Plant Cell Bioprocessing
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Lecture – 14
Secondary metabolism in plant cells – Part 3

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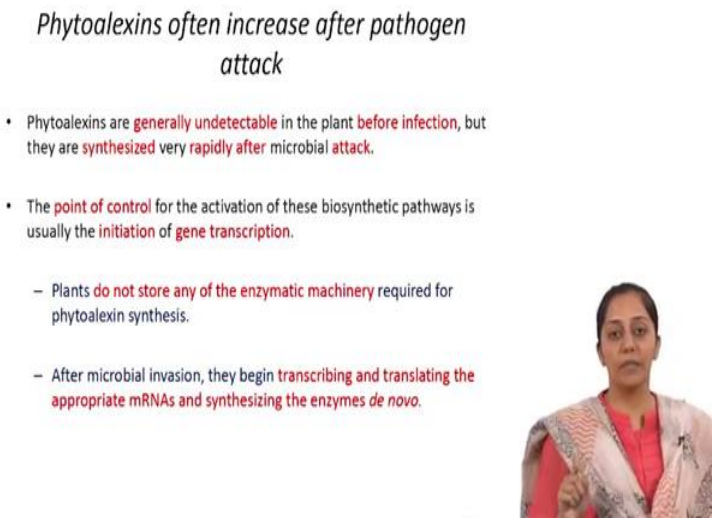

I told you that phytoalexins are secondary metabolites which are not synthesized all the time, but they are species specific, pathogen specific response. Now, this involves therefore a de novo synthesis of the enzymes which includes transcription, translation and all the process starts after the pathogen has attacked.

So, these phytoalexins are a very wide range of secondary metabolites, sometimes which are of very broad range pertaining to a particular taxa or taxonomy or which are very specific to a particular kind of insect or the pathogen which is continuously attacking that particular species. So, they remain generally undetectable in the plant before the infection.

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So, now what other mechanism by which the plant tries to protect itself? You remember I was talking about acquired immunity and innate immunity in plants. So, today , we will be talking about the different acquired and innate immunities in plants. Now, some plants they recognize specific pathogen derived substances.

They can recognize particular antigen epitope of a particular broad taxa; like for example, bacteria which are movable, so, they will have flagella. So, there are epitopes present or the receptor present on the plant cells which can recognize motile bacteria. So, by this, they are able to have a generalized defense against any such bacteria which is motile in nature which comes and attacks the plant.

Now, through evolution they recognize these specific components of their pathogens through which they then develop these specific antibodies which can recognize the antigens and then which can further trigger the secondary metabolism specific to that particular taxa or that species. Now, individual plants we know that they differ in their immune system or their sensitivity or their capability to resist the attack. That depends on two things; one is the array of secondary metabolites and the second is how quickly they recognize and induce the secondary metabolism.

Now, the resistance depends on how plants sense the presence of pathogens and initiate defense responses. Now, the first line of resistance is therefore, provided by a system that can recognize broad categories of these pathogens. Now, these are called as microbe-

associated general molecular patterns MAMPs. Now, these elicitors are evolutionary conserved pathogen derived molecules such as some structural component which will initially come into contact their cell walls.

So, therefore, you will see that through evolution, with their contact, the plants learn. So, there is a memory in the plant, a way through which it is learning and creating its own defense for future. So, structural elements for fungal cell wall for example or the bacterial flagellum.

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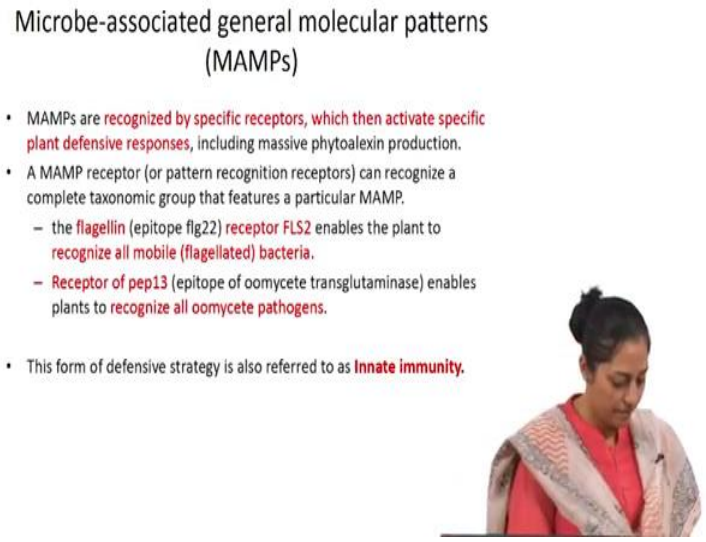

