Plant Cell Bioprocessing
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Lecture - 15
Secondary metabolism in plant cells-Part 4

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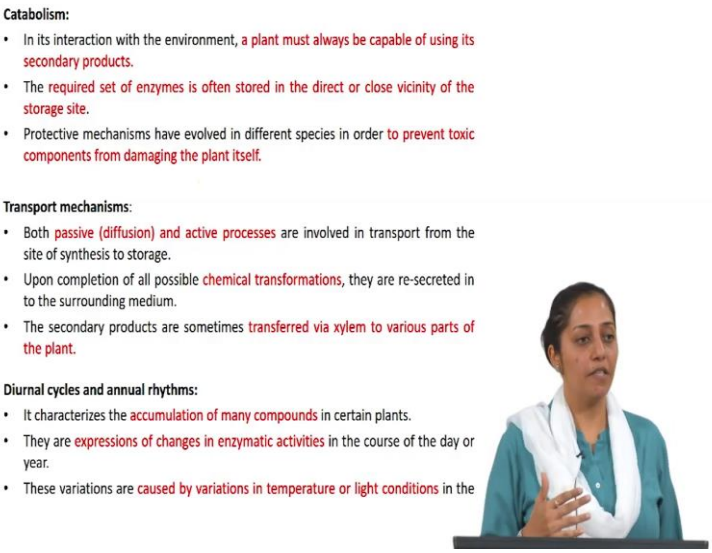

So, we were talking about Secondary Metabolites and about their catabolism. We also spoke about that they generally have turnover processes. The rate of synthesis also depends on their rate of catabolism or metabolism which may include the rate at which they are getting transported to the storage sites or it may also include the rate at which they are getting biotransformed for detoxification and storage subsequently or directly being used in pathogen attack. So, the rate of synthesis is also dependent on the rate of metabolism of the secondary metabolites.

So, in its interaction with the environment a plant must always be capable of using secondary metabolites. So, generally, we have spoken about this that the site of storage may not be the same as the site of synthesis. So, it is transported generally through xylem to the parts where it has to be stored and we know for example, what are the different ways in which the plant stores it. It may store it in vacuole, it may store it in intracellular spaces or trichomes or glandular structures, hairs, where it is stored or epidermis or cuticle where it is exposed and where it is needed for the line of defence.

Now, protective mechanisms have been involved in order for the plant to protect itself from the toxic effect of the secondary metabolite. So, what kind of protective mechanism did we talk about? One is biotransformation, like glucose moiety addition ie glycosylation. What else? The storage and vacuoles. Or keeping the final step where the precursor will get converted to the toxic metabolite, only once the cell is damaged. So, which means the enzymes of the final step are kept in the vicinity of the precursor moiety. So, transport. Yeah.

Student: Ma'am. So, if the transport is of xylems only? So, if the secondary metabolites is produced at somewhere at the top. So, does it flow and does phloem also takes part in the transport?

So, it generally depends. When we say that at the top, at the top is very ambiguous. Now top is everywhere above the ground level, so it is not that it will only happen at one single point of the plant, it will happen aerially. So, we can generally bifurcate as aerial or underground parts. So, it is a very ambiguous term to even answer that top would mean what.

So, transport and there are active as well as passive processes. So, active means against the gradient also it can flow. Now, for transport mechanisms, passive and active processes are involved in transport from the site of synthesis to storage. Now, when active processes are involved which means some transport proteins would be involved. Now, upon completion of all possible chemical transformations they are resecreted in the surrounding medium which is your xylem or the cell sap. The secondary products are sometimes transferred via xylem to various parts of the plant.

Now, if you remember we were talking about induced systemic resistance and SAR, Systemic Acquired Resistance, in both the signalling molecules were transferred through phloem dependent signal and not the xylem. So, I think both are involved in the entire process in the signal cascade or the PR proteins flow to different directions or even for storage of the secondary metabolites.

