Genetic Engineering: Theory and Applications
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Lecture 35
Applications of Biotechnology (Part 1)

Hello everybody this is Dr. Vishal Trivedi from department of Biosciences and Bioengineering IIT, Guwahati and what we were discussing, we were discussing about generating a genetically modified organism following a scheme where we were extracting the gene host and we are digesting it with the restriction enzymes and at the same time we are also processing the transforming units with the similar set of restriction enzymes.

And then we are putting these two for ligations and subsequent to that ligation we were putting it into the transformations and after the transformation we are screwing the screening and post screening we were getting the positive clones and these positive clones are supposed to produce the protein from the genetically modified organism and these proteins or the other products are going to be economically useful for downstream applications.

So now, todays lecture we are going to discuss about how the genetic engineering is going to help the society and as well as what are the different applications in which the genetic engineering can be used.

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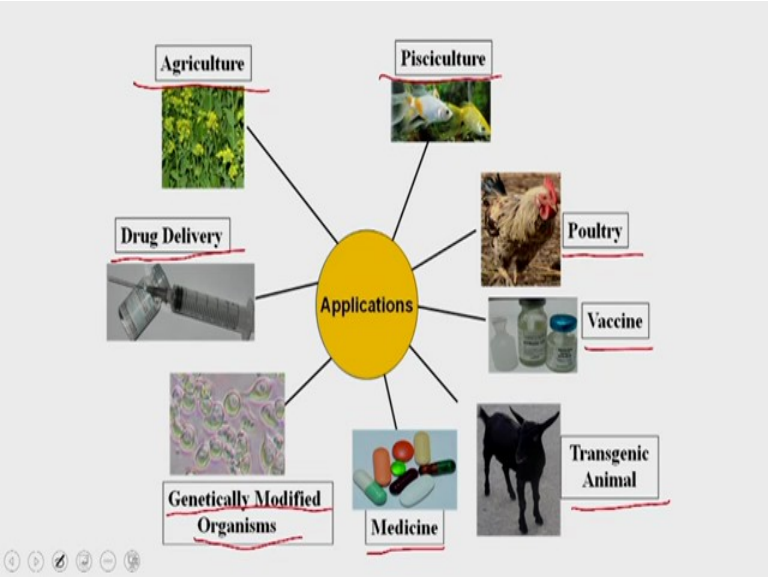

So as I said, the genetic engineering has an application in vast variety of areas whether it is the agriculture or pisciculture or poultry, vaccine, transgenic animals, medicines or whether it is in the application of drug delivery moreover we have already discuss about how to generate the genetically modified organism. So you can see that genetic engineering has lot of applications in diversified fields and now today on we are going to discuss about how the genetic engineering can be used for making an application in the plant sciences.

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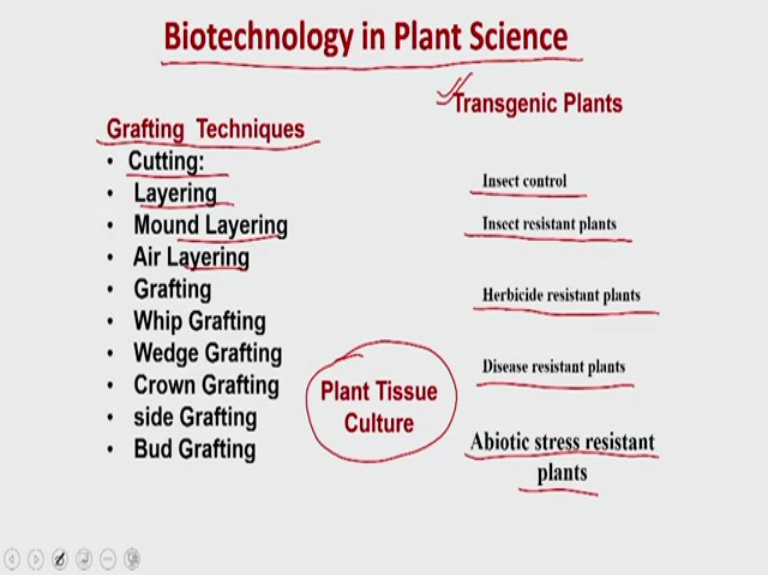

So in the case of plant sciences the genetic engineering in the plant sciences can be used in different fields for example when before the molecular techniques are known, the people are using the grafting techniques to generate the different types of a species or different types of varieties of the plant in these grafting techniques what people were using is they were going with the their own observation that some species are giving good product or good quality whereas some products are actually robust in terms of the disease resistance.

So what they were doing is, they were using the grafting techniques and grafting the good product yielding toing onto the disease resistance plants. So you can see that the cutting, layering mound layering, air layering and all these are the grafting techniques which people have used until they were not having the techniques as well as the ability to modify the same plant in such a way so that it is going to have the different types of features or different types of acquired characters.

So and then once they were having the ability to generate or to perform the molecular biology techniques there could be able to generate the transgenic plants. And then, with the help of transgenic plants they were having the potential to produce the plant which are having the insect control which means they are going to be having the plants which are insect resistant plants, herbicide resistant plants or the disease resistant plants and at the end they are also having the plant which were resistant against the abiotic stresses.

Abiotic stresses means the very high temperature, abiotic means the stresses which are been experienced by the plant, by the environmental factors such as the low level of water or high temperature or high heat and another kind of environmental factors. So with the help of transgenic plants or with the help of the genetic engineering to generate the transgenic plants, they could be able to produce the plants which could be able to withstand the abiotic stresses also.

And then with the help of the development of technique of plant tissue culture they could be able utilize this particular techniques to generate the new plants. So in todays lecture we are not going to discuss about the grafting techniques because the grafting techniques does not require the genetic engineering and it is actually more of a ancient techniques to generate the newer species. In todays lecture we are going to only discuss about how the development of transgenic plants has help the agriculture field and also about the plant tissue culture.

