Plant Developmental Biology
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Lecture – 20
Techniques Used in Lab

Hello everyone. Welcome to the practical demonstration course of Plant Developmental Biology. Today I have Mister Tushar Garg with me, a senior PhD student in my lab. So, Tushar is going to practically demonstrate you some of the practical aspects of plant developmental biology. So, if you can recall our previous classes; so, two very important component or aspect which we consider while studying plant developmental biology is analyzing the expression pattern temporal and spatial expression pattern of the genes.

So, Tushar will show some of the process associated with it. And second important component is you have to genetically modify the plant to understand the developmental biology or the aspect of plant development. So, Tushar is also going to show you or demonstrate you how to make transgenic plant and how to do some genetic manipulation in it.

Hello everyone. So, you have gone through the lectures and you must be knowing that to study plant development biology; the genetic modification of plant is a very essential process. So, for genetic modification, the first thing that we need is an explant. So, explant is any plant material which can be used to make or regenerate callus and those calluses are then transformed using two methods. The first is the biological method and the second is the physical method. So, biological method is the one where we use bacteria and this bacteria contains our construct or the gene which we want to modify and this is then transferred into the callus.

The second one is, where the gene gun like instrument is used for the transformation where the gold particles are used and the gene that we have to transform is coated on the gold particle. And, then this machine sends this gold particle along with the gene into the cells and the cells which are transformed they regenerate and make the new plant.

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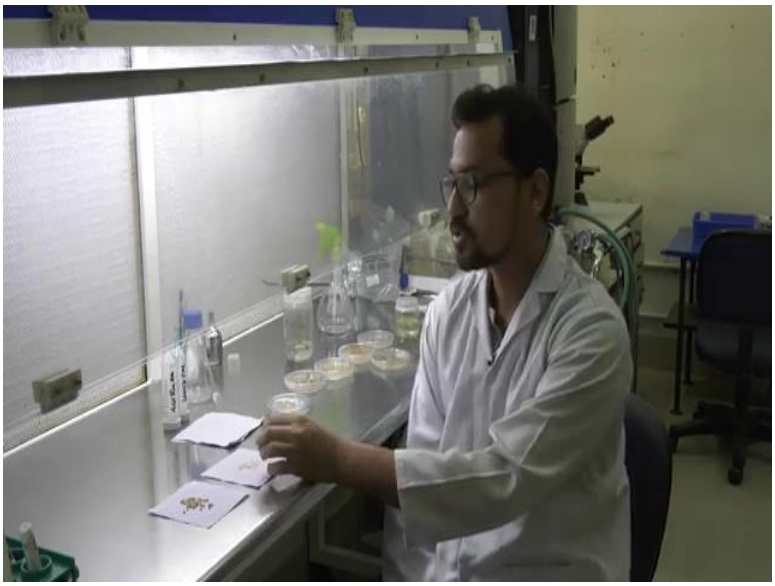

So, first we will be going through the biological method of transformation, where in our lab we use rice as an explant. So, here you can see this is the dehusked dehusked rice and here the embryo; the embryo gives the callus which is under the influence of hormones. So, when you give a certain hormone component then the embryo generates dedifferentiated callus and this callus is further used for our further process. Now, we will see how we proceed.

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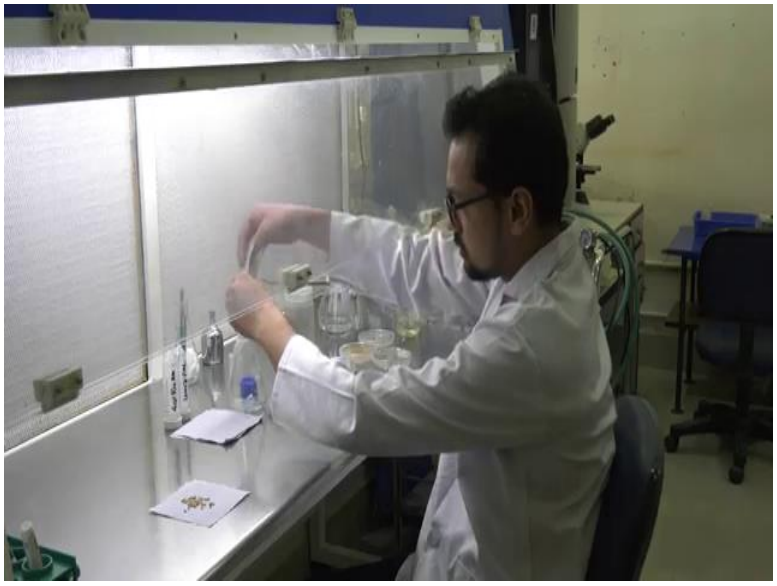