So, one such is the flagellin receptor FLS2 which enables the plant to recognize all mobile bacteria that is one such receptor. Then another as I said is called pep13 which is an epitope for oomycete transglutaminase. Now, it recognizes transglutaminase and then hence it will induce its defense response. So, this can recognize broad range of oomycetes. So, this form of defense strategy comes under innate immunity which through evolution the plant has learned; it is there within.

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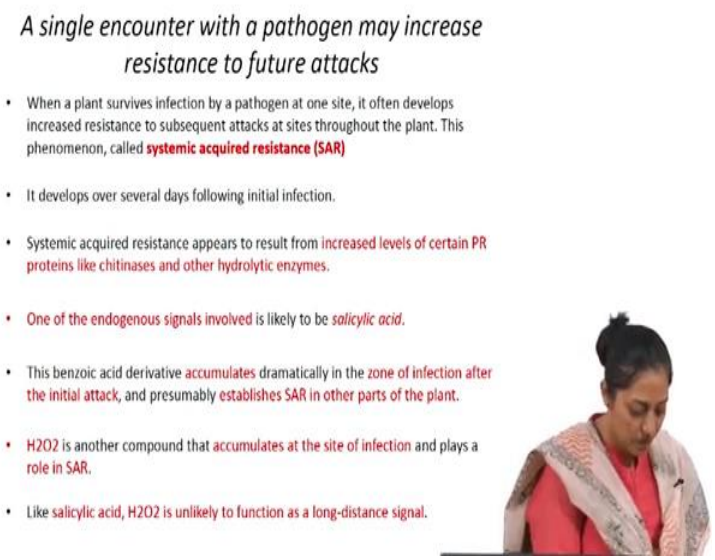

Now, when a plant survives the first infection then it can resist and it has been observed that at its subsequent attacks, the resistance increases. So, their first infection is said to improve its resistance so, they are able to show better resistance during further infections against the similar kind. Now, this phenomenon is called as systemic acquired resistance. Now, what is involved in systemic acquired resistance? It develops over several days; it is not that suddenly after the infection, the plant develops but these are developed after days from the first point of infection.

How does it happen? Now, there are increased levels of certain PR proteins like chitinases and other hydrolytic enzymes in the plants in the process of systemic acquired resistance. Now, one of the endogenous signals involved is likely to be salicylic acid. You remember salicylic acid was one of the molecules involved in the signal transduction pathway. So, now salicylic acid and its volatile components like methyl salicylate are said to be responsible or involved in this systemic acquired resistance.

So, there is a phloem induced signal. So, once the pathogen attacks, there is a phloem signal to other parts of the plant . Methyl salicylate is a volatile component of it and so the nearing plants also would therefore, induce the systemic acquired resistance. By inducing what? By inducing the production of PR proteins.

Now, another such signal is H_2O_2. Now, H_2O_2 is another compound that accumulates at the site of infection and plays a role in SAR. So, this is how it is also linked to as I was

talking about reactive oxygen species. So, at the point of infection it is not that only one defense will be playing a role, but there are multiple modes of defense which come together to protect the plant.

So, it is that hypersensitive response that the same reactive oxygen species or H_2O_2 which is generated is then helping in the SAR also. Like salicylic acid, H_2O_2 is unlikely to function as a long distance signaling molecule. So, you need volatile components.

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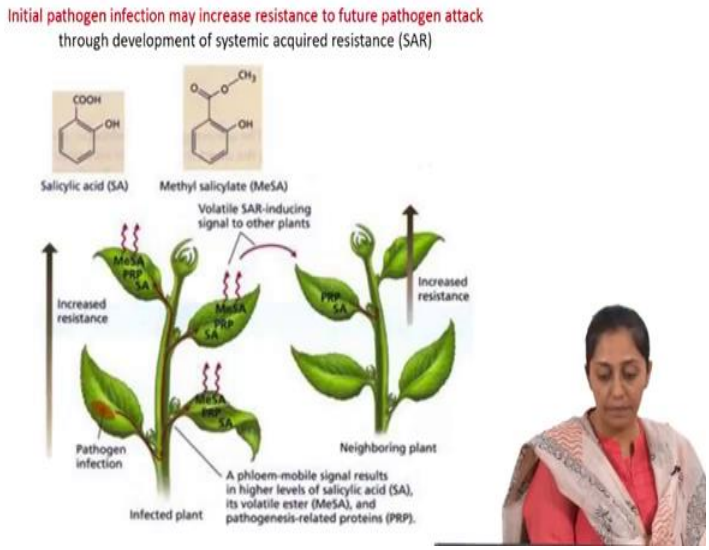