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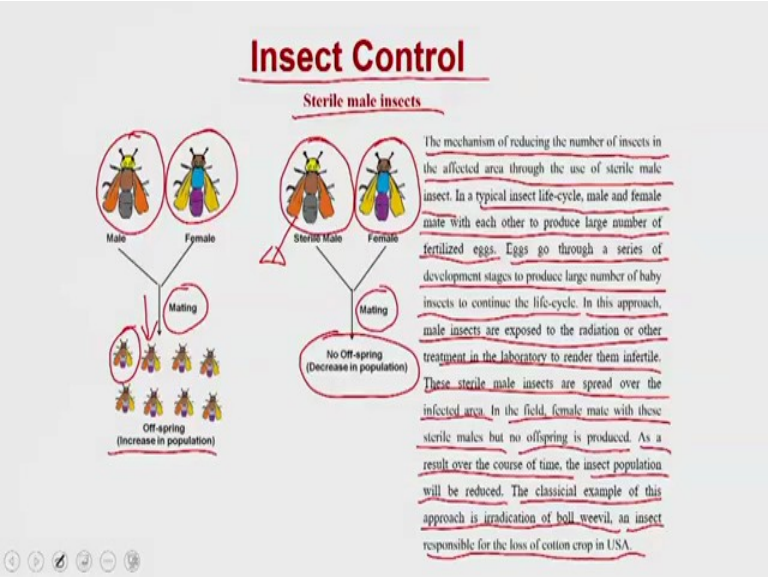

So one of the prime objective of the generation of transgenic plants or one of the major reason why the different types of crops are going to be destroyed because the lot of insects are available which are actually feeding on these plants and one of the prime objective is to control the insects and the control of insects is on the two side. One, you can actually reduce the number of insects which are present or you can actually make the plant resistant for the insect bite.

So in this case what people are doing is, they are generating the sterile male insects which means that under the normal natural conditions you have a male insect, you have a female insect and these two male and female insects when they are matting they are producing lot off springs and that is how they are actually increasing the populations, but what you can do is, somehow if you can convert a male into the sterile male which means if you can generate the male in such a way that if the male is been produced it is going to be a sterile whereas the female can be a non-sterile.

So in that case what will happen is, if the male is sterile it is not going to be mate with the female insects and when since they cannot be able to mate they will not to be able to produce their off spring and hence the result in due course there will be a decrease in their populations. So this idea the is that in which the mechanism of reducing the number of insects in the affected area through the use of sterile male insects in a typical insects lifecycle male and female mate with each other to produce large number of fertilize eggs and these fertilize eggs actually produces large number of off spring.

So eggs goes through a series of development stages to produce large number of baby insects to continue the. In this approach male insects are exposed to the radiation or other treatment in the laboratory to render them the in-sterile or infertile. So this what people are doing is, they are actually exposing the male insects to a radiation or some other kind of chemical treatment and because that the male is losing its ability to become to fertile these sterile male insects are spread over the infected area.

In this field the female when they mate with these sterile males but, no off spring is produced as a result over the course of time the insect population will be reduced. The classical example of this approach is eradication of the boll weevil an insect which is responsible for the loss of cotton crops in USA. So this particular type of idea is already being tested in the USA and people have found that if you generate the sterile male which is actually incapable of doing the mating with the female in the in due course this kind of population is going to be down.

But as a side effect what will happen is that since you have generated the sterile male it is very difficult to predict if this sterile male is not going to transfer its genetic material or if in some cases what people are also trying to do is they are actually making a genetically modified male

which is actually the normal but when it is mate with the female and if that mating off with female is producing the male insects those male insects male off spring are sterile.

So in those kind of conditions what happens is you do not know if the male insect is going to transmit the that particular information with the other kind of insects. For example, if they transit that to other insects which are actually important for pollination because insect are actually feeding on over crops but at the on the other hand insects are also responsible or also important in terms of doing the pollinations.

So because of their important role to be play in the pollination because you do not know if this particular type of insect is also taking part in the pollination of some crops and if these insects are going to be wiped off from the environment probably that particular plant crop or particular plant species also going to be will not be going to propagate because the you are destroying the pollinating agent.

And as a result of this idea was exiting at the beginning when the people have used and tested it in USA and it has actually eradicated the insects which are actually destroying the cotton crops but very soon people have realize that it could have a very serious side effect in terms of the that it is actually going to destroy the normal balance of different types of insects which are available in the environment and which are actually responsible for not only for pollination for other kinds of functions and that is why this idea did not got much attention and this idea was not been propagated in a larger way.

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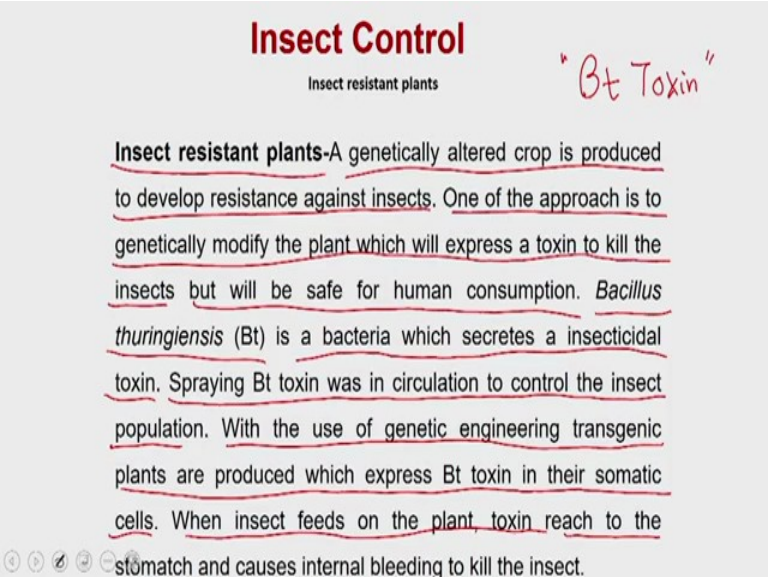

Now the second way of protecting over crop is from the insect is that you generate the insect resistant plants, what you can also do is, a genetically altered crop is produced to develop resistance against the insects. One of the approach is to genetically modify the plant which will express a toxin to kill the insects but will be safe for human consumption.

Bacillus thuringiensis or the Bt is a bacteria which is secretes a insecticidal toxin which is called as the Bt toxin. So spraying Bt toxin was in circulation to control the insect population with the use of genetically engineering transgenic plants are produced which expresses Bt toxin in their somatic cells. When insect feeds on this plants the toxin reach to the stomach and causes internal breeding to kill the insects.

So this is another example where we are actually genetically modifying the plant and we are actually making it resistant for the insects and as a result when the insect feed on these plants the toxin reaches to the insect stomach and actually it is going to kill the, but the same reasoning exist here again that you are actually destroying this particular insects but you do not know if this insect also has the relevance in terms of the pollinating as well as the other kind of environmental control, for example insects are, so we a part of the food cycle and if you destroy these insects you do not know if you are going to affect the birds and all other organisms which are going to feed on these insects.

