selection and mass selection cannot be applied to such type of population, because pureline selection if you recall it deals with the single homozygous self-pollinated plants means, homozygosity should be there. If it is segregating, then that selection will not be effective. While in case of segregating population few methods could be used like pedigree method, bulk method, single seed descent method, those methods could be used in case of means, if the population is segregating in nature. So, what are the methods we are discussing pedigree methods, then bulk method, single seed descent method those things are used for segregating population, but we will be discussing only the pedigree method. Few important breeding methods will be discussed here. First selection we have done, we have discussed pureline, next we will discuss about the pedigree method.

Then individual plants are selected from  $F_2$  and subsequent generations and their progenies are grown and the record of parent to progeny relationship is maintained. So, pedigree means the, means it is the process of keeping the records of parent to progeny; relationship from which parent, which progeny is coming in next generation, from that parent which progeny is coming? Keeping or maintaining this record is highly needed, in this pedigree method. It starts from the F<sub>2</sub> generation and as well as in the subsequent generation also we have to keep the record. So, it was first outlined by Love in 1927. So, let discuss of us the procedure pedigree method.

First of all in pedigree method we have to initiate hybridization. If you recall the Mendelian genetics the last part we have discussed about the hybridization. So, for hybridization of rice what are the things needed, rice is a self-pollinated crop that is known to us. So, within a flower, within a rice flower, the male gametes are produced by the anthers and the female gametes are produced within the stigma means, within the female reproductive parts. So, once the male gametes are produced in the anther, in the form of pollen grains they fall on the stigma of the same flower and then, from the pollen grains the pollen tube forms finally, it reaches the ovary and fertilization is taken place The over there. egg cells are available over there. Ok!

So, in this way within a single flower of rice through selfing the seed could be formed, but if you have to do hybridization first thing what we have to do? We have to do emasculation in self-pollinated plants. Emasculation is the process through which we can remove the male parts, we can remove the anthers, we can remove the pollen grains. So, that selfing could be stopped, could be prevented and thereafter once the emasculation is done next day we can do the crossing by taking the pollen grains from other plants. We can initiate the crossing. So, next procedure is the  $F_1$  generation. So, if crossing is made between 2 plants  $P_1$  and  $P_2$  first generation, first filial generation is known as  $F_1$  generation.

In  $F_1$  generation, we get by crossing 2 different plants and what we have to do in pedigree method, the seed should be place planted. Ok! Later on we will discuss these things once again. Then from  $F_1$  seeds, the  $F_1$  plants are selfed to get the  $F_2$  generation second filial generation.  $F_2$  generation is very crucial because there from we have to start our selection and the selection is done for different simply inherited characters. In  $F_3$  to  $F_5$  generation what will be doing? In  $F_3$  to  $F_5$ , generation basically selection is practiced from within and between the progeny row that is very important point, I will discuss it once again. So, selection is practiced from within as well as between the progeny row.

Next one in  $F_4$  to  $F_6$  generation, individual selected progenies in  $F_3$ , they are planted in multi-row for visual comparison among the progeny rows means a single progeny is being grown in 3 to 4 different rows generally in 3 different rows it is being grown. So, that visually we can see whether all plants are look-alike or not. Then in  $F_7$  generation, gradually we have to move into the preliminary yield trial with 3 different replications. Why replication is done? Replications are done to reduce the soil heterozygosity, to reduce the environmental effect. So, the preliminary yield trial is done in  $F_7$  generation onwards.

Then in  $F_8$  to  $F_{10}$  generation the superior lines are tested in replicated trials at several locations, in multiple locations, trials are conducted as we have done at the last stages of pureline selection also multi-location trial. And then in  $F_1$  generation...  $F_{11}$  generation sorry in  $F_{11}$  generation a new variety will be multiplied for its distribution to the farmers definitely before distribution to the farmer the seeds has to be released ok. So, seed multiplication, seed release process is initiated over there. Ok! So, let me brief the procedure of pedigree method once again. First of all we have to do hybridization over here, 2 plants are chosen plant 1 and plant 2 or we can choose 3 or 4 plants also initially

they have to crossed then the  $F_1$  could be crossed with another  $F_1$  in this way multiple parents could be chosen over here in hybridization process. 2-way-cross, 4-way-cross, different cross could be made.

