Plant Cell Bioprocessing
Dr. Smita Srivastava
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture - 12
Secondary metabolism in plant cells - Part 1

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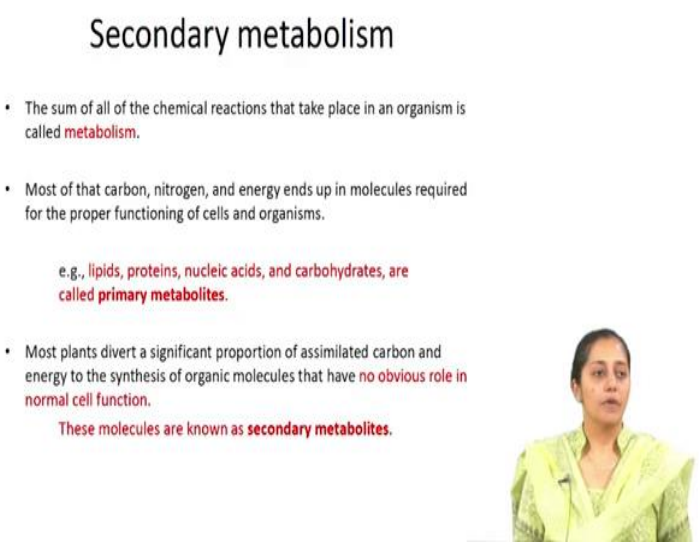

So, from today we are going to learn about secondary metabolism in plant cells. Now comes the commercial applications of plant cell technology. So, before we move on to how to exploit secondary metabolism commercially, it is important for us to know about secondary metabolism in plants, its role and hence we will then extrapolate to commercial applications of it.

So, what is secondary metabolism? Now what is metabolism to begin with; it is the sum of all chemical reactions that take place in an organism, so this forms metabolism whether primary or secondary. Now when most of the carbon, nitrogen or energy which is accumulated in the cell is majorly used for its growth and development, that forms the primary metabolism.

Now, some of the carbon and energy is also utilized for specialized functions which majorly deal with more organized function and differentiation in plants, which deals with its survival in the nature for competition or defense related, all that. This is secondary metabolism.

Most of the carbon, nitrogen and energy ends up in molecules required for proper functioning of the cells and organelles. For example, what are those molecules? We know lipids, carbohydrates, nucleic acids, these form the primary metabolites.

Plants divert a significant proportion of this assimilated carbon pool and energy, to synthesis other organic molecules. So, these secondary metabolites are generally organic compounds, and they actually have no obvious role in general plant growth and development but they have other roles in terms of survival and others.

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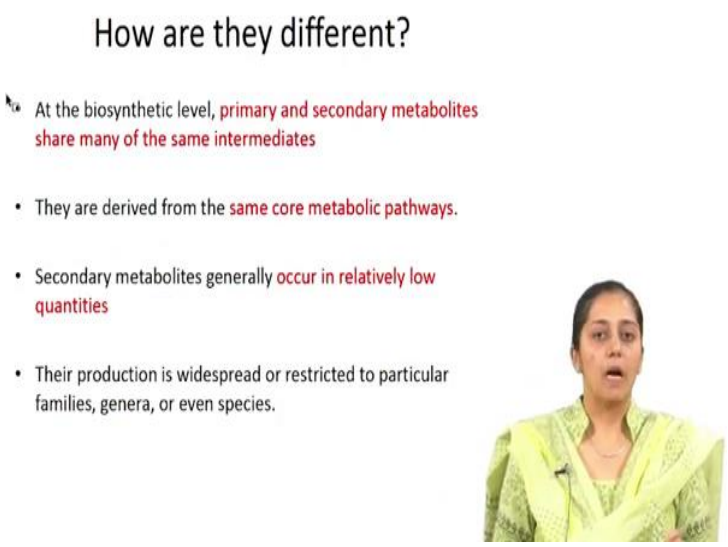

So, how are secondary metabolites different from primary metabolites? At the biosynthetic level, primary and secondary metabolites, generally there are common pathways.

What all pathways and primary metabolism do we know about? Pentose phosphate pathway, glycolytic pathway then?