So as a result this idea also where you are actually generating a transgenic plant to control the insects are also not being taken up with lot of excitement and ultimately we will have tested this idea they could find that the this is working perfectly fine in the fields but because of these kind of environmental as well as other kind of concerns they have limited their usage for generating a transgenic plants for this purpose.

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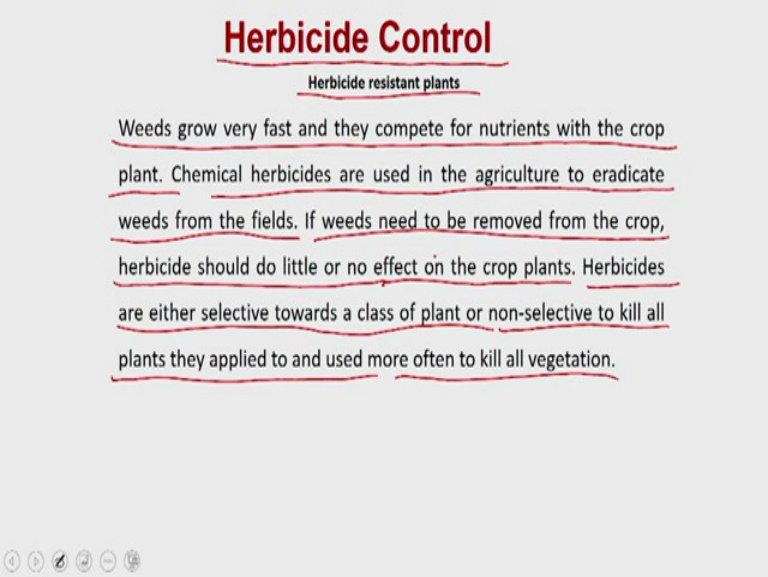

Now the second is, you are actually having controlling the herbicide control. So you have to generate the herbicide resistant plants you know that the weeds grow very fast and they compete for nutrient with the plants so the chemical herbicides are being used in the agriculture to eradicate weeds from the fields. So weeds are the non-wanted small plants which are been grown within the field and they actually grow very fast because they take up the nutrition and so if the weed need to be grow remove from the crop the herbicide should be little or no effect on the crop plants. Which means to remove these weeds what you have to do is, you have to spray the herbicides into the agriculture field.

But this herbicide should not have any effect on to the your crop plants but what happen is that herbicides are either selective towards a class of plant or non-selective to kill all plant they applied to and used more often to kill all vegetation. Which means herbicides are either selective for a class of plants for example the herbicides could be specific for monocots, herbicides could be specific for dicots but or sometime the herbicides are nonspecific they are going to destroy

any type of vegetation and if you use those kind of herbicides in a very large quantity it is not going to kill the weeds but it also going to destroy the plants as well.

So that is why there is a important and there is a urgent requirement to generate the herbicide resistant plant, so that when you spray the herbicides it will only going to kill the weeds, it is not going to affect the crop plants.

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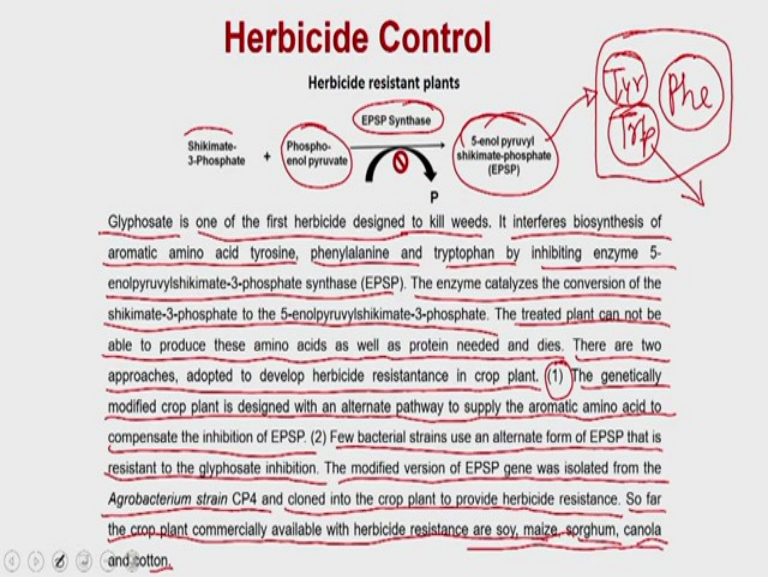

Most of the, so one of the popular herbicide is the Glyphosate and Glyphosate is one of the is designed to kill the weeds its interfere with the biosynthesis of aromatic amino acid tyrosine phenylalanine tryptophan by inhibiting the EPSP that 5 enolpyruvylshikimate-3 phosphates synthase so EPSP synthase is cartelizing a reaction where it is taking a shikimate-3 phosphate with phosphor-enol pyruvate to generate the 5 enolpyruvylshikimate 3-phosphate synthase.

So EPSP synthase is catalyzing a reaction where it is taking shikimate-3 phosphate with phosphor-enol pyruvate to generate the 5 enolpyruvylshikimate shikimate phosphate EPSP and this part actually this particular product has a relevance in the biosynthesis of the tyrosine tryptophan and the phenylalanine and by inhibiting this particular enzyme this you are actually inhibiting or you are actually limiting the availability of these amino acids for biosynthesis of many of the proteins which are actually may be responsible for running the crucial (biosynthesis) crucial metabolic reactions and the enzyme catalyzes the conversion of this to shikimate-3

phosphate to this the treated plant cannot be able to produce these amino acids as well as a protein needed and dies.

So what happen is, when you are treating the agriculture field with the glyphosate it is actually inhibiting the EPSP synthase and as a result of this it is actually not allowing the synthesis of 5 enolpyruvylshikimate phosphate EPSP and since the EPSP is involved in the biosynthesis of tyrosine, tryptophan and phenylalanine it is actually and all these 3 amino acids are the essential amino acids and because of that it could not be the plants could not be able to synthesize many of the essential proteins and as a result they actually dies.

