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-06: Pureline Selection

Hello everybody, this is Dr. Joydeep Banerjee from Agricultural and Food Engineering Department of IIT Kharagpur. I would like to continue our course on Basics of Crop Breeding and Plant Biotechnology. So, today we will discuss about another module, module 2 that is regarding the Principles of Conventional Plant Breeding. What are the different conventional plant breeding techniques are used, we will be discussing here, some basics about plant breeding also will be discussed here. In the first lecture, we will discuss about the pureline selection and pedigree method.

So, the concept covers under this particular module and this particular class, we will be discussing about the evolution of plant breeding, how since the origin of human beings, how the plant breeding has been evolved, how gradually human intervene with the plant species, different plant species, different crop species and how the breeding approaches was conducted and what is the present status and the future perspectives. Then we will discuss about the evolution of maize and rice plants, then we will discuss about pureline selection, a particular conventional plant breeding method, then what are the use or different uses of pureline selection will be discussed here. Then we will discuss about the several advantages and disadvantages of pureline selection. Next, we will move into the origin of genetic variations in pureline, how within a pureline the variations could be arised, it will be discussed in this particular course.
Then gradually we will be discussing into the pedigree method that is another important breeding method in self-pollinated crop, then what are the procedure of conducting pedigree method, how pedigree record is maintained, that will be covered over here and

finally, we will discuss about the merits and demerits of pedigree method. So, we know the *Homo sapiens* have been evolved on this earth approximately at 400,000 to 250,000 years ago before that *Homo erectus* was available. Ok! So, these modern human beings have evolved a long time ago and if we see about the domestication of different crops, some major crops, this domestication process started approximately 10,000 to 5,000 years ago. Ok! So, compared to the evolution of human beings the crop domestication time period is relatively lesser, much shorter. So, why? Initially, the human beings were nomadic, means they used to stay in the caves and they used to hunt different animals or they used to collect foods from different plants, different crops and once a particular place where they used to live, and food is over. So, nomads are basically migrated in nature from one place, as the crop, animal and food source exhausted, they used to shift to a different location. So, thereafter during the course of human evolution, gradually they became food gatherer during that particular period they used to collect the foods from a particular location and after gathering the foods, they used to move to a different place. They were also migrated in, but at that time they started to gather different food crops and finally, the human beings became food producer. So, they became food producer.

So, in this way, human evolution occurred initially they were nomads and the later on they became food gatherer and finally, they became food producer, during this process once they became food gatherer to food producer, during this process they started crop domestication. Some major crops like rice, wheat, lentil those domestication started long years back, and corn, it's domestication also started long year before. So, once the domestication started, thereafter knowingly or unknowingly plants, human beings they started selection of several crops, and during the selection process, they chose some of the genotypes compared to rest of the available genotypes. So, in this way, the plant breeding evolved once we started selection, then the plant breeding came into the picture means we are selecting few individuals from a field, in this way, in a specific direction our plant population could move. Then nowadays, since 1960s, the green revolution started that is the era of modern agriculture, several breeding approaches were conducted by different scientists since 1900 onwards and recently, the molecular plant breeding is going on means application of different molecular markers, then transgenic breeding those things are being

conducted in nowadays.

So, these comes under modern agriculture. So, in this class we will be discussing about the conventional plant breeding methods. So, let us see about the evolution of maize plant. Ok! Maize is known to all of us, it is corn, in this plant male and female gametes are produced. This plant is having tassel wherefrom the pollen grains are produced while in the female part, it will be having silks all over here.

So, after successful fertilization, we can see the cob formation in maize plant because maize cob is known to most of us. So, in maize plant if we carefully see the plant structure a single stalk is available, means a single tall plant is available in each maize plant, but if we go back, if we try to find out the wild progenitor of maize, the scientist found that a particular crop name as teosinte is the wild progenitor of maize. From this teosinte, scientist think that modern day maize has been evolved. So, if you see the structure of a teosinte plant, multiple stalks are there, in each stalk the tassels are formed and the cobs are also formed in teosinte plant, but the size of the cob is very small compared to the cob size in modern day maize. So, in this way knowingly or unknowingly human beings have done some selection.

