Course Name: Basics of Crop Breeding and Plant Biotechnology

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Lecture-43: Application of Plant Tissue Culture (Part -II)

Welcome back, so we will continue again. So, some of the totipotent cells… can be organogenesis, means they can show organogenesis also. Organogenesis means organ formation right. So here also three types are there like caulogenesis their only shoots are formed from those cells, then rhizogenesis their only root tissues are formed from those cells and, caulorhizogenesis over there shoot and root tissues could be formed from this type of totipotent cells. Now in totipotent cells we can see the histogenesis or cyto-differentiation also. Over there only xylem, phloem formation could be mostly observed.

So in this way a differentiated cell can be converted into different types of tissues, due to the totipotency character of plant cell. Now we will start our discussion on callus culture. So callus that is a very important part in plant tissue culture. What is callus? It is the unorganized mass of undifferentiated cells.

So, few things are there, first of all unorganized means a large number of cells are available. So, in this way some unorganized structures could be formed in a callus. It is a mass of cells and undifferentiated. So those cells are not in differentiated conditions ok, means the none of these cells are either leaf tissue or they belong to root tissue. So those are in undifferentiated conditions.

Once the differentiation will be started then different parts of the plant could be generated from this callus. So the callus contains many meristematic nodules invisible to the naked eye, but it develops further to produce the entire plant only on the

availability of suitable condition. So once the suitable condition is obtained then only the cells available within a callus it can form a full grown plantlet. So the organized structures may be formed by one of the three following pathways. First of all the unipolar structure with shoot apical meristem gradually leads to shoot development.

Next one unipolar structure with root apical meristem leads to root development. While from some callus the bipolar structure could be formed leading to the somatic embryogenesis. And once somatic embryogenesis is done, then from that particular somatic cell a full grown embryo will be developed which will eventually convert into root, shoot and full grown plant. So the auxin and cytokinin balance to the solid or semi-solid media which is mostly used in plant tissue culture, it leads to callus formation. And among auxin, 2,4-D that is 2,4-Dichlorophenoxyacetic acid is mostly used for callus induction.

Ok now we will discuss about the callus culture. Three stages of callus culture are mentioned here. First one is induction. In induction, the de-differentiation of explant cells are taken place, means a differentiated cell is being used or tissue is being used. Then de-differentiation means the differentiation process will be stopped of the explant cell.

Then the proliferative stage over there; the cell will divide rapidly the cell divides rapidly and then the morphogenesis stage will be coming. Over there whatever the cell division has been taken place those divided cells will be differentiated and gradually we can see the formation of organized structures means some part will make the shoot part, some part will make the root part, some part will be embryogenic; it will make the whole plant. So over here you can see these are the rice callus ok! Here the de-differentiation stage as well as the proliferative stage are being found. While over here you can see in some callus the green tissues are observed means the shoot growth has been initiated.

Over here you can see in some callus the shoot establishment is being detected. So in this way, different morphogenesis stage will come later on. Now coming to the

organogenesis, means organ formation the formation of roots, shoots, flower buds from the cells in culture in a manner similar to the adventitious root or shoot formation in cutting is called organogenesis. If a plant which is growing outside suppose a Hibiscus plant is growing outside, if we cut a stem after a couple of days we can see some shoot formations on its cut end. In certain plants, after cutting the stem part we can see some adventitious root formation.

So similarly during organogenesis process from the cell different parts will be eventually developed the root, shoot, flower buds gradually and it can be of two types. Two types of organogenesis are observed in plant tissue culture, one is the indirect organogenesis and another one is direct organogenesis. So, next we will see how the indirect organogenesis and direct organogenesis is done. So over here a leaf tissue has been chopped and it has been sterilized and after sterilization and chopping it is being used as an explant in tissue culture medium. So two types of organogenesis could be observed from here. In one mode… through one mode from this tissue several callus could be formed at the cut surface of those tissues or in the old part of those tissue different callus will be developed. And eventually on the media once we will provide proper ratio of auxin:cytokinin, then finally the shoot part will be coming out from those callus. While from some tissue directly we can see the shoot bud formation.

