Plant Cell Bioprocessing
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Lecture - 08
Organogenesis and Regeneration

So, now before we begin the specific requirements of different types of cultures, let us see in order to redifferentiate and dedifferentiate what happens in the plant cells and what is needed. So, whenever you bring an explants; now explant is a tissue or an organ which have you have brought. So, it is a mixture of many different kinds of cells; isn't it? The explant cells are differentiated and are performing a specific function. Now, if you want to bring back the cell into some other form of tissue or organ, first the cell has to be reprogrammed. Although all the genetic information in the plant cells as we say they are totipotent in nature, which means that they have the capacity to redifferentiate into some other form. This means that in turn all the genetic information available for carrying out those metabolic activities is available. It is just that at a particular time and there are only specific sets of genes which are expressed. So, under *in vitro* conditions when you bring an explant, first thing the cells within that explants have to be reprogrammed.

Now, that reprogramming should be involving two things one is cytodifferentiation and the other is organogenesis in order to do regeneration from that explant into some other form.

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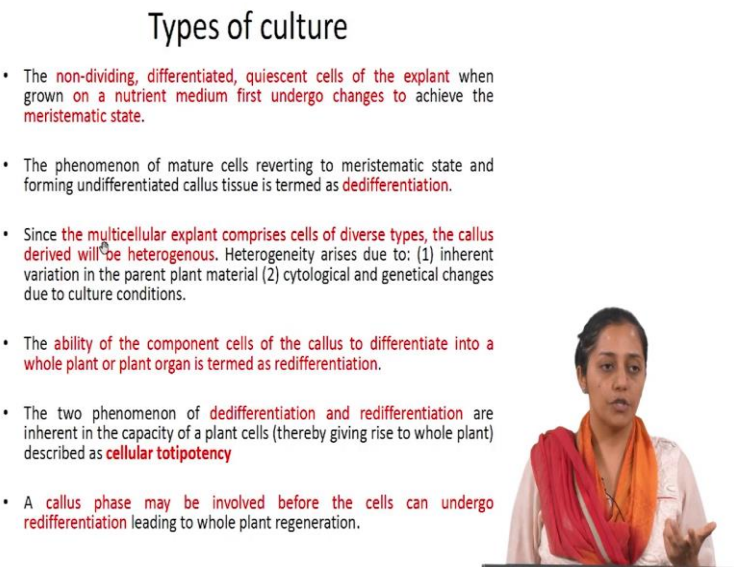

So, what are the kind of cells in an organ or a tissue which you bring out from the nature; these are non-dividing, differentiating, quiescent cells and they are matured. So, now, they are performing a specific function. So, generally these cells of the explant are non-dividing. So, when grown on a nutrient medium, they first undergo changes to achieve the meristematic state. So, these programmed cells or matured cells have to be reprogrammed to come back to their original meristematic state.

Now, the phenomena of mature cells reverting to this meristematic state and forming an undifferentiated callus tissue is what is called as dedifferentiation. So, if you know we were saying that callus is a dedifferentiated tissue. So, the process of reverting back a matured cell into a meristematic cell, is called as dedifferentiation that is what forms that callus blob.

Now, since the multicellular explant comprises cells of diverse type, the callus is therefore, heterogeneous in nature. Now heterogeneity arises due to two factors : one is inherent variation in the parent plant material because there are so many different kinds of cells and the second is cytological and genetical differences. Now all these cells are performing different functions which means that some amount of genetic information is expressed and some genetic information is not expressed in these different types of cells.

Now, the ability of the component cells of the callus to differentiate into the whole plant is what we know as redifferentiation or the totipotency which is the capability to

dedifferentiate and redifferentiate. It is not necessary that the explant has to go to a callus stage before regeneration.