Now, diurnal cycles and annual rhythms are involved in the secondary metabolites synthesis and when we say diurnal cycles which means that they undergo both temporal and spatial variation. Now, this is temporal which means that the synthesis may be following a 24 hour cycle or it may be following an annual rhythm, once in a year, twice

in a year. So, the synthesis takes place. Maybe, if it is playing a role in the developmental stage of the plant then it generally follows the cycles / patterns.

Now, these variations are caused by the variations in temperature or light conditions in the course of the day. So, how do you think that the variations which are following a particular rhythm the plant is able to maintain these rhythms? By manipulating what? How is the plant managing this? Think logically, do not read. Where is the control?

Now, these are enzymatic biosynthetic pathways, enzymes are involved, enzymes are further controlled by the proteins by transcription factors. So, there are different modes where the metabolism is controlled. Now, controlling the metabolism ultimately affects either the activity of the enzymes or the expression of the enzymes. The plant has the handle to manipulate the rhythms. Now, generally, in plants you will see that enzyme activity can be impacted by the common factors. What are the common factors by which you think the enzyme activities can be affected? You must have already learned it.

Student: It is actually pH.

pH, temperature. What else?

Student: Light.

Light can impact.

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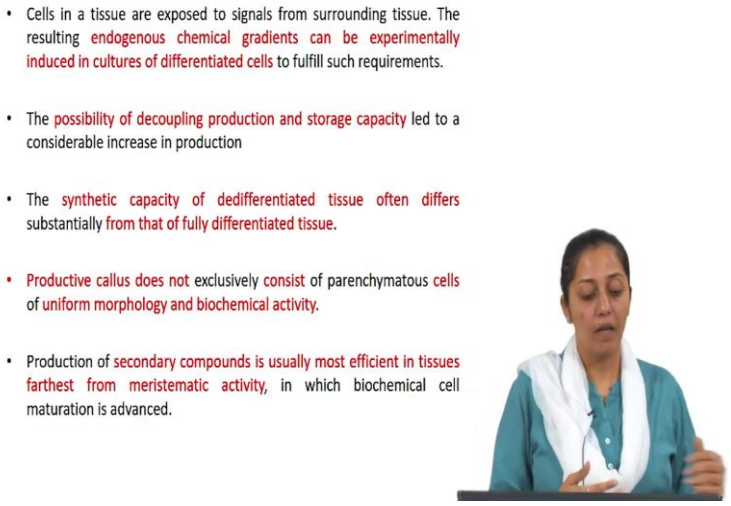

Now, cells in a tissue are exposed to signals from the surrounding tissue. So, endogenous chemical gradients can be experimentally induced in cultures for differentiated cells. So, generally, under *in vitro* conditions when we try to do optimizations for maximum production like for example, we add elicitors, which means stress or we manipulate pH, temperature, RPM.

So, ultimately it is impacting what? What do we check? If you want to dig down the mechanism when we add specially the elicitors, yield enhancement mechanism which means per unit biomass the synthetic capacity of the cell, then we look for the rate controlling steps in the biosynthetic pathway. We see what is the impact of this particular parameter on any of the rate controlling enzymes, whether on their expression or on their activity levels.

Now, the possibility of decoupling production and storage capacity. Yesterday, we were talking about this. Generally, it has been observed that these are all intracellular compounds. Now, in order to improve the production rates if you will increase the rate at which it is metabolized or transported outside will increase the rate of forward reaction which is the rate of the synthesis.

So, either under *in vitro* conditions you have to absorb the product or you decouple the production and the transport to the surrounding medium or you add permeabilizing agents, which can reversibly permeabilize the cell for the product to continuously keep coming out as it is getting synthesized.

So, these are all among the strategies which we will be discussing for improving the productivity of the secondary metabolite in plant cell based bioprocesses. Now, the synthetic capacity of dedifferentiated tissue often differs substantially from the fully differentiated tissue. We saw that when we were discussing that secondary metabolism in plants is a function of cell differentiation sometimes morphogenesis, organogenesis. So, now, it is connected to higher order functions which involves cell differentiation.

Now, therefore, under *in vitro* conditions you would find that the cells in a callus may not be as productive as if you convert those cells into organogenesis or more differentiated or if you see that the callus is more compact or there is more strong cell to cell contact then the secondary metabolite biosynthesis sometimes gets enhanced.