So this part where from those tissue initially callus will be developed and thereafter the shoots will be developed that is the indirect organogenesis. While in those part.. in those tissues where directly shoots will be formed, that is the direct organogenesis. So if we do the tobacco tissue culture using the tobacco leaf disc we can see both type of things the callus occurs over there as well as from some part of the leaf disc the direct shoot bud can be formed also and eventually it could be grown into the plantlets. Now we will be discussing different factors affecting organogenesis. First of all size of the explant that is a very important thing if anyone would like to do tissue culture in tobacco we have to take the medium sized leaf of the tobacco and preferably those plants should be grown in the tissue culture condition; means we have to sterilize the seed first, then after seed sterilization we have to grow, we have to germinate those seeds under contained

condition in control temperature within tissue culture facility.

So once the plantlets will be coming maybe after a couple of months we can use those leaves for plant transformation or tissue culture activity. If the leaves are too much longer or too much larger, then that transformation efficiency will not be up to the mark. If the leaves are too small, then also it could be easily degraded once we will treat it with *Agrobacterium* solution for plant transformation. Then the source of explant, that is another important thing if we have to take the explant from outside we must have to sterilize it thoroughly if the seed tissues are there hard coatings are there we have to use mercury chloride or $HgCl_2$ if soft tissues are there; like leaf tissue, stem tissue.. then we have to use sodium hypochlorite for sterilization purpose, then age of the explant means if the tissue which we are using for organogenesis if it is aged too much then it will not be optimum for organogenesis. Then oxygen gradient quality and intensity of light those things also play significant role in organogenesis and those things vary from plant to plant also.

Then temperature that is another important thing in case of tobacco tissue culture relatively lesser temperature is needed compared to rice tissue culture, in rice little bit higher temperature is needed. The culture medium and its pH that is very crucial in most of the cases we have to use a 5.6 kind of pH then ploidy level and age of the culture those things are considered for different plant species also… for proper.. getting the organogenesis properly getting the organogenesis. Now coming to the applications of organogenesis first of all micro propagation of a large number of identical plantlets could be done as we have discussed about banana, in case of potato also the plantlets could be generated through tissue culture the true potato seed could be generated ok! Then in case of sugarcane also the plantlets could be generated large number of plantlets identical plantlets could be generated through micro-propagation.

Then in case of different orchid species, you know that orchids are vegetatively propagated mostly in nature, mostly it is vegetatively propagated and its growth is very slow. So through micro propagation we can make identical plantlets and in some country

in Thailand, Malaysia.. there the orchid production is highly famous through tissue culture. They raise the orchid plantlets and they used to sell it. Even in certain parts of India also, in southern part the tissue culture of orchid has been highly successful. Then application in commercial research as well as in basic research related to cell biology, genetics, biochemistry those organogenesis things are highly important. So in commercial research suppose we have to produce some specific secondary metabolites that is available in the shoot tissue or that is available in the root tissue.

So through organogenesis we can grow those parts and we can collect those specific secondary metabolites we can purify it and we can utilize it in the industry. Then advantageous characters can be screened from plants rather than full grown plants. During organogenesis we do not need the full grown plant also. If we can grow only the root part if the desirable chemical is available in the root tissue that thing that protocol could be optimized and for our purpose we can utilize it in large scale. This thing has been mentioned over here, production of secondary metabolites from the plant liquid culture ok.

In liquid culture it has been found that once we are giving shaking means once the plant tissue is being grown under shaker in liquid media then its growth become more faster because it will get sufficient amount of oxygen in shaking condition. Then production of virus free plantlets through meristem propagation that could be done also. Now gradually we will move into the somatic embryogenesis. The process in which embryo formation takes place from a single somatic cell or group of somatic cells rather than fertilized zygotic cells. So, in nature in most of the cases we can see fusion of male and female gamete thereafter after fusion after fertilization the zygote is formed and from that zygote eventually the full grown plants are being formed.

