Course Name: Basics of Crop Breeding and Plant Biotechnology

Professor Name: Dr. Joydeep Banerjee

Department Name: Agricultural and Food Engineering

Institute Name: Indian Institute of Technology Kharagpur

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Lecture-42: Application of Plant Tissue Culture (Part -I)

Hello everybody. Welcome to the SWAYAM NPTEL online course on the Basics of Crop Breeding and Plant Biotechnology. So, today under the module, we will start our discussion on the application of plant tissue culture in plant breeding and genetic engineering. So, in this lecture, we will be discussing applications of plant tissue culture. So, these are the concepts which will be covered under this topic. First of all, we will be discussing the importance and applications of plant tissue culture.

What are the importance of plant tissue culture, and what are the different applications will be discussed. Then gradually we will move into the totipotency, it is a character available in the plant cells. Then gradually we will discuss the callus culture, organogenesis, and somatic embryogenesis. Then we will discuss the haploids and anther culture.

And, finally, we will discuss artificial seeds, how artificial seeds are produced, what are its applications those things. So, let us start our discussion on the importance and applications of plant tissue culture. These are the different importance of plant tissue culture. First of all, through tissue culture, a large number of plants having identical features to the parents can be produced by this method. If you think about the seed production, in a particular plant over there the male gametes and female gametes are produced by the flower, and thereafter crossing is taken place, fusion of gametes is taken place and finally, embryo or zygote is formed.

But in the case of plant tissue culture, vegetatively from a particular tissue we can grow numerous plants. So, whatever, we will be getting all the progeny plants will be identical to its parents. Different gametes are not produced, over there means somatic cells will be eventually converted into different plants. Ok! So, whatever was the genetic constitution in the parent, that will be available in the progeny. So, a large number of plants having identical features could be developed through this approach.

Next one adult plants, can be reproduced within a short period of time. If you think about the growing cycle growth cycle of different plants. So, some plants used to take 2 to 3 months, and some plants used to take 4 to 6 months to mature means seed-to-seed formation means, seed is shown thereafter the plant grows gradually and finally, the seed is produced in the plants. So, in this cycle, different time periods are there, but if we grow a plant under tissue culture conditions, there we can optimize the growth condition by providing different nutrients in the media, by changing the light conditions, the temperature conditions, and finally, within a short period of time, we can complete the life cycle of a plant. Then many plantlets can be produced without seeds.

In most of the crops, in most of the crop plants, like rice, wheat, maize, from a particular plant hundreds of seeds are produced. So, their seed production is pretty easy, but if you think about those plants that are reproduced by vegetative propagation. Ok! Suppose someone is working on a jasmine flower, someone is working on sugarcane. So, in those crops specifically through vegetative means the reproduction is done. So, through tissue culture many plantlets can be produced without seeds, because seeds are not formed in means seeds are not used for their reproduction purpose.

So, through tissue culture, we can make an enormous amount of plantlets. In the case of bananas, it has been highly famous also. In bananas, the seed production was found to be lesser due to the availability of triploidy. So, through tissue culture, we can multiply it. Then healthy and disease-free plants can be propagated by this technique.

So, if a plant grows in outside conditions, in natural conditions, then several biotic stress can hamper its yield and its survival percentage. Ok! So, it has been found that the apical meristematic region of a plant, means the tip part of the shoot, they remain free from most of the diseases. So, later on, we will be discussing it once again. So, if we culture that apical meristematic region, then easily we can make healthy and disease-free plants through the tissue culture technique. The next one i.e., very important, i.e., endangered plants can be preserved and conserved by this technique.

Nowadays due to global warming, a lot of plant species are being endangered and some of the species have been extinct also. If you think about high altitude area, means hilly areas there also the average temperature has increased. In different glaciers, the snows are melting. So, globally the temperature shift has been done, and some plants that used to grow in very cold conditions, they are now facing problems and they are becoming endangered. So, through plant tissue culture, we can preserve those plants, we can conserve those species in laboratory conditions under specific temperatures, and specific media compositions, we can grow them and we can maintain them.

The next one is functional genomics study i.e., also a very important thing. So, functional genomics study, what is a functional genomics study? Is the functional characterization of a gene. Suppose if you think about rice plant, there at least 30,000 genes are available. At the NCBI database, the chromosome sequence is available and within those chromosomes maybe 30 to 40 germin-like protein genes are there, maybe 30 to 40 *calmodulins* or *calmodulin-related* genes are there, and 7 different actin genes are there. So, those gene sequences are available over there, but the exact function of those genes whether i.e., that particular gene is involved in some enzyme production, whether it is the part of the cell wall structure, that things are not known.

