Plant Cell Bioprocessing
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Lecture - 09
Somaclonal Variation and Micropropagation

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Somaclonal variation

- The growth of plant cell and their regeneration is an asexual process: involve mitotic division of cell.
- It is expected that the process will produce genetically uniform plants and clonal propagation is possible through callus
- The occurrence of uncontrolled variation during the callus regeneration is termed as somaclonal variation. This leads to variation found in plants that regenerate from cell cultures. A term used for variation found or created through use of somatic cells.
- The cause of variation is attributed to changes in chromosome number and structure.
- Cell and tissue culture undergo frequent genetic changes (anueploidy, polyploidy, gene manipulation and mutation) and these are also expressed at biochemical and molecular levels.

So, today we are going to talk about somaclonal variation, applications, how it is useful, and its demerits in plant tissue culture. We also talk about micropropagation.

Now, first let us talk about the somaclonal variation. So, the growth of the plant cell and the regeneration is an asexual process which involves mitotic division of cells. So, we know now, the ability of the plant cells to regenerate is because they are totipotent in nature. It is because of this continuous division of plant cells, which is because of the mitotic process.

Now, whenever the cell or the callus has to form or the callus has to regenerate into a plant or any organ, the cell has to continuously keep on dividing. Every cell, undergoes hundreds of division cycles, finally, leading to differentiation and the regeneration process. Because of this rapid multiplication there is instability which is, in many different forms. You can see it in, phenotype, in terms of chromosomal instability and genetic insatiability. When I say genetic instability, it would involve in terms of size and number of chromosomes. Then it can also show up as biochemical instability. Which means suddenly you will see some new compounds getting formed or the yield of the existing compounds changing.

Then, I was talking about phenotypic variability. Phenotypic variability - you can see if the regenerants have come after somaclonal variations, you will find that they are not clonal progenies. There will be variation which is visible in terms of maybe flower color, maybe in terms of leaf size, height of the plant, branching patterns. So, these are different kinds of variations which can happen because of this somaclonal variation. So, if the objective is to do clonal propagations of the plant, then somaclonal variation has a demerit. The occurrence of an uncontrolled variation during the callus regeneration is termed as somaclonal variation.

Now, this leads to what? This leads to variation in the regenerants when you are using cells or callus for generating *in vitro* cultures. And because we are using somatic cells, generally in *in vitro* cultures - so therefore, the term somaclonal variation. So, cell and tissue cultures, they undergo frequent genetic changes and these get expressed in the form of biochemical and molecular level changes.

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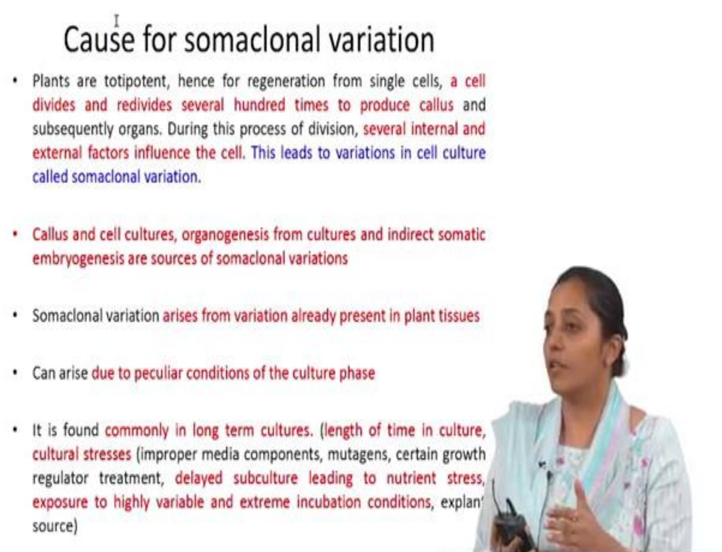

Now, what causes somaclonal variations? We know that the plants are totipotent and as I said every cell needs to divide a number of times before redifferentiation, dedifferentiation, and then again redifferentiation happens. So, because of this, sometimes there is a ploidy change. Sometimes you will find there are karyotypic alterations; when I say karyotypic alterations which means, you will find that there are recombinations happening. Or there may be activation of transposon elements. There can be single gene mutations which might happen. Or, when I say activation of transposon elements, different recombination events might happen which

means that these are those gene elements which can change their relative position in the plant chromosome. When I am saying plant chromosome, I am talking with respect to plant cell.

