Plant Cell Bioprocessing
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Lecture – 01
Introduction to plant cell technology

The relevance of plant cell technology, to industry is as follows.

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Silene stenophylla is a plant whose seeds were found in the permafrost conditions in the Siberian region in the fossils of a squirrel burrow. The plant was revived from the seeds through *in vitro* plant cell cultivation techniques. This is one of the application of plant cell and tissue culture technology, where live plant was revived from seeds which was under permafrost region for 30,000 years. Now, the live plant is able to give rise to new progenies.

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Similarly, plant cell and tissue culture can also be used for conservation of endangered plants and rare plant species. So, some of the plants which are very rare and are under endangered list, can be revived and can be conserved using plant cell and tissue culture techniques. *Ilex khasiana* is a critically endangered plant found in north eastern region of India. This plant was able to be conserved using synthetic seeds and plant tissue culture technology.

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Plant cell and tissue culture can also be used for the production of artificial seeds for large scale shoot multiplication to cater to the increasing demand in the market. Cell immobilization techniques can be used where the embryo is generated using somatic cells, encapsulated in gel beads, and these can be used as seeds to produce plants. A large number of plants can be regenerated through this technique.

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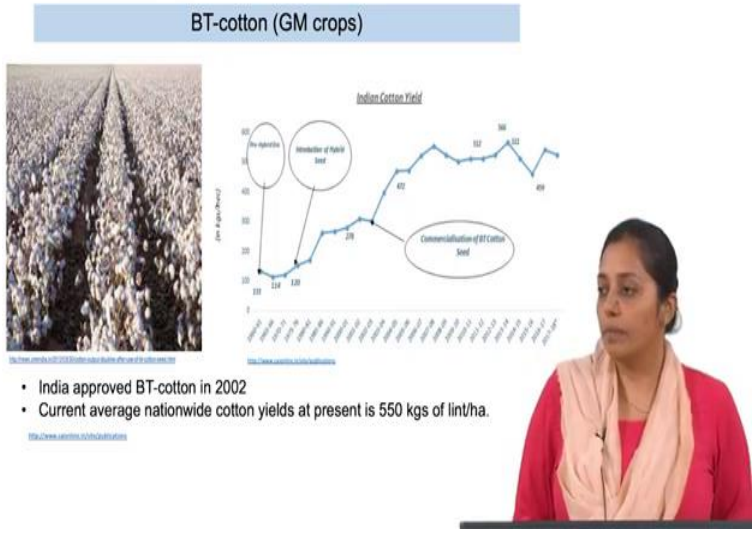

Genetically Modified crops: In order to improve biomass productivity, the growth or the production of medicinal compounds in plants, genetic modification is done. One of the most successful example is the BT-cotton. Almost 60 percent increase was found in the productivity of cotton after introduction of BT-cotton.

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Bioplastics: People have been able to express PHAs - polyhydroxyalkanoates in plants upto more than 50 percent dry cell weight basis. So, there is an opportunity to produce bioplastics from plants. Plants like switch grass which are rapidly multiplying plants or weeds can be used as feed stocks for bioplastics, This can be done through plant cell technology.

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Anticancer drugs: Some of the most popular drugs are vincristine, vinblastine and taxol. Taxol, is an anticancer drug which is produced from yew tree. Camptothecin is another

lead molecule in India which is used for lung cancer and cervical cancer. It is a marketed drug, which is obtained in India from, *Nothapodytes nimmoniana*. It is an endangered plant in western ghats because of rapid uprooting by the industries for the drug.

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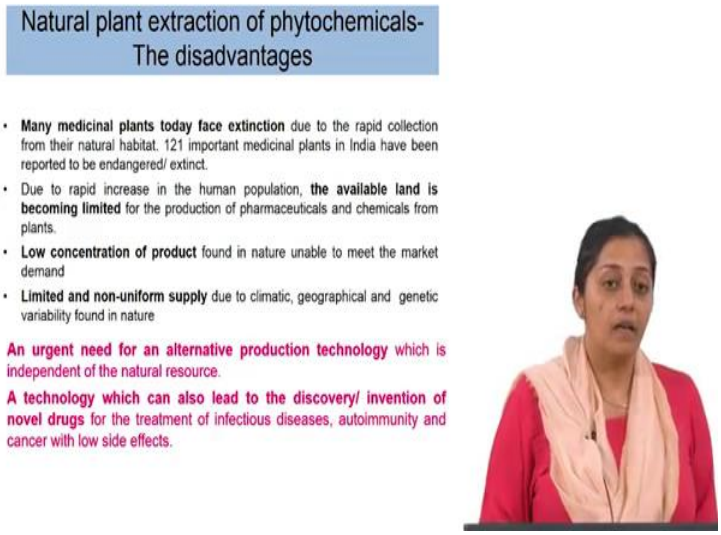