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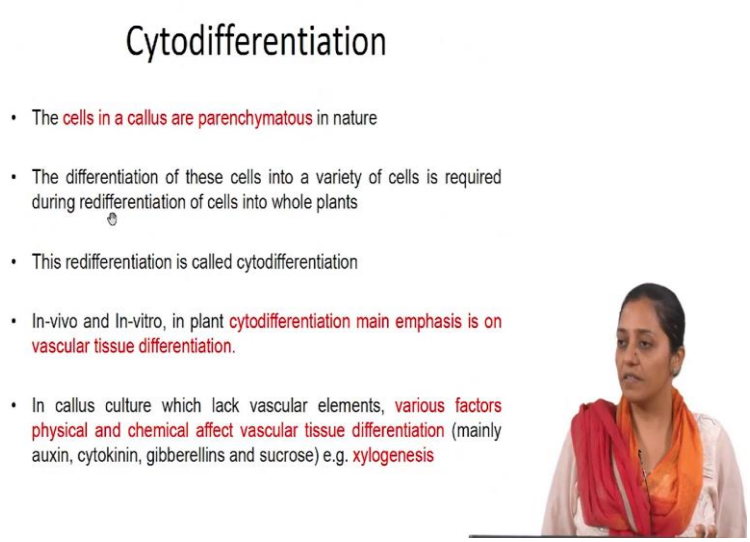

So, now the cells in a callus we know they are parenchymatous in nature which means generally all these cells are parenchyma cells. Now the differentiation of these cells to a variety of other forms of cells needs the cells to be redifferentiated. Now this redifferentiation stage 1 is called cytodifferentiation.

In plant cytodifferentiation, *in vivo* or *in vitro,* the main emphasis is forming these vascular tissue. So, which is for example, as I said xylogenesis forming of phloem and xylem elements through their connections. Now various factors for example, various chemical and physical factors are responsible for this kind of differentiation. For example, you can take auxins, cytokinins, carbon source which is sucrose or gibberellins.

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As I said earlier all genes which are responsible for this process where the cells are totipotent are present, but due to the environmental conditions or the stimuli present *in vitro*, in some of these cells, the genes will get expressed for the reprogramming to happen and the cells to redifferntiate into some other forms of cells and thereby forming another type of tissue. Now for development of an adult organism from single isolated cells from these differentiated tissues, which are generally non dividing and quiescent cells, the differentiated cell first undergoes dedifferentiation and redifferentiation.

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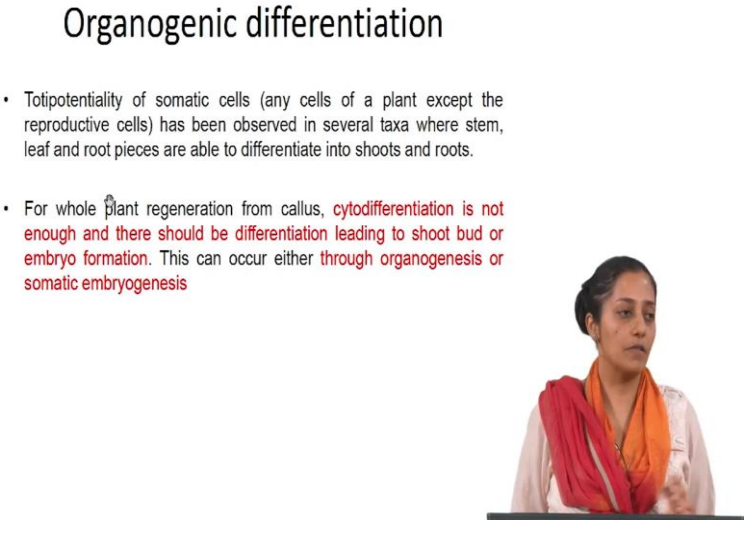

Now, when I say cytodifferentiation, one is that formation of vascular bundles. Now then and in order to regenerate into a whole plant what is needed? There has to be a shoot bud or primordium formation. Now once this is done shoot bud / primordium would form directly and then it is direct regeneration happening or somatic embryos are formed through somatic cells. If somatic embryos are formed then subsequent development of bipolar structures which is shoot primordium and root primordium will form. So, organic differentiation can be of two forms direct formation of shoot bud / root bud or formation of somatic embryos, thereby then forming bipolar structures one leading to shoots and the other leading to root.