So, through functional genomics study, we can characterize a particular gene. So, how can we characterize a gene? For characterizing a gene, generally, 2 different approaches are taken. Eventually, in the next part of this lecture, we will be discussing those things. So, 2 different approaches are taken to characterize a particular gene. The first one is

over over-expression approach means, suppose from sweet peas we have isolated a gene we would like to characterize it.

So, we can over-express that gene in rice plants in a heterologous system. So, in rice that gene might not be available. So, if it is expressed, overexpressed in the rice system its protein production will be, and then that protein may show some features some phenotypic characters i.e., one strategy. Another one is we can reduce the expression of that gene. For expressional reduction a couple of approaches could be taken either we can do the RNAi-mediated gene silencing or the recent method we can silence a particular gene we can do genome editing by CRISPR Cas9 approach.

In this way we can reduce the expression of that gene in its native system, means if we have to silence a particular gene, where from we are taking, we have to silence it there. It means for the characterization of a sweet pea gene, we have to silence that gene in sweet pea system. So, within that plant, its protein or its mRNA will be reduced and once that protein will be minimized, then we may see some phenotype. In this way, the functional genomics study is done. So, why tissue culture is involved there because if we have to do a functional genomics stud, first of all we have to make different constructs the over-expression constructs or gene silencing constructs have to be made.

Then, using those constructs we have to do plant transformation with the help of *Agrobacterium* or direct plant transformation, sorry plant transformation methods. So, then we can develop some transgenic plant to characterize that particular gene. The next one is the preparation of haploids and double haploids. So, through plant tissue culture we can culture the pollen grains, and we can culture the anthers to develop the haploid plants means a single set of chromosomes will be available over there. And once we can get haploid plants by chromosome doubling, we can make double haploids.

So, that the homozygous plant could be generated easily in a short period of time. It is the easiest way to propagate plants which reproduce through vegetative propagation. I have already mentioned this particular part also. So, now coming to different

applications of plant tissue culture. First, of all large-scale production of useful compounds and secondary metabolites by using plant tissue culture could be done.

Suppose, we have found that a particular secondary metabolite is being produced in the root tissue of a certain crop. So, we can do the hairy root culture of that particular crop. So, enormous roots will be developed or we can grow a certain part of that plant where this particular metabolite, is mostly accumulated through tissue culture. And, within a short period of time, by providing enriched nutrients or proper shaking, proper temperature we can make large amounts of plant tissues, as well as, specific metabolites. Next one, the optimization of the micro propagation rate for the multiplication of economically important crops.

For some economically important crops like banana, the tissue culture has been highly famous, and through optimization of micropropagation technique, a large number of banana plantlets, disease free plantlets could be generated easily. And in eastern India as well as in south India, several companies are there that used to produce the banana plantlets through tissue culture, and they used to sell it in the market. The next one is the eradication of systemic disease in plants and the raising of disease-free plants. This part has been discussed earlier and we will be discussing it once again because if we culture the epical meristematic region then gradually, we can reduce the disease occurrence and through tissue culture the disease-free plantlets could be produced. Next one, soma clonal variations are a useful source, the introduction of valuable genetic variations in plants.

You know that mutation is the ultimate source of variation. So once we do this type of cloning of plant parts through tissue culture, through tissue culture a particular plant tissue is reproduced means similar sets of plants are produced, and similar genetic constitution is multiplied again and again. But during this process during this cloning process sometimes some mutation may occur in the genome, and if such mutation occurs during the plant tissue culture, we can tell it as soma clonal variation. And, sometimes it has been found that within the soma clonal variants, some useful traits are available like,

in the case of sugarcane we found that a disease-resistant plant was identified through soma clonal variation. Next one, somatic hybrids, and cybrids they overcome species barriers, and sexual incompatibility and produce hybrid plants with desired combination of traits.