So, this is a picture which demonstrates the systemic acquired resistance. So, upon infection from the site of infection, it induces a flow in signal which further accelerates the production of salicylic acid or methyl salicylate and then it further accelerates or induces or elicits the production of PR proteins. What are PR proteins? Pathogen related proteins. So, this is what is systemic acquired resistance.

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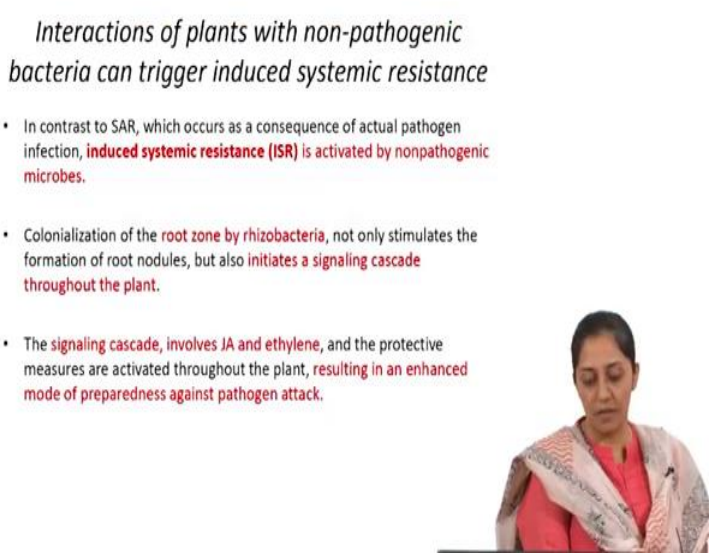

Now, interaction of plants with non pathogenic bacteria also induces resistance in the plant against pathogenic bacteria. So, this comes under induced systemic resistance in the plant. Many plants, although it is a hypothesis, which we are also planning to test is that you have heard about endophytes, you have heard about mycorrhiza and how some of the bacteria live in symbiosis with the plant. So, now, these organisms also first infect the plant. It is not only that one gives but there is a give and take process involved. It will be a mutual beneficial association always.

So, imagine why would plant resist one and why would plant allow other particular organism to grow within. So, there has to be something beneficial from it for the plant. So, that is what we are also testing in our endophyte. So, in lab we also work on endophyte cultures. Now, these are microorganisms which live and reside within the plant tissues and through years of evolution, as the plant has grown that symbiosis has developed.

So, why only a particular species or set of microorganisms, the plant is allowing it to grow and propagate throughout the plant and it is not allowing the others. So, any microbial even to enter and live or reside within the plant, it will have to enter the plant so, it is kind of an infection. So, think in those terms. Now, the plant is allowing that infection and in that process there is always a balance between the merits and the demerits. So, it is because of the mutual benefit, it gives more priority over the demerit

of infection and it is allowing that organism, but what other benefit the plant is getting is this induced systemic resistance.

So, because of that, for example, the signaling cascade were now jasmonic acid is involved or ethylene is involved. Now, salicylic acid is not involved; but now jasmonic acid and ethylene is involved. Ethylene is a growth hormone and it also plays a role in developmental stages of the plant. Now, jasmonic acid is another molecule which plays a role in the signal cascade. Now, jasmonic acid and ethylene are the components which are involved in induced systemic resistance. They increase the production of the secondary metabolites which are continuously being produced in the plant.

So, there were some metabolites which are always present, no spatial or temporal induction of their biosynthesis, but there are phytoalexins which are produced only as a result of infection to the plant. So, this induces systemic resistance and enhances the inherent capacity of the plant to protect itself.