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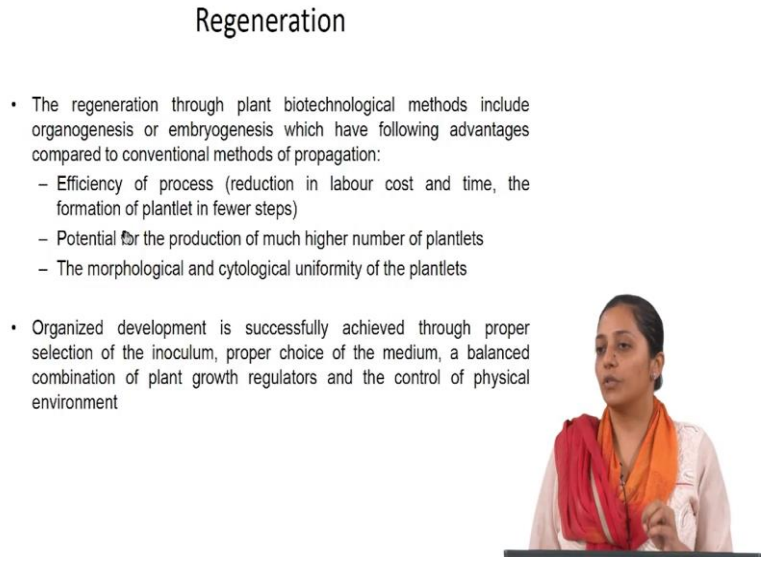

Now, how is regeneration useful in comparison to whole plant reproduction through which progenies are produced? There are three ways in which it is useful. It is more efficient process and you can do clonal propagation of the species which means less time is taken and what else; It has the potential for the production of much higher number of plants. So, the number of plants and the rate of production of the progenesis is also enhanced. So, this is how under *in vitro* conditions if plant biotechnology tools are used for regeneration, then these are the advantages in comparison to natural plant production.

Now, organized development is successfully achieved through proper selection. So, what things have to be kept in mind under *in vitro* conditions for regeneration to happen? You need to have a good starting material which means the inoculum. Then proper choice of

the medium, the media composition matters with a balanced composition of growth hormones which means what kind of the effect of auxins, cytokinins, gibberellins as I said should be chosen have to be optimized and moreover it is controlled physical environment.

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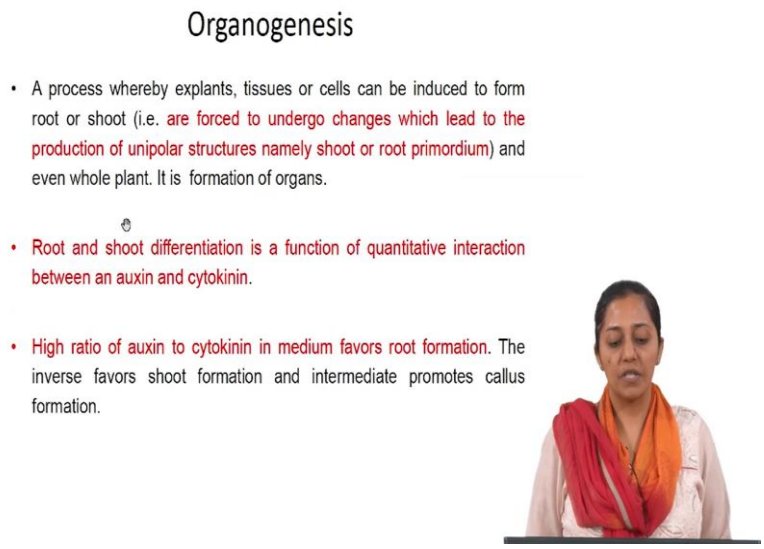

Now, what happens in organogenesis? The cells are forced to undergo changes which can lead to the production of unipolar structures as I said generally shoot. So, once you will see that when shoot multiplication is done for regeneration, it is shoot bud primordium which comes out and you get multiple shoots. Then later you put it into a root inducing medium such that now these shoots will have roots before they are transferred to the fields.