TCA cycle. So, most of the secondary metabolism leads from one or more of these intermediates of the primary metabolism and then it gets diverted into secondary metabolite pathways. So, at the biosynthetic level, primary and secondary metabolites, share many of the same intermediates. They are derived from the same core metabolic pathways which we know as a part of primary metabolism.

Secondary metabolites generally occur in relatively low amounts in comparison to primary metabolites. What can be the reason? Then I will ask why they are found in the stationary phase. So, now, try to reason out.

Why do you think they will be produced in small amounts? So, two things; one is only when it is needed the cell would spend its carbon pool and energy in this biosynthesis and only upto a desired level it will try to use. So, every time the cell would like to conserve its carbon and energy pool.

So, therefore, you will generally observe that secondary metabolites are low volume which means they are present in very low quantities in the plants and the second thing is, there is a wide array. You cannot even imagine how wide is the secondary metabolism in plants. It is not uniform; it can vary from plant to plant in terms of genus, in terms of family. Then genus, till species you can find variations in secondary metabolites. So, for every such wide array of metabolites there will be a carbon flux required. So, the cell would like to judiciously use and prioritize.

Secondary metabolites generally occur in relatively low quantities I said. Then their production is widespread or restricted to particular families, genera and even species.

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Now, what is the role of secondary metabolites in plants? They have ecological functions in plants. So, what kind of functions do they carry out? They prevent the plants being eaten by

herbivores or they prevent the plant from being attacked by pathogens. These pathogens can be fungal pathogens, can be means of microbial origin, can be insects, can be predators or like herbivores, can be mammals, can be humans.

So, they need to have that wide spectrum of defense against all these and then they also need to prevent against environmental stresses. So, they serve as attractants and it is not only defense, but they also carry out higher order functions; like for example, in pollinators - attracting the pollinators or even attracting the predators of the pathogens. So, by using these volatile secondary metabolites they can attract these predators, so that they can get rid of the pathogens.

Then they serve as attractants; like in the form of odor, color, taste for pollinators and seed dispersal. Then sometimes in order to prevent being eaten up, you will find some of the fruits are very bitter, so that humans do not eat. Now they function as agents of plant-plant competition. Sometimes you will see that plants produce exudates in the soil which are toxic to the nearby weeds or plants. This is kind of overcoming the competition for the sunlight and the resources in the soil.

So, they increase the reproductive fitness, how do they increase? By warding off fungi, so that they remain healthy, then bacteria, whatever pathogens may attack them, so that the status of the plant is quite healthy. So, the ability of plant to compete and survive, is therefore, profoundly affected by the ecological functions of their secondary metabolites.

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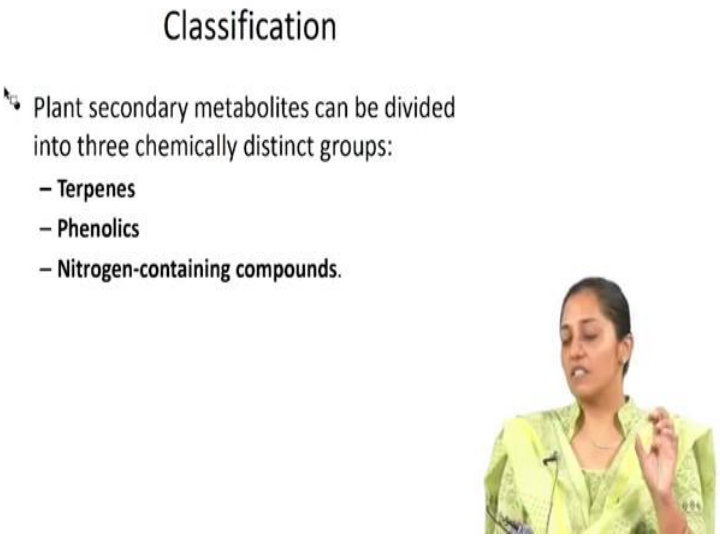

This is the general class, there are many other different subclasses of secondary metabolites, majority of them start from these three major classes of secondary metabolites in plants. What are these three major classes; one is terpenes, then phenolics and nitrogen containing compounds. Your high value compounds which are alkaloids: anticancer agents, taxol and all these, camptothecin or vincristine vinblastine, they come under alkaloids and these alkaloids are nitrogen-containing compounds.

