Wildlife Conservation
Dr. Ankur Awadhiya
Indian Forest Service
Department of Biotechnology
Indian Institute of Technology, Kanpur

Lecture – 33
Other aspects: cryopreservation, seed banks etc.

[FL] Today, we will have a look at two other aspects cryopreservation and seed banks.

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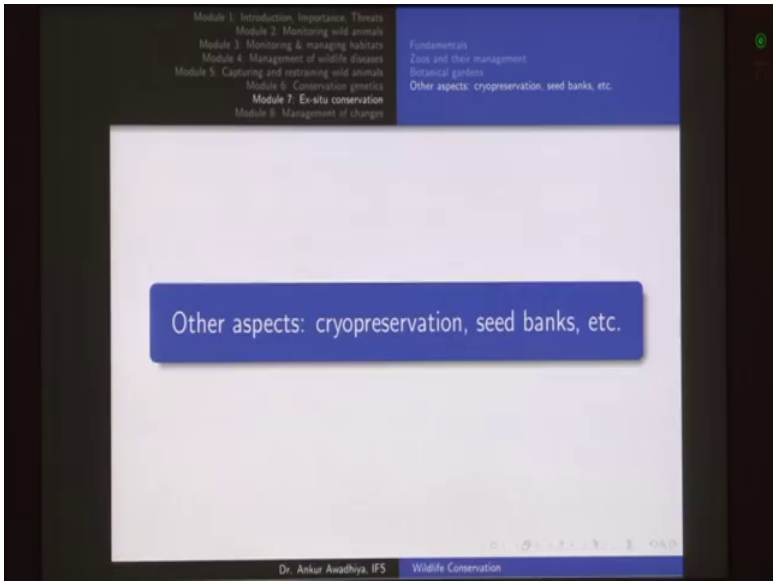

Now seed banks are very important for the purpose of conservation, because if we think about a large size tree and you have a limited area so, you can only have n number of trees in your in your area. But if you could collect the seeds of these trees and if you could preserve them for posterity so, we should be able to regenerate these trees using these seeds. So, seed banks become very important as a measure of ex situ conservation and before we move forward let us have a look at this paper.

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Regeneration of whole fertile plants from 30,000-y-old fruit tissue buried in Siberian permafrost

Svetlana Yashina[a,1], Stanislav Gubin[b], Stanislav Maksimovich[b], Alexandra Yashina[a], Edith Gakhova[a], and David Gilichinsky[b,2]

PNAS

Whole, fertile plants of Silene stenophylla Ledeb. (Caryophyllaceae) have been uniquely regenerated from maternal, immature fruit tissue of Late Pleistocene age using in vitro tissue culture and clonal micropropagation. The fruits were excavated in northeastern Siberia from fossil squirrel burrows buried at a depth of 38 m in undisturbed and never thawed Late Pleistocene permafrost sediments with a temperature of −7 °C. Accelerator mass spectrometry (AMS) radiocarbon dating showed fruits to be 31,800 ± 300 y old. The total γ-radiation dose accumulated by the fruits during this time was calculated as 0.07 MGy; this is the maximal reported dose after which tissues remain viable and seeds still germinate. Regenerated plants were brought to flowering and fruiting and they set viable seeds. At present, plants of S. stenophylla are the most ancient, viable, multicellular, living organisms. Morphophysiological studies comparing regenerated and extant plants obtained from modern seeds of the same species in the same region revealed that they were distinct phenotypes of S. stenophylla. The first generation cultivated from seeds obtained from regenerated plants progressed through all developmental stages and had the same morphological features as parent plants. The investigation showed high cryoresistance of plant placental tissue in permafrost. This natural cryopreservation of plant tissue over many thousands of years demonstrates a role for permafrost as a depository for an ancient gene pool, i.e., preexisting life, which hypothetically has long since vanished from the earth's surface, a potential source of ancient germplasm, and a laboratory for

However, to date, no viable flowering plant remains have been discovered from these ancient permafrost sediments.

Outside the permafrost zone the longevity of seeds in soil has been studied during the last 45 y in many places, including archaeological sites (10, 11). At the moment, the oldest viable seeds with the ability to germinate were radiocarbon dated to the first and eighth centuries of the Common Era. These are, respectively, *Phoenix dactylifera* found near the Dead Sea (12) and *Nelumbo nucifera* found in northeastern China (13).

The burrows from which our study material derived were buried in permanently frozen loess-ice deposits on the right bank of lower Kolyma River, northeastern Siberia (14). These deposits (Fig. 1*A*) are representative of a widely distributed, so-called Late Pleistocene ice complex found throughout the eastern Arctic, which has continuously accumulated during the last 60 ky. This complex comprises icy silts with a network of large syngenetic polygonal ice wedges and is richly fossil bearing (pollen, insects, plants, macrofossil, and mammal fossils). The studied burrows (Fig. 1*B*) were found in three exposures: Zelyony Mys, Stanchikovsky Yar, and Duvanny Yar. The last represents a key cross-section of Late Quaternary environmental history in the eastern Arctic studied by many scientists. All burrows were found at depths of 20–40 m from the present day surface and located in layers containing bones of large mammals such as mammoth, woody rhinoceros, bison, horse, deer, and other representatives of fauna from the age of Mam-

It says regeneration of whole fertile plants from 30000 year old fruit tissue buried in Siberian permafrost. So, what happens in this case is that Siberia is a location in Russia, it is on the northern in eastern portions of Russia and this area is very cold. So, this area has some areas, it has some regions that are a permafrost.

Now, permafrost is a region that always remains under sub freezing conditions. So, the temperature here is always less than 0 degree Celsius. So, the water is always frozen. So, it is permanently frozen permafrost.

Now, in these areas when some exuviations were done, then some fruits were found and when radiocarbon rating of these fruits was done then it turned out the these fruits were at least 30000 years old.

The exact age was 31800 plus minus 300 years. So, in this case when we had when the scientist had access to this fruit tissue, they tried to regenerate these plants and when that was done the plants were again formed.

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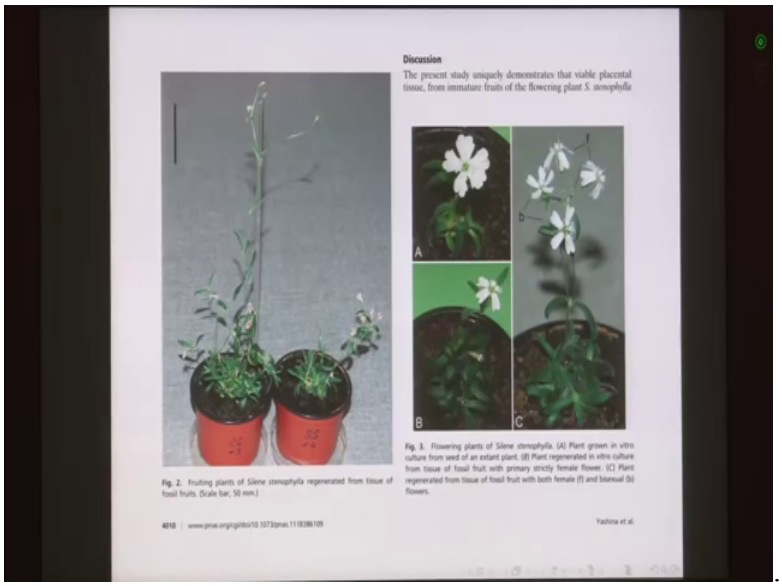

So, these are the plants, they are also giving out flowers and if we look at the flowers from the relative species that is present today will observe quite a number of variations from this old sample.

So, what is happening in this case is that we are having an access to a form of this plant that was existing around 30000 years back and does not exist today. So, this is a very good example of what preservation can do for us. So, this is a form of biodiversity that is lost from the earth, because it was present quite a long back. But, because it was preserved in the form of this tissue, so, we can access this biodiversity even after us span of 30000 years. Now, this was when these tissue were kept in a very cold condition just by nature.

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BREVIA

Germination, Genetics, and Growth of an Ancient Date Seed

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Now, this is another example germination genetics and growth of an ancient date seed. Now, date palms are found in a number of areas speaking in the drier areas.

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And these are 20000 year old date palms seeds that was found in Israel and then these seeds were germinated back. So, this is one of the plant that here through this germination, and then these plants are now growing and they can be propagated.

So, here also the place where these seeds were found these areas were having a number of date palm trees in antiquity. But close to around 800 years back, we started getting historical records that there is no date palm found in these areas anymore. So, at least for the last 800 years, we did not have any of these varieties that were growing here in the antiquity. And the prime reason was climate change, but these date palms seeds were stored in a jar. And around 2000 years back and then because the whole area is very dry and the whole areas is very the seeds were kept in an area where they were kept cool.

So, even after 2000 years people have been able to germinate these seeds and to get the plants back. So, this tells us the power of preserving of the seeds. So, in this lecture, we are going to have a look at this preservation of seeds in a greater detail. How can we use preservation of seeds as a measure of conservation of these species? So, let us begin with what a seed is. So, seed is an embryonic plant enclosed in an outer protective coating.

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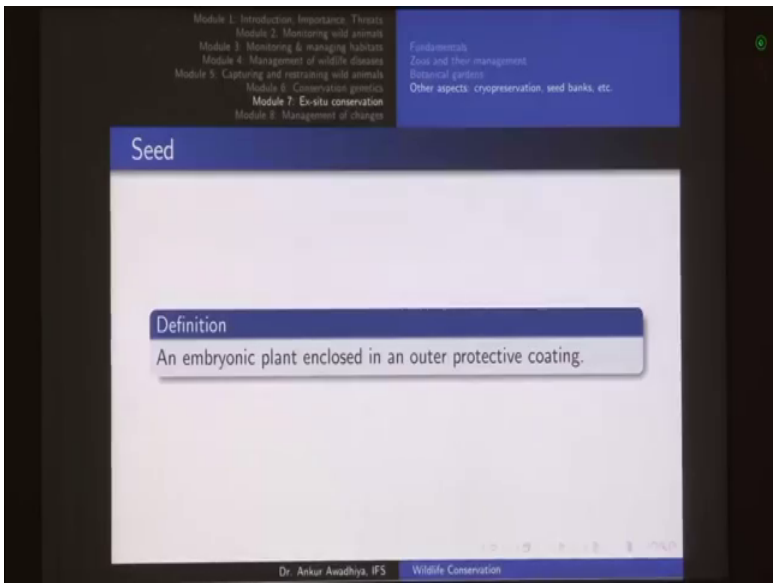

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So, if we look at trees in the forest, so, here will we have a fruit when this fruit opens up, it releases the seeds and then the seeds fall off. So, these are seeds if we cut open a seed then inside we will find something like this. So, we will have a seed coat and the outer area in most of the cases, this is a protective layer. So, it will be very thick and very strong, then we will have some region that has endosperm. Now endosperm is the food source for the embryonic plant.