There are two approaches which people are adopted to developed herbicides resistant in crop plant. In the approach number 1, the genetically modified crop plant is designed with an alternate pathway to supply the aromatic amino acids to compensate the inhibition of EPSP which means the people are generating the transgenic plants, where they are actually putting the alternate bio synthetic pathways so that the plant should not be dependent on this EPSP to provide the tryptophan phenylalanine and tryptophan as a result the even if you treating it the with the glyphosate the only the weeds are going to be die the crop plant is going to be get protected.

The other approach is that few bacterial strains uses an alternate form of EPSP that is resistant to the glyphosate inhibition. The modified any version of EPSP gene was isolated from the agro bacterium strains CP4 and cloned into the crop plant to provide herbicide resistance. So far the crop plant commercially available with the herbicides are soy, maize, sorghum, canola and the cotton.

So the alternate approach is that one of the bacteria agro bacterium strain CP4 is being used to isolate derived version of EPSP and this derived version of EPSP is resistance for the inhibition by the glyphosate and what you8 can do is, you can take this and over expressed that into the plant and as a result the plant is also going to be resistance against the glyphosate and once you use that it is actually going to, not going be killed and it is going to work as it can be able to provide the different types of amino acids and the plant will survive at the end.

So the herbicides resistant plants are available in the form of soy, maize and all these kind of plants.

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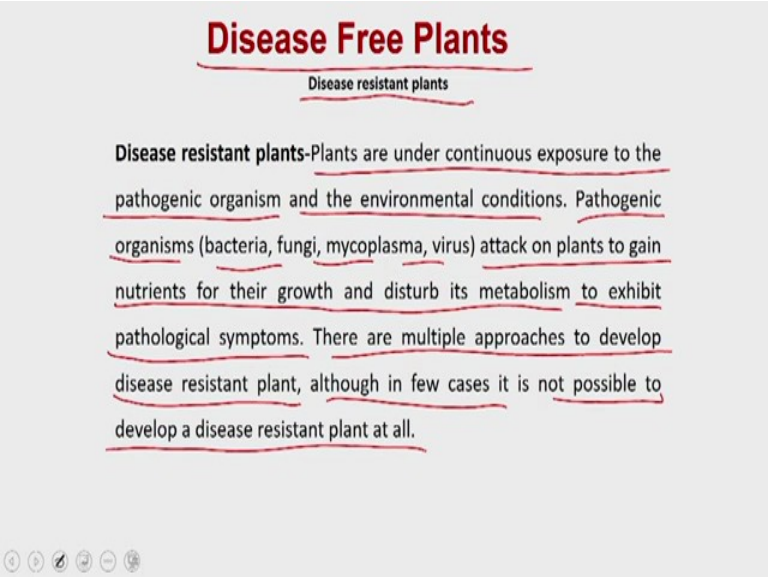

The other aspect is that you want a plant which is disease free plant so in that case you have to generate a disease resistant plants. The disease resistance plants are continuous exposure to the so in one of the approach what people were trying in the beginning is, that plants are when they are getting under the continuous exposure of pathogenic organism and the environmental conditions the pathogenic organism such as bacteria, fungi, mycoplasma, viruses attacks on the plant to gain nutrients for their growth and disturb its metabolism to exhibit pathological symptoms.

There are multiple approaches to develop disease resistance plants although in few cases it is not possible to develop a disease resistant plant at all.

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So what are these approaches? So these are the selected list of disease resistant plants. In the case of French bean you have the Hilda which is actually resistant against the mosaic virus and the anthracnose then you have the beans you have Futura which is actually resistant against the chocolate spot then you have the in cabbage you have stone head F1 which is against the mildew then we have the carrot, carrot you have the fly away F1 which is actually for the carrot fly.

Then we have the cucumber which is bush champion F1 which is the variety and that variety has the resistant against the cucumber mosaic virus. Then we have the pea which is the ambassador and the ambassador is resistant against the powdery mildew or fusarium wilt. Then you have the potato, potato is the very-very important vegetable so you have the 3 varieties, colleen, osprey and milva and all these 3 are having the resistant against the bight and scab, scab and eelworm and the blight resistant.

Similarly, you have the resistant plant for pepper, sweet corn and tomato and all these are actually the varieties which people have to develop the disease resistant.

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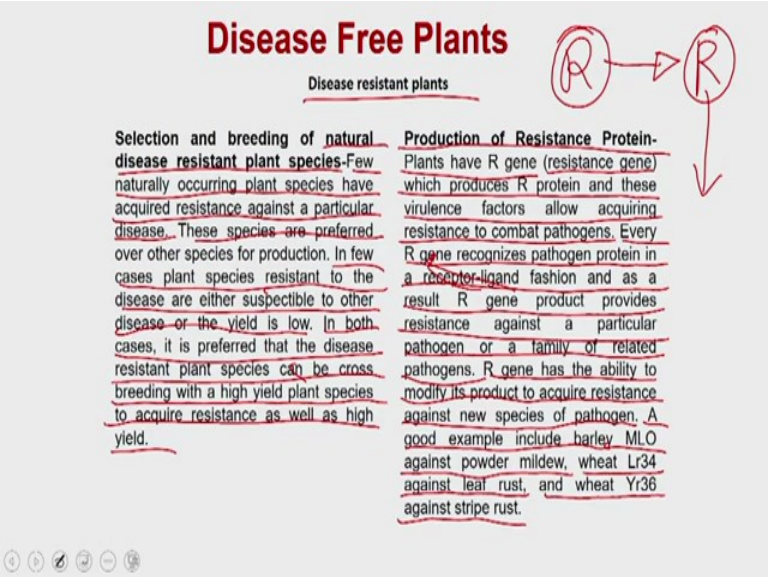

Disease resistant plants can be generated by following the 2 approaches either the selection and breeding of the natural disease resistant plant species. So know that the plants are getting continuous exposure to the pathogenic organisms and once they enter into the pathogenic organism they also acquire the resistant on their own which means they are also having the natural resistant.

So you have few species which are having the resistant against the disease, so there are few resistant plants which are sensitive for the that particular disease. So at the end what will happen is the sensitive species are going to die whereas resistant species are going to survive. So if you breed those resistant varieties you could be able get the natural plants, natural species which are actually going to be resistance.

So in those cases you have the few naturally occurring plant species have acquired resistant against a particular disease. These species are preferred over other species for production in few cases the plants species resistant to the disease are either susceptible to other disease or their yield is low. In both cases, it is preferred that the disease resistant plant species can be breed, can be cross breed with a high yield plant species to acquire resistance as well as the high yield.