So, the first step is where we sterilize the seeds. Now, for sterilization it is called as Surface sterilization. So, in surface sterilization, certain chemicals are used which kills the contamination like bacteria, fungus. So, the most common surface sterilization which is done is using 70 % ethanol.

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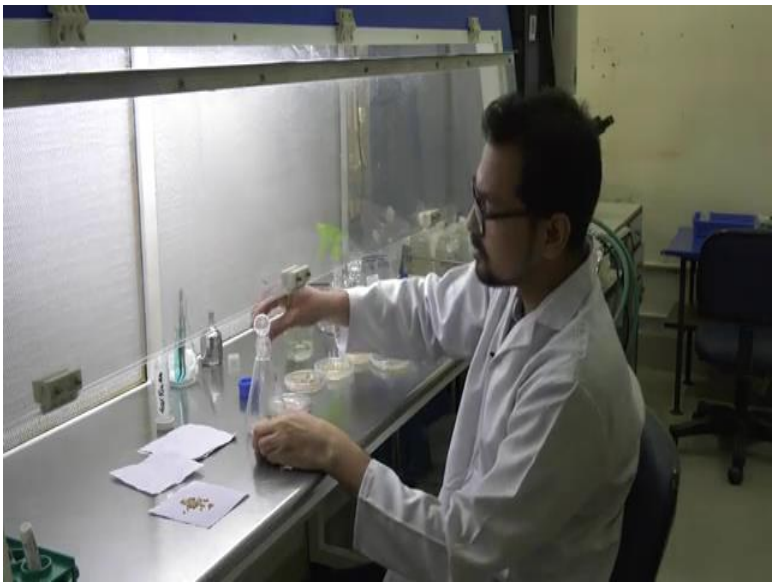

Now, this is done for 90 seconds not more than 90 seconds. So, once 90 second is complete then we use Rin Ala. So, this is Rin Ala which contains sodium hypochlorite. Some of the others chemical agent which is used for surface sterilization is $HgCl_2$. In Rin Ala it is done for 15 minutes.

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So, once it is done so, these seeds are taken on the blotting sheet and then using forceps it is placed on a media.

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Now, this media has auxin. So, auxin is the hormone which causes the embryo to make dedifferentiated tissue or the callus.

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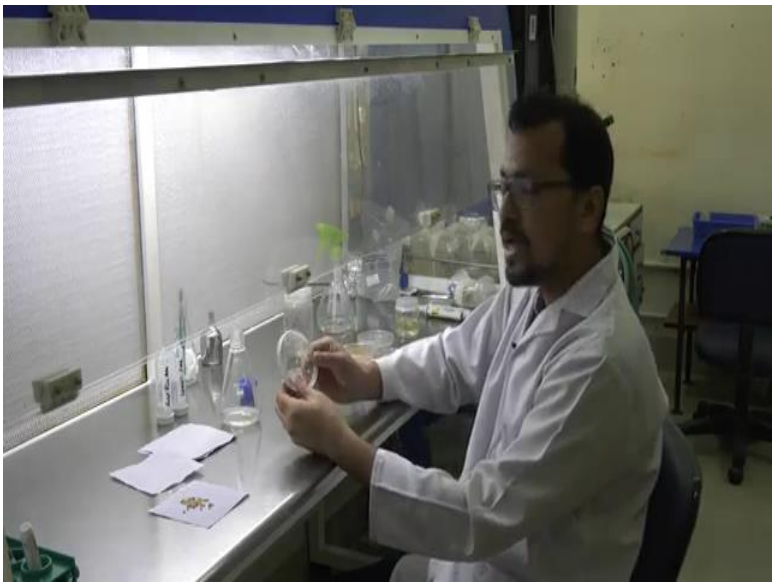

This plate is placed under dark condition for about 30 days. And, once the 30 days is complete, it creates a callus which you can see you over here.