Now, see these are the kind of balancing acts. If the callus or the cell species is aggregating which may be good for the yield of the secondary metabolite. We are improving the yield by making it with much closer contact which would improve organogenesis, biochemical signalling between cell to cell contact would improve, the cell differentiation signals might get induced which is good for secondary metabolite biosynthesis. But where is the limitation?

Student: mass transfer limitation.

Mass transfer limitations. So, the scale up would ultimately get affected, the mixing will get affected which may cause oxygen gradients. So, it is always balancing between the merits and the demerits of any particular strategy.

Now, production of secondary compounds is usually most efficient in tissues farthest from the meristematic activity. So, for higher biomass productivity, you would need that the cells continuously keep dividing, and for secondary metabolite biosynthesis, the word secondary itself says that resting cells would produce better. So, it is a non-growth associated production. So, therefore, decoupling of the production phase and the growth phase is sometimes found to be better in improving the secondary metabolite biosynthesis.

So, for example, like in callus most of the cells will be parenchymatous in nature. Now, you will see that once the cell are meristematic, they are still expanding, growing, multiplying and once the cell reaches a matured state then higher order functions or biochemical activity would appear. So, therefore, it has to be seen that, for inducing callus cultures you need young explants where there is higher meristematic activity but for production of secondary metabolites it would be better in matured state of the culture.

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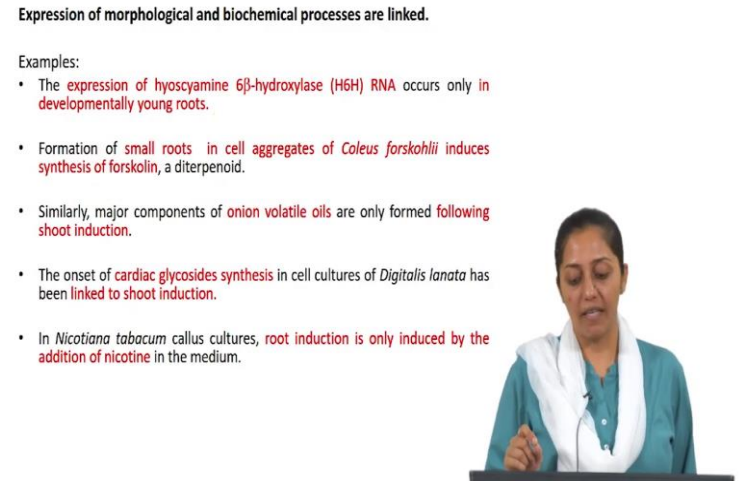

Now, some of the examples. Expression of hyoscyamine 6 beta - hydroxylase RNA occurs only in developmentally young roots. You will observe that some of the secondary metabolites appear only once the shoot is induced or the roots are induced and still when it is in callus or cell suspension phase, the secondary metabolism is not induced and there is no secondary metabolite observed.

Formation of small roots in cell aggregates of *Coleus* species induces synthesis of forskolin or diterpenoid which is of commercial value. Similarly, major components of onion volatile oils are only formed following shoot induction. The onset of cardiac glycosides synthesis in cell cultures of *Digitalis* has been linked to shoot induction. So, these are some of the examples which demonstrate that organogenesis is linked with secondary metabolism. *Nicotiana tabacum* callus cultures, root induction is only induced by the addition of nicotine. So, it is two way. So, nicotine you add and then you see that it has induced what? Root induction or yeah, organogenesis in the cultures.