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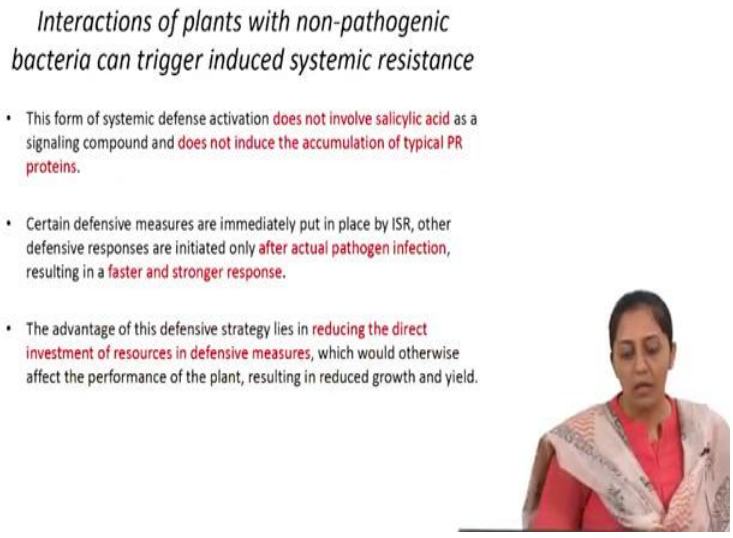

This form of systemic defense activation does not involve salicylic acid as the signaling molecule as I said. It does not induce the accumulation of typical PR proteins and now certain defensive measures are immediately put in place by ISR; ISR was induced systemic resistance. So, this is actually innate.

Now, other defense responses are initiated only after actual pathogen attack. So, that is induced resulting in a faster and stronger response. So, everything comes together, all different modes of actions defense are brought together. The advantage of this defensive strategy would be logically what? Without reading also you should be able to tell me. Why do you think the plant is having first line of defense, second line of defense, having different modes of actions?

Student: It does not want to waste energy.

In the end everything needs carbon and energy and we know how difficult it is for the plant to get the carbon. We have now studied about photorespiration, photosynthesis, the loss the plant goes through. So, the plant has to judiciously use its energy to protect and the priority is growth always, all these come for survival, but ultimately the aim is to propagate, to grow. So, the plant has to prioritize all these together for the ultimate aim. So, therefore, it would not like to waste its carbon and energy all the time in this. So, it will have different stages.

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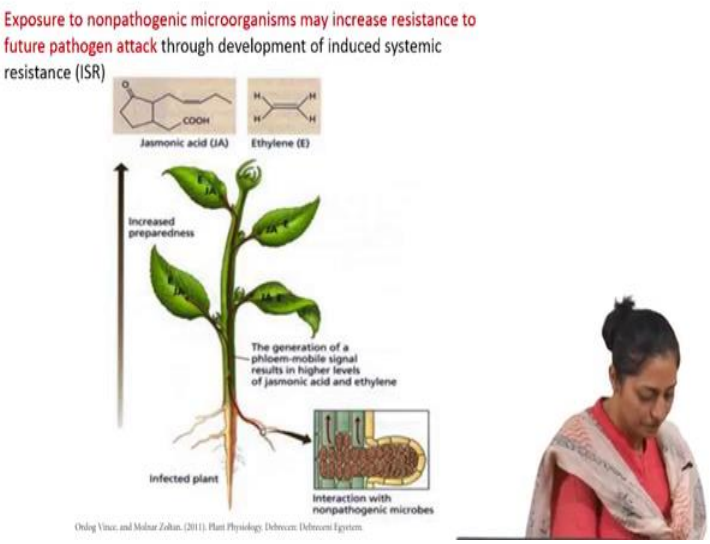

So, this is again a picture which can demonstrate to you systemic resistance, induced systemic resistance were jasmonic acid and ethylene is involved. Again, it is through phloem it spreads to other parts of the plant.

So, they have shown in the root, if you can see that there is a symbiosis between a bacteria. So, it is maybe forming a root nodule. So, because of this the ethylene and your jasmonic acid production is induced which further is involved in the signal cascade to enhance the secondary metabolism. So, you will always find that some plants are found to be very resistant which will have a very high antimicrobial activity; not all plants have the similar antimicrobial activity. That is the very reason when some natural products are used as pesticides, not all; like neem for example, there is a difference in the capability of each plant. So, from where is that coming? It is coming from all these. The different array of secondary metabolites which a plant would produce would depend on its systemic and acquired resistance.