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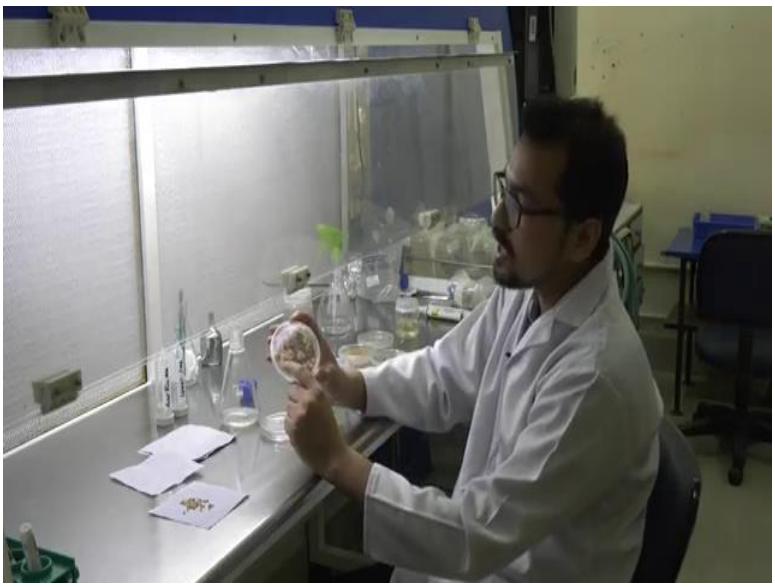

So, these are the calli. Now the cream-colored embryogenic calli, they are selected and placed on the subculture media.

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Basically, it is placed for 2 to 3 days for having a proper amount of growth. So, that it is ready for *Agrobacterium* infection. Now, once my callus is ready for *Agrobacterium* infection so, I use the *Agrobacterium* which I will show over here.

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So, this is the overnight grown culture of *Agrobacterium*. This *Agrobacterium* has my gene of interest. So, this is pelleted down on centrifuge.

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And, then a 5 % sucrose solution is added to it. Now, this is vortexed to suspend in the solution.

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Now, we take the suspended *Agrobacterium* in the flask, and put our callus in it. In this way we take all the calli and put acetosyringone in it. Acetosyringone is basically a signaling molecule, which helps *Agrobacterium* in its growth. Now, we keep this for 15 minutes on a shaker and after that, we take out we throw the media, and we take the callus and put it on a co-cultivation plate.

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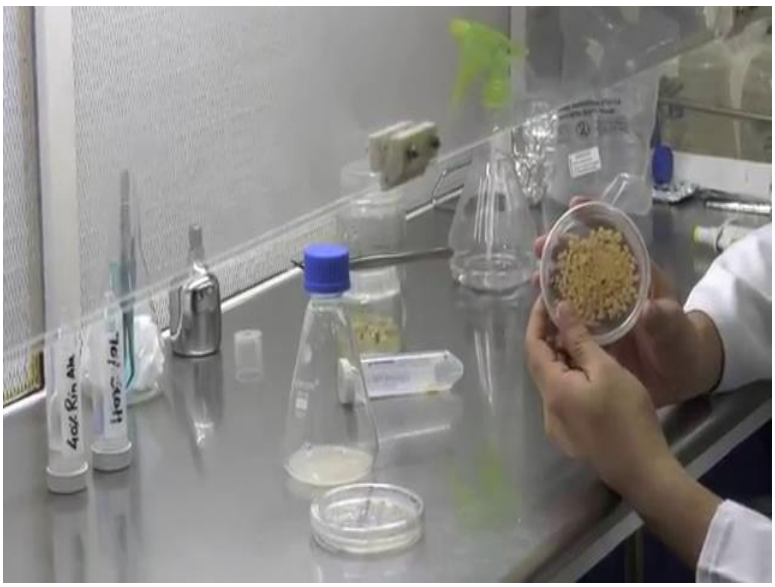