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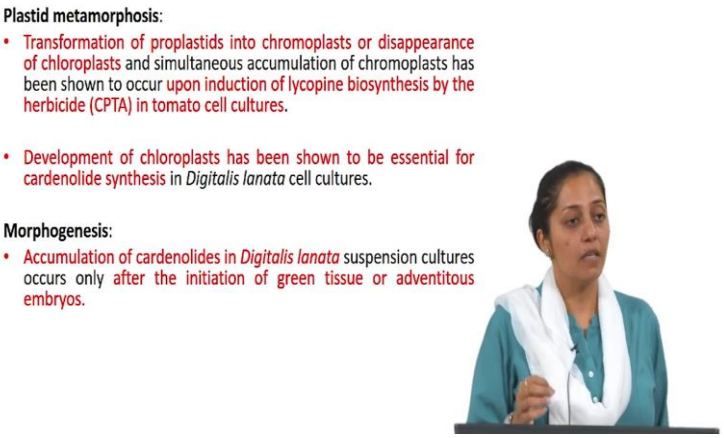

Now, plastic metamorphosis also sometimes has been observed to be linked with secondary metabolism, where they have observed in lycopine biosynthesis. Where, adding a herbicide in tomato cell cultures lead to induction of lycopine biosynthesis. With the herbicide , the chloroplast started diminishing and they started converting into chromoplasts that red color pigment started appearing.

So, in nature what might be happening is that as the lycopine synthesis has to happen for the red color to come up, the tomato or the fruiting or the maturity of the fruit to happen, the chloroplasts start getting diminished, which means at the proplastid stage , the pro proplastid rather than getting converted to chloroplast start getting converted to chromoplast.

Now, developmental of chloroplast has sometimes it is the other way round. Like we are observing an alpha tocopherol as the more green is the callus, the better is the alpha tocopherol yield. So, it is linked to chlorophyll biosynthesis, even in the biosynthetic pathway there is a link between chlorophyll biosynthesis and vitamin E biosynthetic pathway. So, similarly accumulation of cardenolides. So, it is morphogenesis effect example. Accumulation of cardenolides in *Digitalis* cultures has happened only after the initiation of the green tissues or adventitious embryos.

We have observed in *Viola odorata* also that the cyclotide biosynthesis is stable in an organ culture like somatic embryos. When you induce a fresh callus, they show that the

cyclotides array is there but with subsequent years of subculturing the biosynthetic capacity is lost. So, this is an example which shows us that organogenesis is linked with the secondary metabolism. Now, the same callus if you converted it into somatic embryos the cyclotide array is revived.

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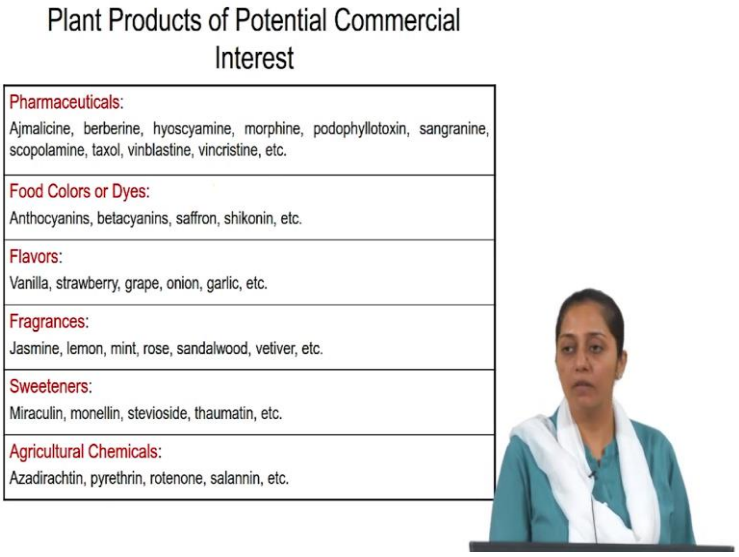

So, these are some of the examples of plant products of commercial interest where it ranges from pharmaceuticals, then food colors, flavors, fragrances, sweeteners, artificial sweeteners and agriculture chemicals, like pesticides or even fertilizers.

