**Plant Cell Bioprocessing**
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**Lecture - 13**
# Secondary metabolism in plant cells -Part 2

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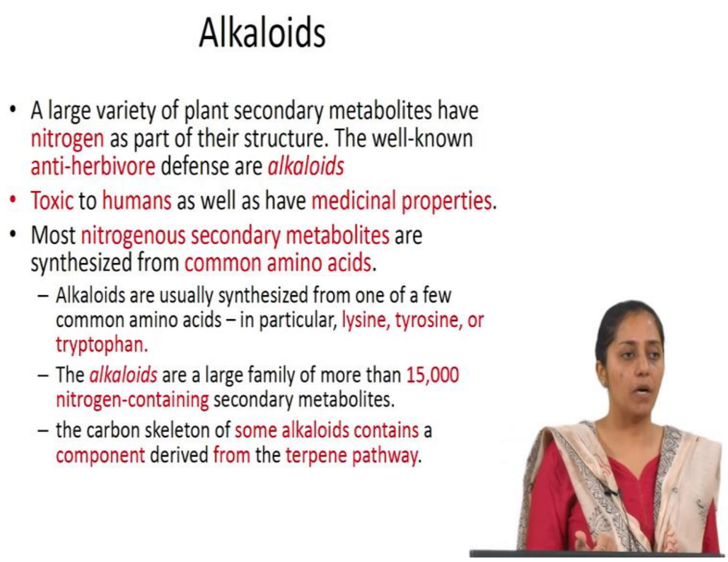


We studied about secondary metabolism, its role in plants, what are the different functions of secondary metabolites; for example, what are the different functions of secondary metabolites in plants?

Student: Defence.

One is defence.

Student: Specialised function.

In specialised functions like ripening, attracting pollinators, completing the cycle and what?

Student: To overcome competition.

To overcome competition for survival, ok. So, then we went on to see what are the major class of secondary metabolites in plants; what were the major class of secondary metabolites found in plants?

Student: Nitrogen containing compounds.

So, nitrogen containing compounds , terpenes and what?

Student: Phenolics.

Phenolics. So, we then studied in detail about these three classes; now we were at alkaloids. So, alkaloids is one of the nitrogen containing compounds, there are almost 15000 different types of alkaloids present. So, now, in these we also have other nitrogen containing compounds which are not that well known or commercially utilised, but have a function to play in the plant defence. So, talking about alkaloids, alkaloids are generally used as antiherbivore in the plants system and they are toxic to humans. Most nitrogen for secondary metabolites are synthesized from amino acids. So, for alkaloids , their backbone will be amino acids.

Majorly what three amino acids? Lysine, tyrosine or tryptophan. Now the alkaloids as I said these are almost 15000 present in the plants and in some, the carbon skeleton may also be from terpenes. So, now, alkaloids like for example camptothecin it belongs to monoterpene indole alkaloids. So, which means that it's structural moiety also has the nitrogen containing moiety as well as the terpene moiety.

So, there are many such secondary metabolites, as I mentioned in the previous class that what were the different pathways; from primary metabolism then it went on to secondary metabolism, shikimate pathway was involved, MEP pathway was involved, mevalonate pathway was involved. So, either singly these pathways are leading to products or combining; the two pathways are combining to give intermediate subsequently dealing to different types of secondary metabolites, so which means that the array is so widespread.

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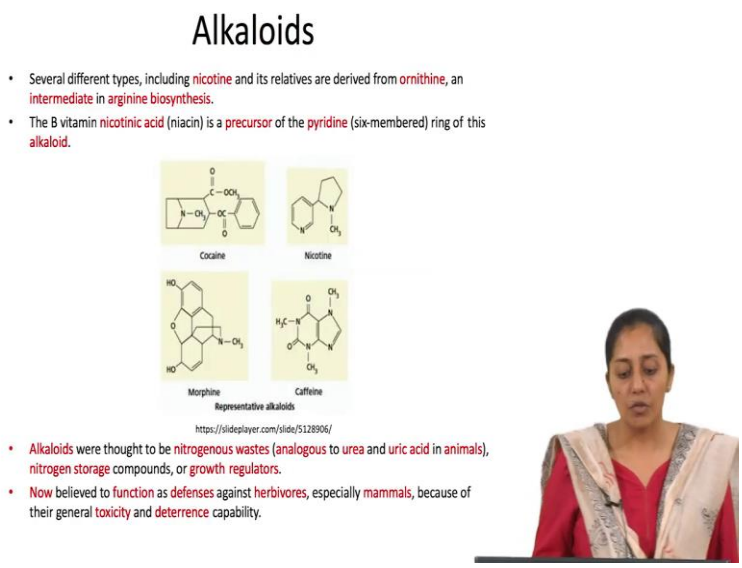


So, apart from the alkaloids which we know then there are other alkaloids where arginine for example, is involved. So, this is nicotine, your cocaine, morphine, caffeine these are also parts of alkaloids and they are used as deterrents for mammals, animals, for humans. So, they act as either as feeding deterrents; they directly act as toxins or they will disturb the digestion process of the pathogen or the herbivore.