But over here the embryo formation takes place from a single somatic cells means a body cell it may be in the leaf tissue it may be in the root tissue there from ultimately the zygotic cell is being formed the embryo is being formed not the zygotic cell the embryo is being formed. The term embryo is generally used for embryos developed by somatic

embryogenesis. Stewart in 1958 and Reynard in 1959 they discovered the process of somatic embryogenesis in carrot that is *Daucus carota*. So, how did they do the procedure was as such the carrot explant was used and it was placed in nutrient media and after sometimes by using proper phytohormones they found the callus formation and eventually they observed the embroider formation from those callus means the somatic cells available in those tissues were initially converted into callus means unorganized mass of undifferentiated cells and from those callus eventually a couple of cells became converted into embryoid structure and then from those embroyider cell the embryo formation took place and eventually from that embryo the root shoot part emerged and full grown plants were developed. This is the process of somatic embryogenesis somatic cell will be converted into embryo.

Though the somatic embryo are without membrane, seed coat, endosperm it is fully functional. So, in somatic embryo you can see membranes are not there means seed coat, endosperm which are mostly available in a normal seed which is formed by crossing male and female gamete ok! but it is fully functional because here we are providing sufficient nutrients to grow the plantlets. Then we are discussing the process of somatic embryo formation, first of all the inductive phase is there the embryonic competence is acquired by the somatic cells which then proliferates as embryonic cells ok. So, the somatic cells eventually converted into embryonic cells. Then 2,4-D a form of auxin 2,4-Dichlorophenoxyacetic acid is necessary to induce embryogenesis formation of pro-embryogenic masses is also taken place then upon transferring to media with no or low auxin the pro-embryos start to developed into mature embryos means 2,4-D is needed for initiation of embryogenesis or at the beginning stage of callus formation ok.

Then gradually we have to reduce the auxin once we have to develop the mature embryo. The Murashige and Skoog media supplemented with varying concentrations of 2,4-D leads to callus induction in different crops as well as in rice also. Then coming to the expressive phase means the embryo has been almost developed ok initiated. So then once it start to express itself different structures are formed in development, we can see the globular structure, we can see the heart shaped structure, we can see the torpedo

shaped structure and these are the different developmental phase and at the maturity we can see the cotyledonary structure or germination. So this is the somatic embryogenesis in nutshell.

Suppose from here we have taken the leaf tissue as explant in the culture medium we are adding proper amount of hormones like 2,4-D thereafter the callus formation is taken place. So two steps are there, one is indirect organogenesis they are from callus eventually we can get the organ development, but here we are discussing about embryogenesis embryo formation. So from those callus the embryoid could be formed. Similarly direct embryo could be formed from those leaf ok. So once the embryos are formed we can see this different developmental stages the globular structure, heart shaped structure, torpedo structure and the cotyledonary stage means the shoot buds are coming or leaf parts are coming and then finally, we have to grow it in the media by cutting the tip part and eventually we can make the root formation of different shoot buds or different plant shoots ok! the rooting will be done and finally, we can get the mature plant.

So these are the factors affecting somatic embryogenesis. So first of all, type and character of explants earlier also we have discussed. So what type of tissue what age of tissue we are using it will determine the somatic embryogenesis, then several plant growth regulators promoting the somatic embryogenesis within auxin 2,4-D is used mostly within cytokinin, zeatin is used then ABA abscisic acid is used sometimes then plant growth regulators earlier we have discussed about plant growth regulators which basically promote somatic embryogenesis. Now we will be discussing plant growth regulators which mostly inhibit somatic embryogenesis like BAP, kinetin, gibberellin they basically inhibit somatic embryogenesis ok! So mild amount of nitrogen in media promotes somatic embryogenesis while excessive amount of nitrogen inhibits somatic embryogenesis those are few facts, few factors which regulate the somatic embryo, its growth.