So that, the cob size could be increased from teosinte into modern maize. So, let us see the cob structure and grain structure. So, if you see about the cob structure in teosinte, only 2 rows of seeds are available over there, while in case of maize, 6 to 12 rows of seeds are available in each cob. Ok! Here, only 2 rows were available, after selection finally, we are observing 6 to 12 rows are available in each maize cob in the modern corn. If we see the grain structure, in case of teosinte, for each and every seed, a hard seed coating is available, this is the hard seed coating.

We have to break this coating then we have to get the grain, the grain size is very small. While, if we see the grain structure of cob of present-day maize, then the coating size is very less, while the grain size has enlarged a lot means, we need more food, right? So, the selection has been done in this particular direction. So, in this way, the maize has been

evolved since a long time, it's cob size has been increased also, initially it was smaller, several fossils were available. Therefrom, we came to know that initially the cob size was very small, gradually due to selection, the larger size cob were available.

Now, let us discuss about the evolution of rice. Just if you try to visualize the earlier scenario once the human beings were nomadic in nature, at that time the rice, whichever was available most of them were wild type, and in the wild rice, the shattering type character is available. What is shattering type character? Shattering type behavior, if high wind is there then the grains may fall down from the plants, that is the shattering feature during harvesting if the plant is shaken, then the seeds will fall down. So, at that time, human beings were not food producer. So, the plant had to think about itself, it's progeny.

So, it was shattering type in nature. So that, the seeds once will be matured, it will fall down and next generation will come, but later on, once human beings became food gatherer or food producers, they started selection for this type of rice plant which is non-shattering type means during harvesting also the seeds will not be lost. Ok! So, once the farmer will try to harvest this particular plant, they would like to harvest the seed from this particular plant, from each and every panicle the seeds will not be released easily after harvesting, they have to do threshing. So, this is the evolution of rice. So, in this way, by knowingly or unknowingly, human beings have started plant breeding since long time.

Now, let us discuss about few famous conventional plant breeding procedure. First, we will discuss about pureline selection. So, let us discuss about some basic features of this particular selection. First, pureline selection basically means individual plant selection, means from the field a large number of plants are available, we have to identify few individual plants and it will be analyzed by different procedure, which we will be discussing. So, individual plant selection is pureline selection, then it is defined how a pureline can be defined.

It is defined as the progeny of a single homozygous self-pollinated plant, three points are very important the progeny of a single homozygous self-pollinated plants. Suppose, these

two types of plants are available, it is being grown as well as it is being grown in next generation. So, pureline is nothing, but the progeny of a single homozygous self-pollinated plant. In case of self-pollinated plant, pureline selection is done. So, it should be self-pollinated homozygous in nature and from a single plant, we have to do the selection.

Next, all individuals are of same genotype in pureline. So, suppose it's progeny are being grown, again from here, again we are growing the progeny. What will be the genotype of this plant? All will be capital A capital A and in it's progeny also will be having capital A capital A in all the plants . Ok! Suppose, we are rejecting this one, we have rejected this one earlier. So, all individuals are of same genotype in pureline because it is coming from the same mother plant.

And now, another thing is there, variation is only environmental, that is another important point. So, variation may be available in pureline, how? Suppose the genotype of all these 5 plants are capital A capital A, genetically they are same. Now, these plants are being grown in a particular field. In this field, we are putting irrigation in this direction, we have given irrigation. So, if irrigation is given from this side, this plant will be getting more amount of water, while this plant may face water scarcity.

Some phenotypic difference could be arised, means this variation is due to the environmental factor. So, in pureline, whatever the variation is there that is due to environment. Next, a large number of plants selected and harvested. So, initially a large number of plants are selected and harvested then the progeny rows are evaluated to select the best progeny as a pureline. So, this plant is being harvested and ultimately, it's progeny is being grown.