When natural plants are used for extraction of such high value molecules, the disadvantages can be as follows:

1. Extinction of species due to over exploitation

2. Downstream processing is difficult because the whole plant has ‘n’ number of other molecules

3. Seasonal and geographic variation of growth and secondary metabolite production.

A project on cyclic peptides called cyclotides is being carried on in the lab. Cyclotides are small proteins produced in plants which have plethora of therapeutic activities and potential. The same plant from different sources - Himalayan region, Ooty region, plants procured from Ooty and cultivated for three years in the horticulture at IIT madras, were characterized for different types of cyclotides in the plant material. Interestingly, it was found that the plant material from different sources had very different array of cyclotides. This shows that there is high impact of climatic and geographic variability. The micro flora which is present around in the soil also impacts the kind of secondary

metabolite or the expression of secondary metabolites in the plant. There was a difference in cyclotides in different parts of the plant. A certain kind of novel cyclotides was found in the horticulture grown plant which was absent in the one grown in Ooty.

An industry cannot work at this pattern, it depends on consistent product quality and quantity. Natural plant extraction hence has a demerit associated, because there can be variation with location and season. For example, Azadirachtin is a bio pesticide obtained from neem. It is majorly obtained from the seed which has the maximum content. Seeds are available only twice in a year. So, one has to to wait for a year long and then collect the seed. So, demand and supply gap can come up. So, an *in vitro* cultivation technique will therefore have higher productivity because there is less space and time requirement.

There is also a demand for land with the growing population. There has to be a priority drawn between agricultural crops and medicinal plants.

Also, with low concentration of the product, downstream processing is difficult, because there are ‘n’ number of molecules present in the plant. Now, imagine the same carbon flux is being diverted towards array of different metabolites. Under *in vitro* conditions the plant cells are amenable to process optimization. Process optimization can help in diverting desirably the metabolic flux towards the compound of interest.

Limited and non-uniform supply: This can be because of the climatic and geographical variability in nature. So, therefore, an alternative strategy using plant cell technology where under controlled environment plant systems can be grown and it is possible to produce the desired metabolite. This is the advantage of plant cell and tissue culture.

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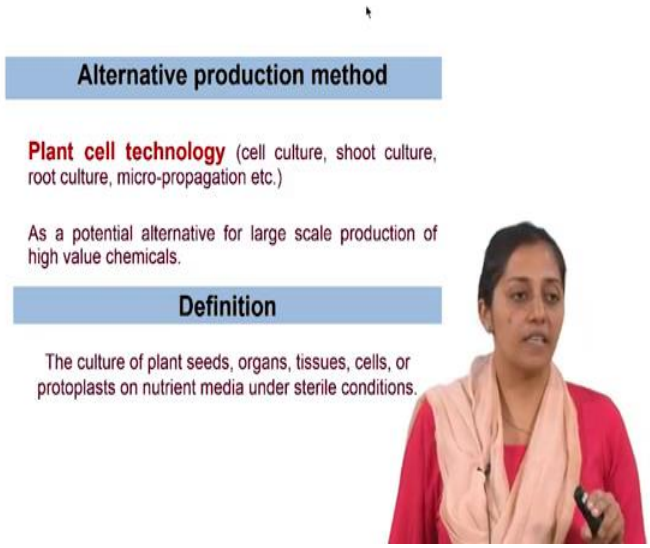

There are different forms of in vitro cultures which come under plant cell technology. To name a few, cell culture, organ culture, tissue culture and protoplast culture. Organ culture includes somatic embryos, simple roots grown by multiplying the roots only, or hairy roots induced by *Agrobacterium* mediated transformation. So, these are different forms of cultures which can be used for large scale production of the high value chemicals.

Protoplasts are cells devoid of the cell wall. They are required as it is easier to manipulate and do hybridization with protoplasts, because cell wall has been removed.