So, these get activated; now, why this is happening? This may happen because of many reasons. For example, it depends on the genetic makeup of the explant, the ploidy level of the explants which has been used. Then it also depends on the culture conditions. Sometimes if there is very high concentration of phytohormones that can also lead to this. There can be stress conditions. Those who work in plant tissue cultures, it is recommended that the time of subculture, the uniformity of the inoculum which you use - which means the quality of inoculum has to be taken care of. The reason: to avoid these somaclonal variations.

If the objective, is to produce a secondary metabolite, the product quality and the product quantity has to be kept consistent. If you need to have reproducible results, these have to be taken care. The inoculum which you use for subculture has to be same in quality and age, and the culture conditions have to be kept same. Sudden changes in conditions can also lead to epigenetic variations. So, when we say somaclonal variations, some of the epigenetic variations like for example, DNA methylation can happen which is also a an example of somaclonal variation.

Then, even addition of chemicals like colchicines. Now, this may bring about a change in the chromosome number and the ploidy levels. So, that can also cause somaclonal variations. But generally, you would not use mutagenic agents in the medium. So, when you are doing plant tissue culture, there may be sudden changes in the culture conditions or if you may delay the subculture cycle for very long periods. So, the variations happen when there is a very long term, it takes time, it does not immediately happen. But if there are stress conditions continuously provided for a number of subculture cycles, then this can lead to somaclonal variations in the culture.

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Methods of assessment

- **Phenotypic parameters**
  - Quantitative (leaf size, plant height, etc.)
  - Qualitative (branching pattern, flower color, etc.)
- **Physiological parameters**
  - Protein patterns by electrophoresis, or total content
  - Secondary products formation, e.g. alkaloid and steroid, etc.
- **Genetic parameters**
  - Chromosome number and structure (inversion, deletion, etc.)
  - alteration in DNA segments
- Not all variations are stable, phenotype (observable characteristics) not inherited, epigenetic variation (changes due to mechanisms other than change in DNA sequence), carry-over effects of the culture conditions, probably change in phenotype due to change in DNA methylation (a biochemical process, an epigenetic gene regulation) in culture

Now, what are the methods of assessment? How will you come to know that there has been possibly a somaclonal variation in the culture? So, there can be three methods, one is you will see a change in phenotypic parameters. You can find out from that. Now, in phenotypic parameters, there can be two ways: quantitative, qualitative. Now, quantitative, people measure plant height. So, actually you should see similar phenotypic parameters, but if you see a change in the leaf size or a plant height or the length of internodal regions, then that is an indication.

Now, qualitative for example, in flowering patterns, the color change happening. Then physiological parameters – which means functional parameters. Now, the minute there is a change in functional characteristics, you can see a change in the protein characteristics. That means the differential expression of proteins or even the total protein content can be seen on the gel electrophoresis.

Now, then, your secondary metabolite. Like for example, we saw in *Viola odorata*, I was mentioning in the earlier classes, that we were working on class of plant peptides called cyclotides from a medicinal plant called *Viola odorata*. Now, what we observed was when the plant was brought *in vitro* we could generate the *in vitro* plantlets. We did a study on the array of cyclotides being produced from the same plant material which we got from the horticulture which was being maintained at IIT, Madras.

We saw some novel cyclotides coming out. This means that some of these variations which might have happened is because of the *in vitro* conditions which may be stress to the culture or

which may have caused these variations ultimately then leading to variations in its biochemical synthesis. It is not that the genes were not present earlier, but maybe these genes were not expressed. But because of the conditions which were provided inside the lab it lead to expression of the genes of other cyclotides.

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Now, what are the factors which can affect somaclonal variation? The source of the explant, which means the genotype of the explant or the ploidy levels. In plants it is well known. So then medium composition, which means as I said if there is higher amounts of phytohormones or plant growth regulators then it can act as a stress. For example, BAP and 2, 4- D, they can bring about karyotypic alterations. So, please remember when I say genetic variability it can happen in terms of chromosome number, shape and size.