From this progeny, ultimately, the best progeny we have to release as a pureline variety. Next one, let us discuss about few characteristics of this pureline selection. First of all, the plants with a pureline have same characteristics as the mother plant. All the plants within a pureline have same characteristics as the mother plant. Next one, variation is environmental and non-heritable.

You know that the variation is environmental. I had told earlier, it is not heritable in nature. And third one, pureline can become genetically variable with time. How it can be genetically variable with time we will discuss later on. Now this particular person Johannsen, he basically gave pureline theory for the first time. So, he did his experiment on princess bean that is *Phaseolus vulgaris*.

So, let us discuss what did he do in his experiment. So, first of all, Johannsen collected the commercial seed lot of *Phaseolus vulgaris*. And from the market, in the commercial seed lot, he found that several size seeds are available. Let us assume, he has collected the seed lot and some seeds are like this, some seeds are like this, some seeds were like this, while some seeds were like this. In this way different sized seeds of princess bean was available within the commercial seed lot.

If you see, this one is the largest one, this one is the smallest one and in between different seeds are available. So, in this way ultimately, he separated it and he developed plant from each and every line and in this way, he generated 19 different lines based on the average seed weight. The average seed weight or the mean seed weight of the first line was 640 milligrams. While, the average seed weight of somewhere in line number 13, it's average seed weight was 450 milligrams. While in the least one, line number 19, the average seed weight was 350 milligrams.

In this way, initially, he classified the total seeds available in the seed lot. Then he planned his experiment in two different ways. In first approach, he has grown this particular plant. So, the plant grown from the seeds formed this particular plant and once the harvest was ready, he has done selection. He took the largest seed and he took the smallest seed from this harvest.

So, here over he has done some and what selection he has made? He took the largest seed from here and he took the smallest seed from here. In next year, this largest seed was

shown, the plant was generated. He collected the harvest from that plant and again he took the largest seed from there. While, from here, he has grown this particular plant, it's seed was harvested and he took the smallest seed from here. In this way, from here, he identified the largest seed, from here, he identified the smallest seed and this experiment he conducted for 6 generations.

He conducted this experiment for 6 generations and after that he took the average seed weight of that particular plant. And he found that the average seed weight over here was 683 milligrams. While, in case of the smallest seed, the average seed weight of this plant, the plant which was grown over here its average seed weight was 690 milligrams. So, this is the part of one experiment. In another experiment, he basically conducted taking the line number 13.

Here, he distributed the seed in 4 different lots based on the seed weight and he classified it into 100 milligrams classes. These 450 milligrams, its progeny, means once the seed was formed in this particular plant, it was divided into 100 milligrams classes. In first one, he took 200 milligrams, in next one, he took 300 milligrams, then in third one, he took 400 milligrams seeds and in last one, he took the 500 milligrams seeds. And those seeds were grown and its progeny were evaluated and finally, he took the average seed weight once again. He found that over here, the average seed weight was 475 milligrams over here, the average seed weight in the progeny was 450 milligrams.

In this line, the average seed weight was approximately 451 milligrams and over here, the average seed weight was 458 milligrams. So, in this way, he planned these different experiments initially after dividing the seeds, based on its size, he planned these different types of experiment. Ok! So, if you see carefully from this particular seed where the average seed weight was 640 milligrams, over here, in 2 progeny lines from the largest one as well as from the smallest one, its average seed weight was 680 and 690 milligrams they were close statistically, these 3 were close. While over here, if you compare these 4 things with this particular one, they are also not statistically too much different. Ok! So now,

initially we will discuss about the pureline selection method. Ok!

So, before discussing pureline selection method first, as we have done the Johannsen's experiment. So, let us discuss about the what are the conclusions Johannsen made. First conclusion he made that homozygosity is increased due to selfing, means selfing improved homozygosity. Next one, selection is not effective within pureline, he basically told this as a pureline. So, if we try to do some selection that will not be effective that was his conclusion, Johannsen conclusion.