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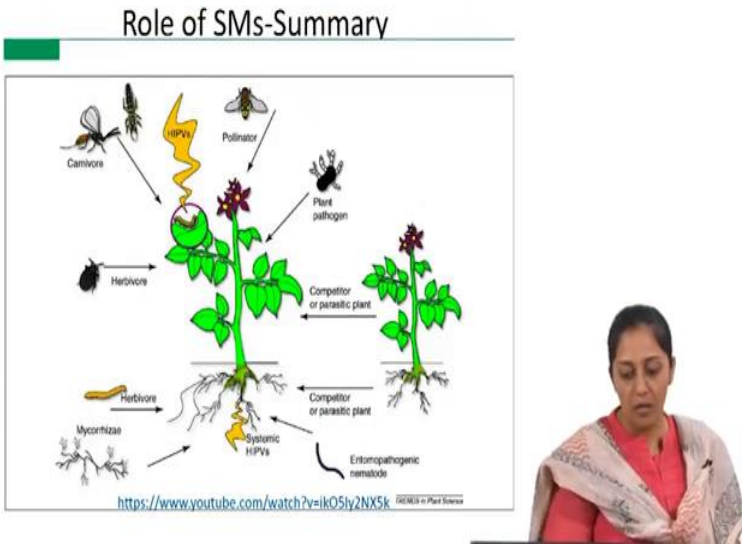

So, this is the summary of what we have learned up till now. So, the plant has to protect itself against the herbivore or carnivore is involved or is involved to protect against HIPV any guesses? So, it is herbivore induced plant volatiles.

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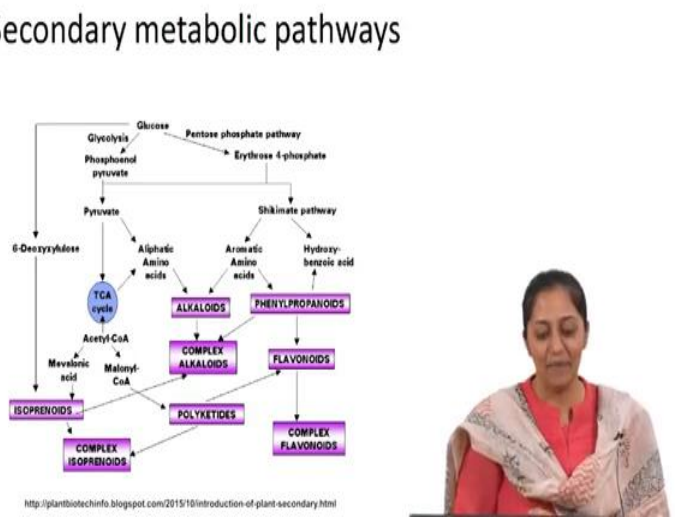

So, secondary metabolic pathways, again to just to reiterate that there is a broad range of secondary metabolites including classes like isoprenoids, complex isoprenoids. So, as I told you that it begins with the primary metabolism, then major secondary metabolic pathways where MEP pathway, shikimate pathway, mevalonate pathway, these are in DX5 pathway they may be involved for the backbone of those secondary metabolites.

Then from these intermediates major classes can further come together to form further more complex metabolites. Now, why do you think the plant is finding a need?

For combining further branching it out.

Student: May be to stabilize the compound.

Can be, what else?.

Student: It is like it has very basal structure like fungal structure and then just by changing the functional groups or adding some more you can change the entire property of the metabolic for different.

Property in the sense, bioactivity.

Student: Bioactivity.

If you see the chemistry of the mode of action of these complex metabolites, specially plant based products are very complex large molecules organic compounds. Now, their mode of action depends on a particular moiety or a functional group. It is not that every moiety has a different role to play. So, it is kind of increasing the diversity of defense in the plant or maybe to protect the plant itself. So, that is the very reason possibly why the secondary metabolism is so widespread in plants.