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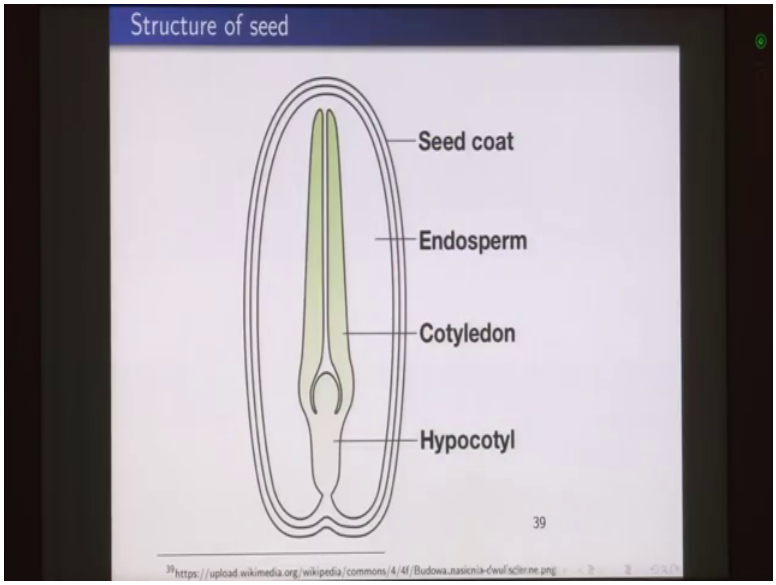

So, when the seed falls on to the ground it will not be able to perform photosynthesis right away. So, there is some amount of stored food that is kept for the embryonic plant. And then this is the embryonic plant these are cotyledons or the embryonic leaves, this is hypocotyl or the embryonic stem. So, when this seed falls into the ground and when it gets right conditions right amount of moisture, right temperature, then this plant will grow out of this seed.

And while doing so, it will utilize this endosperm for the initial period still; it is able to throw a leaf out. And once this leaf has come up then, it will be able to perform photosynthesis and gain energy from the sun from that point onwards. Now, the important point will note here is that, because seeds have this stored food in the form of endosperm.

So, they also act as food for a number of organisms including us. So, when we are eating the rice when we are eating wheat, what we are eating is the seed of the rice or the wheat plant. Now, similarly out there in nature there are a number of organisms that eat these seeds and some organisms even collect these seeds for posterity such as ants or squirrels.

Now, if we want to conserve these seeds, we will have to protect them from these animals. And we will also have to protect them from the microorganisms, because microorganisms also want to eat all of these. So, how do we do that? So, we begin by collection of good seeds.

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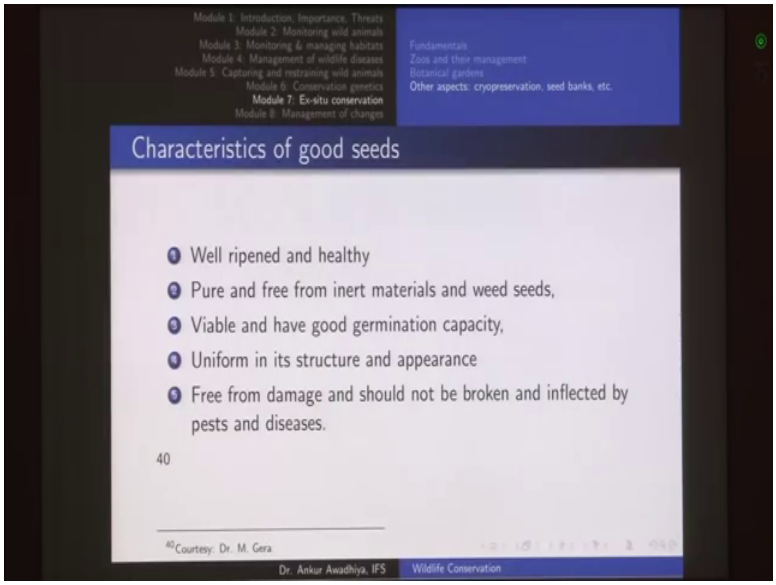

So, what do we mean by good seeds? The good seeds are these characters; they are well ripened and healthy. So, you should not collect your seed that is half ripened, because in that case the embryonic plant inside might not be well formed and it might not be able to germinate later on which is our prime purpose when we are creating the seed bank.

Then they should be pure and free from inert materials and weed seeds, because finally, later on we are going to plant these seeds back in to the environment. So, we do not want any weeds to come to with at when it is also should be free of inert materials. So, inert materials include thing such as soil or things like husk. Now why should we have seeds free from inert materials, because these inert materials might also be harming some microorganisms then these seeds should be viable and have good germination capacity viable means that this seeds should be living..

So, for instance if you take a seed and if you say dig the seed, so because of the high temperature the embryonic plant inside would die. And when that plant dies then we will not be able to generate this seed any longer. So, when we are collecting good seeds for our seed banking purpose then they should be viable and they should have a good germination capacity. Now, it should be uniform in its structure and appearance.

Why uniform, because in a number of seeds that are infested or infected will observe some amount of structural abnormalities. So, when we seeds are uniform, then it gives us an indication that these seeds are healthy then they should be free from damage and should not be broken and infected by pests and diseases. So, free from damage means that when we are processing these seeds. So, in a number of situations, we will find that we have collected some fruits and then these fruits have seeds inside.

So, these seeds have to be taken out. So, we will have to remove all the pipe that is inside and then when we have this seed out then we will dry it may be put it into some machine for some breeding purposes. Now when we are doing such activities there should not be any mechanical damage to the seeds, because if there is a mechanical damage. And remember that the seed coat is a protective covering.

So, if there is a damage to this protective covering then the seed is very much susceptible to further infections or infestations. So, it should not be broken and it, it should not be infected with pests and diseases.

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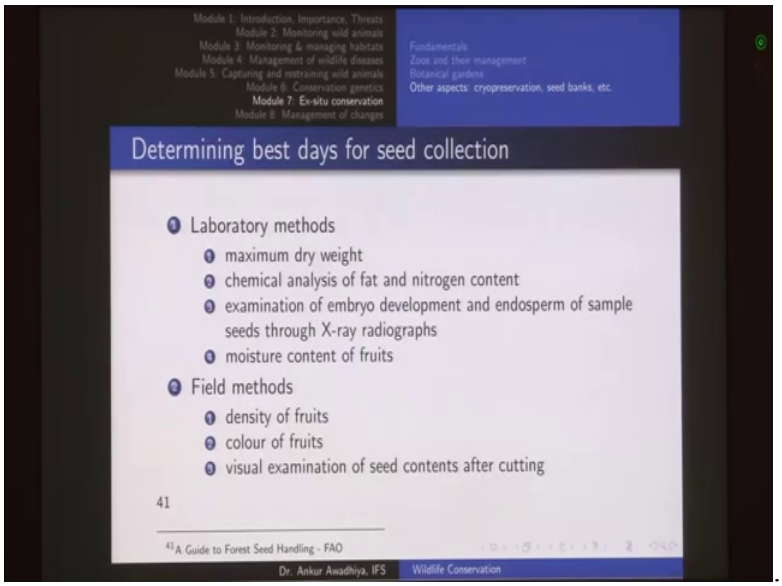

So, when we are collecting the seeds what are the best days for a seed collection, how do we know that our seed is right enough to be collected? So, there are laboratory methods and then there are field methods. Now laboratory methods include things like the maximum dry weight. So, for instance, if you look at a fruit so, initially we will have a flower. Now in this flower, it is converting into a fruit. So, there will be a small size fruit then this fruit will grow bigger and then finally, this fruit will attain the maximum size.

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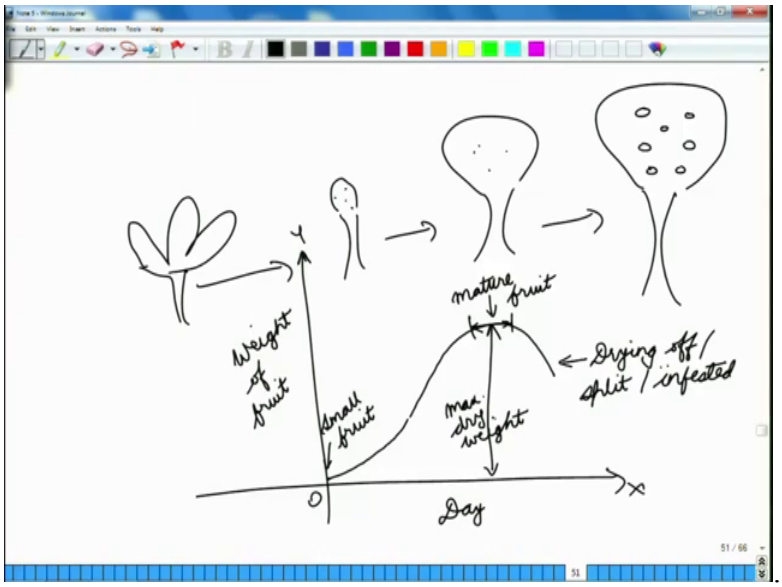

Now, we would find some small seeds here as well, but only in the larger size fruits will be having the seeds that have reached the maturity. So, when we look at the maximum dry weight as a laboratory method, what we are doing is that we take a sample of the fruits. So, basically we begin by saying our day and we have the weight. So, in this graph at days the row will have a fruit with a very small weight and then slowly and steadily the weight will increase and then later on this weight may remain constraint and then it will start falling.

Now this is the weight of fruit, because initially we had a very small fruit then at this point we have the matured fruit. And then at this point this fruit is drying off or it has split or it has become infested. So, there are some other organisms say ants or monkeys that has that has started eating away the fruit. So, in the laboratory method when we say that our fruit should have reached the maximum dry weight we want our fruit to be in this range.