So, co-cultivation means, here we have bacteria as well as the callus. Now this co-cultivation plate is placed under dark condition for 2 to 3 days and after that these callus;

so, you can see here there is little amount of growth of the bacteria. Now, this callus is taken again in the flask and washed using water. So, enough amount of washing is done. So, that there are no *Agrobacterium* left, there are no threads coming out of the *Agrobacterium* and then it is placed on a selection media.

Selection media is the media which contains our selection marker; over here suppose my gene of interest which I have included in the *Agrobacterium* and it has been if my callus has been transformed. So, along with the my gene, there is selection marker such as hygromycin. So, when I grow this callus on hygromycin plates, so this, callus which are transformed they will grow. But, those which are not transformed they will die due to the hydromycin which is an antibiotic.

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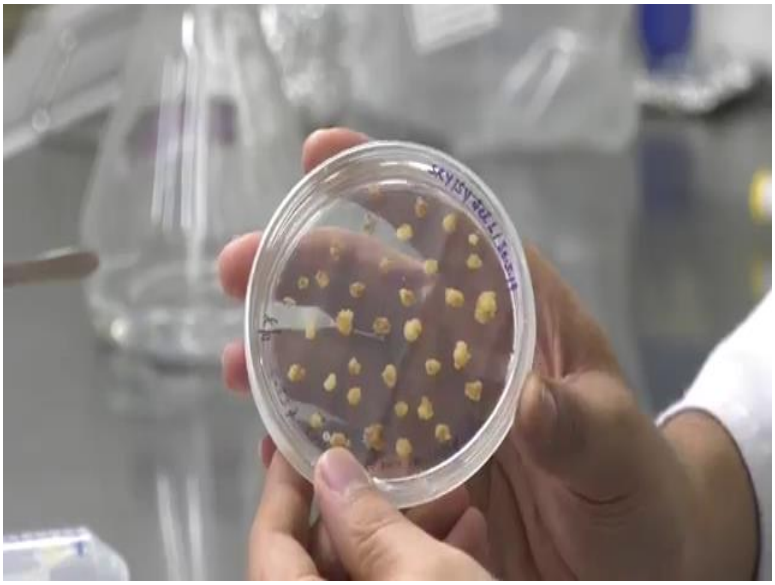

So, once placed on selection media, here you can see some of the calluses are dying whereas, some are not dying. So, those which are not dying they give out new cells and which proliferate and to form a regenerated calli which once it is ready and enough growth has taken place we place it on a shooting media. So, shooting media is a media which has high cytokinin to auxin concentration, under the influence of high cytokinin the callus forms shoot apical meristem and regeneration takes place.

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Now, here you can see with time this bottle has been placed under light condition and with time you can see that there are green colored tissue coming out. These are basically shoot apical meristem where new organs are being formed. Now, once this greening is enough and there are enough organogenesis occurred.

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So, this is taken on a rooting media. So, this rooting media has high auxin concentration, auxin helps in the initiation of root formation. So, here you can see this greening plant has enough shoot and roots are also coming out once this plant is about this the length of

the bottle. So, from here it is transferred into the soilrite for hardening, which we will see in the greenhouse. Now, we are done with the biological method of transformation then, now we will see the physical method of transformation.

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So, I will just show you the machine over here which is called as gene gun. Gene gun is used for the physical method of plant transformation. Here the principle for gene gun is that, the gold particles or the tungsten particle they are coated with the gene of interest.

The gene or the construct that we were transforming into the bacteria, here those construct are coated on the gold particles.

And, this gold particle is forced on to the callus; it means that it is projected onto the callus with lots of pressure or a force. And, then these gold particle insert inside the plant callus where it goes and integrate with the genome of the plant or the callus. Now, we will take this callus and it will go through the process similar to that of the biological transformation. So, it will go through the process of selection, then regeneration, then rooting and further hardening.