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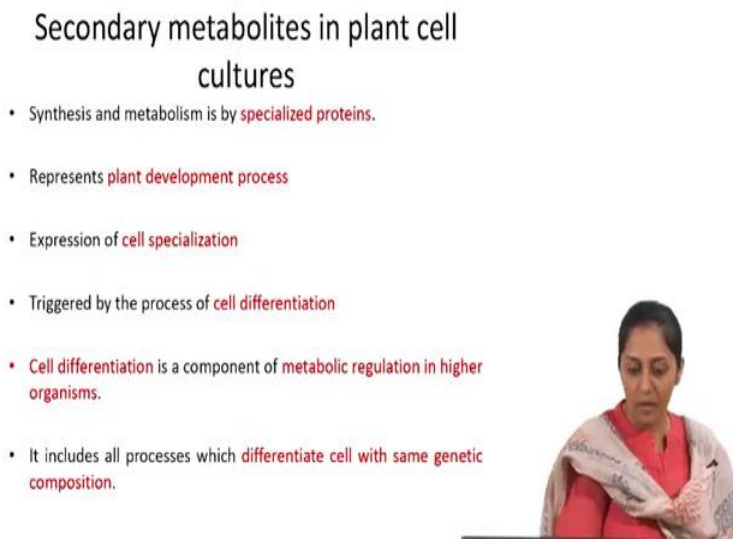

So, secondary metabolites, synthesis and metabolism in plant cells is by specialized proteins. The secondary metabolism majorly represents the plant developmental process. It is an expression of cell specialization. Now, all these points are important to know, why? So that when you are developing a plant based bioprocess for the production of a specific secondary metabolite, you will be able to know that what is needed for that bioprocess.

Whether a simple undifferentiated cell or homogeneous dispersed cell would work or you need aggregation also to happen or you need organogenesis to also happen for that particular metabolite. So, when you know these aspects and you know your secondary metabolites and how is it and when is it and where is it getting produced in nature you can accordingly device your bioprocess development; like some particular secondary metabolites are not produced all the time. They are only found when in a particular tissue, like they are only found in roots or they are only found when the leaf a new leaf

starts coming out, like an onion. I will be talking about those volatile components. So, then if you know what is happening in nature in order to produce that *in vitro* you will have to create similar conditions.

So, for expression of cell specialization, secondary metabolism is now triggered by the process of cell differentiation because it is a higher order function. So, it is generally associated with cell differentiation; the yields will be higher when the cell differentiates. Cell differentiation is a component of metabolic regulation in higher organism.

Now, it includes all process, when I say cell differentiation, it is from the cells to the tissues then to the organs and then to the entire organism, that is differentiation. It includes all processes which differentiate cell with the same genetic.

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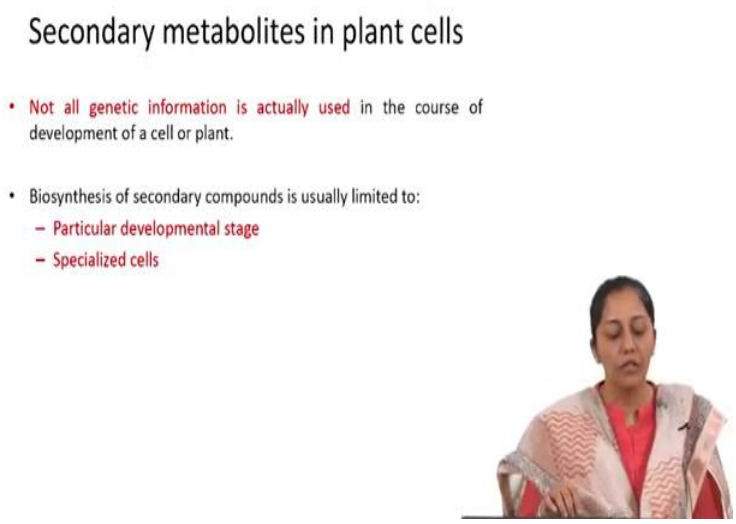

Although they are totipotent in nature,with the genetic machinery is available, not all genetic information is utilized at all the time. So, biosynthesis of secondary compounds is limited to therefore, particular developmental stage most of the times or specialized cells.