So, this is the maximum dry weight and when we are using this laboratory method, we want to ensure that our fruits have reached this weight. Next is the chemical analysis of fat and nitrogen content. Now this is important, because in a in a seed that is well developed will be having an endosperm that is well developed. So, it will be having quite a heavy amount of fat and even nitrogen inside to act as initial starting points for the embryonic plant.

So, we can perform a chemical analysis of the amount of fat and nitrogen that is there in the seeds. Next, we could go for examination of embryo development and endosperm of sample seeds through X-ray radiographs. Now in this case what we are doing is that we take a sample of the seeds from the fruits and then we perform an x ray examination of those to understand whether these parts have fully developed or not. If they have fully developed they; that means, that are fruits are now ready to be collected for seeds.

Next you have moisture content of fruits. So, in a number of cases the moisture content will also tell us whether this, these fruits are ripened fully or not then you have the field methods. So, field methods include density of fruits or also weight of fruits colour of fruits. So, for instance in a number of fruits will observe a change in colour from green to say yellow or green to red and that will tell us whether these fruits are matured fully or

not and also a visual examination of seed contents after cutting. So, what we are referring to, here is that in a number of seeds such as bamboo seeds.

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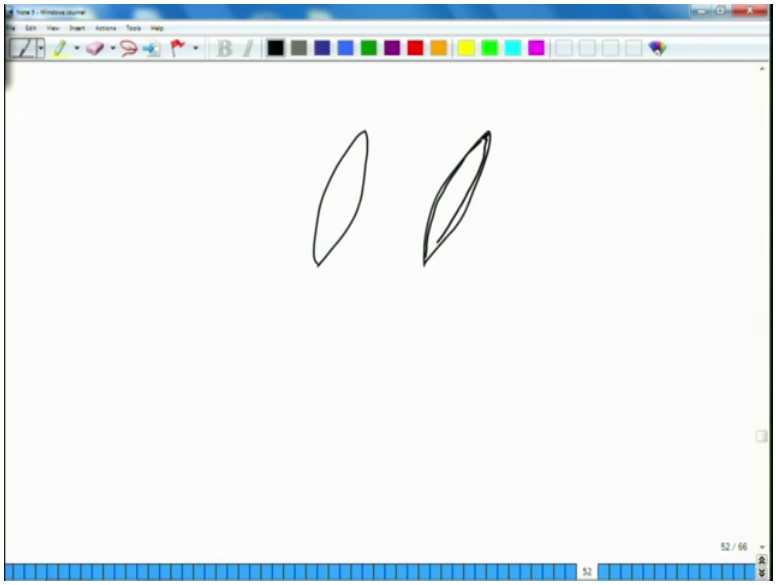

So, you will have this husk, but inside there will not be any seed and in certain situations when this fruit is completely developed will have a seed inside. So, you can always take a few samples out and observe what is inside the seed, do you have your embryonic plant inside or not? So, that can also be done.

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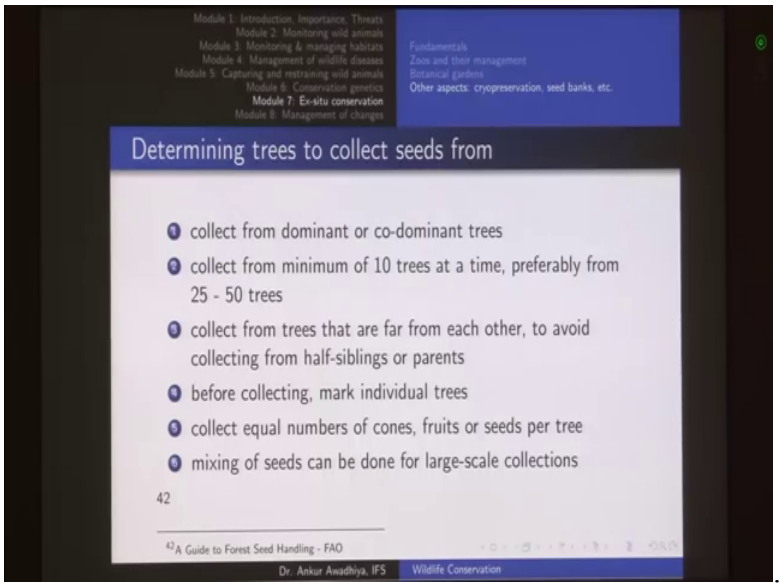

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Next, which seeds should be collector seeds from? So, we want to develop a seed banking facility. So, which trees should be collect seeds from? So, the first point is that you should collect from dominant or co-dominant trees. Now dominant or co-dominant trees means trees that are the highest that are there in that particular stand. So, a dominant tree has come out and is getting sunlight from all the directions. So, you should take seeds from dominant or co-dominant seed, because they are the largest size trees. And they are also considered as having the best genotype then you should collect from a minimum of 10 seeds at a time preferably from 25 to 50 trees..

So, you are not collecting just from one particular tree, because you want to have a diversity of genotypes that has there you should collect from trees that are avoid collecting from half siblings or parents. Now, in this case what we are referring to is that if you have a tree and if you try to understand that if there is a fruit or a seed here and when it gets dispersed what is the maximum distance that it can go out.

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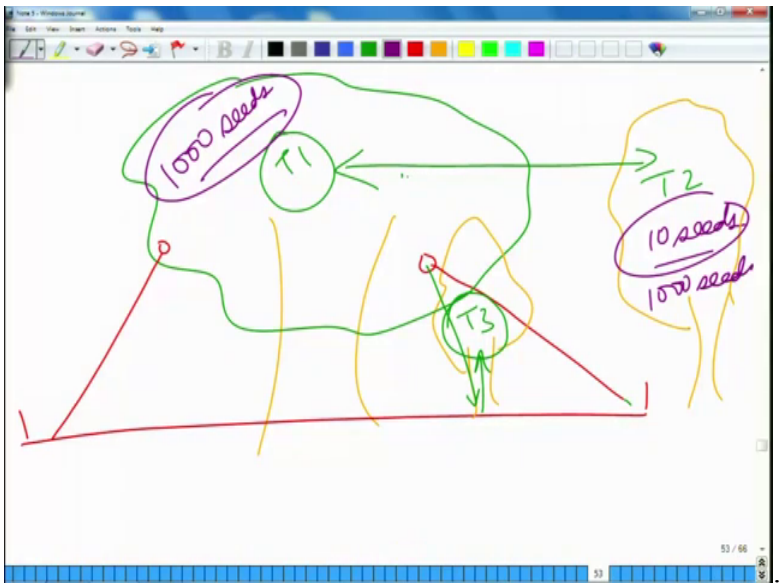

So, for instance if this is the maximum distance to which you can have the seeds or the fruits of this plant or this tree. So, the next tree that should be collected should be outside of this distance. So, this should be the next tree. Why? Because if you are collecting from a tree that is here when this particular tree. So, let us call this tree 1, tree 2 and this is the third tree.

So, if this is within this distance. So, it is quite possible that T 3 is the progeny of T 1. So, essentially if say this tree was a female tree and it was getting problems; so, these fruits got these flowers got pollinated we form the seed and then this seed fell here and this form r T 3.

So, in this case when we are collecting seeds from T 1 and T 3, the amount of genetic diversity between both of these collections will be less, because they are related to each other. Whereas when we are collecting from T 1 and T 2 then, because they are very far from each other there is a reduced possibility that both of these trees are related to each other.

So, in that case the amount of genetic diversity between both of these will be much greater. So, you should collect from trees that are far from each other provide collecting from half siblings or parents. Now, before collecting mark the individual trees and create a record for these collect equal numbers of cones fruits or seeds per tree. Now why this is important, because essentially what we are doing is a sampling procedure. So, for instance, if we collected say 1000 seeds from here and say 10 seeds from here then this will not be representative of what is being found in the nature.

In nature, we are having one tree that is T 1 and one tree that is T 2. So, if we are collecting 1000 seeds from here we should also be collecting 1000 seeds from the second plant. So, this is what is being said here, collect equal number of cones fruits or seeds per tree whatever your collecting. Next mixing of seeds can be done for large scale collections. So, it is not necessary that you should keep the seeds of T 1 separate from those of T 2; you can mix all of these depending on the conditions.

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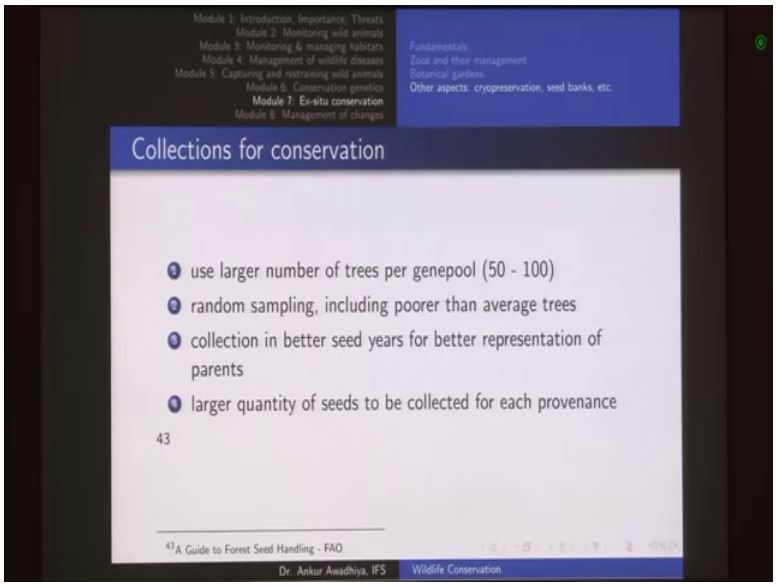

Now, when we are specially collecting for seed banking purposes then we generally increase the, the number of seeds of fruits that needs to be collected. So, in case of 25 to 50, we generally say that is the even mode number of trees per gene board 50 to 100 trees. And in this process dominant and co dominant trees are preferred, but we also might go for a random sampling including poorer than average trees, because even those poorer trees might be having some genes that are important for biodiversity.

So, it is quite possible that are tall and dominant trees are having less amount of disease resistant, but are shorter trees are having a better disease resistance. So, when we are collecting for conservation purposes only. So, in that case a random sampling is preferred including those of poorer than average tree. Next, collection in better seed years for better representation of parents; now what is this saying? So, when we talk about a seed here so, essentially if you have these trees in the forest.