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So, the process of hardening is carried out in the greenhouse where we provide the optimal light, temperature and humidity condition. So, the plant that we saw in the bottle, that is taken in a pot which has soilrite in it. So, initially the plant is covered with plastic so, that there is 100 % humidity condition. So, that the plant does not come under stress and after a few week this polythene is cut, as you can see over here and once the plant is healthy and has good strength then it is transferred into the clay pot. So, here you can see this is a transgenic line which is about 4 months old and then the seeds mature and here the seeds dry.

The dried seed is then collected and used for selection. So, we take the seeds and grow them on the 1/2 MS plate containing hygromycin. So, hygromycin acts as a selection marker and only those plant will grow or the only those seeds will germinate which has

hygromycin in it. Now, we will see how transformation is done in a model dicot plant which is *Arabidopsis*. So, similar to the procedure in rice we also surface sterilize the seeds of *Arabidopsis* and these sterilize seeds are placed on the 1/2 MS plate.

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So, this is the 1/2 MS plate where the seeds were put and after about 2 weeks the plants are of this length. Now, once my plant is having enough root and healthy shoot this is transferred on a soilrite.

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Now, once on soilrite this plant grows of this height in around 1 month and this is my fully grown plant which you can see here it has silliques, flowers. For the transformation what we do in a younger stage when the flowers are not even opened that is it is just a floral bud. The *Agrobacterium* which is having my gene of interest is used for a transformation.

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So, for that what we do first we pellet the Agrobacterial culture as we did similarly for rice.

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Now, this pelleted culture is then suspended in sucrose solution along with silvett-77, silvett-77 is a chemical which allows the *Agrobacterium* to stick to the *Arabidopsis* plant.

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Now, that the suspended culture is then applied on the *Arabidopsis* using the pipette.

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So, I will put the culture on the buds. Previously people use to completely dip the plant in the culture and, but now that method is not used anymore. Now, this plant is placed

under 100 % humidity condition and once the plant forms the new seeds, it can have both the kinds of seed.

The first one can be the non transformed one and the second one will be the transformed one. These seeds are collected and grown on 1/2 MS plate with hygromycin. So, hygromycin will be my selection marker over here. Once, the seeds grow only those plants will generate which will have my gene or my selection marker.

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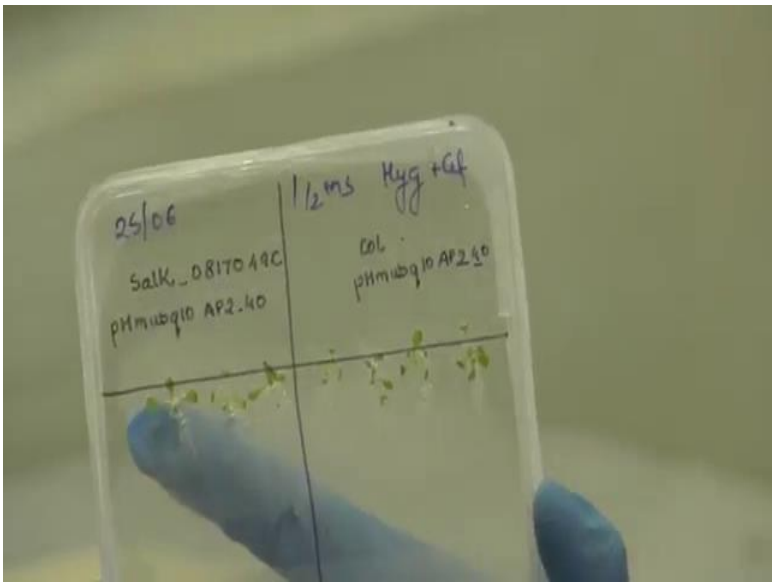

So, here this is a transformed *Arabidopsis*. Here you can see that the plants which I have taken, it is the transformed one and they are growing healthy. So, these are the transformed *Arabidopsis*. Here we have placed DR5 promoter and downstream of DR5 promoter we have placed GFP. So, wherever there will be auxin maxima DR5 will get activated and hence GFP protein will be made and a green signal will be observed. So, we will take this and see under the microscope how and where the auxin maxima is formed. So, we will take this *Arabidopsis* and place it on a slide.