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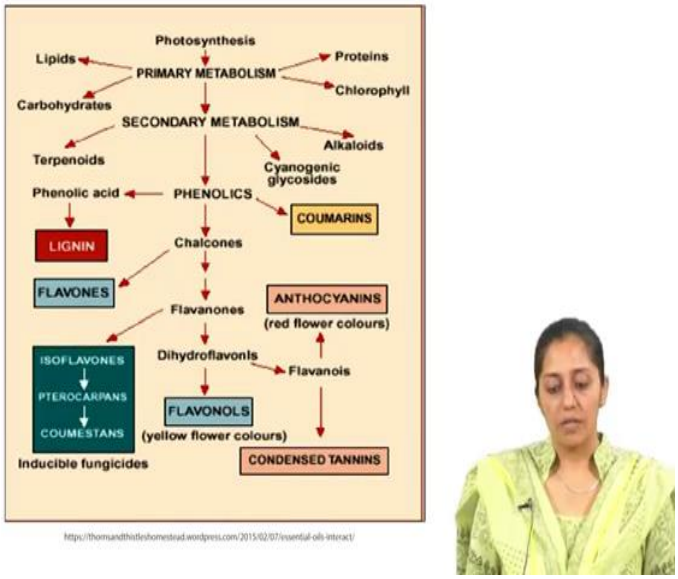

So, this is the general picture which shows that it starts from primary metabolism and then some of these intermediates get diverted towards the formation of secondary metabolites. So, you will find that shikimic acid pathway or pentose phosphate pathway or MEP pathways, they may independently produce some of the secondary metabolites or some of these intermediate from two different pathways can come together to give rise to the new secondary biosynthesis pathway.

So, you will find different types of secondary metabolites: coumarins, anthocyanins, flavonoids, alkaloids, terpenes. So, there are n numbers.

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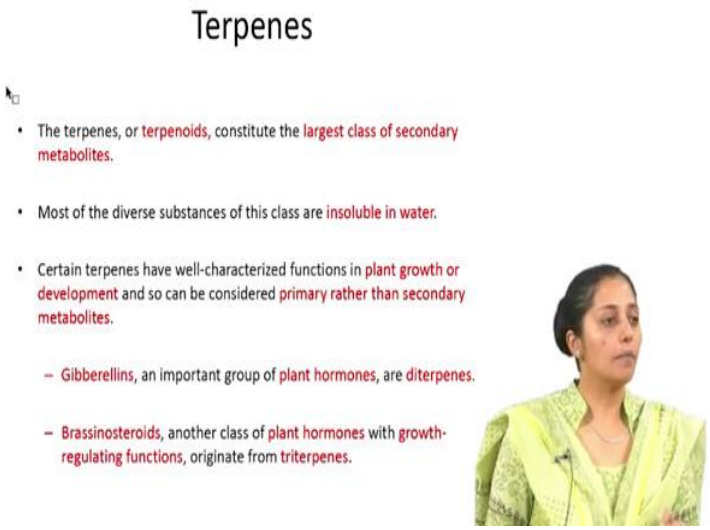

So, let us talk about terpenes. What are terpenes? They constitute the largest group of secondary metabolites present in plants. Now one of the most popular biopesticide which is azadirachtin, I gave an example also is a sesquiterpenoid. So, its structure comes from terpene and then it is a sesqui - means because of the structure - the number of carbons and the number of rings involved - terpenes and its derivatives - is formed.

So, the terpenes or terpenoids constitute the largest class of secondary metabolites in plants. Now most of the diverse substances of this class are insoluble in water. Then certain terpenes are well-characterized function in the plant growth or development and so can be considered as primary rather than secondary. Some of these terpenes can also play a role in the growth and development. So, therefore they are classed as primary rather than secondary.