So, these are the Johannsen conclusions. Next conclusion is selection is not effective within pureline. Third one, selection is effective within the original population that is very important point, selection is effective within the original population. If you consider this as the original population, which was collected from the market. Ok! Their selection was effective. And fourth one, variation within a pureline is due to environment. Fourth point is that variation within a pureline is due to environment, because he found some seeds are largest some seeds are smallest. Ok!

If you try to find out a pod formation in beans, those seeds which are growing over here, they will be larger, while the seeds which is being grown over here that will be smaller due to the physiology of the plant, right? So, in this way, some environmental effect could be arised due to which, the variation may be there within a plant also, but those are not the genetic factor in next generation, it will be same like its mother plant. So, let us discuss about the pureline selection method. So, in pureline selection, first we have to grow the homozygous self-pollinated plants in the field. Ok! Whether it is homozygous or not that is not known to us, but in the self-pollinated plant we have to do this pureline selection.

During this process, we can identify whether it is homozygous or not. So now, from the field we have to identify few better plants, 200 to 300 plants are selected based on the phenotype. Suppose we have selected plant 1, we have selected plant 2, we have selected plant 3. Next in next generation, we have to do individual plant to progeny row means their seeds are harvested separately and it will be grown over here like the progeny of P_1

plant, the progeny of P_2 plants in this way. We have to grow the progeny of different plants and undesirable plants could be rejected.

If it is undesirable, we can reject it. Then, we have to repeat these things once again to check whether the lines are homozygous or not. If it is heterozygous, then we can see the segregation because according to the Mendel's law, we know that if heterozygosity is there, upon selfing the segregation will be available right. Next, gradually, we have to move into the preliminary yield trial. In preliminary yield trial, earlier it was being grown in a particular row. In preliminary yield trial, basically, the plants are grown in a field where a number of rows are grown and, in each row, different plants will be available.

So, in this way each and every line will be grown in a small field during the preliminary yield trials process. Like over here, if the plants are being grown in row only, suppose, this is line 1, this is line 2, this is line 5. The plants which are growing close to the irrigation source, it will show a particular feature while, which is away from the irrigation source, it may show some drought features. Ok! It may encounter some drought, but if we grow this particular line in a field like this, and if irrigation is done by the side of each and every field, each and every plot, then we can get the average value of the plants. Ok! Means, the environmental effect could be minimized and over here also, during preliminary yield trial, we have to reject the inferior one.

Next, whatever is the superior, we have to reduce it drastically, whatever the superior lines we are observing, we have to grow it in replicated trial at different locations to produce disease free plant. So, few things are there replicated trial, different locations. Suppose someone is planning to develop some pureline rice variety in Uttar Pradesh. So, the soil or environmental condition in UP and West Bengal might differ. So, if we compare that particular line in West Bengal, also if we compare that particular line in Tamil Nadu also.

So, in different soil condition and in different agro-climatic condition we can evaluate that genotype. So, the different location trial or multi location trial is done, as well as in each location, we have to grow each variety, each line in different replication, like this is

line 1, this is line 2, this is line 3. Again, we can grow line 2, line 1, line 3 we can grow line 3, line 2, line 1. In this way, in different positions of the field, we can grow our targeted lines. So that, the environmental effect could be minimized, further the soil heterogeneity could be minimized.

And if we identify some elite lines over here, finally, we have to go to the seed collection from the base progeny and eventually, it will be released either by state varietal release committee or central varietal release committee, based on the performance of that variety. Now, what are the uses of pureline selection? First of all, we can release these pureline selected individuals as a pure line variety. It can be used as a parent for hybridization because in this pureline selection, if you see carefully, no crossing has been made between any plants. All the plants were selfed, because it is self-pollinated in nature, we did not attempt any cross, just from the field we have identified few individuals, its seeds were collected, progenies were grown and therefrom due to selection, eventually we found a particular line that might be suitable in different locations. So, it could be used as a parent for hybridization also and third, it could be used for mutation study or other biological research.