So, in organogenesis shoot bud / primordium formation is the objective when you are trying to regenerate into new plantlets. Now root and shoot differentiation is a function of quantitative interaction between auxins and cytokinins. So, last class also as I said that depending on what species are you working whether dicot, monocot, herbs or tree species, the effect of the same auxin may vary. Now high in general what has been found is that higher ratio of auxin to cytokinin would lead to a production of root and lesser ratio will induce shoot formation.

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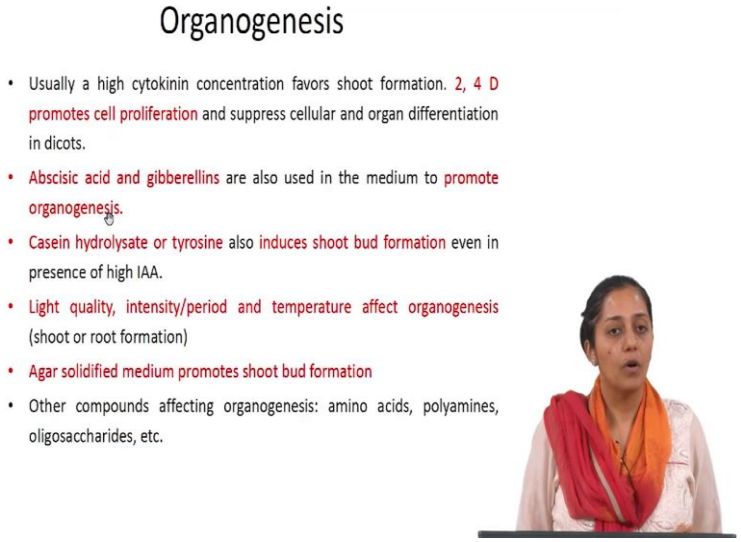

Now, usually high cytokinin concentration favors shoot formation as I said and 2, 4 D is generally used for cell proliferation, but it suppresses cellular and organ differenciation in dicots. So, these things you can note down although this is specific, but in general how it helps that when you are working in with a species for which no information is available, this can be the starting point.

Now, 2, 4 D promotes cell proliferation and suppresses cellular and organ differentiation in dicot plants. I hope you know what are monocots and dicots; monocotyledonous dicotyledonous; cotyledons; that much you people know. Now abscisic acid and gibberellins now what as abscisic acid and gibberellins? These are plant growth regulators / plant hormones.

Now, they are also sometimes used for promoting organogenesis in the medium. Now sometimes complex nitrogen sources like casein hydrolysate and amino acids are also known to affect shoot bud formations. Now light quality, intensity, period which means photoperiod, then temperature can also affect organogenesis in *in vitro* cultures.