So, then the F<sub>1</sub> plants will be grown, the F<sub>1</sub> seeds are space planted then from their seeds after selfing will get the  $F_2$  progeny. So, by selfing will get the  $F_2$  plants from  $F_2$  our selection will be started then in F<sub>3</sub> to F<sub>5</sub> generation this is very crucial time here selection is practiced within and between the progeny row and variation within the progeny row is vanished as we will progress towards the  $F_5$  generation why I will explain later on. Then in F<sub>6</sub> generation the individual selected progeny is in F<sub>5</sub> are planted in multi row for visual comparison. Ok! Basically in  $F_5$  to  $F_6$  generation this is done. Then in  $F_7$ generation ultimately preliminary yield trial is done in different replications to reduce the soil heterozygosity and in F<sub>8</sub> to F<sub>10</sub> generation the superior lines are tested, multilocation trials are conducted and in F<sub>11</sub> new variety development or seed multiplication is done. So, let discuss this procedure once again. us

Suppose we have taken two different parents, one is parent 1 and parent 2; its genotype was capital A capital A small b small b and its genotype was small a small a capital B capital B we had these two parents in our hand. So first, in first generation we have to make cross between these two plants and we will get  $F_1$  generation that is first filial generation. So, what gamete will be produced from here capital A small b gamete should be produced from here only one type of gametes will be produced while from this plant 2 small a capital B gamete will be there right. So, if these two gametes will fuse in  $F_1$  will be getting capital A small a and capital B small b. This is heterozygous condition for two genes; the heterozygosity is there none of them are in homozygous condition. Some genes may be in homozygous condition, but heterozygosity must be prevailed as we are making cross between two different parents that is the basis of selection in pedigree method.

Now in  $F_1$  generation basically no selection is done because all the plants will be having same genotype; all of the plants will be having capital A small a capital B small b

genotype. So, selection will not be effective then we will go to the next generation that is  $F_2$  generation all the plants will be selfed and we will go to next generation. So, next generation we may get different combinations like capital A capital A capital B small b, we may get capital A capital A small b small b, we may get capital A small a capital B capital B; these different combinations could be there right. Means if you make a checker board by selfing these things... this is this will be similar to the dihybrid cross two genes are involved over there. So, 16 different combinations will be observed in  $F_2$ .

So, what we have to do in  $F_2$  generation? Individual plants are grown, space planted, the plants will be grown in spacious way and their seeds are collected separately because its seeds will be collected separately... its seeds will be collected separately. So, then its seeds means all the plants are selfed the plants are space planted and selfed. So, we will move into the  $F_3$  generation. In  $F_3$  generation what if the seeds we are observing from  $F_2$ that will be grown in plant to progeny row means plant to progeny row will be grown from here whatever the seeds we are getting from  $F_2$  it will be grown in different lines in  $F_3$ . So, there from if you just consider this line, its produce, suppose is being grown over here ok.

So, what could be the combination here, some lines will be of capital A capital A capital B capital B; some lines will be capital A capital A capital A capital B small b; some plants will be capital A capital A small b small b right? because for capital B and small b heterozygosity is available over here. So, in its progeny capital B small b will be segregating this is the segregating population. So, this type of variations will be available within this line, therefrom we can select a particular individual. Here from, we can select another individual; suppose from here all the progeny will be having capital A capital A small b allele will be produced from here only single type of not allele single type of gametes will be produced. So, all progeny will be same for this line. So, whatever we are making the selection over here, in  $F_4$  generation, again we have to grow plant to progeny row.

So, if you carefully see earlier we have mentioned that from  $F_3/F_4$  in this generation we have to start selection within the lines and among the lines within the row and among the row. So, within this particular row here you can see different genetic combinations are available. Suppose this plant... suppose this plant was chosen from here. So, in its progeny also we can get 3 types of plants like capital A capital A capital B capital B, capital A capital A capital B small b and capital A small b small b isn't it? If this plant is selected, in its progeny in  $F_4$  generation we can get this 3 types.

So, within a row we can do selection, because it is a segregating population, even across the row means in between different rows also we can do selection. Here we will be having some plant capital A capital A small b small b here we may get some plant where small a small a small b small b could be there. So, within row selection and between row selection is done. Thereafter we will go to the  $F_5$  generation. In  $F_5$  generation onwards we can grow the progeny of each selected plants; the progeny of each selected plants is grown in multiple rows. Suppose the plants which was capital A capital A capital B capital B is being grown here, the plant capital A capital A small b small b is being grown here, the capital A small a capital B small b is being grown here.