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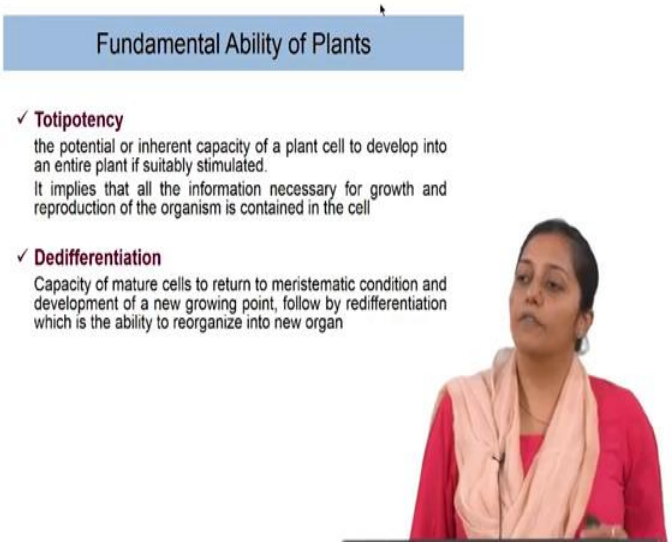

So, what is so different about plant cells which has been exploited in plant cell technology? A very good advantage which is totipotency.

Totipotency comes from the cell theory- a cell arises from a cell. So, all the genetic information of the plant is present in a cell. So, totipotency is the ability of any plant cell, to reverse to its multiplication stage even if it is organized and performing a specialized function.

So, it is the potential or the inherent capacity of the plant cell to develop into an entire plant which is being exploited. So, even from a single cell, by rapid multiplication and reprogramming of the genetic machinery the entire plant can be obtained. This could mean that all information required for generation of a plant is present in a single cell, but not all information is expressed at a given time point. The cell has all the information, but some cells perform one function while the other perform another function. It does not mean that the genetic machinery is absent, but it's about whether the cell is expressing the genetic information.

To explain the fact of finding novel cyclotides in the same plant variety, although it was the same plant brought from Ooty and transferred to IIT Madras. This could mean that the genetic machinery for the novel cyclotide is present but must have been encrypted. It is the epigenetic factors or the environmental conditions which might have been able to induce the expression of these silent genes.

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History of plant tissue culture: It began, in Europe and US parallelly during the world war. They were able to continuously culture the carrot cells in nutrient medium and keep it live. Later, while using coconut milk, they found that there is a compound in the coconut milk, which was the first auxin to be discovered, Indole Acetic Acid (IAA). It is said to play a huge role in expression and keeping the cells live.

It was initially thought that IAA was the only compound to be used for *in vitro* cultures. With the advent of microscopy and analytical techniques, it was also found that apart from auxin, there is some other compound present in the coconut milk which is critically affecting significantly affecting the in vitro cultures.

So, that lead to the discovery of kinetins. The initial study which took place in plant cell technology dealt with the nutrient requirements of plant cells. Later the development of different forms of cultures, such as what lead to roots, what lead to shoots was studied. The importance of growth harmones was understood thereby.

Then studies helped in developing predefined medium compositions such as MS media and Gamborg medium. These predefined media vary in their nutrient composition. Depending on the kind of *in vitro* cultures to be induced, these pre-defined media work well irrespective of the species. So, MS, Gamborg's and White's medium are very popularly used predefined nutrient media for *in vitro* cultures depending on the objective, whether you want to do root culture, shoot culture or callus.

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Two major hormones that impact *in vitro* cultivations are auxins and cytokinins. Auxins are said to be root promoting hormones, and cytokinins are shoot promoting hormones. This is the general trend, but sometimes it may vary. So, general trend is that if the auxins and cytokinins concentrations are kept equal, it may lead to dedifferentiation of the explant which may lead to callus formation.