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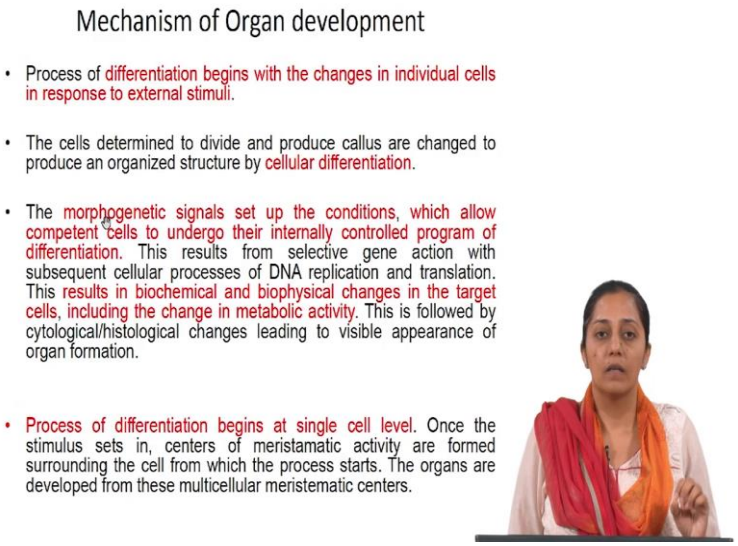

So, how does the organ development begin? Differentiation begins with the changes in the individual cells in response to the external stimuli. So, generally what happens is that if suppose there is a cell which is competent enough for dedifferentiation to happen. So, there are competent cells which are ready for differentiation; dedifferentiation which means meristematic which have the ability to quickly go through meristematic state. So, it begins from there around that cells there develops many meristematic centers.

So, from these meristematic centers the development of organogenesis takes place. So, there is a cell which is determined to undergo differentiation and dedifferentiation around that cells after multiplications, there are some more meristematic centers which get formed and these meristematic centers together will lead to formation of your shoot bud or root bud primordium.

Now, the morphogenetic signals setup the conditions which allow competent cells. So, these are called as competent cells. So, not all cells and sometimes it is very species specific sometimes you may try out many hormonal combinations, but you will see that you are not getting successful organogenesis happening.

It depends on the nature of the explant, the age of the explant and the season in which you have collected the explants; the cells in that explant may not be competent for coming back to a meristematic state and then redifferentiatiating. It also depends on the environmental conditions which you have given. So, there are multiple factors which are

responsible for successful regeneration to happen and even the genotype and the type of explant which you have used will be playing a crucial role in this.

So, why did I say because, there has to be cells which should be competent enough to undergo these reprogramming. So, morphogenetic signals set up the conditions which allow competent cells to undergo internally this controlled program of differentiation to happen.

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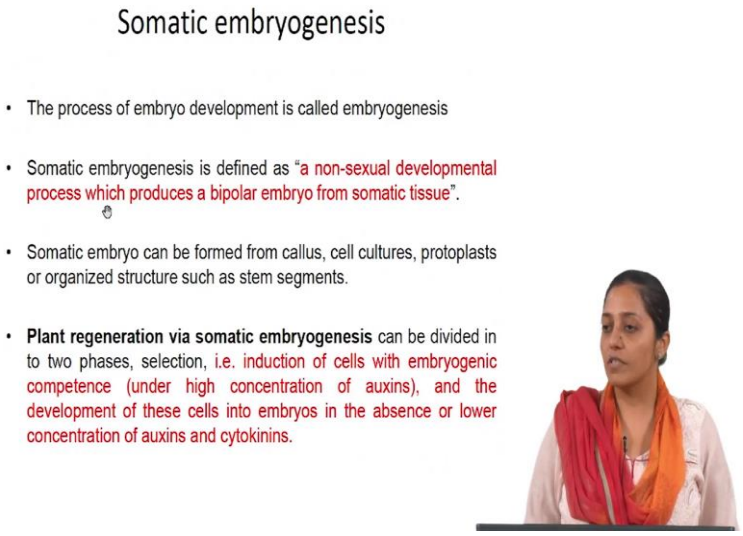

Let us talk about somatic embryogenesis. The process of embryo development from somatic cells is called somatic embryogenesis. Now when we say somatic cells are non germ cells and the cells do not participate in the sexual reproduction of the plants.