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And, see it under the microscope.

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So, here at the tip you can see that the signal for GFP is high which means that an auxin maxima is present over here. So, in this fashion we can use this method to temporarily or spatially understand the gene expression pattern also. So, in the lecture you have seen how the temporal and spatial expression for gene is checked, the method is called as *in situ* hybridization. So, I will show you how we prepare the sections or the tissue for *in situ* hybridization.

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This is a wax block which has a stem base of the rice embedded in it. So, we have taken the stem base of the rice, gone through the steps of dehydration then through xylene series and finally into the wax. These wax blocks, are then used for sectioning.

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So, this is a Microtome. Microtome is the machine which makes thin sections, here we will be making 8 micrometer sections of the stem base and then those sections will be taken on the slides and fixed to the slides. We make a 8 micrometer section using this

microtome, here you can see the ribbons are formed. Now, these ribbons will be taken on the slide.

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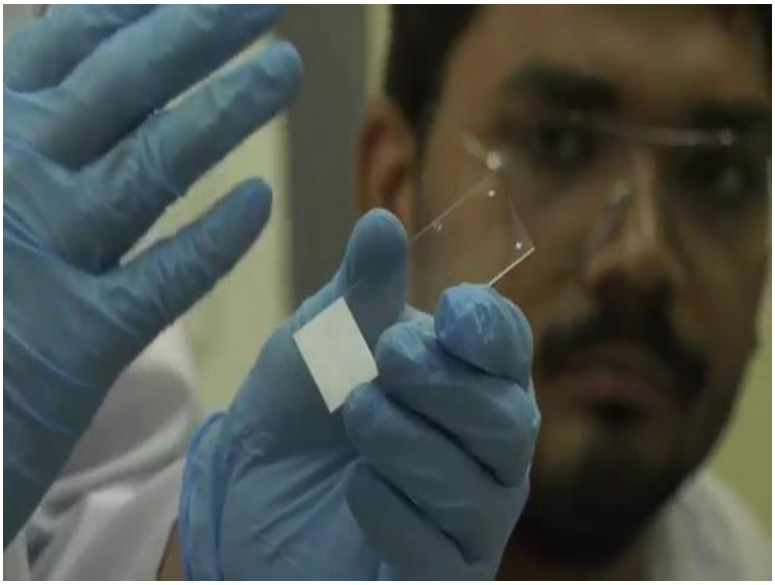

So, the slides used here are poly-L-lysine coated slides. So, poly-L-lysine basically creates a positive charge on the slide and it helps the tissue to stick to it. Because, you have seen that through the steps of *in situ* hybridization, the slides goes through various amounts of steps and there is a chances of that tissue will fall off. Therefore, the positive slides are used and the tissue does not fall off.

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Now, we will use nucleus free water. So, that our RNA is not degraded.

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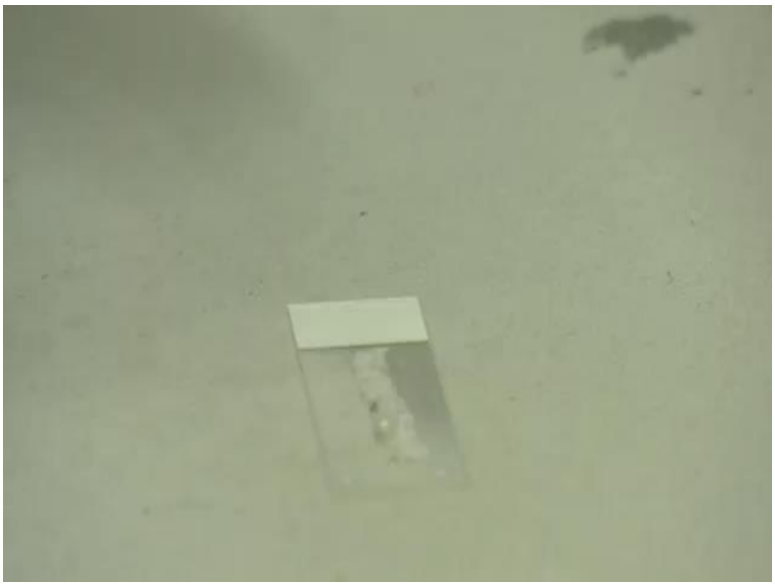

And we will pour few drops over here, now this ribbon will be taken and placed on the slide.