So in these kind of cases what happen is sometime when the plant acquired the resistance against a particular disease either it gets susceptible for other disease or in some cases its production got very low which means the yield is going to be very low but it is going to survive that happens

because it is actually producing or it is actually investing lot of energy into the survival pathways instead of for the production pathway.

So because of that if you have such kind of situation where a plant species is resistance against a disease but its productivity is low, what you can do is you can do a cross breeding with a high yielding crops and with these kind of cross breeding it is going to give you the new species which is going to be disease resistance and at the other hand it is going to give you the high yield.

The other option or other approach is that the production of disease resistance plant, you know that the plant have a R-gene which is actually the resistance genes which produces the R-protein and these virulence factors allow acquiring resistance to the combat position. Every R-gene recognizes pathogen protein in a receptor ligand fashion and as a result the R-gene product provides resistance against a particular pathogen or a family of related pathogen which means what you are saying is that every resistance is mean because of the R-gene and if this R-gene is actually producing a R-protein and this R-protein is having a ligand on to the pathogen and that is how it is actually giving the resistance in that particular plant.

So R gene has the ability to modify its product to acquire resistance against new species of a pathogen. A good example includes barley MLO which is against the powdery mildew or the wheat Lr34 which is against the leaf rust and the wheat Yr360 (36) against the stripe rust. So these are the 3 examples where the people tried over expressing the R-gene which is actually resistant gene which is going to produce the corresponding protein and that corresponding protein are going to recognize the ligands which are present on the pathogen.

In some cases, you can actually modify the R-gene in such a way so that it is not going to only recognizes the pathogen but it also can recognize the other species of the pathogen so that you can actually bring the multi-drug resistant plant and as a result it is going to be resistant. It will acquire the resistance against not only one disease but also against the multiple diseases.

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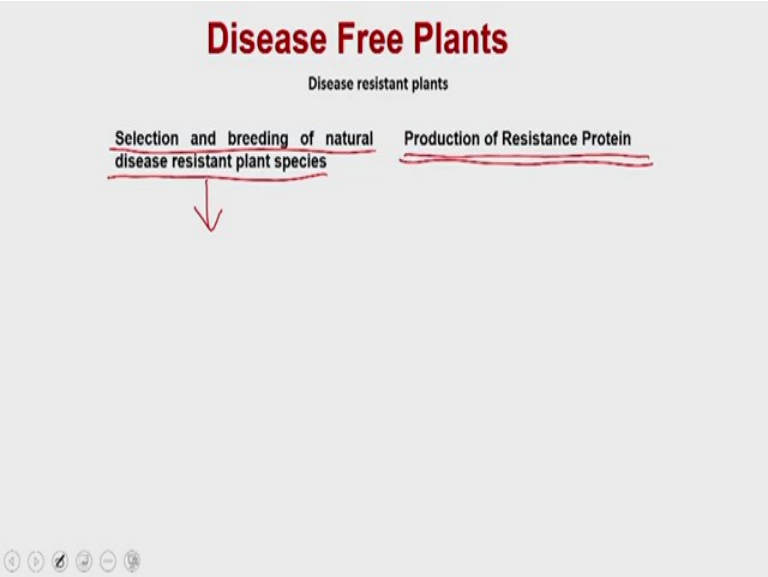

So, respective of whether you follow this (species) approach which means selection and breeding of natural plants or whether you use the production of resistance plants both are these approaches are having the advantages as well as the disadvantages this is the species where you a disadvantage that it is very-very time consuming and many of the time you do not know what is the mechanism through which the particular plant species has acquired the resistance.

So in those cases sometimes it happens that when you realize that okay this particular plant has species has resistance it could be possible that the resistance may revert back and it becomes sensitive for the that same species in case you have actually cross breeding this with the high yield plants. And all so on.

Similarly, in the production resistance protein (you are) in this particular approach where you are using the production of resistance protein this is time consuming process it actually first of all you also have to know that what are the genes are important for providing the resistance and then you have to take out these protein, genes from the corresponding plants and then you have to over expressed into the different plants and generate the transgenic plants and all that, and that is a very-very challenging task to generate the transgenic plants.

Irrespective of these approaches the ultimate thing which going to terminate is at in both of these cases you are going to use the help of other techniques such as the plant tissue culture.

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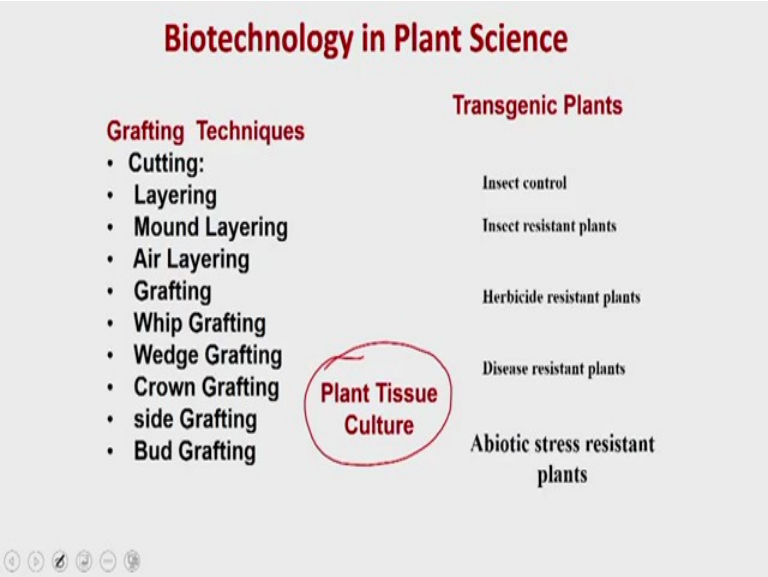

So, now we are going to start discussing about the plant tissue culture, so plant tissue culture is a technique which is been evolved so that is allows you to generate the disease free and the clonal propagation of the same plants without going through the breeding cycles because the advantage of not going into the breeding cycle is that it is going to help you to bring the no variability in the genome which means the cell line is going to be remain appear throughout the process of the plant tissue culture.

On the other hand there are plants which are actually facing problems in breeding through a natural process. So those plants also can be propagated using the plant tissue culture and plant tissue culture is a systematic way in which you take up the some portion of the plant and then you develop the plantlets from their and then you take those plantlets and grow them to a plants and then ultimately you can takes to those to the fields and use them for the downstream applications.