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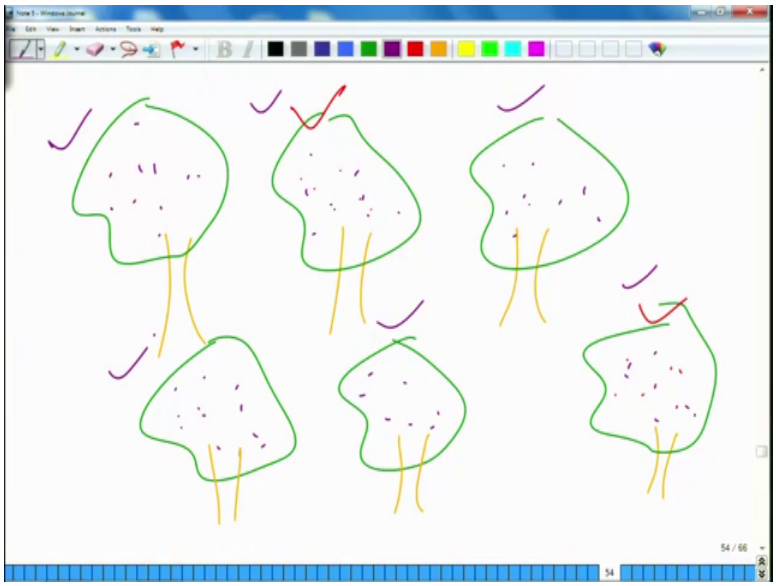

Now the seeding can happen in two ways, one is a sporadic seeding. So, in this sporadic seeding, what happens is that in a particular year this tree might be having fruits and this tree might be having fruits, but the other trees are not having fruits or it is possible that a number of these trees are having fruits, but the number of fruits that are being given out are very less.

So, typically in the forest, we observe that in a period of say 3 to 5 years there is one year in which will have the maximum amount of fruiting and seeding. And then this will remain subsided for under that 2, 3 more years and then it will again peak up; so, will observe a graph such as these.

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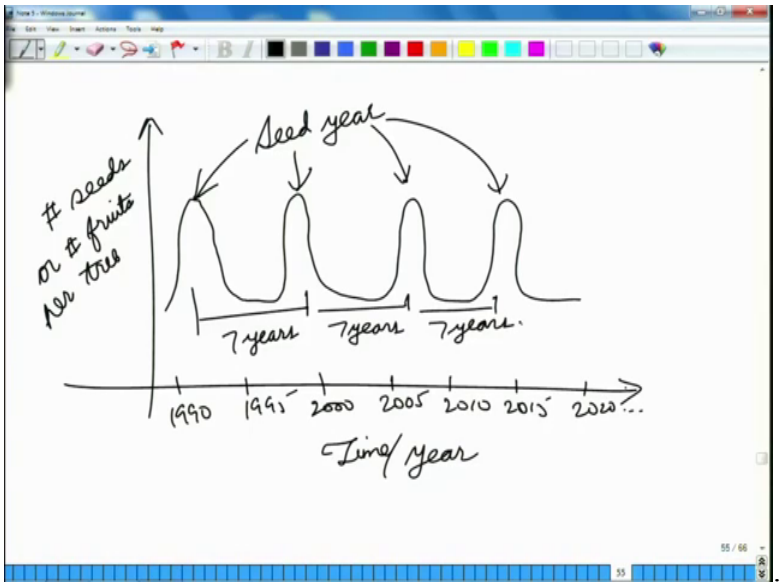

So, here we have the number of seeds or number of fruits per tree. And here we have the time or let us call it the year. So, year, say we have the year 1990, 1995 see, 2000 2005, 2010, 2015 and so on. Now, what happens is that typically we observe that there is a peak and then will have a break, then another peak and then another break and then another peak and then another break and so on. So, these years in which we are having the highest number of seeds of fruits per tree are known as seed years. So, this is one seed year, this is another seed year, this is another seed year, this is another seed year and all of these seed years show a certain periodicity. So, essentially if this period is say 7 years then this period will also be around 7 years, this period again will be around 7 years.

So, when we are collecting in better seed years what is happening is that we are having more number of a fruits per tree and also more number of trees that of fruiting. So, essentially we have a larger number of seeds when we have a larger number of seeds then the amount of diversity is also greater, because in our previous situation here, if we collected in this particular year we will only have the diversity of these two trees, but when we are collecting from our good seed year, what is happening is that this trees also floating this tree is also floating, this tree is also floating, this tree is also floating, this tree is also floating and this tree is also floating. So, in that case, will have in the diversity of all the trees together; so, which is by a good seed year is preferred for the

collection of seeds next larger quantity of seed to be collected for each provenance. So, a provenance means a region.

So, for instance in the case of teak you can have take from Madhya Pradesh, you can have teak in Kerala, you can have teak in Tamil Nadu, you can have teak in Karnataka. So, all of these are different locations from which you can have the teak seeds and we are when we are collecting for the purpose of conservation. When we are collecting to create a seed bank, we want to have as many seeds as possible. So, that for each sample for say every species like teak, if we have say around 1000 seeds then it is normally observed that the viability of the seeds goes down with time.

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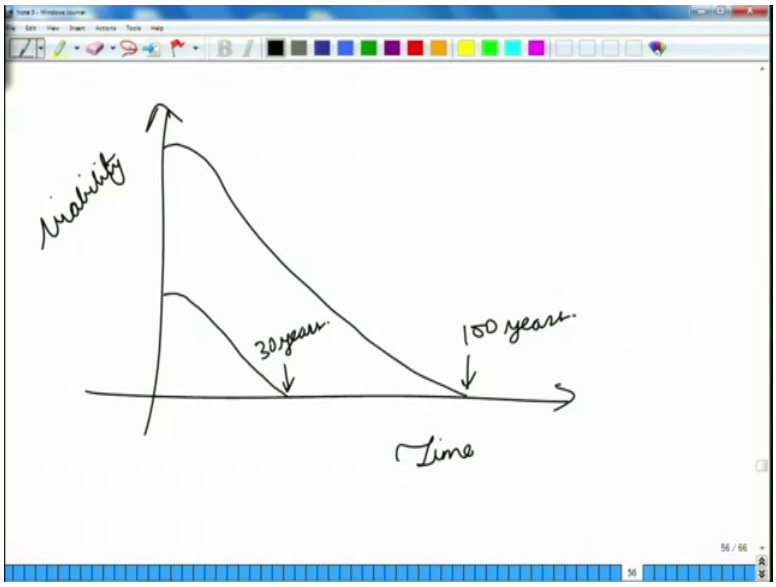

So, this is viability and this is the time. So, right after collection, you will have the maximum viability, but then later on it will go on decreasing at. Finally, it will reach a viability of 0 after say in this case let us say this is 100 years so, this is the viability curve. Now, if you are collecting a very less number of seeds then with this graph you might have a zero viability at the age of say 30 years. So, we always want to have a larger number of seeds for provenance. So, that even after a long period, even after a very long period has elapsed, we still have some seeds that are viable and that we can use to propagate the species.

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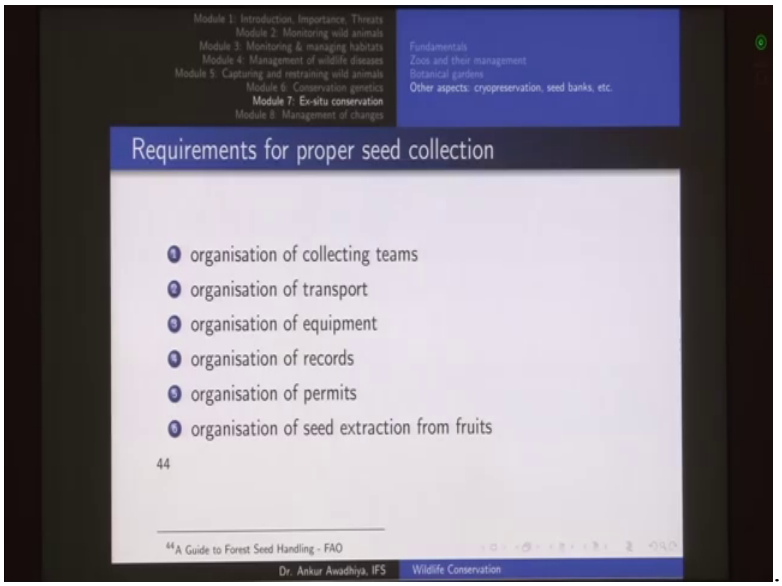

Now, when we are doing the collection, you will need to organize a number of things we need to organize collection teams. So, these collecting teams have to be taught about how this collection needs to be done, what are the, the do's and do not's that should be done. So, for instance if there is a tree and if there is a seed that has fallen down in to the ground. So, after it has falling in to the ground, it might be covered with dirt, it might be having some fungi to get that with this dirt. So, this seed should not be collected whereas, seeds that are still there on the trees are better. So, things which is these have to be taught to all of these collecting teams, then organization of transport, because you do not want to keep seeds out there in the field, we need to bring them to our facility as soon as possible.

So, that they are preserved in a better manner. So, organization of transport is required you might require some equipment. So, equipment for climbing of trees equipment for plucking of branches equipment for depulping of seed all these equipments are may be required organization of records.

So, for every sample you need to know what is this species, where this is species was collected, when was this particular sample collected and so on organization of permits. So, this is especially important when you are moving your seeds across the international boundaries. So, for instance, if you want to send your seeds to say Norway, so, there might be an number of permits that are required especially from the biodiversity point of

view and then organization of seed extracting from the fruits. So, once you have, you have brought your seeds or fruits to the facility. Next you need to extract the seeds and process them.