In F<sub>5</sub> generation, we are growing in multiple row the plants what have been selected over here, it is being grown in multiple rows. In F<sub>6</sub> generation also we will do grow the plants in multiple row whatever will be selecting from here it will be grown in multiple rows. Now, if you carefully see in its progeny, all plants will be having capital A capital A capital B capital B is not it because it is homozygous, means it was homozygous for both the genes. Over here all the plants will be capital A capital A small b. But over here again we can see segregation some plants will be capital A capital B capital B some plants may be capital A capital B small b, some plants may be capital A capital B small b. These different combinations may be there. So, if we see such type of heterozygosity, if the phenotype of all the plants are not uniform then we will reject it in F<sub>6</sub> generation because until F<sub>5</sub> homozygosity will be at maximum level.

So, if any row, if in 3 rows they are not showing similar type of phenotype then the heterozygosity might be there, then we can reject it. So, next we will move into the  $F_7$  generation.  $F_7$  generation the preliminary yield trial is started in different replications. So, whatever we have identified from here that will be grown in different replications R1, R1, R2, R2, R3. So, in this way different lines could be grown in replication in preliminary yield trial within a small field. Then ultimately, we have to go to the multilocation trial for 2 to 3 years multi-location trial and ultimately, we have to go to the seed multiplication.

So, this is the process through which the pedigree selection is done. So, now let us discuss what are the different uses of pedigree method? So, it is most commonly used method for selection from segregating generation. From the beginning we are discussing that in the segregating generation that is if we have to do selection after crossing 2 different plants, if you start the selection from  $F_2/F_3$  generation. So, then this is one of the important method which could be followed in self-pollinated crops. Next one, combination breeding that is another important thing let us discuss what is combination breeding?

Suppose we know that Swarna a rice variety, that is famous means that is very popular, that is cultivated all across the India, I have mentioned earlier. So, good consumer preference is there while we have another rice variety. In Swarna we have good yield is also available the yield is also pretty good. While we have another variety let us assume Gobindobhog this is a small grain aromatic rice it is used for pie preparation mostly it has sweet aroma. So, suppose a farmer is trying to introduce the aroma genes or aroma character from Gobindobhog into Swarna. So, the target of the breeder is to get the produce like like Gobindobhog. Swarna and the aroma get

So, it is a kind of combination breeding means he would like to get both of this characters both of the important features available in this two genotypes. So, he can go to pedigree method through pedigree method he can choose this two plants he can make  $F_1$  and in  $F_2$  onwards. He can do different selection process. So, he can identify those plants

which will be having significant amount of yield like Swarna and the aroma could be tested easily by the means if you take the seed of that particular genotype if you just take a smell you can know whether it is aromatic or not, in Swarna you will not get any aroma. So, in this way the combination breeding could be done for pureline selection, Next one is transgressive breeding that is very through pureline breeding method. important thing, but discuss here. we are going to over

So, what is transgressive segregation? So, before describing transgressive segregation let us make a cross between the two plants. Suppose we had one plant of capital A capital A small b small b and it is being crossed with another plant its genotype was small a small a capital B capital B. Now, let us assume capital A; if capital A capital A allele stays together then the value of the yield suppose we are providing some specific value to the yield. Ok! Let us assume if this two allele stay together a variety making 12 quintals per hectare is produced. If capital A is available as a single copy there, we can get 10 quintals per hectare; while if capital A allele is not at all available the yield become 6 quintal per hectare.

Suppose A gene control the yield per plant, somehow A gene is associated with the number of tillers available in rice plant. If two alleles are there more number of tillers will be there, if one capital A allele is there relatively less tillers will be there; if no capital A allele will be there then the tiller number will be pretty less. So, ultimately the yield could be reduced while for B gene, if capital B capital B is available then what we may get suppose the length of the panicle is 10 cm. If capital B is available single copy means capital B and small b ... there the length become 8 cm length of the panicle of the rice. And if small b small b genotype is there supposing the length become 5 cm. The panicle length if become smaller the seed will also be produced less. Ultimately it will hamper the yield also is not it. Suppose these two variety was initially crossed and capital A is a positive allele while capital B is also a positive allele in one plant. We had capital A capital A in homozygous condition while the negative allele of B gene was available 1. in plant