For example, some of the plant growth hormones like gibberellins. Gibberellins are an important group of plant hormones and it is a diterpene moiety. Now brassinosteroids, another class of plant hormones with growth regulating functions, takes care of the primary aspect of the plant growth and development. So, it is also termed as primary metabolite but it originates from triterpenes.

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So, generally you will observe that most of these high value terpenes, are plant defense related triterpenoids or terpene molecules. Now terpenes, they act as toxins and feeding deterrents to many herbivores, insect and mammals. That is the very reason why Azadirchtin is used as a biopesticide; it acts as a feeding deterrent to the insect pest.

Monoterpenes, it also depends on the mode of action. Suppose if it is a wide spectrum, like Azadirchtin has a wide spectrum of antifeedant activity, so it can cater to a large range of insect pests. So, that is the reason why neem is being used against even mosquitoes. Then it is also used for pathogens or for biopesticides in crops to remove insect pests, so it has a wide range of applications.

Now, monoterpene esters are also called as pyrethroids, this is also a class of biopesticides. Found in the leaves and flowers of chrysanthemum species which shows insecticidal behaviour. It is also used as biopesticides. Now you can see and make out that, the commercial application is very closely associated to its application in nature, so that can give you a cue or an indication to what kind of commercial application, a particular secondary metabolite may have.

Now in conifers: monoterpene accumulated in resin ducts, found in the needles and trunks that is for what? Once it is in the bark region, then it can prevent either the pathogenic attack or even the herbivory.

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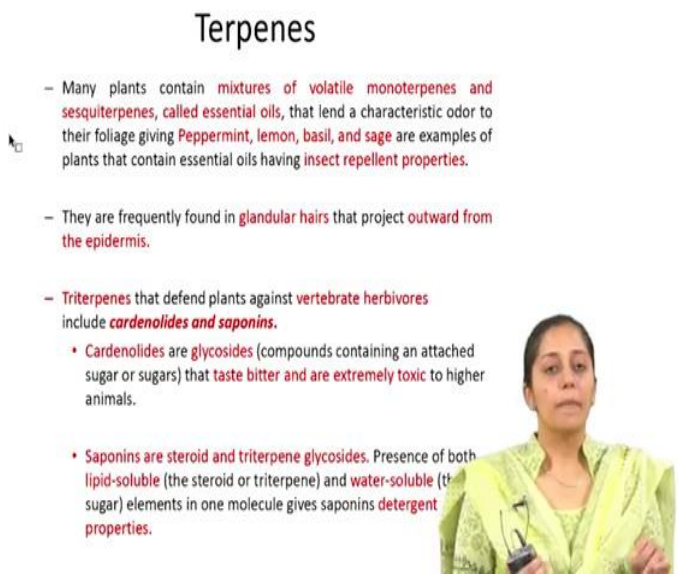

So, many plants, they contain a mixture of volatile monoterpenes and sesquiterpenes and these are called as essential oils and they are highly expensive. So, you will find that these essential oils are very expensive in market and they are not found in very large amounts. I had gone to Coimbatore and there in the shop I went to a tea plantation where a factory where they were showing. So, there was a shop in which they were selling these essential oils. So, there was a essential oil which caught my attention, it was just 2 ml; 2 ml bottles which were being sold, so tiny one, and then I started asking what is the use of this. So, they were said it is a very expensive, very good oil for acne treatment and then for, which means antimicrobial activity and all.

So, by the time I was about to reach the counter to buy that tiny stuff, I was asking why it is so expensive, why in such tiny bottle. So, to find the plant, and they started to telling me it is very difficult. So, that got my attention because we work on plant biotechnology. So, because of the availability, the demand is high and the availability is less. So, the minute I could reach the counter to buy it, the stock got over. So, they just had two bottles and those too someone picked up before me.

So, that really has got my attention that essential oils are expensive, and they can have large commercial applications in cosmetic industries, as your antimicrobial agents, and so on. They are frequently found in where, glandular hairs or in projections of the epidermis, epidermal hair or even in trichomes. Trichomes are also found on the surface of the leaf, on the epidermis

projections, where these secondary metabolites get accumulated in the form of bulbs. Now why do you think these essential oils are getting accumulated on the surface?