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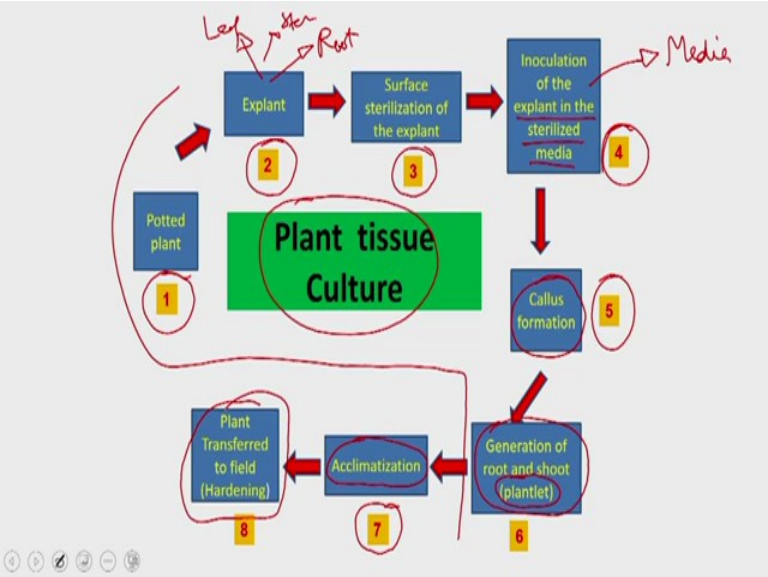

So the a typical plant tissue culture protocol requires following steps first is you are going to select this source of explant which means either the potted plant or the plant which you can take up from the fields then you are going to select the explants which means explant could be the leaf, explant could be the stem or in some cases the explant could be the root.

So you can choose which part of the plant you would like to use as a explant then what you are going to do in the third step? You are going to do a surface sterilization of the explant then in the fourth step you are going to do the inoculation of the explant in the sterilized media which means for this particular step you are also have to prepare the media which is required for the plant tissue culture, once you have done with the forth then you are supposed to go to the fifth step.

Which means once the (plant) explant will grow you have to, it is going to form the callus, once the callus is formed then you have to modify the growth conditions in the step number 6 so that it is going to generate the root and shoot which is actually going to give you the plantlets and then once the plantlets are being generated then you can take up to the acclimatization steps where the plant is going to be acclimatized for the external environment.

Because up to the step 6, this step 6 you are doing it in a controlled lab conditions where everything is under the control whereas the acclimatization steps you are going to do that into the outside. Which means you are going to do that into the green house where the conditions are not that going to be under control and you are going to acclimatize that particular plant for how to

survive in a natural environment and ultimately you are going to transfer these plants to the field for the hardening, so that the plant is also going to acclimatize how it is going to what are the problems it is going to face when it will go to the fields and once you have done with the hardening steps these plants are ready to be propagated into the fields and that can be, they can be used in fields for further application in the plant science.

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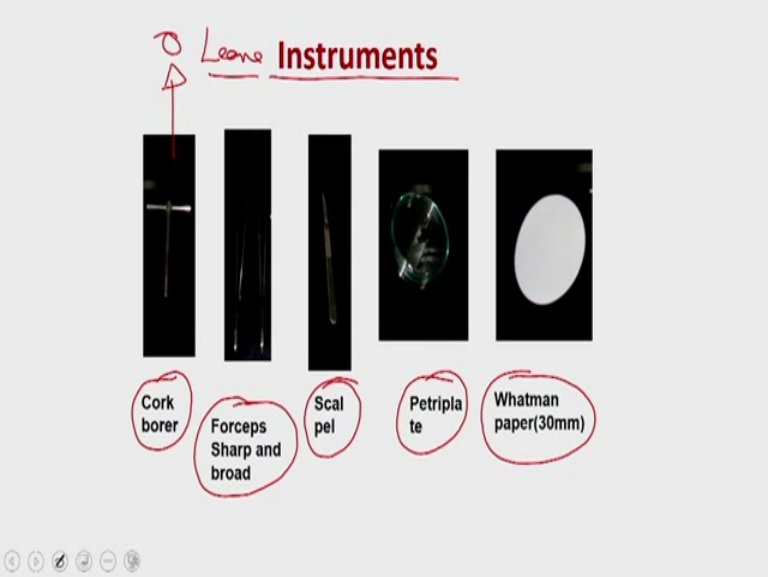

So for tissue culture you have require lot of different types of instruments, so what are the instruments you require? You need a cork borer, a cork borer is a instrument which actually requires to cut the disk from the leaves. So in this particular examples we have shown how to do the plant tissue culture from the leaf but if it is a any other explants like stems or leaf stems or the root you can use the different types of instruments.

Then you need the forceps which are going to be different types of forceps either the sharp forceps or the broad forceps these are the forceps which you need to pick up the plant parts then you need a scalpel, scalpel is required for cutting the explants if it is a root or stem then you need the petri plates, petri plates to keep the explants and also you need a Whatman filter paper so that you can actually drive the explants before you inoculate them into the inoculation media.

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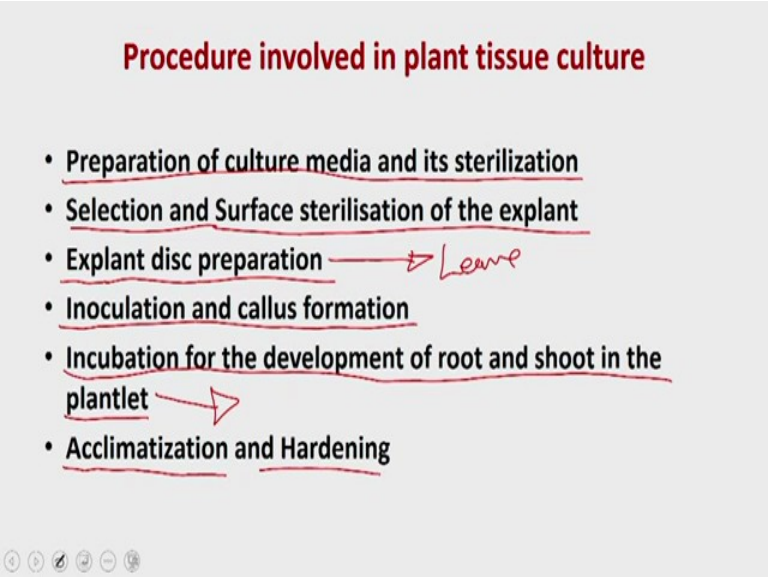

So for procedure what are the procedure are involved in a plant tissue culture? In the step number 1 you have to prepare the culture media and then you have to sterilize this culture media. In the step 2 you have to select the explants and then you would have to do a surface sterilization of the explant then you have prepared the disc from the explant. In this particular examples since we have taken a leaf, we are preparing a disc from the leaf then you have to do a inoculation of this disc and form the callus and then we have to do a incubation for the development of the root and shoot in the plantlets.