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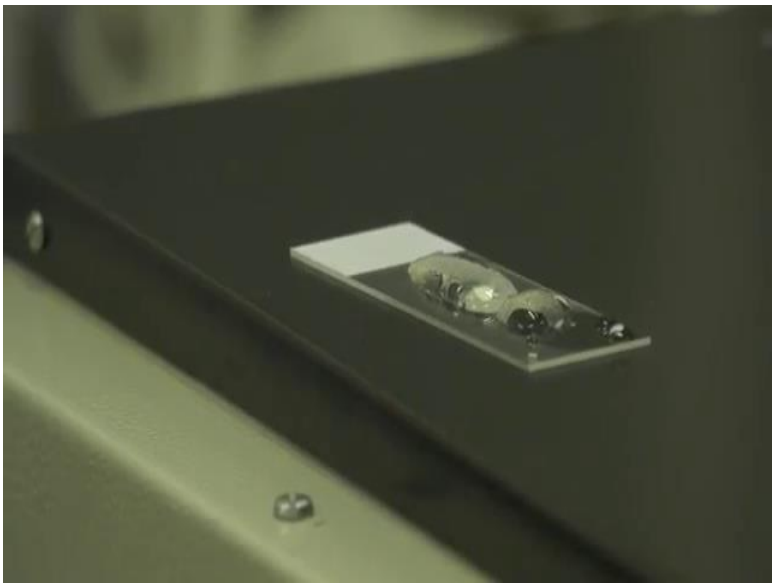

Now, we will place the slide on the hot plate which is at around 40º celsius. So, as the water will heat the wax will, the ribbon will starts spreading. The excess water is removed using the tissue paper.

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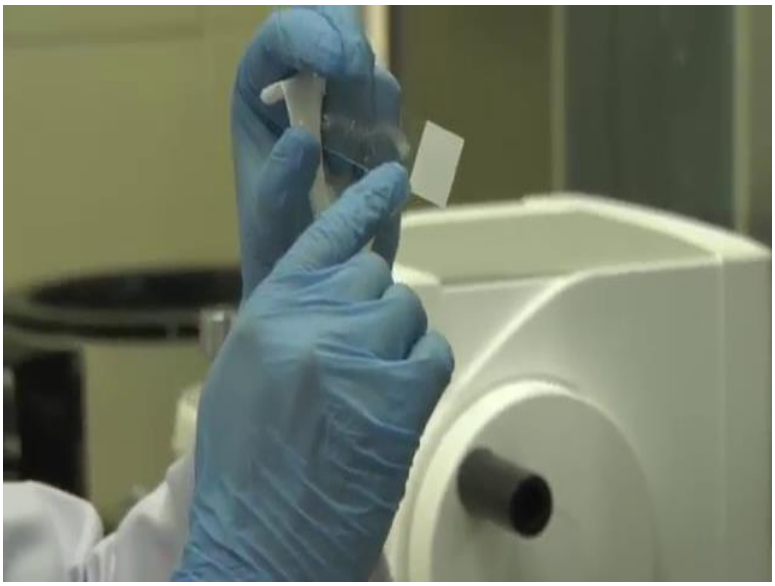

So, here you can see that my tissue is spread evenly on the slide.

Now, this slide is kept overnight on the hot plate so, that the tissue sticks to the slide completely. The next day you will have the tissue completely stuck to the slide as you can see over here and now this slide is ready to go for the process of *in situ* hybridization.