Now, somatic embryos can be formed from callus, cell cultures, protoplast or any kind of organized structures from the plant. Now plant regeneration via somatic embryogenesis can be divided into two phases: one is induction of cell with embryogenic competence. Now this happens when you have very high concentrations of auxins in the medium and the development of these cells subsequently into embryos once vascularization, which means cytodifferentiation and organogenesis both have taken place. Now, organogenesis means coming up of the shoot and the root primordium.

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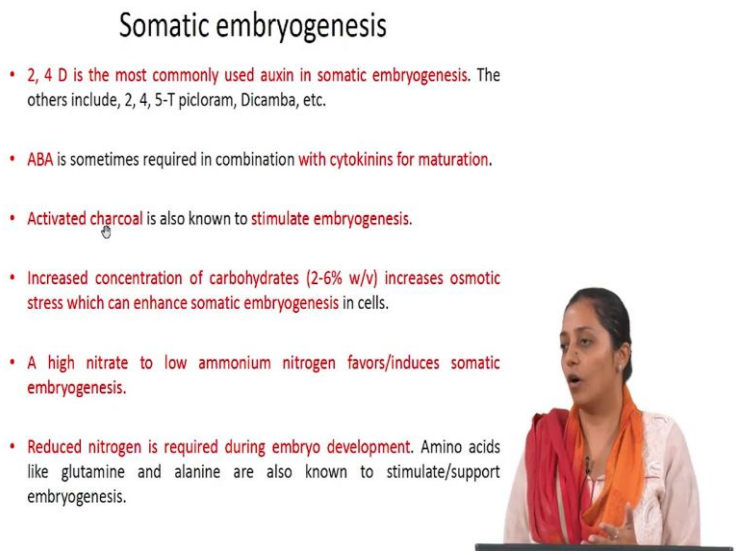

So, for subsequent growth you need a combination of cytokinins and auxins. Now 2, 4 D is the most commonly used auxin in somatic embryogenesis sometimes thidiazuron or zeatin these are other kinds of cytokinins which may also help in subsequent growth and development of somatic embryos because even in the development of somatic embryos there are different stages involved we will take up that later once we come to somatic embryos.

They are first globular structures, then torpedo, heart shaped structures though this is a gradual development. So, for this gradual development you need variation in the nutrient. So, abscisic acid is used in combination with cytokinins for maturation of the somatic embryos. Now sometimes in some species even activated charcoals can impact the induction of somatic embryos.

So, it is the stimuli which can induce either the determined cells for this maturation to happen or some of the cells will be induced to undergo embryogenic changes. So, a high nitrate to low ammonium nitrogen also has been found to favor somatic embryogenesis.

Reduced nitrogen is required during embryo development. So, induction is one thing and subsequent development is another. So, it is not necessary what has worked for inducing will work for subsequent development. If you see that once callus has been induced or somatic embryo has been induced, you may find that keeping in the same medium does

not lead to further growth even if it has lead to successful induction. So, which means that and it needs revision of the media.

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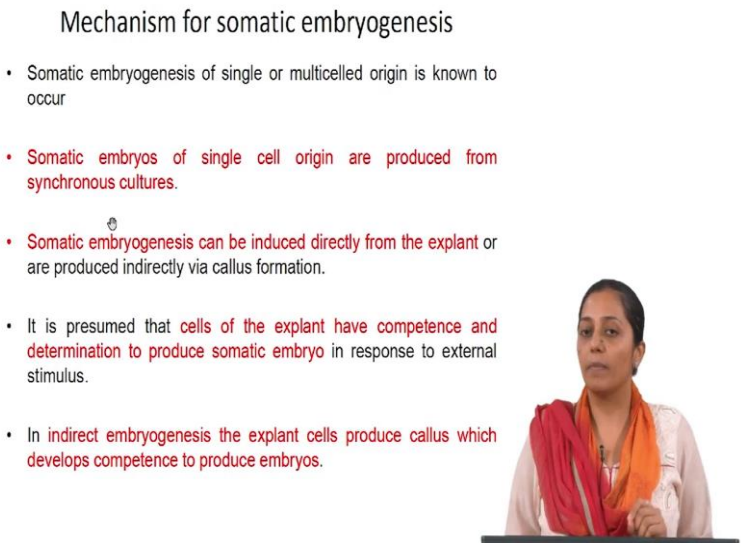

Somatic embryogenesis of single or multi-celled origin is known to occur. Now they can occur even from single cells or multiple cells together. Somatic embryos of a single cell origin are produced from synchronous cultures. Synchronous cultures means all the cells have the similar metabolic activity.