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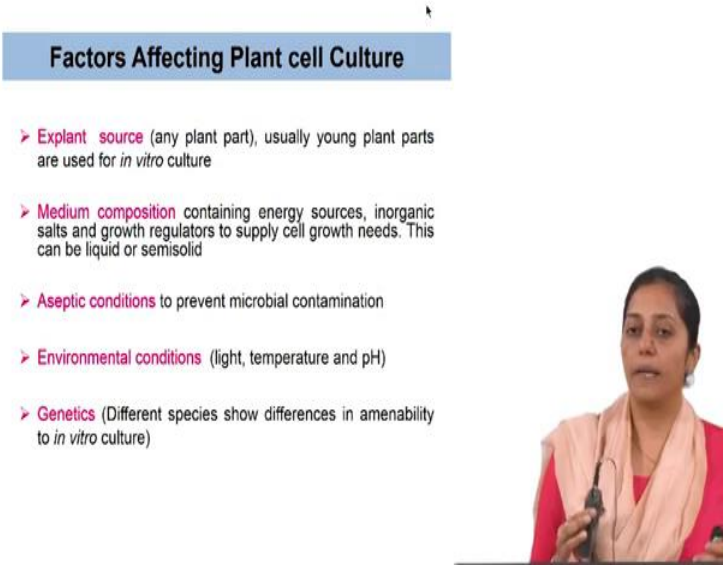

Factors affecting plant cell culture: Explant source and type is an important factor for development of *in vitro* cultures. Young plant parts are generally used. This is because they are more amenable to reprogramming.

Medium composition such as carbon and nitrogen source, environmental conditions, aseptic conditions, light intensity (as they are plant cells), affects the plant cell culture.

Genetics: Particular auxin/cytokinin which leads to callus formation or root generation in sunflower will may not work with *Viola odorata*. So, the kind of harmones to be used is species dependent. Thus optimization of what kind of hormones and their concentration is important.

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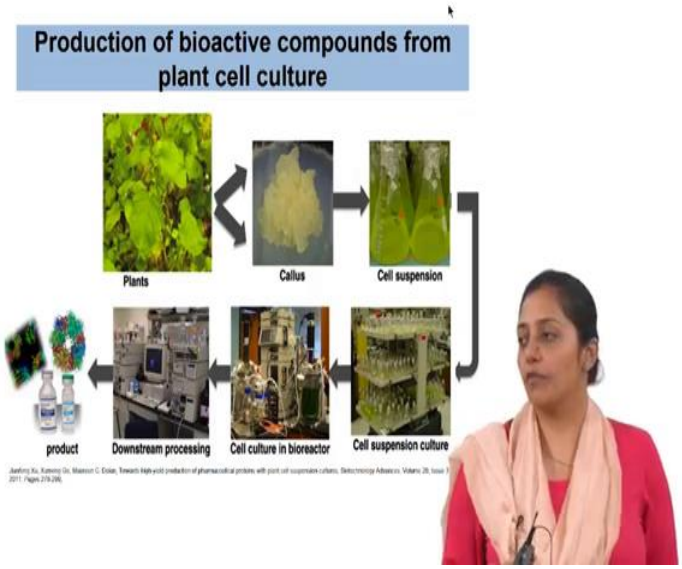

This is a small flowchart of how plant cell fermentation is done. One can use *in vitro* developed shoots or plant material from outside as explants. When explants are used from outside one must do surface sterilization which means that the contaminants will be removed from the surface. Then the explant is brought to a dedifferentiated form which is called as callus. When the callus is put in suspension, the cells are dispersed. To obtain a fine suspension, subculture and filtration is to be done, so that the aggregates are removed.

The increased number of subculture cycles required in inoculum preparation or in suspension culture development will facilitate in bringing the cells uniform. If cells are

passed through sieves of uniform size continuously, we can assume that whatever passes through will be uniform in size. Therefore synchronous cultures having uniform shape, size and metabolic activity can be obtained. Optimization strategies can be implemented on cell suspension cultures.

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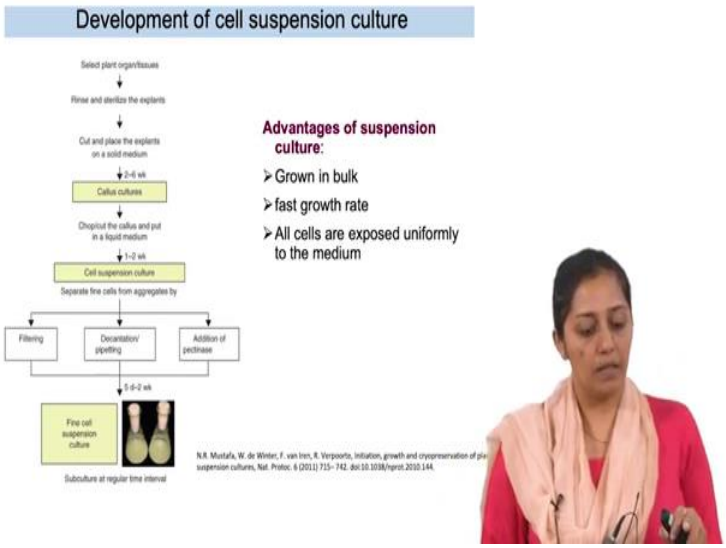