Now, for example, your nicotinic acid, the backbone, in case of nicotine it is obtained from ornithine; ornithine is a precursor as an intermediate in arginine biosynthesis. Now ornithine is a non proteinaceous amino acid. Now B vitamin which is nicotinic acid is a precursor of the pyridine moiety of this alkaloid nicotine. So, what is pyridine moiety? The nitrogen containing ring, so it is $C_5H_5N$.

So, now this is pyridine moiety which is obtained from the nicotinic acid. Now alkaloids were thought to be nitrogenous. So, initially it was thought like that because they were nitrogen containing compounds. So, people were of the belief that either they are acting as nitrogen sources for the plant, compounds which are stored have stored nitrogen; or they are nitrogenous base like urea or uric acid in mammals or they might be acting as growth regulators.

Later it was found that they are performing higher functions of defence in the plants, by either having toxicity function or as feeding deterrents.

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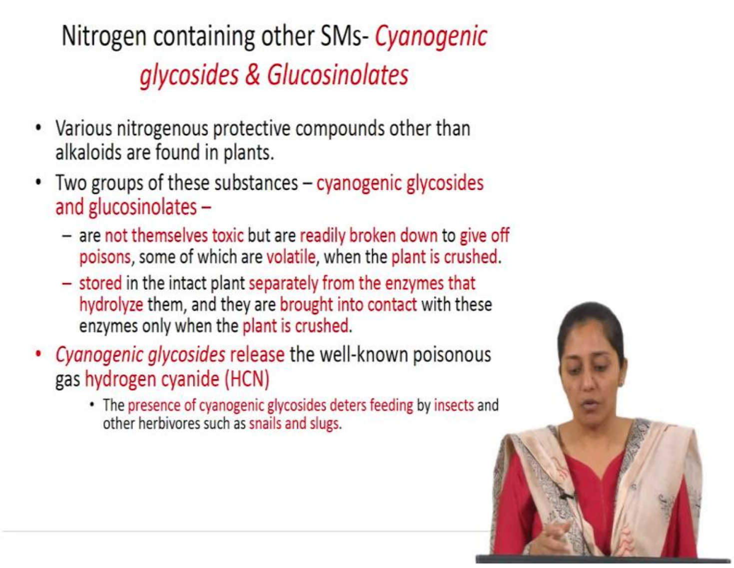


Now, nitrogen containing other secondary metabolites they are cyanogenic glycosides and glucosinolates. Now cyanogenic glycosides which means that; sometimes for the plants as we were discussing for it to become non toxic they are joint with as conjugates, with a sugar moiety, so for example, this cynogenic glycoside.

Now, once the cell gets ruptured; these conjugates will be exposed to the enzymes which are in the vicinity after hydrolysis. And the final break down to such toxic compounds which can be volatile like; for example, hydrogen cyanide, which gets released, which can either directly damage the pathogen, or can help to trigger the signal cascade mechanism in the plant itself.

So, two groups of these substances cyanogenic glycosides and glucosinolates. Now they are themselves not toxic, but are readily broken down to give off poison; for example, I was saying hydrogen cyanide; now which may be volatile when the plant is crushed. Now, they are stored in the intact plant separately from the enzymes that will hydrolyse them as I said to perform the final toxic breakdown product.

Now cyanogenic glycosides they release hydrogen cyanide which is a well-known toxic compound. Now the presence of cyanogenic glycosides deters the feeding by insects and other herbivores like snails and slugs. So, this is like it flows through generations and so it is kind of acquired deterrence.