Now, somatic embryogenesis can be induced directly from the explant or can be indirectly from the callus phase. So, in case when the callus phase is involved , that is once the explant develops into callus, then you do a variation and it develops into somatic embryos that is called as indirect somatic embryogenesis and when directly an explant which means a leaf giving rise to directly somatic embryos then it is called direct somatic embryogenesis. It is presumed that the cells of the explants have the competence and determined to produce somatic embryos.

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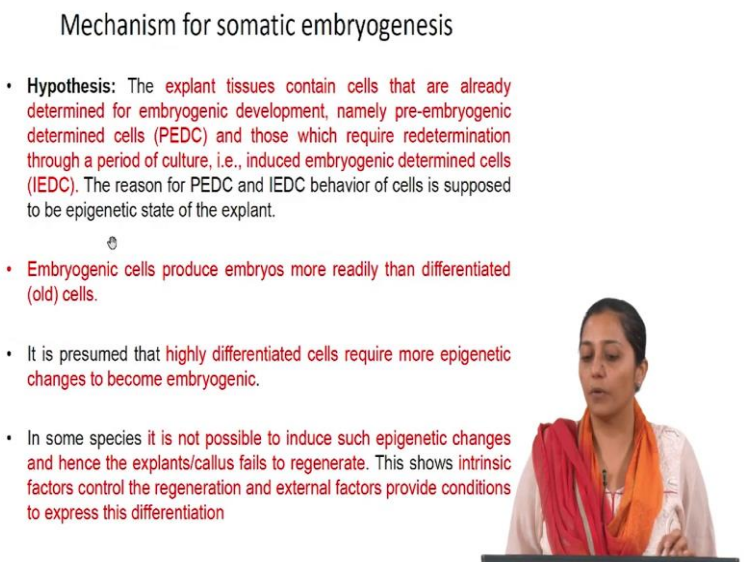

Now, there are two kinds of cells . The explant tissue contains cells that are already determined to embryogenic development. So, these are called as pre-embryogenic determined cells and these group of cells are already determined that if given the chance, they will form somatic embryos others are called as redetermination through a period of culture which means induced embryogenic determined cells which means they can be brought into somatic embryogenic stage, but after reprogramming which means keeping them under the right stimuli so as to reprogram them into somatic embryogenesis which means that it will take much longer time.

Now, embryogenic cells they produce embryos more readily than the differentiated explants or induced embryogenic competent cells. So, it is presumed that highly differentiated cells have more epigenetic changes. So, when we change the environmental parameters or we give stimuli then what is happening? The cells are undergoing these genetic changes; genetic changes in the sense, the genetic machinery which was not expressed starts getting expressed. It means which was cryptic is now expressed leading to some DNA replication and translation thereby leading to biochemical changes, histological changes and cytological changes which leads to the morphogenetic events like somatic embryos.

Now, it is presumed that highly differentiated cells require more epigenetic changes. Now what do you understand by epigenetics?

Epigenetics can bring about a change in the gene expression; now in some species it is not possible to induce such epigenetic changes and it depends on as I said in the nature of the explant and also depends on the conditions which you give *in vitro*. If those conditions are not adequate enough to bring them into induced embryogenic stage or to bring about such epigenetic changes then you will not get successful results.