Student: Insolubility.

That is the reason the plant will put it on the surface, how is insolubility related?

The question was - why do you think these essential oils: will be found on the surface of the leaf or on the surface epidermis?

Student: Protection from transpiration

If it is volatile, it will help, then if it is nonvolatile it has a role to play in attracting, so if either color should be responsible that should also show up or the odor should be responsible that should also be on the surface. Then if it is acting as a defense mechanism, then should it be inside or should it be on the surface?

First level of defense. See the plant is not moving, like we can move our hands and legs. So, if the attack is at one point, it cannot move. It has to make sure that the rest of the parts of the plant are now geared to overcome the pathogen attack. So, if the compound is on the surface it will be first line of defense, it will be ruptured and then the secondary metabolite is released.

So, that is the one of the reasons of having these in the form of trichomes or glandular hairs.

Now, triterpenes defend plants against vertebrates, herbivores include cardenolides and saponins. I do not expect you to remember, but at least I would like to tell you what are the different wide range of secondary metabolites and they can have varied commercial applications.

Now cardenolides are what? Glycosides; now glycosylation is the addition of sugar moiety to reduce the toxicity. So, even here cardenolides are glycosides, compounds containing sugar or sugar alcohols that taste bitter and are extremely toxic to higher animals. So, some of these glycosides taste very bitter and can act as a feeding deterrent or can be very toxic to the feeders, the herbivores.

Now, saponins are steroids and triterpene glycosides. So, as I said earlier that there is wide range of secondary metabolism. So, some of these intermediates can be directly getting used up in the final product in the biosynthetic pathway but some may have one or more involvement

in more than one secondary metabolic pathways; like for example, saponin. Saponins are steroids and triterpene glycosides. So, there is triterpene moiety and there is a steroidal moiety, so presence of both. So, which means what? It will be both water-soluble, water insoluble, so it can form micelles.

So that can improve its diffusion through the membrane. Now I will talk about it later. Some of the secondary metabolites, how do you think the plant protects itself from the toxic action? So, one is they should be modified and stored, or detoxified and stored and when required, the required final step to again bring it back to the original compound is carried out and so the enzymes are stored in the vicinity. They are stored generally in vacuoles.

So, for example, I said in this case, if it is has to be stored in tonoplasts in the form of micelles it can easily pass through the phospholipid layer but once it comes inside, it is an acidic environment, so which will again transform and it will find it difficult to return back. So, these are ways in which the plant would protect itself.

But suppose this was stored in leaf or stem part and gets ruptured by the attack.

So, now, this cell is ruptured. So everything will come out from the vacuole although the cell has died and whatever enzymes there in the vicinity will also come and the final step can take place with that intermediate and the enzyme which can lead to the production of the final toxic metabolite. So, it is not that it is very quick and you will also find it is not quick for wound response by the plant. The cell which has died or the tissue which has been affected may not come back again, may not be revived but, the objective is to prevent its spread to the other parts of the plant or even to the neighboring plants, so that also forms a part of secondary metabolism.

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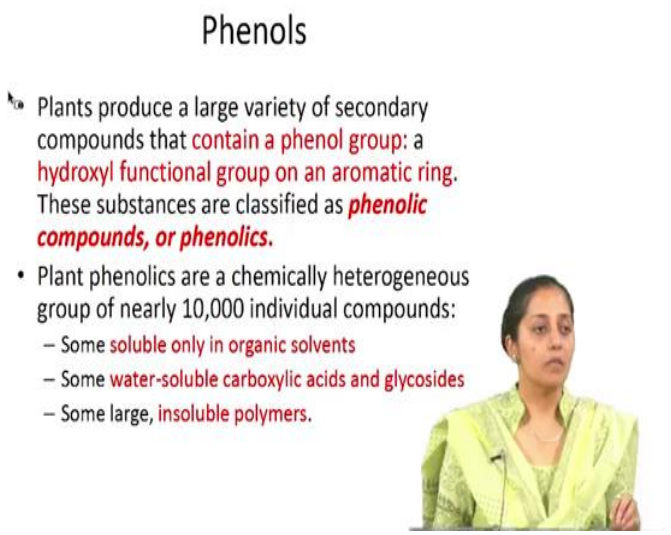

So, the other class as I said was phenols. What do they have? We know a phenol group has an attached functional group OH.