This is another topic that means a somatic hybrid could be made the somatic cells, from two different species could be fused and if those two species are not compatible to mate naturally then by making a somatic hybrid we can transfer its genome part. Now, these is a few facts first of all embryo culture helps in overcoming seed sterility and dormancy. If we think about distant hybridization if a cross is attempted between *Oryza sativa* and *Oryza nivara,* then sometimes this type of situation may arise that, as those two species genus is Oryza, but the species is different as those two species their chromosomal structure their genetic structure might vary. So once the hybrid is formed the chromosome pairing is not up to the mark. So, in that case, we have to do the embryo culture the embryo rescue technique could be done.

So, that the seed sterility or dormancy problem could be minimized. Next the production of synthetic seeds via somatic embryo differentiation for commercially important plants it will help to achieve increased agricultural production. So, later on, we will be discussing about, synthetic seed production also. And next one is plant tissue culture aids in producing genetically transformed plants, it has been already discussed in the functional genomics part. Then triploids and polyploid plants can also be prepared by tissue culture technique for use in plant breeding horticulture as well as in forestry.

So, now we will discuss the production of disease-free plants through plant tissue culture. So, there first the meristem culture is done in the apical meristematic part, I was talking about the meristem culture, is a method of plant propagation involving the growth of meristematic tissue, that can effectively eliminate viruses and systemic pathogens. Systemic pathogens, means a pathogen suppose a bacterial infection has occurred in the plant leaves. So, eventually, it will be that particular bacteria will be propagated throughout the plant. Similarly, if a fungal infection initiates in the root part gradually its

pore and its different hypha it transmits throughout the plant system and it will infect the plant totally.

But, if we do the meristem culture in the meristem part basically, it cannot reach easily the meristematic growth is too fast. So, using the meristem culture, we can make a disease-free plant-like production. Then micropropagation a subset of tissue culture conducted in a controlled environment allows rapid and genetically identical multiplication of plants on artificial nutrient media. So, this technique is widely utilized in the commercial realm especially for species that are propagated vegetatively. Now, these are the steps for generating pathogen-free plant leaves through meristem tip culture.

First of all screening of parent material for viruses and similar pathogens. First, we need to screen whether the virus or similar pathogens are available in the plant material through real-time PCR, or through ELISA, different techniques could be used to check the availability of viral genome in different parts of the plants. Then applying, thermotherapy or chemotherapy, if disease-free material is unavailable. If the virus-free material is not available then we can apply these things, the temperature treatment or chemical treatment, to make the disease-free material. Thereafter surgically excising the meristem tip in sterile condition.

So, we have to excise the sterile meristematic part using the surgical blade or scalpel sterile scalpel then culturing the tip along with a few leaf primordia on the suitable medium. So, along with the plant tip, a couple of leaf primordia is also taken. So, that a shoot could be developed easily. Ok! And finally, the plantlets are generated from there. Then monitoring the plantlets, for pathogen presence means again we have to check it whether the pathogen is present there or not maybe by doing different molecular test.

And if we get the disease-free plantlets eventually, it will be transferred into the soil, and maintaining a stock for pathogen-free nuclear plants means some plants are maintained in the tissue culture condition and their propagules are transferred into the soil for subsequent growth. And subsequently mass propagating the virus-free plants through in

vitro techniques. So, in this way, the virus-free or disease-free plantlets could be done following these different steps. Now gradually we will move into the totipotency. So, what is totipotency? It is the ability of a single cell to give rise to all of an organism's cell types including extra embryonic and embryonic tissues is known as totipotency.

Totipotent cells can help to produce a fully developed functioning organism, since they have the capacity to specialize into any type of cell in the body. So, let me describe, it in a simpler way. So, in plant tissue, in plant cells, the totipotency is observed, in animal cells the totipotency is not available. So, in plant tissue or in plant cells what happens? Suppose a tissue we are taking from the leaf tissue. So, in the leaf tissue whatever the cells are there different palisade parenchyma type of cells are there.

So, those are developed cells, right? So, from those developed cells through totipotency first we can stop the differentiation means i.e., known as the de-differentiation. Thereafter once it will be de-differentiated, then we can start its re-differentiation, which means a cell which has been differentiated, first we can stop its differentiation process. Then it could be multiplied a number of cells could be formed from there, those are not differentiated. Thereafter we can start differentiation. So, the leaf tissue, the root tissue the stem tissue, could be formed from a single cell i.e., the thing associated with totipotency.

So, in totipotent cells, these different things are observed somewhere, we can see that embryogenesis means, from some cells, the embryoid is formed and from those embryoid, the complete plantlets could be generated.