So at this stage you are going to vary the combination of the hormones in such a way so that it is going develop the root and shoots and then ultimately you are going to do the acclimatization as well as the hardening steps.

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So let us start with the first step, so in the first step you are going to do a preparation of the culture media, the preparation of a plant tissue cultures media requires many components such as it requires the MS media which is called Murashegi media or MS media that MS media and then you MS media requires the major salt or the minor salts.

So the minor salt requires like ferrous sulfate or sodium acetate and minor salts, minor salts are requires such as Dk,i borate, manganese, zinc, molybdenum, copper and cobalt and the major salt what is required is the ammonium nitrate, potassium nitrate, calcium chloride, magnesium sulfate and hydrogen phosphate you need a carbon source, so in this case people are normally using the sucrose as a carbon source.

One you prepare the MS media then you can have the, you can put the gelling agent and as a result a MS media is going to be form a thick layer.

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This is the once you culture you have prepare the culture media is requires the sterilization, so sterilization can be done if you remember we have already discussed about the sterilization in our one of the demo videos. So you can use the similar kind of procedures to sterilize the media, the only difference is that do not have to add these sucrose while you are doing the sterilization you can add the sucrose later on in a septic conditions.

And you can do autoclave at 120 degree Celsius for and 15 Pascal and once the culture media is prepared you can see that the culture media will solidify to give you a slant so it is going to be like this so, that actually if you prepare a slanting media it actually going to give you large surface area for inoculation.

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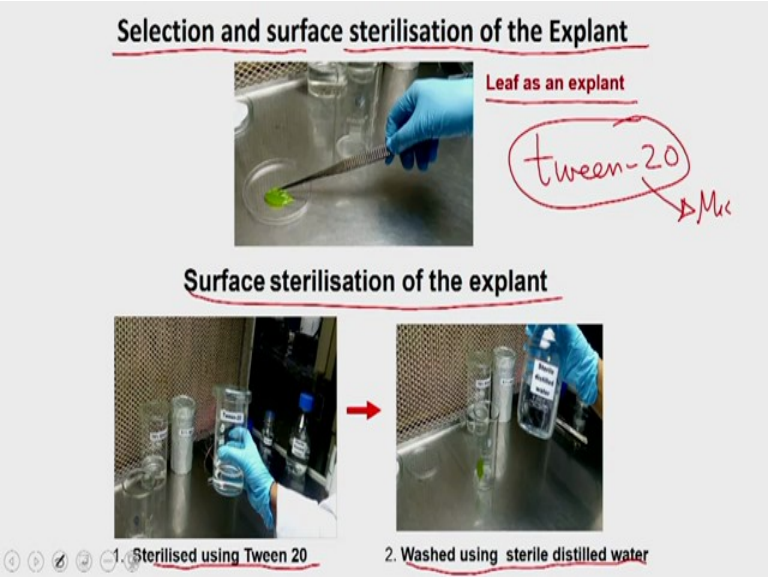

Once your media is prepared then you can use a selection and the surface sterilization, so in this particular example we have taken leaf as an explant you can do a surface sterilization of the explant so what you can do is, first wash the leaf with the tween 20, once you have done with the washing with the tween 20, so tween 20 is a detergent so that tween 20 is going to destroy the microorganism.

So the purpose of sterilization is that you do not want the microorganism to grow while you are generating the plants. So you wash the plants so the explant with the tween 20 and once you have done with the tween 20, then you wash using the sterilized distilled water.

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After the tween 20, you sterilize again with the 70 percent using 70 percent ethanol and the 70 percent ethanol is also been done only for the purpose that it is going to destroy the microorganism and then you wash using the sterile distilled water and ultimately you are going to sterilize explant with the help of the mercury chloride, the mercury chloride is a poison.

So it is going to kill the microorganism but since the mercury chloride is poisonous in nature it have to be handled very carefully and then ultimately you wash the explant with the sterile distilled water and that actually is going to make the surface of the explant very sterile, so that now it is free of microorganism so you can use that for tissue culture.

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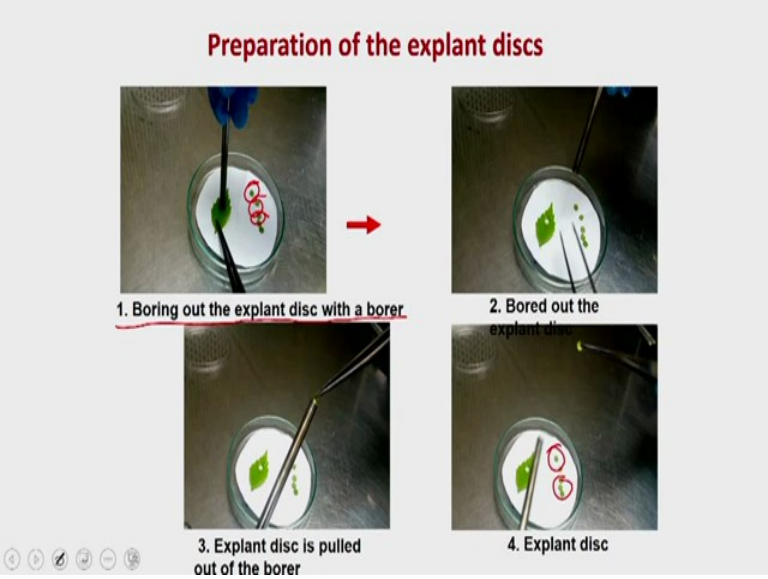

After that you have to use the borer and with the help of the borer you can actually make the disc so we can see that lot of disc we cut it from the leaf. Now you take these one of these disc and so these are the bored disc what we have prepared.