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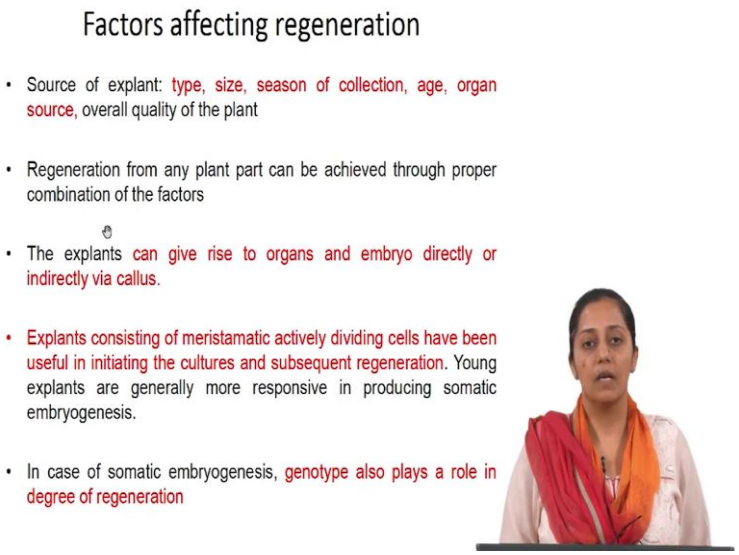

Now, what are the factors which can affect regeneration? It is the type of the explant, sometimes the size of the explant, season of collection, age of the explant. So, younger plants and younger explants they can easily get into and can be manipulated and are more conducive for epigenetic changes. Reason because they are still dividing cells and the majority of the cells will be at a meristematic stage because they are younger and have still not completely differentiated and organized to carry out a matured function. Now they can be easily manipulated.

The explants can give rise to organs and embryos directly or indirectly via callus. So, as I said what happens is that, there may be a single cell which is determined to undergo cytodifferentiation and organogenesis; as it divides there are multiple meristematic centers around that region and these meristematic centers undergo reprogramming . This leads to your shoot or root primordium formation.

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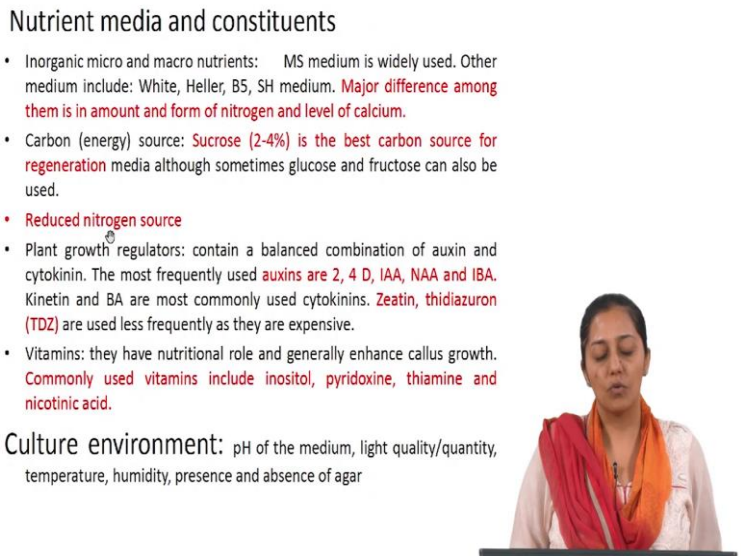

So, what kind of nutrient media helps in regeneration? In organic micro and macro nutrients major difference as we said yesterday is that although defined media can be used, but there is a difference in these. So, if it is high ammonium to nitrate. So, generally what induces somatic embryogenesis; when there is a very high nitrate to ammonium ratio or suddenly you give very high concentrations of sucrose; so, or if you can increase the concentration of auxin.

So, although dependent on species but in general it may be noted if no information is available these things can be tried and rest of the environmental factors anyways can play a role in generating these organized structures; ok, that is it. I will stop here today.