While in plant 2 the negative allele of A gene is available and the positive allele of B gene is available. Now, let us make a cross in  $F_1$ . In  $F_1$  will be getting capital A small a capital B small b. Ok! So, if F<sub>1</sub> is capital A small a it is yield is supposed to be 10 quintal per hectare and it is panicle length will be moderate 8 cm. Earlier it was 10 cm panicle length, here the panicle length was 5 cm and here we are getting panicle length 8 cm. Ok! It is not exceeding its parent, right? The  $F_1$  for the panicle length is not exceeding this parent any one of this parent while if you consider the performance of F<sub>1</sub> in terms of yield here yield was 12 quintals per hectare, here yield was 6 quintal per hectare and here yield is 10 quintals per hectare isn't it? Here also the yield is not exceeding any one of this parent. Now, if we go to next generation that is F<sub>2</sub> generation for both of the gene the heterozygosity is there the combination is capital A small a capital B small b both of the  $\mathbf{F}_1$ it? genes are in heterozygote condition in isn't

So, what type of gametes will be produced? Some gametes will be capital A small a some gametes will be capital sorry capital A small b, some gametes will be capital A capital B, again small a small b and small a capital B this 4 types of gametes will be produced. Ok! So, if it is fused upon fusion of these gametes random fusion of these gametes ultimately some plants will be having capital A capital A capital B capital B, some plants will be having capital A small a capital B capital B, some plants will be having small a small a capital B small b small a small a small b small b and so on. In this way different combinations will be observed in F<sub>2</sub> in F<sub>3</sub>. Different combinations will be observed isn't it? So, these are the segregating generation from F<sub>2</sub> onwards, it is starting. So, if within a segregating generation we get some individuals that outperforms both the transgressive parent then it is known segregates. as

In the segregating population if we get some individual which is outperforming both the parents over here the yield is supposed to be 12 quintals per hectare again it is panicle size panicle length will be also 10 cm. So, ultimately overall it is yield will be more it will be more than 12 quintal per hectare. Ok! So, it will exceed both the parent 1 and parent 2. So, this is the transgressive segregates in this way, through pedigree method we can identify the transgressive segregates also. Now pedigree record another important

thing that is the part of this particular pedigree breeding method.

So, what is that? First through this pedigree method we have to maintain the detailed record of the relationship between the selected plants and their progeny. Ok! So, this record keeping process of the plants and its progeny is known as the pedigree method, pedigree record. Ok! So, what are the advantages of this pedigree record? First of all each progeny in the every generation can be traced back to the  $F_2$  plant from which it has been originated. Because if you try to recall the pedigree method, in  $F_1$  no selection was made from all the plants seeds were collected and in  $F_2$  different seeds were shown individually, each and every seeds were space planted. So, if we collect the record therefore, we can trace back to the  $F_2$  plant.  $F_2$  particular plant from which plant this progeny has been originated.

Next one, it helps to find out whether two individuals may share some allele in common if they are related by descent. Suppose if you recall in F3 generation different lines are grown. Right from F3 generation the plant to progeny rows are being grown. So, suppose in first row we have selected two plants. So, some allele may be common in between these two plants as they were being grown in a particular row. Ok! So, next third one is allelic composition of the final population could be predicted also if there is such history in its ancestry ok.

Suppose we are observing a particular line, their husk color is brown or husk color is black while in rest of the lines the husk color is straw colored. Then ultimately we can predict it that from which ancestry it is coming? From which parent it is coming? Now there are two ways means two different strategies are taken to maintain the pedigree method, two systems are followed basically. First one is pedigree based on the location of the progeny row in the field, next one is pedigree based on the serial number of the selected plants ok. First one is based on the location of the progeny row and next one is serial number of the plants. It is plant specific another one is row specific.

So, let us discuss about the pedigree based on the location of progeny row in the field.

Ok! So, for each and every pedigree record in the pedigree number a first number is given there, a 4 digit number is placed; the first 2 digit basically corresponds to the year on which the cross was made. Suppose this cross was made in 1982 so, we can write it as 82 and thereafter number 12 is there. The 12 determines the number of cross attempted in that particular year, from which cross we are conducting the experiment. Ok! So, the cross number 12 made in year 1982 is being analysed over here ok. So, now individual plant progeny row in  $F_3$  if you carefully recall the pedigree method in  $F_2$  no row was there, single plant was grown. From  $F_3$  onwards plant to progeny row was grown.

So, here individual plant progeny row in  $F_3$  and later generation are assigned row number based on its location on the plot. Ok! Maybe it is growing in third row, maybe it is growing in seventh row. So, based on that we have to mark it. Then in progeny in  $F_4$ and later generation is identified by its row number in that current generation proceeded by the row number of the progeny row of the previous generation from where it was selected. So, suppose this is the pedigree number suppose this is the pedigree number we got few  $F_2$  plants. These are plants no row is there. These are different  $F_2$  plants. Its seed has been grown in this particular row its seed has been grown in this particular row.