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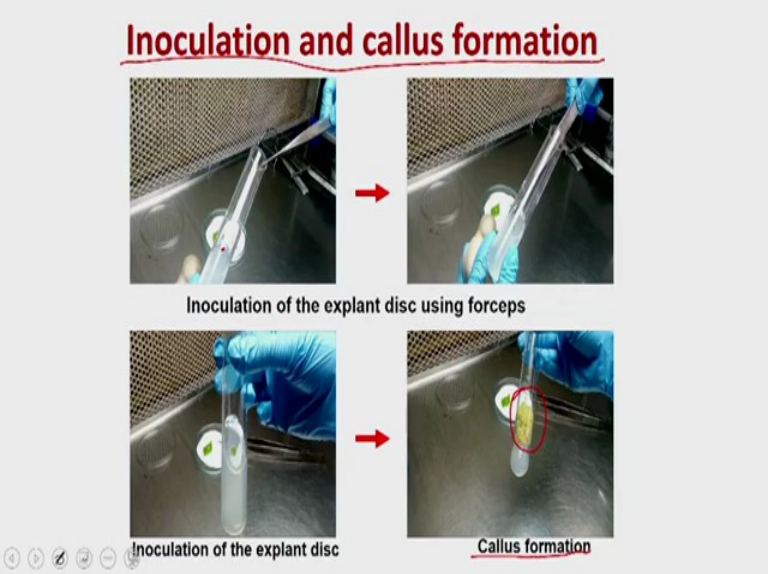

And in the next step you have to do inoculation in callus formation. So what you have to do is, with the help of the sterile forceps you can take one of the this disc and put it into the culture media or inoculate that into explant into the explant disc into the media. And once you inoculate you have to leave it and after inoculation you have to leave it for few weeks and in this period

what will happen is, that inoculated disc will take up the nutrition from the media and it will grow and ultimately with the help the combination of the hormones it is going to give you callus, this callus now can be differentiated with the help of the different types of hormones which was responsible for generating the shoots as well as the root and that actually we will give you the plantlets.

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Now once you do that the plant is going to generate the shoot on the top and the root at the bottom and now this plant is good enough to transfer it to the green house for acclimatization.

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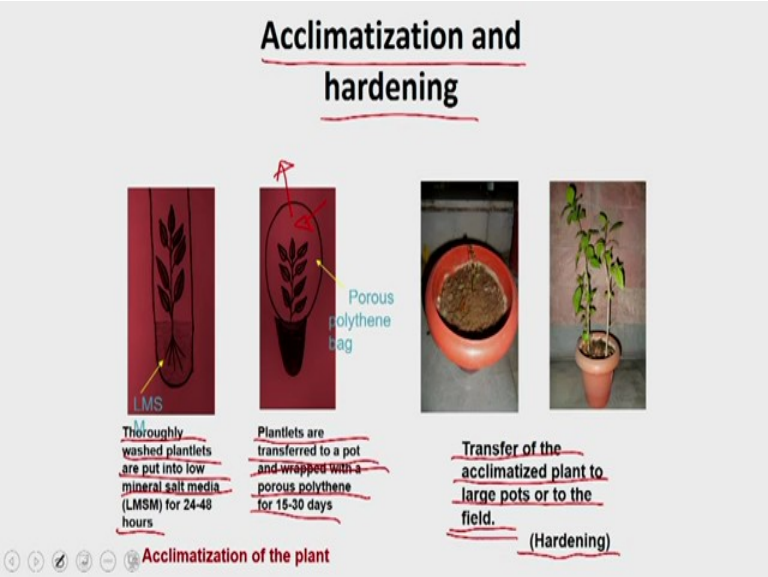

So next step is that you do the acclimatization as well as the hardening of these plants so, you thoroughly wash the plantlets and put out into the low mineral salt media, for 24 to 48 hours and then the plantlets are transferred to a pot and wrapped with a porous polythene for 15 to 30 days since you are making it covering it into a porous polythene bags that usually allow the entry and exit of the gases but it will not allow the entry of microorganisms, so the plant will still be under the control condition.

But, it can actually get acclimatized to the environmental conditions and then if you keep them for 15 to 30 days they will get acclimatized for the external environment and then what you can do is, you can transfer the acclimatized plant to the large plot or to the field. And once you transfer them to the large plot they will get not only acclimatized for the environmental condition they will also get acclimatized for the microorganism as well and then once you show that they are surviving in the pot then you can transfer them into the fields for other kind of downstream applications related to the plant tissue culture.

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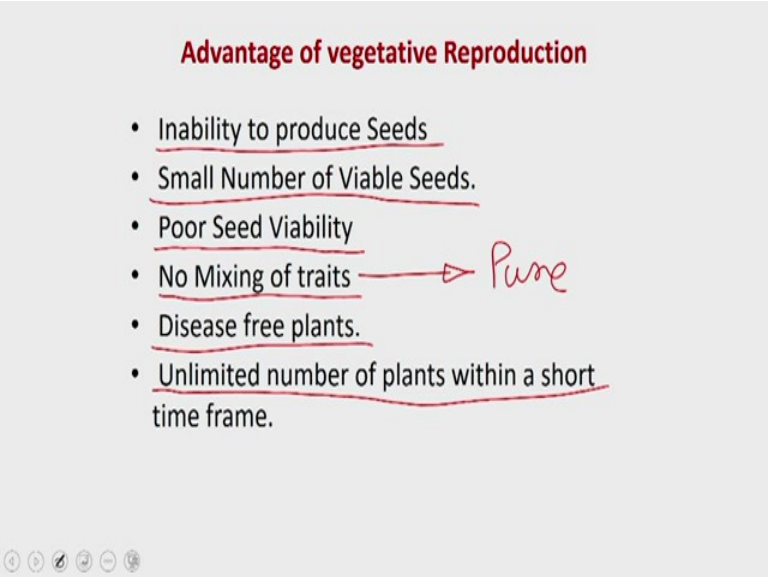

Why we are doing this plant tissue culture? Because the inability to produce seeds, because we are doing it for the plant which are inability to produce the seeds or if they are producing the less number of viable seeds or even if they are sometime they are producing the seeds but they are, their seeds are very poorly viable or you do not want the mixing of traits which means you want the pure cell lines or pure lines which means you do not want a mixing of the genetic material, lastly you want the disease free plants

Because the plants what you are going to grow into the plant tissue culture technique are going to be disease free and ultimately the plant tissue culture can give you a unlimited number of plant with the short time frame. With this we would like to conclude over lecture here in our subsequent lecture we are going to discuss about the role of genetic engineering in the animal sciences as well as in the fisheries and as well as the other areas Thank You.