Other parameters pH we have to use 5.6 to 5.8, the temperature mostly 26 to 28 °C, but it

will vary from plant to plant, the relative humidity 40 to 80 %, the light intensity should keep 5000 to 8000 lux and light duration generally 16 hrs. light and 8 hrs. darkness is followed. Now gradually we will move into the haploids ok! So, why haploid are important to us? The haploids are generally referred to cells which consist of single set of chromosome most of the crops rice, then maize those are diploid in nature ok.

So, in haploid single set of chromosomes are available while in diploid two sets of chromosomes are available. So, generally the gametic cells, that is the cells available in egg or sperm cell, I mean the cells available in sperm …. in egg cells those are termed as haploid cells; sperm cells, egg cells. So, what are the importance of haploids in agriculture? First of all in haploid single set of chromosome is there, if a single set of chromosome is there whatever may be the allele, it will be available in single copy may be capital A is there, may be small b is there, may be capital C is there. So, those things are available as single copy. So, if we do chromosome doubling of those haploid plants.

So, we can develop homozygous genotype very quickly ok! If you think about the cross pollinated crop there getting a homozygous genotype is next to impossible because in each and every generation the pollen grains may come from any different parents. So, developing homozygous genotype is very difficult over there. Now another one is double haploid it can rapidly create plants with desired traits in one generation avoiding the need for multiple rounds of breeding. Once we have discussed about the reverse breeding approach, reverse breeding strategy… therefrom through reverse breeding we could develop two different parents if we recombine them, we can get a very good hybrid ok! Means in reverse breeding we started our experiment from the hybrid field or from the heterozygous heterogeneous population and there from we tried to make its parent in such a way that if those two plants are crossed those two parents are crossed we can get that suitable F_1 or suitable heterozygous plant. So, over there also we discussed about the haploid and its importance. So, the double haploid is very much needed to develop the suitable desirable plants in one generation ok! The suitable homozygous plant could be generated and eventually it could be utilized in breeding program. So, it enables

easier selection of recessive traits due to absence of dominant allele, masking that is another important thing.

Suppose, we have a diploid plant here capital A and small a alleles are there, capital A is there the small a is also there. So, we cannot tell that capital A is responsible for this particular trait and small a is responsible for this trait ok. Means which trait is controlled by A gene we cannot easily tell as both the alleles are there. Now if we get only capital A in haploid and by chromosome doubling, we can make A A capital A capital A, then they are all the dominant alleles are there in homozygous condition. Similarly we can make small a small a plants also through haploid and subsequently double haploid production.

Then if we compare capital A small a and small a small a plants then we can identify the character of the recessive allele easily because the capital A allele is not masking the effect of small a over there. We will be discussing how to produce haploids. So, in-vivo formation for spontaneous formation of plants, but at low frequency means in nature the haploid sometimes are produced, but at low frequency. Then it could be done by induction through treatment with physical and chemical agent means different physical agent or chemical agents could be used to develop haploid plants. Then we can attempt the interspecific hybridization followed by chromosome elimination.

In barley, it is found by interspecific hybridization, crossing between two different species and after the cross somehow the chromosome elimination is taken place and we can produce haploid also. Other options are there by parthenogenesis. Parthenogenesis, it involves the development of an organism from an unfertilized egg ok! We know that within the ovary the egg cells are produced if the organism is produced from the unfertilized egg ok! That is the parthenogenesis and unfertilized means no male gamete is fusing there.

So, it can form haploid. Then apogamy, it refers to development of new plant from cells other than the fertilized egg means like the synergies, antipodals those cells are also there

within the embryo sac ok! The plants are developed from those cells then it is known as apogamy, there also we can see the haploid formation because single set of chromosome will be there. Then chromosome elimination occurs in somatic cells where specific chromosomes are eliminated or inactivated that is also sometimes occurs.