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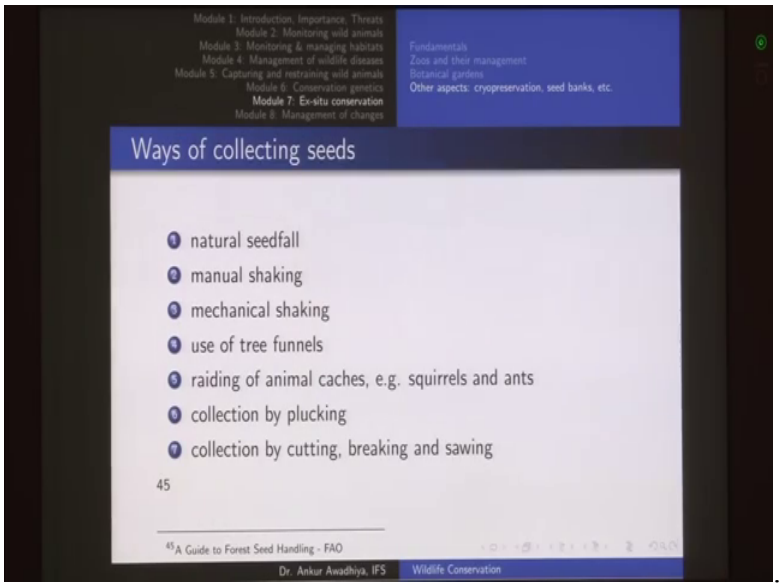

Now, what are the ways of collecting the seeds? So, you can collect seeds after they have fallen out naturally. So, which is known as the natural seed fall, the second one is manual shaking. So, you can go to a tree and you can shake that tree. So, that all the fruits come down and then you can collect them or you can make use of equipment for mechanical shaking of trees or you can make use of tree funnels or tree funnel is an interesting concept. So, here what we do is that if there is this tree that is now giving out the seeds what we do is that we construct a funnel around this tree and this funnel is normally make made out of cloth.

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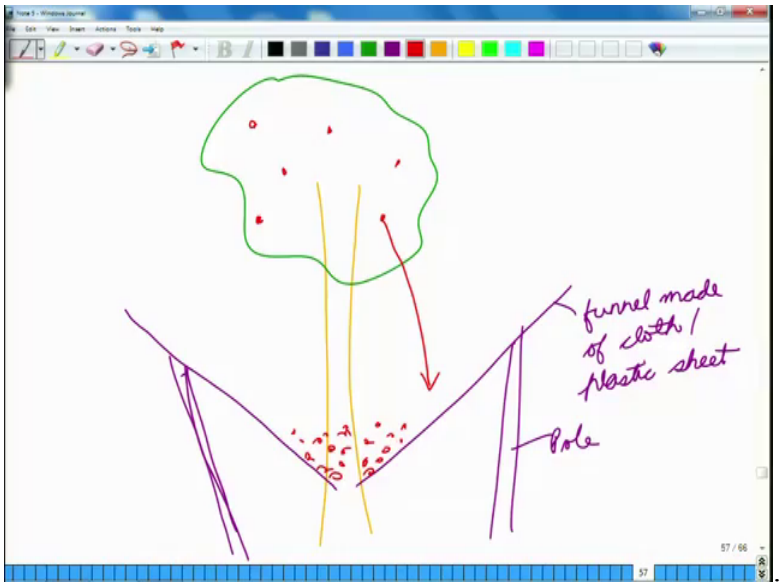

So, these are say some poles. So, this is a pole and this is the funnel made of cloth or a plastic sheet. Now what would happen in that case is that there is any fruit on the tree, let us represent it by a red colour. So, these are the fruits and when these fruits break open or when these fruits fall down. So, they will fall on this funnel and then get collected here. Now, this is especially important for those fruits or seeds that has small in size, because in the case of these small sized fruits or seeds your fruits and seeds might dispersed to our very large distance. And then once, they have mixed up with the with the dirt or the soil; that is surrounding these trees when it become very difficult to collect them back, but then with this mechanism, your seeds will fall in to the funnel, they are not getting in contact with the soil. And so, they are not getting into contact with the microorganisms.

So, once your seeds have collected here, you can take them away. So, this is specially used for conditions such as cones. So, in the case of pine cones there seeds are very small so, this is one thing that could be used. Another thing is raiding of animal caches like those of squirrels and ants.

Now these animals squirrels and ants they collect a number of fruits and they collect a number of seeds and then they bring them to their own homes. So, do (Refer Time: 26:09) and then they collect them there or they store them there for weathers that are not very good.

Now, in these cases if you raid a squirrel caches so, what will find is seeds from a number of different trees surrounding those areas. So, that is that could also be one-way that is that can be used. Normally, we do not go for these because the places where these animals store the fruits and seeds are generally damp. And in those damp areas these are very high probability that you can, that you could have a fungal infestation. By then talking back about our first example the Siberian permafrost, there the fruits were collected from one of a squirrel cache. So, that is one example where we collect it's seeds or fruits from a very old sample, another is collection by plucking.

So, in the case of plucking you can climb a tree and then you can pluck different fruits. Once, they have matured or you can have collection by cutting breaking and sawing. So, in this case, you can cut a branch or you can pull a branch to break it or you can saw a branch to break it and once this branch is cut down, you can then collect the fruits from these plants.

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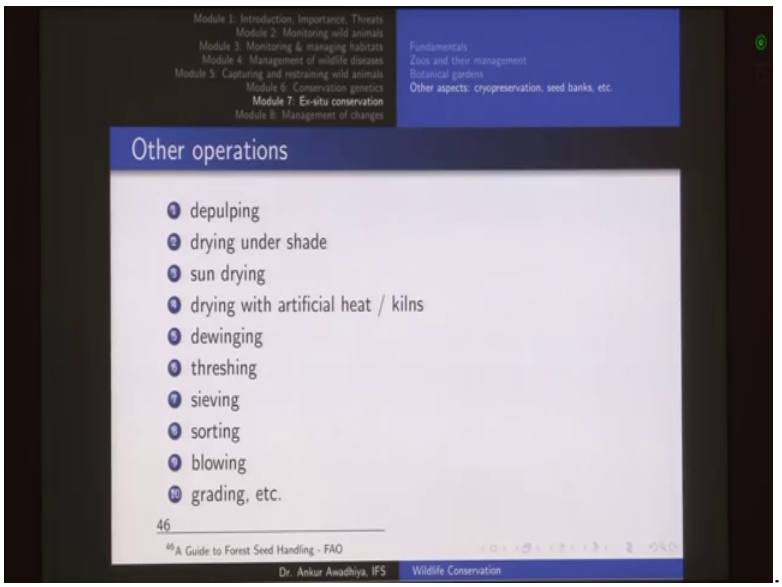

Now, once your fruits have been collected, there are a number of other operation that need to be done, one is depulping. So, in this case you have separating the pulp out from the fruit. Now pulp is a soft tissue, it has a lot of moisture and so, there is a larger chance that your seeds might get infestate, if they are surrounded by the pulp. So, we remove this pulp, this can be done by hand by soaking the fruit in water or by using the mechanical device. Next softer pulping, you could go for a drying under the shade or a

sun drying depending on the species, depending on the seeds you will, you will have to dry the seeds.

Now why is drying done, because typically our seeds have a larger moisture content and at larger moisture content the probability of the seeds getting infected or infested becomes larger. Where as in the case of dried out seeds there is hardly any microorganisms that will grow there. Now a case in point here would be the date palm seeds that we saw earlier. So, even after 2000 years, they were able to threaten their viability, because they were stored in a cool and dry place.

Now, in certain situations if you need to dry these things very fast or when the weather is not supporting, we can even go for drying with artificial heat or using kilns. Now in the case of some seeds you have wings attached to the seeds. So, in this case you will have a seed. So, this is a seed and then there is a wing attached to it. Now why are these wings attached to the seeds, because remember, you have this tree. And this tree had a fruit and this fruit had a seed.

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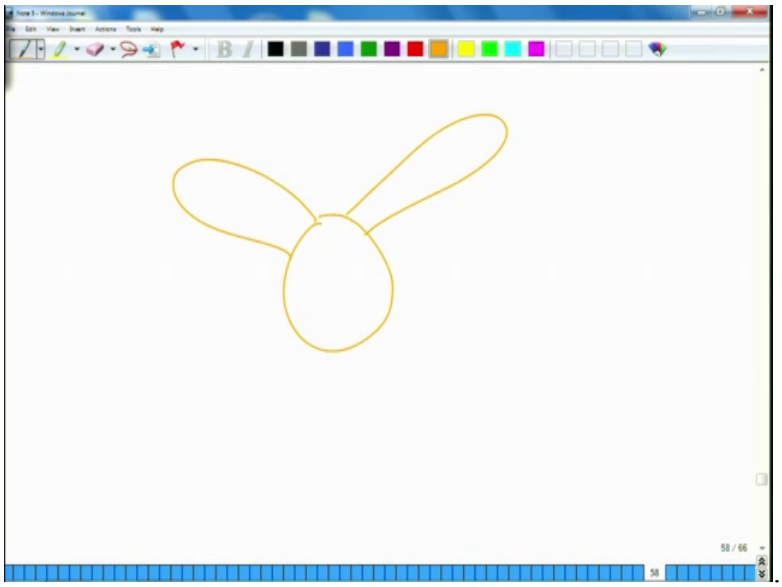

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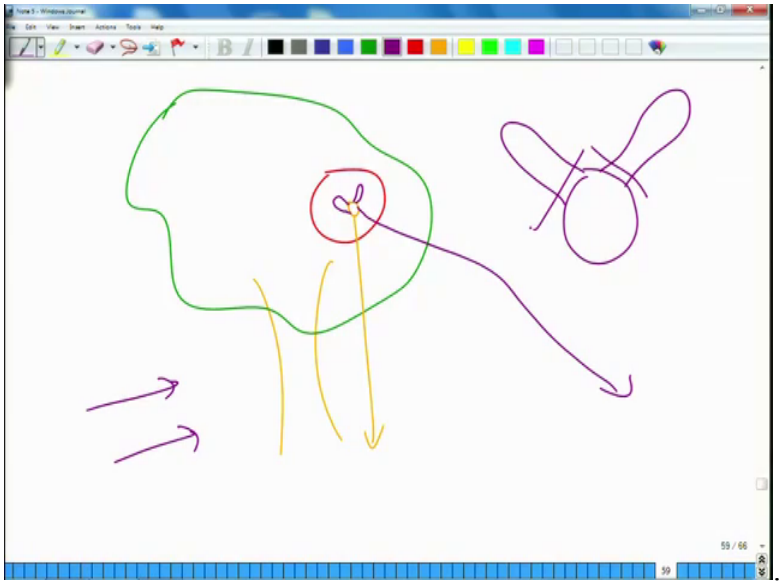

Now, if this fruit breaks open the seed will directly fall down whereas, if this seed has wings attached to it. So, if this seed has these wings then when there is even a small blow off wind these seeds will be able to disperse to a fiber of place. So, just for facilitating dispersion there could be a number of seeds that have these wings. Now once, you have collected these seeds there is no purpose of having the wings together. And besides if you have a seed with the wings.