So, these are the advantages and disadvantages of pureline selection. First, among advantages, it is developed from the available genotype, means suppose, we have a rice germplasm, it is growing in the field, rice is self-pollinated in nature. From the field, we have started selection, we have identified few individual plants, their seeds were evaluated and ultimately, we have identified a pureline variety. So, it is developed from an available genotype, no crossing has to be made. Next one, it is attractive to the farmers and consumers, as it is developed from an available genotype, suppose, it was developed from Swarna rice genotype.

Swarna rice genotype, which is grown all across the India. Ok! So, the farmers also know that this variety has this amount of produce, it can tolerate the environment in this particular condition and what is the water requirement of this crop and at what time the Swarna rice

will mature, these informations are available with the farmers. So, the farmers can believe the pureline which will be generated from here easily. Next one, from the consumers perspective also it is good, because the consumers will think that we know the seed type of this particular variety. So, it could be easily accepted by the consumers, some consumers will prefer sticky rice and some will prefer non-sticky rice. Ok! So, if a particular variety is known to the consumer previously, any pureline selection has been done from there will also be accepted easily.

Next one, it is stable as tested for many years we know that Swarna rice variety is grown all across the country. Now, again suppose we have identified some lines from here it is being grown in preliminary yield trial, replicated yield trial, multi-location trial and for these years it is being tested. So, it is stable and it is highly uniform and easily certified as it is being developed from a single homozygous self-pollinated plant. So, it should be highly uniform, the genotype of all of the individuals will be same and it could be easily certified. Ok! Suppose over here, one plant had this genotype, and another plant had this genotype, this one had this and this one had this genotype. Ok!

We have done selection process and ultimately, we have gone for this particular individual. Ok! So, all of them will be having same genotype in its progeny. So, it could be certified easily no variations is expected. So, now let us discuss about the what are the disadvantages of pureline selection. First of all, new genotypes are not created by pureline selection.

So, whatever is available over there, we are selecting from here, no new creation is being made, no crossing is being made. Next, improvement is limited to test population. Suppose, someone is attempting to do pureline selection by taking a particular rice variety which is growing in 5 bighas area. There may not be any variation, no genetic modification might be there. So, the selection process will not be too much effective until and unless some variation is there, all plants will be same because rice is a self-pollinated crop. Ok!

Next one, improvement is limited to the test population, next one, it may have limited adaptability. Suppose, from this plant we have generated the pureline. So, this pureline

now is being popular in all the fields in UP it is being grown. Now, suppose, a particular fungal race has been evolved or a bacterial race has been evolved. A disease epidemic condition has been created and that race can attack this particular genotype capital A capital A, capital B capital B.

Suppose, it can attack this genotype profusely, it can cause significant loss in this particular genotype. We know that for each and every disease incidence, some gene-to-gene hypothesis might be there, some genes from the pathogen and some genes from the crop might play some important role in this initial disease recognition process, ultimately disease progress process. So, if capital B capital B gene is responsible for that type of susceptibility. So, within this pureline all the plants will be having same genotype.

So, all the crops may be damaged. So, its adaptability could be limited. Ok! If multiple genes will be there like if genotype will be capital A capital A small b small b or capital A capital A capital B small b. If these different combinations are there, at least farmers can get some seeds from that one. In case of pureline all will be homozygous, all will be having same genetic constitution.

So, its adaptability is less. Next one, no further improvement is possible from pureline variety. Ok! Suppose, we got this one. So, within next 3 to 4 years, we may not expect any further improvement because all the genes are in homozygous condition. Ok! So, if it is in homozygous condition in next generation, or in next-to-next generation no genetic changes could be occurred.

So, no further improvement is possible. Now, that is another important story what we are going to discuss that how genetic variations are obtained in pureline. Ok! If you just recall the Johannsen's experiment he started the experiment with the commercial seed lot of princess bean. He found different size seeds, means some genetic variation was there. So, how genetic variations could be obtained in pureline? Ok, so now, suppose a 5 kilometer radius area is available, radius area is available, all the farmers are growing different rice varieties. Ok!