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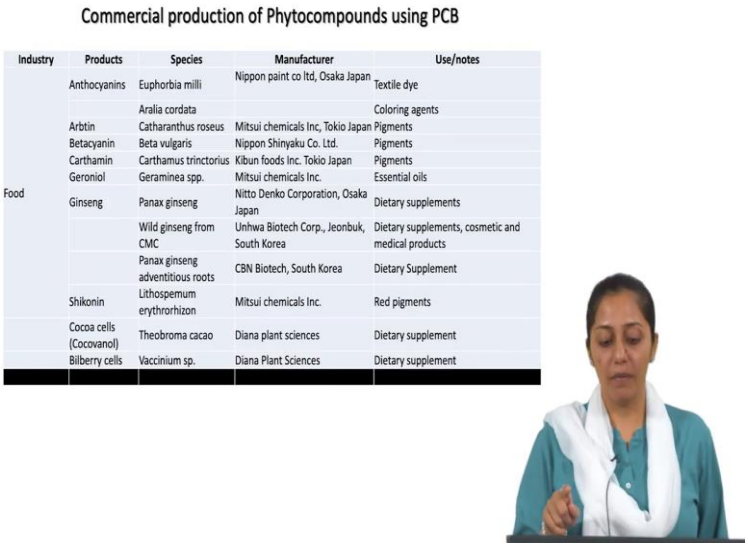

Now, some of the commercial productions which are happening for the phytochemicals using plant cell based bioprocesses are these. Your anthocyanins, betacyanins, so these can be pigments coloring agents, flavoring agents. So, there are different kinds. Betacyanins, carthamin, geronoil, ginseng can be used for good health. Shikonin, it is a coloring agent. Cocoa cells, bilberry cells, so these are different kinds of products which have been commercialized by Japanese firms, by German firms, by US, by Israelis. So, they are already there in place and are being used for what all uses? If you can see the table as textiles, as coloring agents, as pigments, as dietary supplements for as nutraceuticals, so as cosmetic agents.

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Pharmaceuticals	Berberines	Coptis japonica	Mitsui chemicals Inc.	Anticancer
		Thalictrum minus		Antibiotic, Anti-inflammatory
	Echinacea polysaccharides	Echinacea purpurea	Diversa, Ahrensburg, Germany	Immunostimulant, Anti-inflammatory
		Echinacea angustifolia		
	Paclitaxel	Taxus spp.	Phyton Biotech Germany	Anticancer
			Samyang Genex Seoul South Korea	Anticancer
	Podophyllotoxin	Podophyllum spp.	Nippon oil, Tokio Japan	Anticancer
	Rosamarinic acid	Coleus blumei	Anattermann & Cie. Gmbh, Cologne, Germany	Anti-inflammatory
	Scopolamine	Duboisia spp.	Sumitomo Chemical Co. Ltd, Tokio Japan	Anti-cholinergicum
				Antimuscrnic
				used in treatment of motion sickness, nausea and intestinal cramping

These are pharmaceuticals which are getting produced commercially. Anti-cancer, anti-biotic, anti-inflammatory, then for the treatment of motion sickness, nausea. So, then pharmaceuticals like berberine, then paclitaxel is well known it is the taxol drug which is used for anti-cancer, vincristine, vinblastine, then even your podophyllotoxin.

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Cosmetic	About fifteen active ingredients for cosmetic use	Cell suspension and hairy root cultures of different species	Sederma, Le Perray-en-Yvelines, France	Develop Cosmetic active ingredients
	Active ingredients for cosmetic use	Suspension and hairy root	Mibelle, Switzerland	Develop cosmetic active ingredients
		Malus domestica		
		Solar vitis		
Heterologous proteins	Human Glucocerebrosidase GCD enzyme (Taliglucearse alfa)	Carrot suspension cultures	Protalix BioTherapeutics, Karmiel, Israel.	ELELYSO Plant cell expressed form of the GCD enzyme for treatment of Gaucher disease
	Vaccine against Newcastle disease virus NDV	Tobacco suspension culture	Dow Agroscience, LLC, Indianapolis, USA	First tobacco cell based vaccine approved by the FDA against NDV in poultry
	Human proteins	Physcomitrella patens suspension cultures	Greenovation Biotech BMBH Freiburg, Germany	α-galactosidase for Fabry disease and β-glucocerebrosidase for Gaucher disease. Both are in phase I of clinical trials
	Glycosylated recombinant proteins	Hairy roots	Root Lines, Amiens, France	Rhizo prot platform
	Lactoferin, albumin, Lyzozyme & Transferrin	Oryza sativa	Invitria, USA	Recombinant human enzymes from rice cells

Now, heterologous proteins. In heterologous proteins the interesting part is that the plant cells are also replacing CHOs in some cases as production platforms. Now, CHOs are increasingly used as for production of recombinant proteins, human proteins as vaccines or for animal vaccines or even as anti-bodies and bio-similars is one big area where CHO cells are used.