So, the, the later processing might become difficult. So, for instance, if you want to grade these seeds. So, you for instance you wanted the bigger size seeds in place of this smaller size seeds, because the, the larger size seeds are more matured; they are having more amount of endosperm. Now in that situation when we are putting these seeds through a grader, when these wings may become and block the holes or block the seeds. So, in those situations we may go for a dewinging operation. So, both of this wings are taken off.

Then you could have, threshing and sieving operation. So, as to remove all that husk or all the broken parts of the wings or the broken parts of the fruits that are together. So, you can have this threshing and sieving operation or a blowing operation, then we can go for a sorting and a grading operation. Now in sorting and grading, we do two kinds of things, one is a visual examination in which we ensure that your seeds are not having any defects, especially they are not broken down. Mechanically, there seed coats are intact; they are not showing any signs of infestation..

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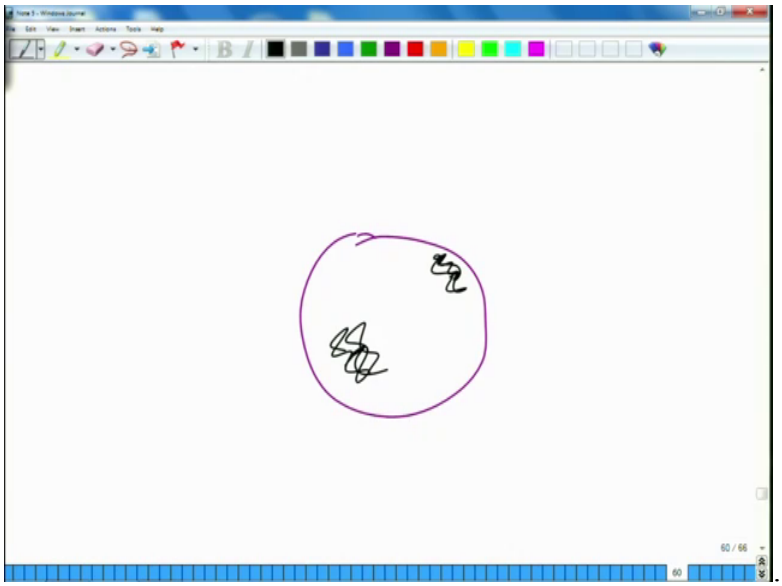

So, for instance if you have a seed, if there is a seed and then there is a black colored spot here and a black colored spot here. So, this might show that there is some amount of disease that is coming up on the seeds. So, there is some amount of microorganism's growth that is going on in the seeds. So, in the grading operations and in the sorting operation, we remove this seeds. The second sort of grading operation is when you sort your seeds by size. So, in this situation what happens is that you have a sieve hole.

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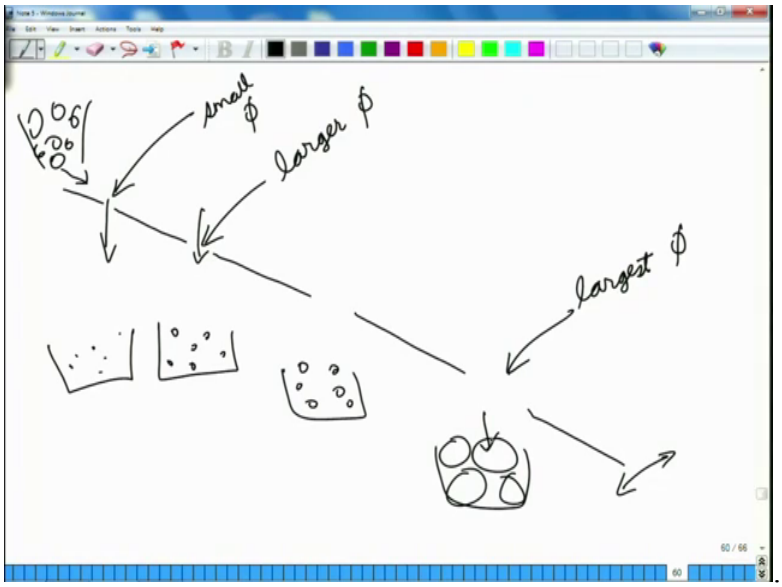

So, this is a sieve in which you have hole that are there in different gradation. So, this one is a small diameter, this one is a larger diameter and this one is the largest diameter. Now, when you put all your seeds here and this particular sieve is shaking in this manner.

So, now when all of these seeds fall and when they move down all the smaller seeds will come out through this hole and collect in one bucket. The larger size seeds will come down from this hole and collect in this bucket then this bucket and then this bucket. Now these seeds are the largest sized seeds and these one are very small sized seeds and then these are the medium size seeds.

Now, because these seeds have the largest amount of endosperm and because, these seeds have reached the complete maturity. So, the amount of viability in these seeds and also the, the amount of regeneration that will observe from these seeds as much greater typically than these smaller seeds. So, because of this we can go for sorting and a grading operation. So, these are different operations that need to be carried out.

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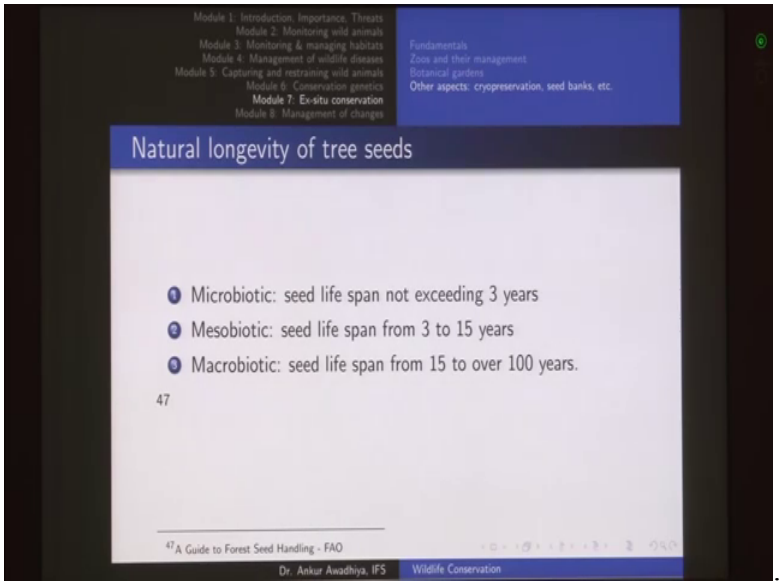

Now how long does a seed last? What is the longevity of a seed? So, depending on the natural longevity of tree seed, we divide them into three categories micro biotic in which the, the seed life span does not exceed 3 years. So, micro is very small, very small life medium size life and very long life or large size life. So, Mesobiotic is 3 to 15 years and Macrobiotic is 15 to over 100 years so, which has a very long life. Now why do different

seeds have different life forms? So, we can understand that by looking at these this classification.

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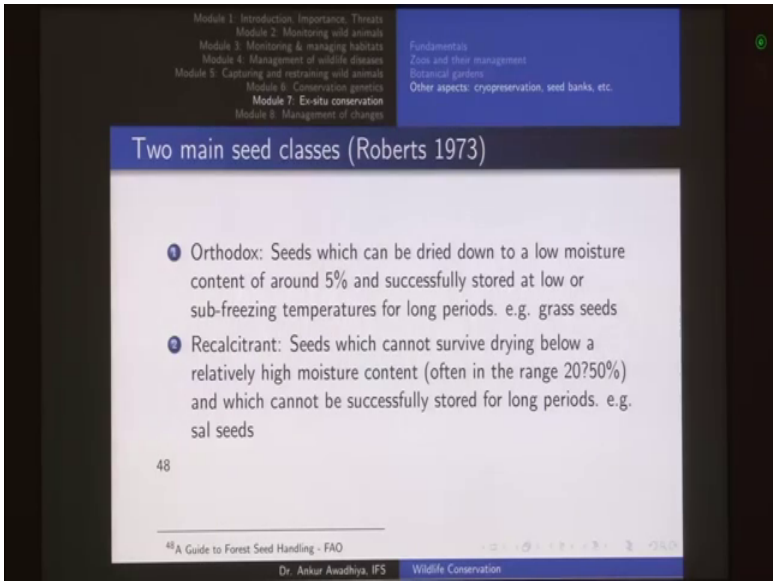

So, different seeds can be classified as orthodox seeds or recalcitrant seeds. Now Orthodox seeds are seeds which can be dried to a low moisture content of around 5 percent and successfully stored at low or subfreezing temperatures for a very long period for example, grass seeds. Now typically, in these seeds the amount of fat content is very less or the amount of oil content is very less. So, what happens is that if you have any amount of oil in a seed.

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So, the oil when it comes in contact with oxygen, then it becomes a Rancid oil. Now Rancid oil means that that this oil has undergone oxidation. In the case of oxidation a number of wants have been broken and after breaking an oxygen atoms have come into the structure. So, essentially the purpose of the oil is lost. Now, if you have those seeds that have very low amount of oil, you can dry these seeds and then keep them for a very long period. So, these are known as Orthodox seeds. So, even are a seeds; such as wheat or rice come in this category.

So, you can store these seeds for a very long time the second category goes by the name of recalcitrant seeds, seeds which cannot survive drying below relatively high moisture content often in the range of 20 to 50 percent. And which cannot be successfully stored for long period example, sal seeds. Now these seeds have a very high amount of oil content. And so, when you are drying these seeds completely or you or you cannot dry them to, high level of, of dryness because these typically have quite a heavy dose of oil. And during this process of drying the oil also becomes Rancid. So, these are recalcitrant seeds and you cannot store them for a long period of time.