So, once the slide is dried overnight as I have shown you. The slides are now dewaxed using xylene and then goes through a series of step as you have seen in the lecture. And, then at the end once the slide is probed and the signal is detected, it is fixed using entellan and a cover slip is placed on it.

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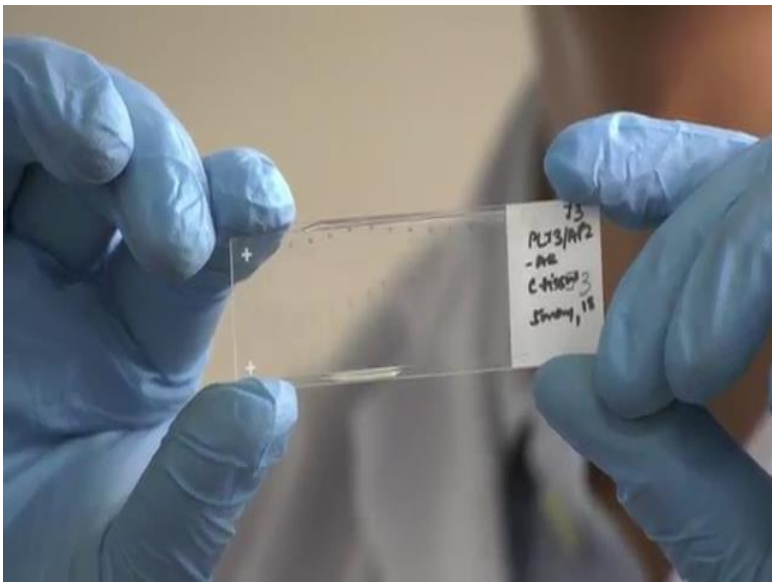

So, the slides look like this. If you can notice there are some blue spots over here which is basically the signal, this is the color reaction. So, therefore, a color has developed and it is seen in particular tissue only.

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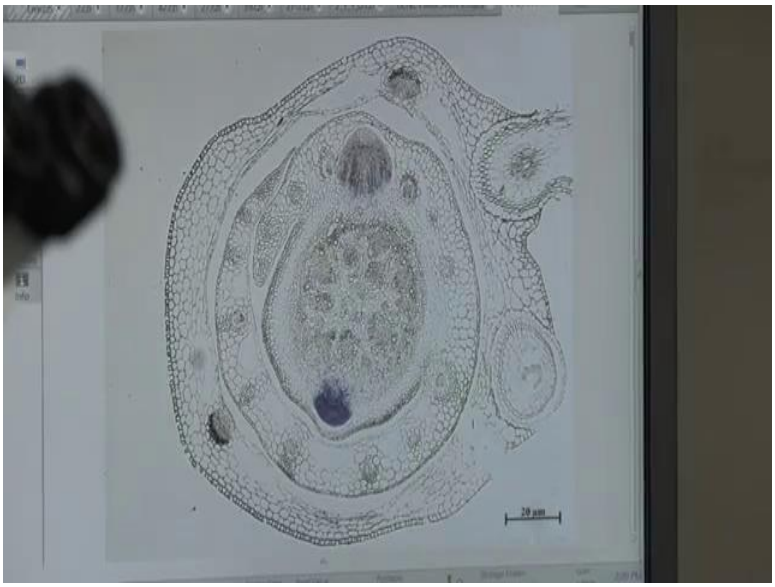

So, now here you on the screen you can see. So, this is the rice crown tissue and this is a primordia which is at a younger stage whereas, this is the primordia which is at older stage. So, you can see how differential expression of gene is taking place; in the younger tissue you can see the intensity, the color signal is higher which means, that the gene is

highly expressed in the younger stage whereas, when the primordia is old the expression of the gene goes down and therefore, the color signal is lower. Therefore, *in situ* helps us to understand the differential expression pattern of the gene in the tissue, that is why called as *in situ* in its own place.

So, these are the few basic techniques that we use in the study of plant developmental biology. And, I hope that this session will help you in understanding the topic much better.

So, this practical demonstration is going to be the last class of the plant developmental biology. So, we hope that you have learnt something in this field and we wish you all the very best for your examination and future career.

Thank you very much.