So, that forms the phenolic group. Now these compounds, are chemically heterogeneous and almost 10,000 different phenolic compounds are produced as different secondary metabolites in plants. Now some are soluble in organic solvents, some are water-soluble when they are in the form of carboxylic acids or glycosides, and some are insoluble polymers.

So, why do you think there are these different forms? This is nothing but biotransformations; functional groups getting added, derivatives, saturation, unsaturation which might change the chemical structure and its biological action in the plant.

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So, phenolics play a variety of roles in the plant. What are the different roles? As defense against herbivores and pathogens, they can also act as mechanical support. Now how do you think this phenolic compound can act as a mechanical support?

They can form a structural component. In attracting pollinators and fruit dispersers, the colored pigments of plants usually provide visual cues that help to attract pollinators and seed dispersers.

Now, some of these compounds may also absorb the harmful UV radiation. In *in vitro* culture sometimes we find that some of the species, because of large exudation of phenolic compounds it becomes very difficult to grow them *in vitro*; that is because of the toxic nature of the phenolic compounds which get released in the medium.

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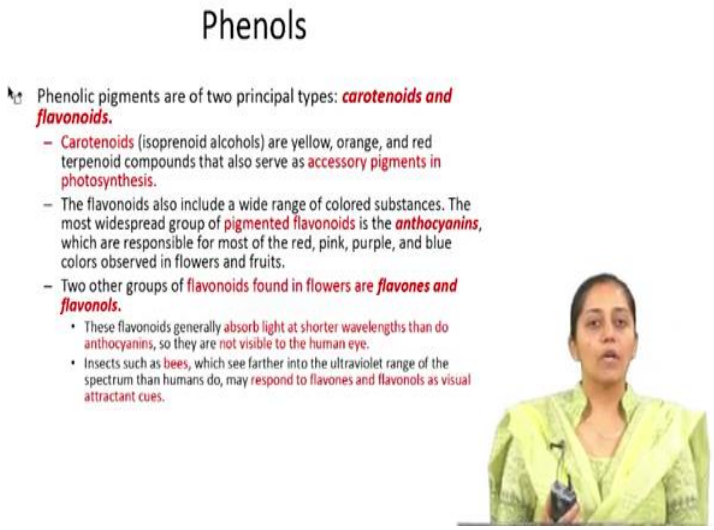

So, phenolic pigments are of two principle types; one is carotenoids and flavonoids. Now, carotenoids, these are isoprenoid alcohols; for example, there are different colors - yellow, orange, red, terpenoid compounds that serve as accessory pigments in photosynthesis. So, it is not only green color or your chloroplast, sometimes you will find that there is a transformation from chloroplast to chromoplast. Sometimes in *in vitro* cultures in order to enhance the production of lycopene, some elicitors were added but it was also observed that once the lycopene synthesis started happening, the chloroplast started getting transformed reduced and the proplastid which is the previous stage of forming different types of plastid, started getting converted to chromoplast.

Far UV range which is the lower wavelength, are not visible to the eye but some of these secondary metabolites can also capture light energy which is in this shorter wavelength area.

So, the flavonoids also include a wide range of colored substances. The most widespread group of pigmented flavonoids is anthocyanins.

These are commercially very important molecules, secondary metabolites. Now which are responsible for most of the red, pink, or purple color of the fruits and flowers. Now the two other groups of flavonoids that found in flowers are flavones and flavonols. Now these are those secondary metabolites which are also used, not only for helping in pollination but they have other defense related activities and they can also help in light absorption, because they can absorb light energy in the shorter wavelength.