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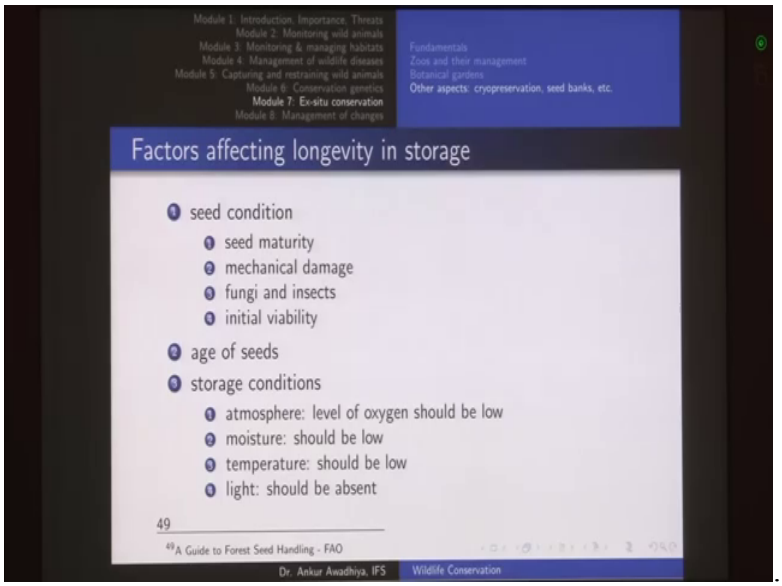

So, this is the natural variation, but then the longevity of seed also depends on how it is being stored? So, even in the case of orthodox seed such as a graph seed, you can have a situation in which your seed does not have a long longevity. For instance, if you store it in a damp place so, that fungus grows on it or if you have collected these seeds before they have matured or if during processing these seeds of broken up. So, what are the factors that affect longevity storage? So, they we have the seed condition, the maturity mechanical damage fungi and insects and the initial viability. So, these are the four initial conditions then the age of seeds. Now longevity also depends on the age, because typically the viability goes down with the age.

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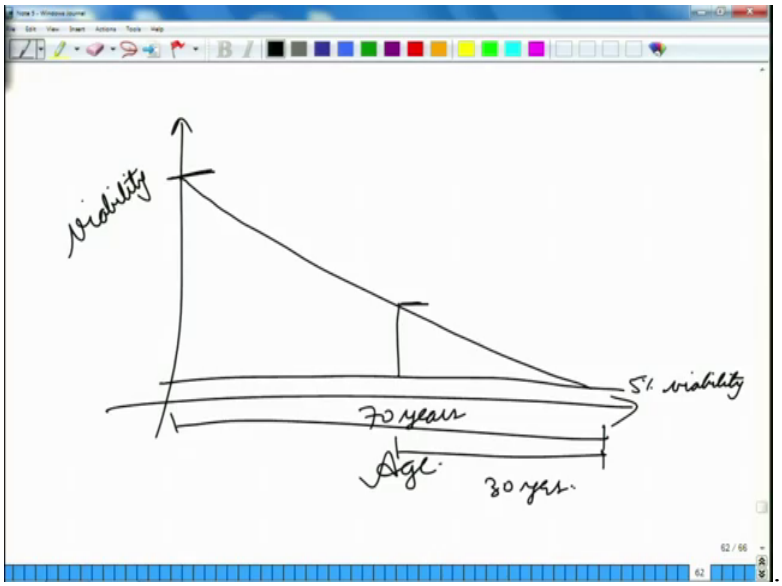

So, here you have the viability and here you have the age. Now the viability will typically go down with age. So, for instance if you consider your longevity as viability of greater than 5 percent, so, this is 5 percent viability. So, if you have these seeds, if you have started at this point then your seeds will typically last for this much period, let us say that this is 70 years, but then if your seeds have already aged till this point. And then you are considering the, the longevity of these seeds then this age might only be 30 years..

So, the longevity in the storage also depends on the age of the seeds. So, the near the seeds are the better it is and then it also depends on the storage conditions the atmosphere. So, the level of oxygen should be low not only, because oxygen can oxidize any of the oils that are there in the seed, but also because oxygen is required for respiration. And if your seeds are expiring so, they might be they might end up using all the endosperm. The amount of moistures should also be low, because with moisture and, and good amount of oxygen, you can have more amount of respiration.

And also, if there are any pest or any microorganism, they will also able to be survive well, temperature should be low. So, for instance in both our earlier cases, in the case of the Siberian permafrost, the seeds the fruit was always kept below 0 degree Celsius. So, it was always kept frozen. So, when something is frozen, then if they cannot be any life going on. And even in the case of the date palms, they were stored in grass which are

kept in a cool position and then they should not be any light here, because again in the in the case of light you can have some seeds that might start germinating or you might support some other life forms. So, light should also be absent. So, in most of the situations, when we are creating a seed bank what we do is that we, we collect the best of the seeds like collect them when, we do not keep them for a very long period of time and then we seal them hermetically in a glass container say an amp wood.

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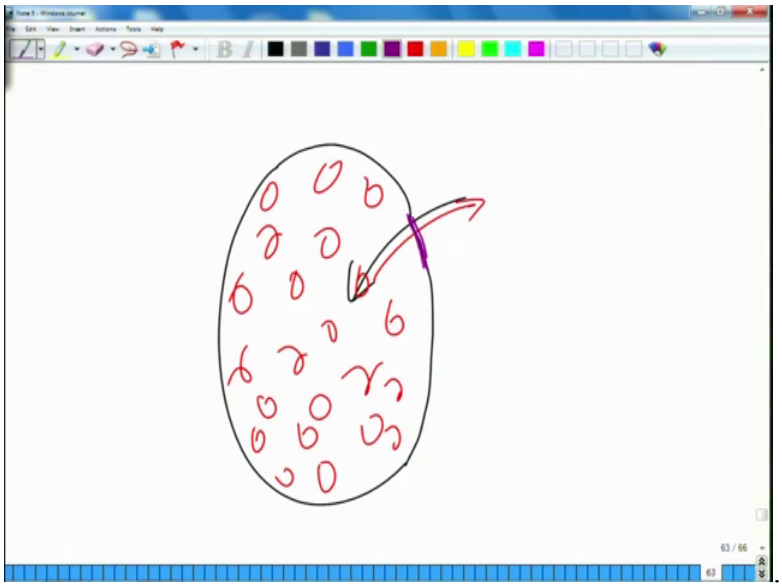

So, what would happen in that case is that you would have a glass ample. In this ample, you would put all the seeds inside. So, now, you have all these seeds and once your ample was full, you will then remove the air from inside. So, the air is removed out and then this portion has hermetically sealed. So, it is now completely fused and so, the your seeds do not have any more access to oxygen. And then these ample are stored in dark conditions and at low temperature and typically they are stored at subfreezing temperatures less than 0 degree Celsius.

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So, this is one example of one such storage facility. So, this is the Svalbard Global Seed Vault in Norway and this location is chosen in a way that we do not have any earthquakes in this region, there is no tectonic region activity, this area is on a very highland. So, that if there is a global warming at all the ocean levels rise even then this area will not be in underrated. And then this is one area that is always under the permafrost and even then the inside temperature is cooled even further. And even if all the power supply goes off then the outside is already subzero and it will take a number of centuries to reach a temperature of 0 degree Celsius. So, this is one way in which the seeds are stored.

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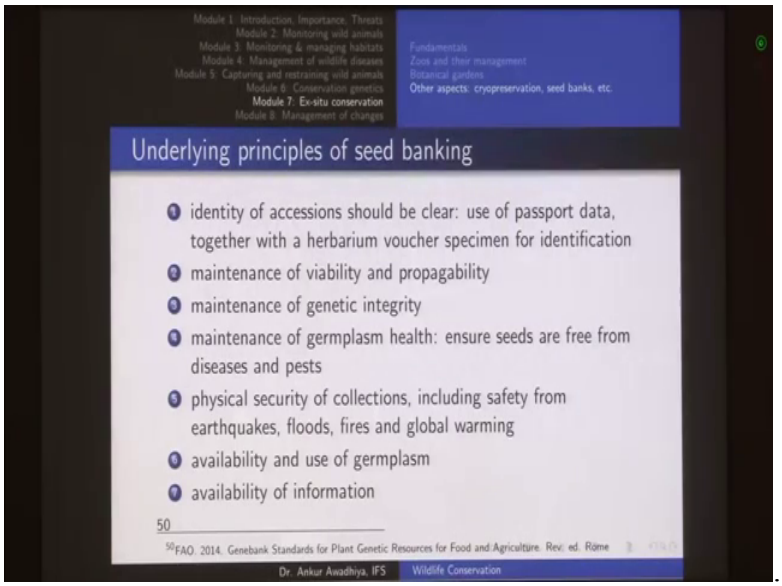

Now, the underlined principles of seed banking are these if you have a seed bank and if you do not know which seed is rich and it does not make any sense. So, identity of accessions has to be very clear. So, we make use of passport data together with a herbarium voucher specimen for identification. Now what is a vouchers specimen? So, in the case of a voucher specimen, you would have a sheet of paper in which you would have a pressed flower.

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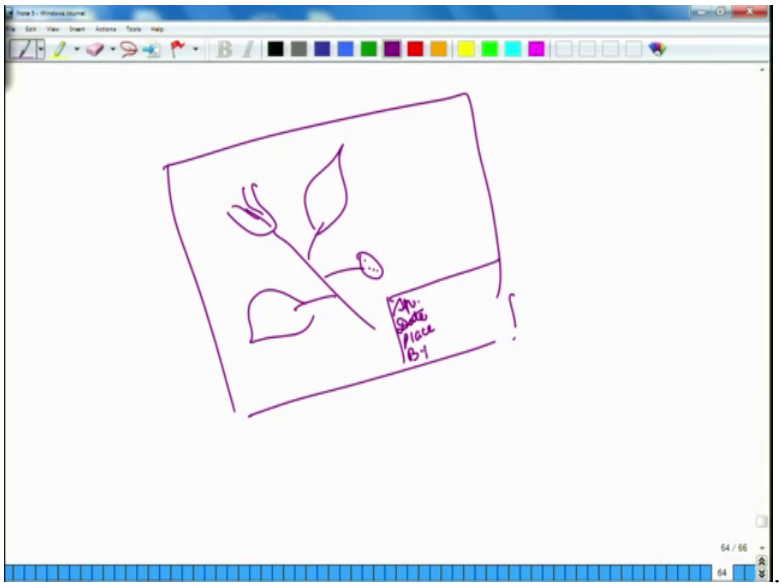

So, this is a pressed flower or dried flower with a leaf or may be a fruit. And all of these things are dried and all of these are pressed just like a herbarium sheet. So, when you have this sheet and then you will also have the identification details. So, the species the date of collection the place of collection collected by whom and so on. So, all these information are there. So, this becomes herbarium vouchers specimen. So, this is always supplied along with the seed; so, that we can have clear identification of what this species tells. Next, if you have stored your seeds and these seeds die off then it does not make any sense. So, we need to maintain the viability and you also need to maintain the propagability..