Development of cell suspension cultures: The explant is placed on solidified medium with predefined hormonal conditions. It will dedifferentiate into a callus form. This callus is transferred into a liquid medium of desired composition with hormones and suspension culture is obtained.

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This is one of the endangered plants for which the callus could be generated. This is from a north eastern part of India.

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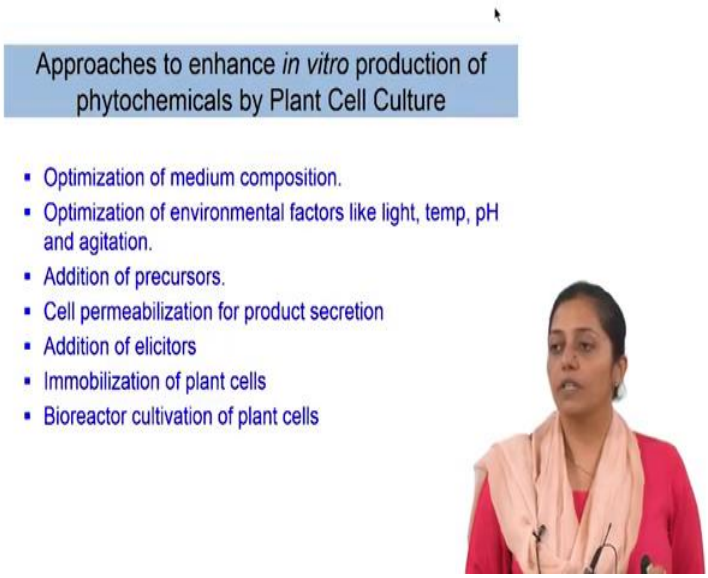

Strategies to optimize the plant cell bioprocess:

Media optimization: One cannot vary different media components one by one as it will be time consuming. Plants are slow growing and it will take time to arrive at an optimum medium composition. So single factor experiments can be used to identify the most

significant and crucial component that affects the culture. Then design of experiments can be used to identify the optimum medium composition.

Single factor experiments are useful to know the range in which the optimization can be done. A wide range is suggested so that one can be sure to have an inflection point. In this range, there must be a low and a high value such that that one works positively and the other negatively. Once the positive and negative end is identified, it can be used in statistical design of experiments.

Suppose A, B, C, D are four crucial factors. Design of experiments, can help identify how A, B, C, D is interacting with each other and how it is impacting objective function which may be biomass or your product yield. Once the interactive effects ate identified and taken into account, it will help optimize and predict the most optimum A, B, C, D value in the range selected earlier leading to maximum growth or maximum product formation depending on what objective function has been used.

So, this is how media optimization is done with less time and minimum number of experiments, to give better results. Now, optimization of environmental factors can also be done in the similar fashion.

Addition of precursors: Precursors are intermediates in the biosynthetic pathway of the product. For example, let us take camptothecin or taxol. There are biosynthetic pathways involved where there will be ‘n’ number of intermediates connecting to the central metabolism and secondary metabolism. Adding intermediates which is close to the desired product can lead to increase in the rate of forward reaction to the desired product. The intermediate which is closest to the product and is readily taken up by the cell is selected. When they are utilized by the cell, there will be an enhancement in the rate of forward reaction, and therefore, leading to increased product formation.