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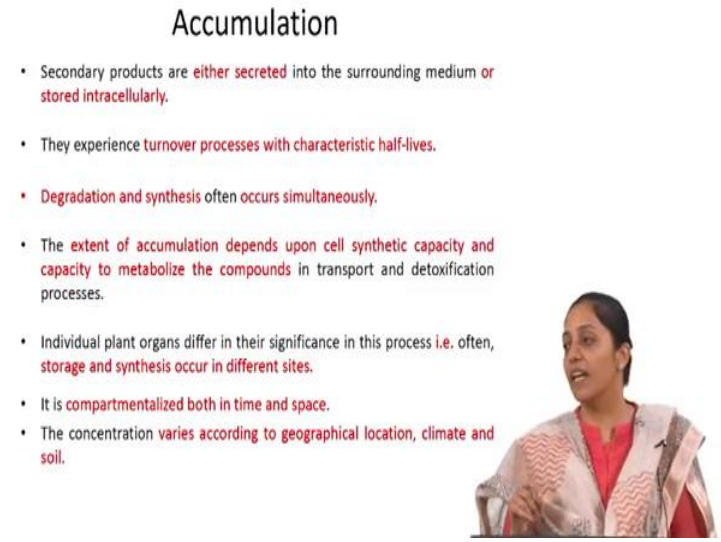

Now, where do they get accumulated? Now, for secondary metabolites as you know, there are specific synthesis sites, storage sites and generally synthesis is not the same as the storage site, why?.

But, they are getting synthesized, is it not?

They undergo?

Student: The site where it produces might be far from the first line of consumption.

That can be a reason. Yes, what else? There are also secondary metabolites which we know that the plant will judiciously produce only when it is needed it and will accelerate the machinery. So, a plant would not like to have it all the time because they may be toxic. So, there are turnover processes. So, the metabolism, the rate of synthesis and there is a rate of utilization which also includes the rate of transport if it needs to be stored somewhere.

So, there are turnover involved in secondary metabolites process. So, therefore, generally as I said one of the logical reasons can also be to drive the reaction forward.

Student: (Refer Time: 19:40).

So, that is the reason why you will find sometimes adding resins for adsorption of the product helps in increasing the rate of synthesis of the product. So, that can also be a

reason. So, now, they experience turnover processes with characteristic half lives of some of these toxic secondary metabolites. Now, degradation and synthesis often occurs simultaneously.

The extent of accumulation depends upon cell's synthetic capacity and the capacity to metabolize the component. So, when we say metabolize the components this also includes further modifying it for transport to the required places or modifying it for detoxification. Individual plant organ differs in their significance in this process. So, you will find that storage and synthesis occur in different sites.

It is and sometimes the synthesis also is compartmentalized. Now, the concentration also varies according to geographical location, climate and soil. So, it is nature dependent.

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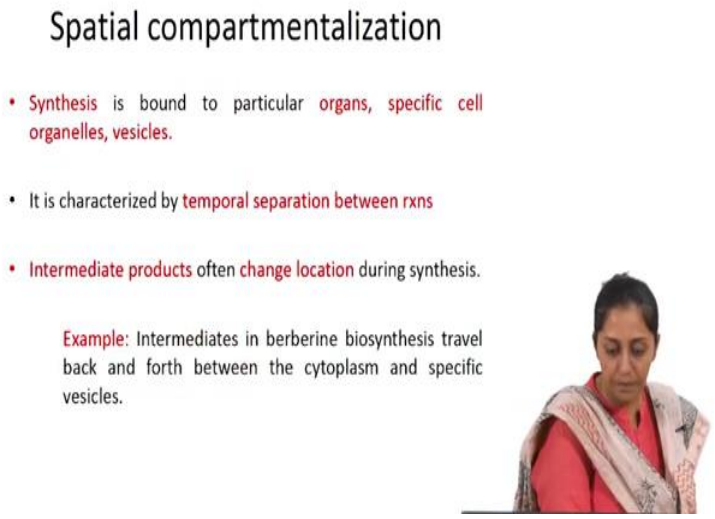

Now, spatial compartmentalization: synthesis is bound to particular organs, specific cell organelles or vesicles. So, synthesis need not be like for example, in camptothecin, there are reports and literature which say that if you try to extract camptothecin you will find that you will be able to do it best and maximum yields from the bark, but when synthesis has been observed there are literature reports which indicate that the synthesis happens in the root. So, which is an example that, the site of synthesis may be completely different from the site of storage.

So, example intermediates in berberine synthesis travel back and forth between the cytoplasm and specific vesicles. This is for an example for spatial compartmentation of the secondary metabolite.