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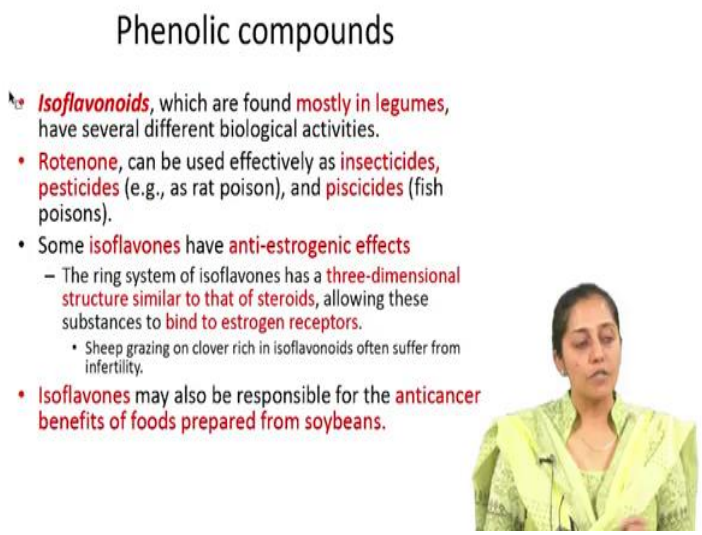

So isoflavonoids which are found mostly in legumes have several different biological activities. Rotenone can be used effectively as insecticide or for piscicides which is killing the fishes. Isoflavones may also be responsible for the anticancer benefit of food prepared from soybeans.

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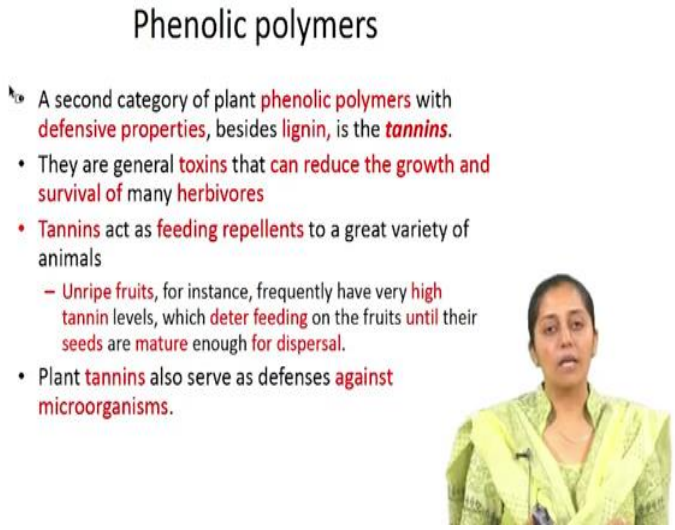

Now, phenolic polymers: now these phenolic polymers have defensive properties and are called as tannins. They are general toxins that can reduce the growth and survival of many herbivores. Now tannins act as feeding repellents to great variety of animals. Unripe fruits for example,

now the plant would not like anybody to eat the unripe fruit until unless the seed is ready for the next stage.

So, it then produces more amounts of bitter compounds. Where will the plant produce more amount of such compounds? It will produce inside the fruit, so that it can deter the herbivores. You will find that I also spoke that Azadirchtin in the plant is found maximally, in the seeds, what can be the reason? So, as to act as a feeding deterrent. It is bitter in taste, you will find the neem leaves are very bitter and the seed is also very bitter but the ripe seeds, they are full of sugar.

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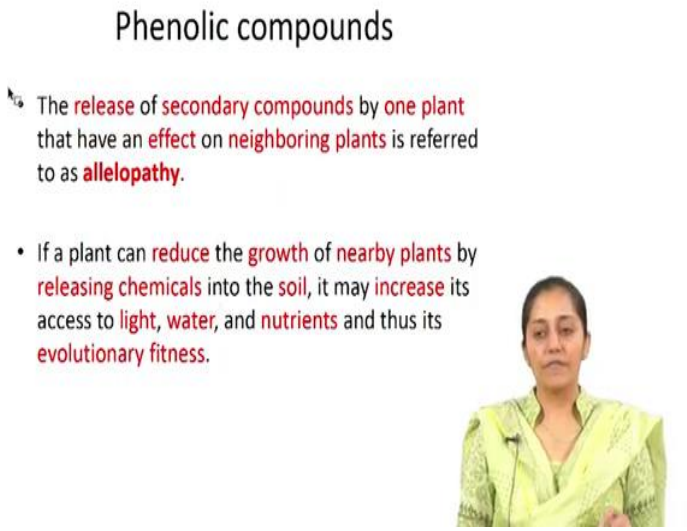