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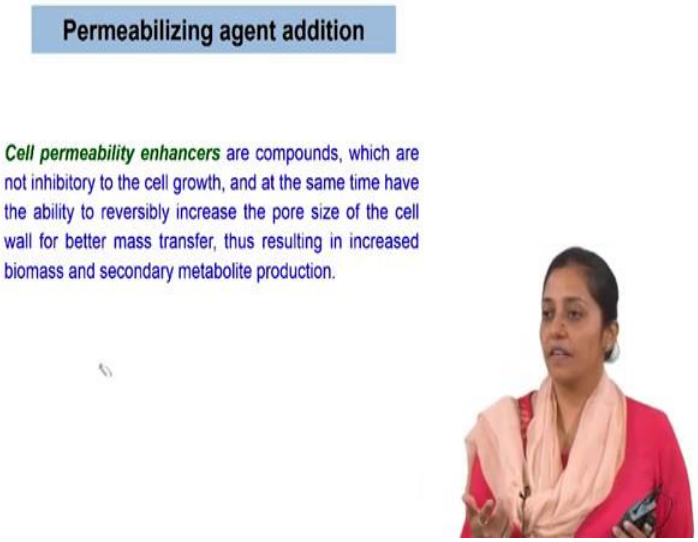

Permeability enhancers: Permeability enhancers are compounds which can reversibly permeabilize the cell, provided the cell viability is not impacted. There is an increase in mass transfer across the cell membrane causing a sink for the product outside the cell. This drives the reaction forward increasing product productivity.

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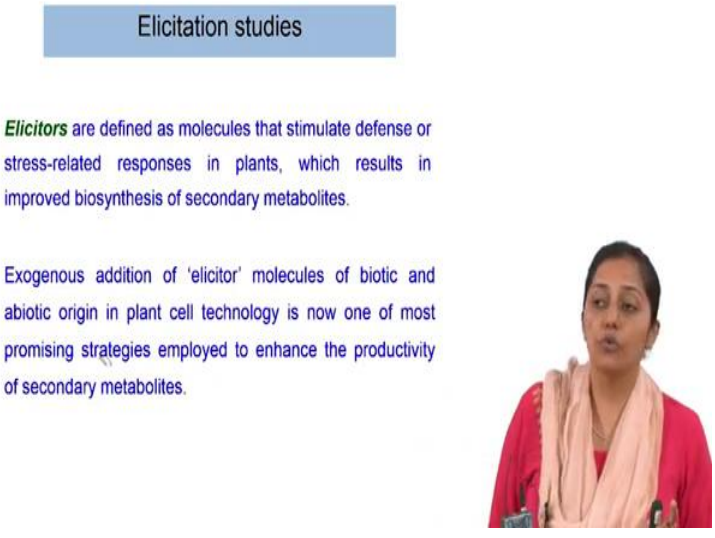

Elicitors are the most promising strategy which can be used to improve the product yield in plant cell technology. Elicitors are molecules which stimulate defense or stress-related responses in plants. Now, majority of these high value compounds are phytoalexins, that

is defense related compounds. So, if such molecules are added exogenously, defense responses in the plant can be induced. The elicitor can be a signaling molecule which can lead to a cascade effect thereby leading to an enhancement in the secondary metabolism. It can also be a component the plant is already immune to, which can increase its defense response. For example, fungal components or any pathogenic component which can give the signal to the plant cell and induce the defense machinery.

Plant cell immobilization is also used to improve the productivity of metabolites.

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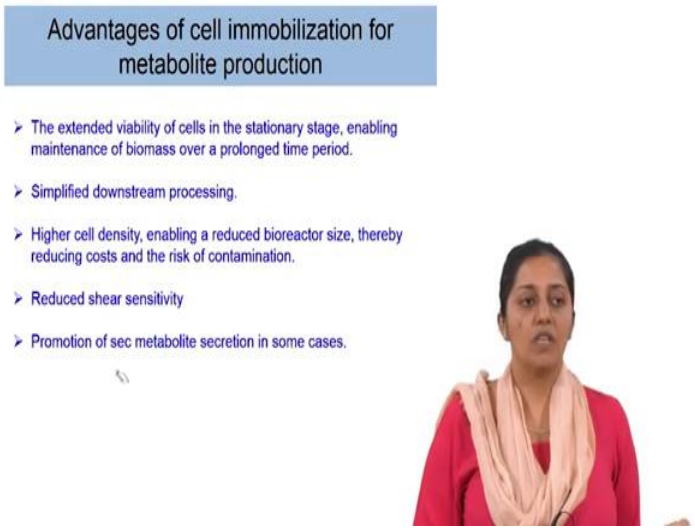

So, these are some advantages. In case the species is shear sensitive, encapsulation helps to overcome the shear forces.