What other factors? Culture period, generally long-term cultures. You keep them for very long under a condition then you will see these changes happening. The subculture period can be a factor, and the subculture frequency. So, it is recommended, keep things consistent whatever conditions are being kept.

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Molecular basis of variation

- Variants in tissue culture may arise due to single gene mutations in cultured cells.
- Changes in cytoplasmic genome have also been observed
- Single gene mutation responsible for somaclonal variation relates to activation of transposable elements (DNA sequence that can change its relative position in the genome of a cell)
- Variations have been reported as a result of insertion of plasmid like DNA in the mitochondrial genome of cell cultures
- Somaclonal variation may also be due to molecular changes caused by mitotic crossing over (homologous chromosomal segments are accidentally paired in asexual cells) in regenerated plants.
- Changes in organelle, DNA, isoenzyme (enzymes differ in amin acid sequence but catalyze the same chemical rxn) and prote profile correlate with the occurrence of somaclonal variation

Now, what is the molecular basis of this variation? Now, this can arise due to single gene mutations which can happen in the cultured cells, which can be because of the conditions which you provide. Then changes in the cytoplasmic genome might happen which can also be a part of somaclonal variation.

Sometimes it is also observed that some plasmids get extra chromosomal material getting integrated into mitochondrial genome and bring about a change.

Then as I said activation of transposable elements. What are transposable elements? Transposable elements, are small DNA sequences which can change their relative position in the chromosome. So, as recombination happens because of this jumping, these are called jumping genes. So, that can cause a genetic variability.

Now, somaclonal variation, can also happen because of the mitotic crossing over. Generally, crossing over is a natural phenomena to bring about, to increase genetic variation in the progenies, which is a well-known part of meiosis. But the cell division is happening because of mitosis, the cells are continuously dividing because of the process of mitosis. If during this continuous division some crossing over happens, which means the gene transfer in the homologous pairs of the chromosomes, then this can cause a genetic variability which means chromosomal instability.

Now, changes in organelle, DNA, protein variation. So there can be ultimately a change in enzymes. You can have same enzymes, same sequence, but their functionality might change . There can be a difference in the amino acid sequence, but they catalyze the same reactions.

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Application

- Useful for crop improvement by selecting variants for desired character such as disease resistance, salt resistance, environmental stress tolerance.

Now I hope you can make out how can we exploit somaclonal variations. Because there is a chance by inducing epigenetic variations, by changing culture conditions, you can bring about a change in its phenotypic parameters and their physiological parameters it also gives you a chance to make a desirable change in the cell line. So, this can be therefore used for choosing a disease resistant cell line or salt resistant, drought resistant plants.

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Micropropagation

- Multiplication of plants through plant tissue culture
- In plants even highly mature and differentiated cells retain the ability to regress to a meristematic state.
- The vegetative method of propagating plants is termed as micropropagation or cloning tissue culture or growing *in vitro*
- Application in medicinal plant preservation, floriculture and forestry.
- Differentiated cell → Dedifferentiation → Redifferentiation → A new plant
- Micropropagation is defined as production of miniature planting material in large number by vegetative multiplication through regeneration
- Rapid multiplication without unwanted somaclonal variation

Now, what is micropropagation? Micropropagation is multiplication of plants through plant tissue culture. In plants, even highly mature and differentiated cells retain, the ability to regress to meristematic state. So, the vegetative method of propagating plants is termed as micropropagation or cloning tissue culture.

Now, what are the different applications? For example, if you need to preserve medicinal plants then this can be of use. Under *in vitro* conditions you can generate large number of plantlets of the same genetic makeup and then those can be planted. But this is again a very slow process. This is all being done under controlled conditions. So, very gradually they need to get acclimatized to environmental outside conditions and then they are transferred. So, what is required? The differentiated cell, undergoes dedifferentiation, redifferentiation, and then a new plant is obtained.

Now, let us see what are the different kinds of micropropagation methods.

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Methods for micropropagation

- **Axillary budding**: Development of shoots from pre-existing meristems on nodal region ensures genetic stability of the regenerants and is termed as axillary budding.
- Principal method of propagation at industrial level with high standards of homogeneity
- Variation developed during multiplication does not exceed than that found with conventional methods

So, development of shoots from these pre-existing meristems or nodal regions ensures genetic stability of the regenerants and it is termed as axillary budding. Now, similarly this is also a method to develop virus free plants. As we were talking about in the last class, these are meristem regions which are rapidly dividing and so there is less chance that the virus can get propagated from the infected areas of the plant to these regions at the same rate as the cells are dividing. And moreover, on top of it, the vascular bundle is still not formed, so the chances that the virus can propagate to these regions is missing or is less.