Now, propagability is the property of these seeds being able to propagate the species later on; so, essentially if you have a seed that is viable, but the plant that it produces is infertile. So, it will not be called a, a high level of propagability. So, the seeds should be in a position to in that they are living, and they are also able to propagate the species. Next, you need to maintain the genetic integrity. Now genetic integrity means as we have seen an our fundamentals lecture that after a while the amount of genetic variations may go down. So, we need to maintain that. So, how is this maintained?.

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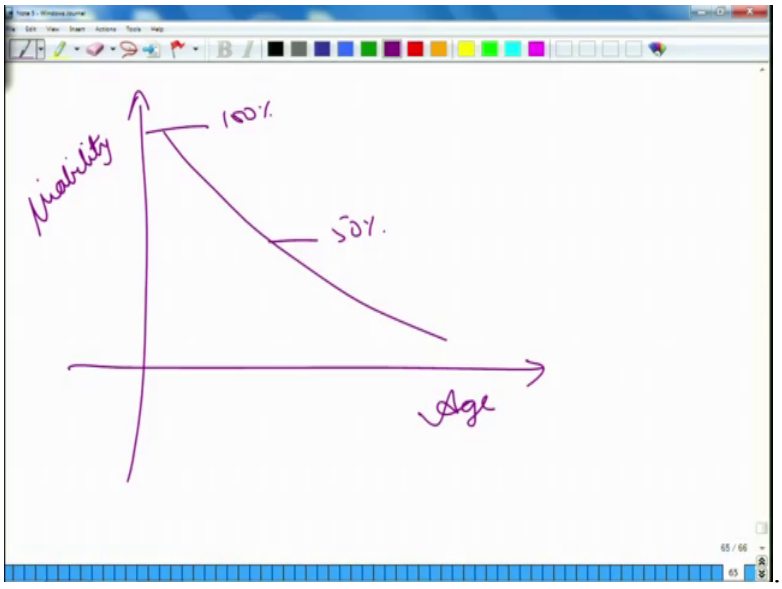

So, for instance after your seeds have gone down in the viability so, you need to. So, this is the viability and this is the age. So, after your seeds have gone down in viability. So, here it was 100 percent and say this was 50 percent.

So, at 50 percent, you want to have more seeds in to your facility, because you still do not have a situation of domes day. So, you can still collect these seeds back. Now you have got two options, one option is that you take these seeds out, you grow them in the field produce plants out of them and then recollect those seeds and then place them back again. The other option is that you collect these seeds right from the natural sources so, you collect them back from there forest. Now, if you are you are replanting these seeds. So, the amount of genetic variation will go down after every researcher planting, because you have this is small collection of seeds, this small collection which will give out the small collection of plants and you will only have breeding amongst these..

So, very soon in breeding might crop in, but if you are collecting these seeds right from the nature then the amount of genetic integrity is much greater. So, when you are maintaining a seed bank, you also need to ensure that the genetic integrity is maintained. Next is maintenance of Germplasm health. So, you need to ensure that the seeds have free from diseases and pests. Next is physical security of the collection including safety from earthquakes, floods, fires, global warming and even terrorist activities these days So, they you need to have a good amount of security in this area.

Now if you have this germplasm and you are just turning it in that you are never going to use it, it does not make any sense. So, there needs to be a, a process of availability and use of this germplasm. So, if somebody wants to have these seeds what are the, the protocols that what would have to be followed, these need to be very clearly defined and also the reliability of information.

So, availability of information means that if you have a seed bank then you should also have a procedure through which people might come to know what all seeds are there, what all seeds are required, what are the ages of those seeds, what are the provenances of those seeds and things like that. So, all of these things are being taken care of in Svalbard Global Seed Vault..

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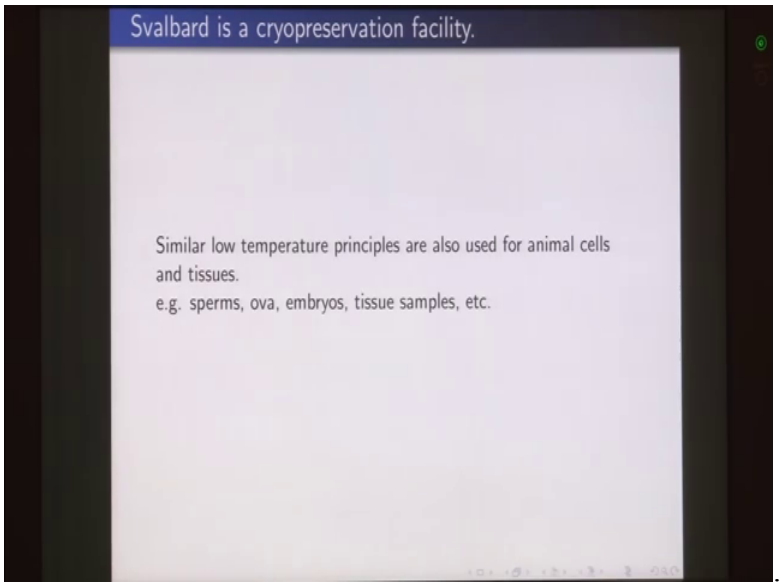

Now here we talked about the plant cells and the plant seeds, but then prevalent low temperature principles are also used for animal's cells and tissues such as sperms, ova, embryo tissue samples and so on. So, for instance in the beginning, we saw that using the fruit tissue plants were raised back from 30000-year-old samples. Now, if you have animal tissue and you can make use of cloning you can recreate this animal later on. If you can store it at a subzero temperature at freezing temperatures or if you have stored sperms of ova together, you can fuse them, them together later on and create an embryo or you can just store embryos as such. So, all these things are not just applicable to plant cells or plants, we are also applicable to animals.

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And so, we have also created a number of cryopreservation facilities for animals. So, this is one such facility. So, these are all large size chest freezers in the, which we are keeping different samples of animals.

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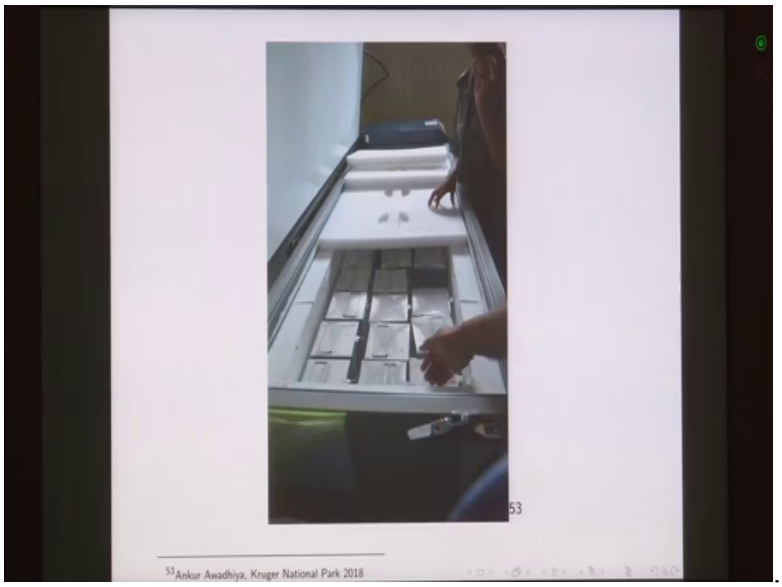

So, if you open here of these this is how little look. So, with this lid open, you have these insulating materials and then you have all these chests in which you have a different number of samples are kept. So, how does this sample look?

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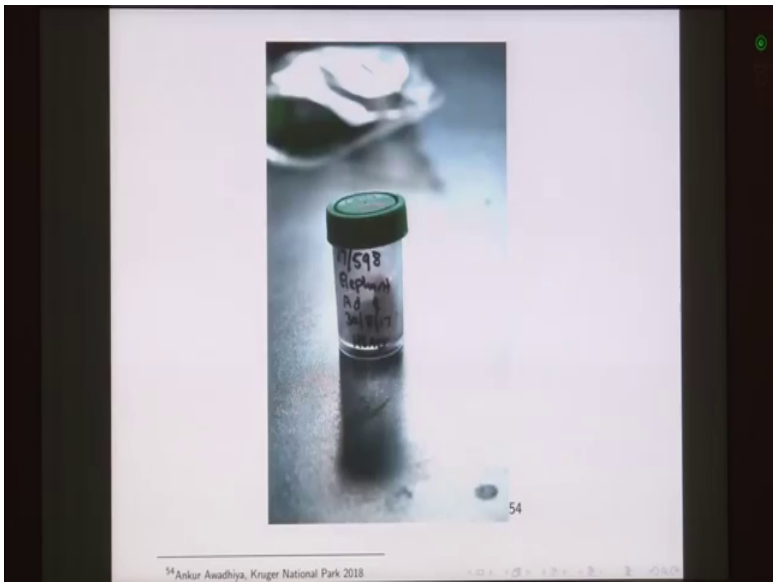

So, this is one sample when we have taken it out, this is a plastic wire which is air tight and this has an identification code. So, it says 17 by 598. So, this is an elephant adult female and then this was collected in almost 2017 and this tissues from the head region. Now such types of facilities are important, not just for preserving of these variations over a long period of time, but they also become very crucial when you need to understand any diseases. So, for instance, if in your in your area you find out, but there is a particular infestation of say tuberculosis.

Now you want to ask, when did tuberculosis actually come in to my park? So, in that case, if you have one such facility you can go back to all of your stored samples. And then you can investigate when was the first instance when you are finding a tuberculosis bacteria in the sampling or if you are finding antibodies against tuberculosis in the blood samples. So, things such as these can also be done. So, cryopreservation is extremely important for plants as well as animals and not just for their propagation, but also for as a as a repository for biodiversity. And also as the repository for further scientific studies. So, that is all for today.

Thank you for your attention.