Some farmers are growing Swarna, some farmers are growing Gobindobhog, it is a small grain aromatic rice, some farmers are growing Pokkali. So, Pokkali is a salt tolerant rice. Now, suppose, over here in between of all these fields, a particular threshing floor is available. So, in this particular threshing floor, all the farmers can bring their harvest and they can do threshing.

So, after threshing, the seeds will be bagged. So, during this bagging process, some seeds of Pokkali might be mixed with Swarna, some seeds of Gobindobhog might be mixed with Swarna. So, in this way mechanical mixture could be arised in different self-pollinated crops during harvesting, during sorting the seeds. Ok! So, in this way, some new genetic constitutions could be arised within a pureline population. Next one, we will be discussing about natural hybridization. Ok!

In self-pollinated crops, certain amount of natural crossing could be occurred. The pollen grains from a different variety, which is growing in the next field can come into a particular field. Suppose, two different varieties are being grown over here too in two different fields, and due to some insects, some natural hybridization could be occurred even in case of self-pollinated crops also. If some pollen grains come from this particular variety, definitely it's genetic constitution might be different. So, a new gene or a new allele will be coming over here. In next generation along with this type of seeds, some seeds will be there that will be having some different allele which is coming from V_2.

In this way, due to natural hybridization, some new genetic constitutions could be generated. Third one is mutation. Mutation is inevitable, natural mutation is inevitable and what is the rate of natural mutation? 1 in a million means, in 10^{-6} individuals one mutation could be occurred and mutation is the ultimate source of most of the genetic variations. What are the different alleles developed for each and every gene that is developed due to mutation? So, suppose in a particular field a rice variety is being grown for years after years. Let us assume not rice, a mung bean variety is being grown years after years, some farmers, they are growing this particular mung bean variety.

Mung bean is also self-pollinated in nature. So, pureline selection could be arise over there, it belongs to bean family. So, suppose its genotype was capital A capital A capital B capital B. So, all the plants were supposed to be capital A capital A capital B capital B. Due to UV rays or due to some reason, in a particular plant the mutation has occurred in a particular chromosome. So, due to mutation in one chromosome the mutant allele has been formed or a new allele has been formed.

Mung bean is self-pollinated in nature. So, this plants will be selfed in next generation, if we find the progeny what type of progeny will be obtained? Capital A capital A capital B capital B, will be getting this capital A capital A capital B small b and capital A capital A small b small b. These three types of progenies will be obtained from here. How? From this plant what will be the gametes formed? One gamete will be capital A capital B one gamete will be capital A small b. So, similar types of male and female gametes will be there, capital A capital B and capital A small b, right? So, if the checkerboard is made, we will be getting capital A capital A capital B capital B, here from we will be getting capital A capital A capital B small b and here from we will be getting capital A capital A capital B small b and here from will get capital A capital A small b small b.

So, these three types will be generated from here. Ok! So now, if we start pureline selection from here, definitely some plants will be having capital A capital A capital B capital B, some lines will be having capital A capital A small b small b both are homozygous and this one is heterozygous, let us reject it. So, in this way, the variation may be obtained in pureline through mutation also. And last one, through chromosomal aberration, through chromosomal aberration means once the chromosomes are separated, homologous chromosomes are separated during meiosis process, what we have discussed during the Mendelian genetics. Then also, we may see that sometimes, in one gamete or at one end two homologous chromosome has gone, while in another pole none of the homologous chromosome has reached. In this way, the chromosomal imbalance could be arised, may be a chromosome may not be separated properly or two chromatids may go to a pole or none of the chromatids may go to another pole.

There also, we may lead to chromosomal aberration i.e., some part of the chromosome may be deleted during the process of segregation, meiosis or those things. There also, we can lead to chromosomal aberration means variations in chromosome number. Ok! So, if variation in chromosome number is available, then also, within a pureline some new genetic combinations may be observed and some new phenotype could be observed.