So, over here from  $F_3$  onwards we can trace it. Suppose in  $F_3$  generation among this particular cross in 1982 the twelfth number of cross was made and maybe in fifth row in  $F_3$  generation, maybe in fifth row we have chosen a particular plant. So, its description will be the progeny available in the fifth row in  $F_3$  plot this is  $F_3$  plot in fifth row suppose this is the fifth row 1, 2, 3, 4, 5, 6 these are different row, in fifth row this plant is available. In  $F_4$  suppose this number is given, what does it mean? It means the progeny available in the fourth row in  $F_4$  plot,  $F_4$  plot in fourth row its progeny is available which was selected from the progeny of the fifth row in  $F_3$  plot. Suppose this plant was selected in  $F_4$  generation its progeny was grown in plant to progeny fashion and in fourth row it available.

Here from we are identifying another one. Ok! So, in this way we can make the pedigree record. According to another method means which is based on the serial

number of the selected plants, here basically the first process is same and according to the next process after dash what will be put? Each selected individuals in  $F_2$  generation is given serial number. Earlier from  $F_3$  generation in the field the row number was given, but here in  $F_2$  generation the plants are given a serial number and the individuals in subsequent generation is given the serial number of that particular generation preceded by the serial number of the plants of previous generation from which it was derived. In this way we can trace it back like progeny obtained from plant number 5 selected from  $F_2$ . Here also suppose these are different  $F_2$  plants from plant number 5 we have grown the progeny in  $F_3$  generation.

Its progeny have been grown in  $F_3$  generation. Suppose from plant number 5 we have grown this line. So, it will be mentioned in this way that is plant number 5 selected from  $F_2$ . Its progeny is being grown. In  $F_4$  generation also it could be marked in this way, the progeny from the plant number 4 selected from the  $F_3$  progeny which was derived from the plant number 5 selected in  $F_2$ . Ok! Suppose fourth plant is selected from here, here different plants were available 1, 2, 3, 4 in this line and in next generation it has been grown. Ok! So, in fourth generation we can mark it in this way, the progeny from the plant number 4 selected from the  $F_3$  progeny which was derived from the  $F_3$  progeny from the plant number 5 selected from the  $F_3$  progeny which was derived from the  $F_3$  progeny from the plant number 5 selected from the  $F_3$  progeny which was derived from the plant number 5 methods.

In this way basically we can do. Then we can maintain the pedigree method. So, what are the merits and demerits of pedigree method? In merits first it provides maximum opportunity for the breeder to use his skills for selection of desirable plant from segregating generation. It provides maximum opportunity for the breeder to use his skills for selection of desirable plants from segregating generation. Ok! Means based on the phenotype, based on the segregating individual he can identify the most potential one. Then transgressive segregates for yield and other quantitative traits may be Ok! in addition the specific recovered to improvement in trait.

Suppose farmers target was to get the normal yield and the aroma of the Gobindobhog along with that, he may get some transgressive segregates also. The breeder may often be

able to obtain information about inheritance of qualitative trait from pedigree method. Like the sheath color, flower color those qualitative traits that is controlled by a few genes could be easily depicted, easily calculated, through this pedigree method. Then plant progenies with visible defects and weakness are eliminated at early stage. Next what are the demerits of this method? First it is very time consuming and tedious process to maintain pedigree record.

In each and every year once you are selecting something you have to maintain the record that is tedious. Then selection among and within a large number of progeny in each and every generation is laborious and limit the number of cross. If a breeder is attempting 30, 40 crosses then maintaining its progeny will be a huge problem. A lot of land, labor is needed, huge time could be involved there. Ok!

Then success of this method depends on the skill of the breeder. If the breeder is not skillful the success will not be occurred, will not be obtained. Because no opportunity... no opportunity is there for natural selection. Ok! The breeder has to identify based on his trait selection ok. He has to choose the genotype in  $F_2$ ,  $F_3$  different lines. Then selection for yield in  $F_2$  and  $F_3$  is ineffective because at that time the plants are not fully homozygous, they are in segregating condition, right?

So, full effective selection is not possible. Then if care is not taken properly to retain a sufficient number of progenies, valuable genotypes may be lost in early segregating generation. That is very important point. So, if proper selection is not taken suppose this population has been lost in early generation. Somehow we have not selected. Then at later generation we may not found it. Ok! Suppose we have only selected this one then suppose we have selected this one.

So, in next generation onwards capital B allele will not be available. So, initial selection is of prior importance. So, these are the references of this particular class.