Now, there are examples where some are in human trials, but some plant based recombinant proteins which have been produced using plant cell based bioprocesses are now approved by FDA and are now marketed. So, some of the examples are for your Gaucher's disease. It is being produced using carrot cell suspensions and it got approved by FDA in 2015 and is now marketed it with protalix, it is an Israeli based company which first made it and now it is being sold. Then vaccine against Newcastle disease virus. It was being produced using tobacco cell suspension culture and it is marketed by Dow Agrosciences. And similarly, there are many others.

So, this is just to show you people that there is so much of scope and what the world is gearing up to and why do you think that it is on the rise? So, one, is it is a green technology. So, immune response against the plants the chances are less. So, it is a sustainable greener, environmental friendly technology. So, please remember yield now on would be referring to $Y_{P/X}$ which means the amount of product per unit biomass, and

productivity would be volumetric productivity which is the product titer. Product titer is what?

Student: Concentration.

Product Concentration per unit time. So, we will be working around in all these strategies either it will be impacting both the things: the product yield and the productivity or it will be impacting overall productivity. By improvement ; if it is not impacting product yield, but still the productivity can be high , because of, time. So, from where is that coming? Probably, generally these are intracellular products. So, when we say product titer what does it mean? This will include what two parameters?

Student: Amount and volume.

So, from where is this amount and volume coming? When we say productivity in an intracellular based product it will be suppose grams per liter per time. Now, this gram per litter per time is a club of what two things?

How do you measure gram per litter per time? How would you measure? Think that ways.

Student: Amount of product in total volume before the extraction. No. From the total volume.

So, how do you get the total amount of product?

Student: From different.

Student: Concentration.

What concentration? Think a little math is involved. How would you calculate? Start from the scratch. I said product productivity would be; assuming it is an intracellular product, so she said it will be extracting from the biomass. So, how will you calculate? So, how will you get to grams per litter per unit time?

Student: Total amount of biomass which we had initially, will come in denominator and after all the extraction steps.

So, for extraction every time you will have the entire biomass.

Student: No.

For extraction?

Student: No, no.

How, how will you do it at a experimental level? Think that ways. You are running a shake flask or you are running a reactor, what would you do? You will every time harvest the entire biomass and then you will dry it, then you will extract using the entire bio mass?

Student: No, that is this if in 1 ml I have 1 mg.

Ok.

Student: That is 1 mg per ml to what would be there in 1 litter. So, it will be.

Right. So, here 1 ml would be there, ml is what then?

Student: ml is cell bio mass, the total mass.

Will it be volume, cell biomass?

Student: Ok. It will be weight by weight.

Weight by weight. Now, this weight by weight is called what?

Student: Yield.

Yield.

Student: Yield

So, one is yield.

Student: Ok.

The other would be?

Student: Rate of productions.

Rate of what?

Student: The reaction time

Student: The μ.

So, this μ is specifies what?

Student: The kinetics of the.

Of?

Student: bio mass.

So, now it will take care. Now, this was if you think mathematically it is gram per gram biomass multiplied by the concentration of the biomass. So, this will give you the product titer, isn't it. And then the time in which that product concentration of the biomass is achieved. So, that is what is overall volumetric productivity. When you have μ it becomes?

What does it called when you have μ? What will it be called?

Along with the yield what will it be called?

Think about it this.

It is simple maths. I want you to represent your product after evaluation as the volumetric productivity or the product concentration. How would you determine? Just think on those lines, then you will be able to figure out that what should be your two important responses when you are optimizing a process; what will be your two objective functions. ok.

So, we will continue tomorrow.