Now, the principle method for propagation in industry is axillary budding.

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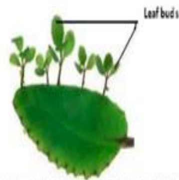

- **Adventitious budding**: *De novo* formation of adventitous buds (not from pre-existing meristems) may occur directly from the tissues of the explant, e.g. from leaf petiole or root segments, from internodal region of stem explant.
- They also form indirectly from callus (as it is an unorganized structure therefore prone to nuclear instability during rapid cell division.
- Chances of genetic instability increases when intermediate callus is formed

Now, what is adventitious budding? Adventitious budding is directly from the explants, from the organs you can see de novo meristems induced. It means that they are not from the existing meristematic region, but new meristematic regions get formed and directly from the explants you see organogenesis happening. So, it is de-novo formation of adventitious buds - which means not from the pre-existing meristems but may occur directly from the tissues of the explants.

Now, they also form indirectly, it depends on the species. Sometimes it may not happen directly for the same conditions you are trying - it depends on the genetic makeup, the species on which you are working. So, if direct is not possible then generally it becomes more conducive if an indirect phase - which means the callus phase is involved.

Even in somatic embryo induction, you will find that, because it is so much dependent on the species response, depending on the objective it is preferred to use a callus phase and then regenerate into organ cultures.

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Methods for Micropropagation

- Somatic embryo development: somatic embryos occur directly on explants or more frequently in callus cultures (indirect embryogenesis). Indirect embryogenesis involves callus or cell culture. Hence, lead to somaclonal variation in the regenerated plant population.
- This method does not ensure genetic stability
- They are used for large scale production of synthetic seeds using bioreactor technology through encapsulation when very high standards of genetic stability are not required.

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Now, as I said axillary budding, then adventitious budding, you can even do micropropagation using somatic embryos. Now, somatic embryos we know as synthetic seeds. They can also be used to generate large number of plantlets in smaller amount of time and space.

Now, what is a demerit here? Now, because it is through somatic embryos and if you are using an intermediate callus phase (indirect somatic embryogenesis), then there will be genetic variability in these plantlets. If the purpose is not clonal propagation, if the clonal or the genetic stability is not a requirement, if it is being used for say fuel, if you just need to do forestry or if you need to propagate a weed just for the sake of biomass to be used for biofuels – just generating biomass, then clonal propagation might not be a need.

So, if you can compromise, synthetic seeds is a good way of multiplication of those plants. So, somatic embryos can be used for production of synthetic seeds. So, if you can see in the picture these are gel beads. So, it is nothing but, like you do plant cell immobilization - similarly somatic embryos are immobilized. So, what is critical here? The viability of the somatic embryos in the gel.

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Advantages

- Small space requirement for producing hundreds of planlets
- Plantlets produced under sterile conditions are free from insects and microbes
- Virus elimination possible producing a large number of virus free plants
- Reproducible system as conditions are well defined and controlled
- Production is unaffected by seasonal variation
- No care required between subcultures like watering and weeding in vegetative propagation
- Used for difficult to root plants by conventional methods

So, what are the advantages? Small space requirement in case of micropropagation. Then plantlets produced are free from insects and microbes because they have been under controlled conditions. Then virus elimination is possible. Why? You know now the reason because we are making use of the meristems to bring about the micropropagation. Then reproducible system as the conditions are being maintained - under controlled environment everything is being done, so it is reproducible. Then production is unaffected by the seasonal variations as which might happen in the natural conditions of propagating a plant.

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Stages involved

- Stage I: Selection and establishment of aseptic cultures
- Stage II: Multiplication of Propagule
- Stage III: Plantlet regeneration
- Stage IV: Preparation and transfer to field

So, these are the stages which will be involved; selection and establishment of an aseptic culture, then multiplication of the propagule, then plantlet regeneration and preparation and transfer to the field. The step of preparation and transfer to the field is a long drawn. It takes time.