Hello students, I am Marsha Rodrigues, Assistant professor in Botany from Carmel College of Arts, Science and Commerce for women.

Unit 2. Plant tissue culture technique and the name of the module is “Establishment and incubation of cultures” and this is module number 10 of Unit 2

The module outline
- Ideal conditions for incubation of cultures
- Set up of culture room
- Establishment of *in vitro* cultures.

These are the learning outcomes of this module.
- Lists the ideal conditions required for *in vitro* cultures.
- Describes the setup of the culture room.
- Explains the establishment of *in vitro* cultures,

The steps in plant tissue culture technique are as follows:
- Preparation of culture media.
- Preparation of ex plans for *in vitro* culture.
- Establishment and incubation of cultures, and
- Maintenance of cultures and subcultures.

Students, in the previous module I explained to you about preparation of explants for *in vitro* culture. That is the selection of explants, isolation of explants, surface sterilization of the explant and finally the inoculation of this explant on the nutrient medium.

In this module we will look at the third step in plant tissue culture technique, that is, “Establishment and incubation of cultures”. The last step that is, “Maintenance of cultures and subculture” which I will teach you in my next module. Students, in this slide you can see an image and, in the image, there is a culture vessel which is inoculated with seeds. So, in the previous module, in the last part we had learned about inoculation of explant. So, here we have a culture vessel which is inoculated with seeds. Now, culture vessels, as I told you in the previous module can be a bottle or it can be a Petri plate or a test tube containing nutrient media. And here we have a culture vessel that is a bottle and it is inoculated with seeds. Now, this culture vessel which is inoculated with seeds as the explant, has to be given the ideal conditions to grow. So this bottle that is the culture vessel along with the inoculated explant, has to be transferred to another room of the tissue culture laboratory, that is, the culture room, where all the growing conditions or the ideal conditions that are required for this explant to grow are provided.

Before we move on to the culture room, we will first learn about the ideal conditions for incubation of cultures. So, the three ideal conditions that are required or the. The physical environment that is required to support or to facilitate incubation of cultures are: the first one is temperature. The temperature has to be 25 degrees plus or minus 2 degrees. Second is the photoperiod that is the amount of light that is required for the explant to grow. Normally 16 hours of photoperiod is given to the explant. And the last is the relative humidity that is around 60 to 80%.

Now this relative humidity is maintained within the culture vessel, because in the nutrient medium there is water that is present. In this slide you can see an image that is the culture room and you can see on all the three sides there are racks that are kept and they are painted white in colour. And each rack has got a fluorescent tube, and the cultures are kept on these shelves.

As I said in the previous slide, three ideal conditions have to be provided, that is temperature photoperiod and relative humidity. The temperature is regulated by using an air conditioner and that's how you provide 25 degrees plus or minus 2 degrees. Next, is the photoperiod wherein artificial illumination is provided by fluorescent tubes and these fluorescent tubes are placed for each shelf. The third one is relative humidity. As I said in the previous slide, relative humidity has to be around 60 to 80%. In case if there is any excess amount of humidity that is present in the culture room, then this excess humidity is taken out by the air scrubbers. And the culture room has racks or shelves on which the culture vessels are placed.

In case there is liquid culture, then this liquid culture, which is in the flask has to be placed on the rotary shaker because liquid cultures require aeration. Now we will learn about the establishment of *in vitro* cultures. So once the explant is inoculated on the nutrient medium, then that culture vessel is then brought into the culture room, wherein all the ideal conditions are provided, such as temperature, the photoperiod, and the relative humidity.

Photo period depends upon the type of the explant that is used. Some explains require less amount of light, some require darkness for growth. In case of callus, initially it requires a dark period followed by photoperiod. During incubation of cultures there are certain events that take place. The first is absorption of the nutrients.

As the explant grows and as you provide the ideal condition the explant starts to absorb the nutrients that are there in the nutrient medium. And as nutrient absorption takes place the cells begin to enlarge that is there is increase in size. Cell enlargement takes place and cell division takes place. The cells begin to divide and finally there is callus formation. Callus is a mass of undifferentiated parenchymatous cells.

Students in this slide, the first image is the inoculated explained. Now, as I told you, the culture vessel can be a bottle, it can be a Petri plate or it can be a test tube. So here we have the explant which is inoculated on a Petri dish containing the nutrient medium and after three to four weeks of incubation we will see the growth of callus tissue. So, the second image is of the callus tissue that is the undifferentiated mass of parenchymatous cells.

So, students in this module we have learned about the establishment and incubation of cultures. wherein we learned about the ideal conditions that are required for incubation of cultures, that is, the temperature, the photoperiod and the relative humidity. We also learned about the culture room, the setup of the culture room, and all the ideal conditions that are provided in this culture room. The culture room also contains racks or shelves and rotary shaker. And finally, we learned about the establishment of *in vitro* cultures wherein different events take place during incubation of the cultures.

These are the references that I have used to prepare this module, that is Plant tissue culture by Mishra and Plant tissue culture by Singh. This is an additional reference that is a web link.
Thank you, students.