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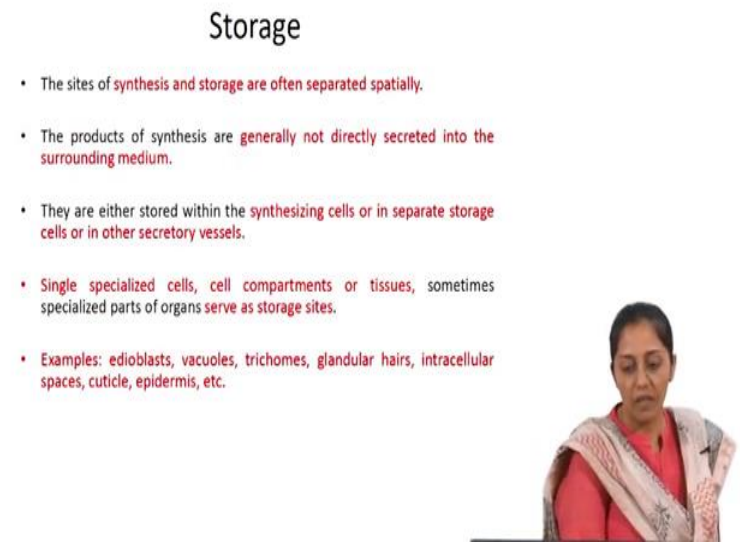

Now, for storage, there are different places and there are different forms in which the product can be stored. They can be stored on the surface – surface in the form of trichomes or even in the intracellular spaces empty spaces like for example, for Azadirachtin production, we were working on hairy roots. So, if you do a cross section of the hairy roots, you can do histochemical assay and find Azadirachtin spots are colored under the microscope and they get stored in the intracellular spaces of the root matrix.

Then, if you can see trichomes – trichomes are hair like glandular structures on the plant leaf or the stem; then edioblasts, these are specialized plant cells which are meant for storage only. So, they are only meant for storage of either pigments or wax or oils or your secondary metabolites. Then glandular hairs, trichomes, cuticle, epidermis, there are different parts or tissues or specialized organelles where you will find these secondary metabolites will get preferentially stored.

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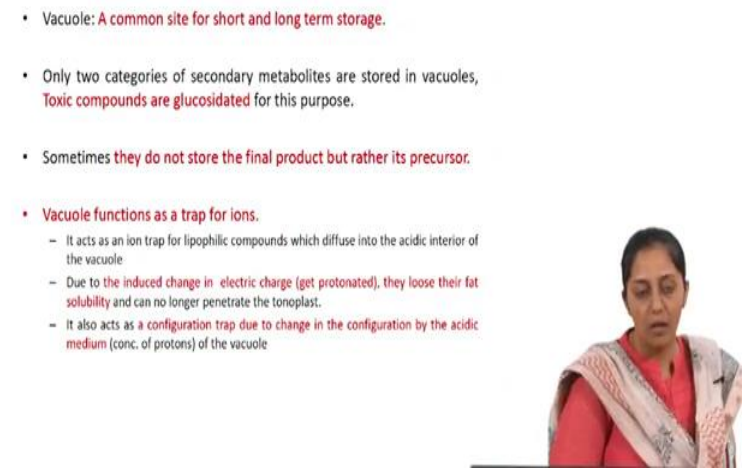

So, vacuole it is a common site for short and long term storage. The compounds are stored in vacuoles inside plant cells for protection. Now, only two categories of secondary metabolites are stored in vacuoles. Toxic compounds are glucosidated for this purpose. So, when glucose moiety is added which is one of the biotransformations for detoxification.

Sometimes they do not store the final product, but rather precursors. We have studied about it, where the enzymes for the final product are in the vicinity such that the intermediate compound as soon as the cell ruptures and comes in contact with the enzymes to give the final product which is toxic metabolites for the pathogen.

So, vacuole functions as a trap for ions. Now, how does vacuole functions as the trap for ions? When we were studying about the different organelles and their functions we studied about it. It is something to do with the tonoplast. The molecule is facilitated and it is generally not active process while entering. It enters, but going out is active or it is not allowed, how? We have studied this. How is it happening? Go back and read.

Student: Because of the ion.

But, I said it is a non-active process while coming in generally, what facilitates that?

Student: It says that interior of vacuole is acidic.

Right.

Student: Ions can come inside.

Tonoplast is what?

Is it no there is no membrane across the vacuole? There is.

Student: There is.

Membrane is made of?

Student: protein

Cell membrane.

Student: lipids.

So, what will allow easy passage? It has to be?

Student: Hydrophobic.

Ok.

Student: It has to be hydrophobic.

Think.

Student: some portions in

I heard hydrophilic also.

So, you need to think.