So, the release of secondary compounds by one plant that have an effect on neighboring plants is called allelopathy. If a plant can reduce the growth of nearby plants by releasing chemicals into the soil, it may increase its access to its competition. So, it would like to reduce the growth of the nearby plant, so secondary metabolism also plays the role there. Now how do you think that in all this the plant will protect itself; one is the storage, what else?

All the compounds will be stored. There are so many compounds which the plant is preparing. How much can vacuoles hold? Thousands are getting produced.

So, everything is not getting stored in just vacuoles. Some are in the open spaces also in the plant. Some particular tissue or you will find that it is in cortex cells, directly accumulating there. You can visualize them in a tissue section, but then how do you think the plant protects

itself? Let us take camptothecin. I will take that as an example. So, camptothecin, it is a topoisomerase inhibitor, that is why it is a very good candidate for anticancer activity.

It is cytotoxic in nature. It is also present in plants, so then how do the plants protect itself? The topoisomerase of the plant gets mutated such that the mode of action is different. See the mode of action of topoisomerase in mammalian cells is different than in the plants. Now that is how plant protect itself from the cytotoxic effect of camptothecin.

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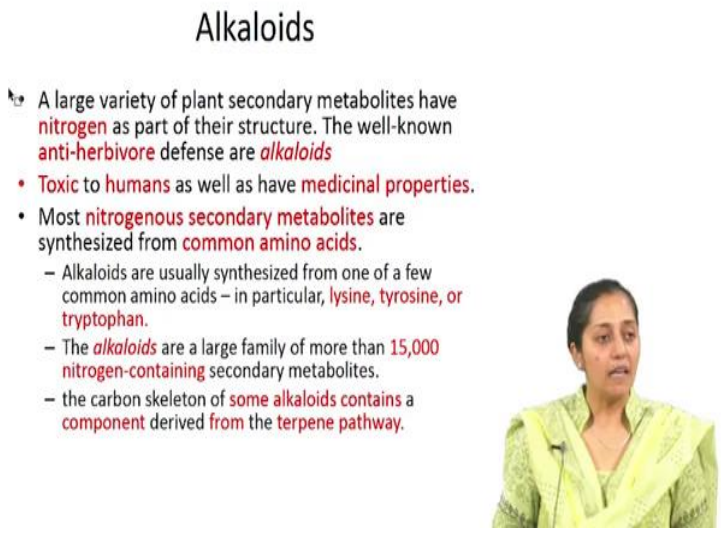

So, alkaloids; So, a large variety of the plant secondary metabolites, they have nitrogen as part of their structures. So, nitrogen containing organic compounds, they form alkaloids. Now the well-known anti herbivore defense, these are called as alkaloids. Now most nitrogenous secondary metabolites are synthesized from common amino acids. So, most of these alkaloids, are formed from amino acids. What are these? Lysine, tyrosine or tryptophan.

The alkaloids are a large family of more than 15,000 nitrogen-containing secondary metabolites. Now can you see 10,000 phenols, 15,000 alkaloids and the largest group was triterpenes much more than that. So, imagine the wide array of secondary metabolites the plants are producing. So, how much carbon and energy are getting utilized apart from growth into secondary metabolites. So, no wonder the plant has to judiciously prioritize the carbon flux into this wide array of secondary metabolites. How does the plant prioritize then?

The enzymes which are involved in the biosynthesis they get impacted by external factors. Maybe temperature, light or elicitors. So, that is how the plant is managing its resource. We will see it later as I go on that there are a class of secondary metabolites which are called as phytoalexins. Phytoalexins are not present all the time. They are synthesized de novo when needed, but the trick is, the synthesis is very fast.

There are also constitutive secondary metabolites which are present all the time. So, these are different ways in which the plant is prioritizing.