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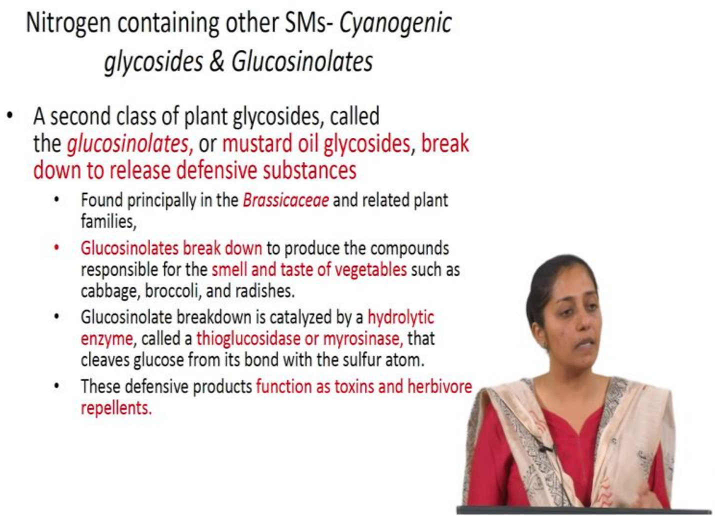


Now, the second class of plant glycosides I was talking about is glucosinolates, which are also called as mustard oil glycosides. They are present in plants like your cabbage or your radish which has a very different taste, strong taste. So, it acts like a feeding deterrent for the feeders. So, glucosinolate break down is catalysed by a hydrolytic enzyme which is thioglucosidase or myrosinase; that cleaves glucose from it’s bond with the sulfur atom.

So, it is generally present as conjugates; the conjugates may not be toxic, but the final break down may lead to the impactful compound. So, they generally lead to bad smell or a strong smell and taste in the fruits or in the plant parts.

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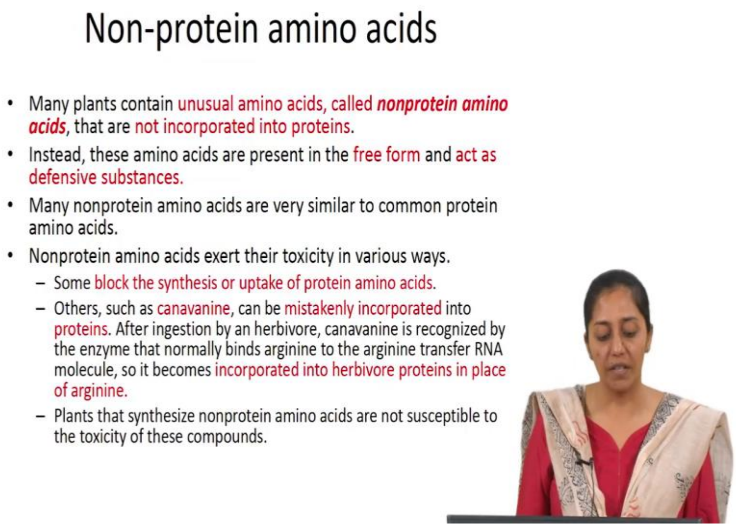


Now, non protein amino acids, many plants contain unusual amino acids which are called as non-protein amino acids; which get incorporated in the proteins. So, for example, canavanine. Sometimes these non-protein amino acids can also replace the amino acid in the metabolism of that herbivore. So, this may then disturb the metabolism of the herbivore; for example, it is written here canavanine which can replace arginine.

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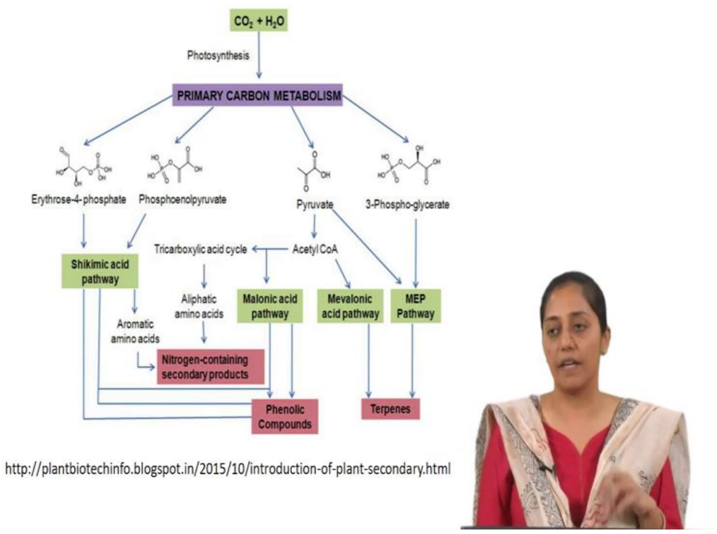

So, this is what I was talking about primary metabolism leading to secondary metabolism; in secondary metabolism the major pathways involved shikimic acid, mevalonic acid pathway, MEP pathway, which is methylerythritol phosphate pathway.

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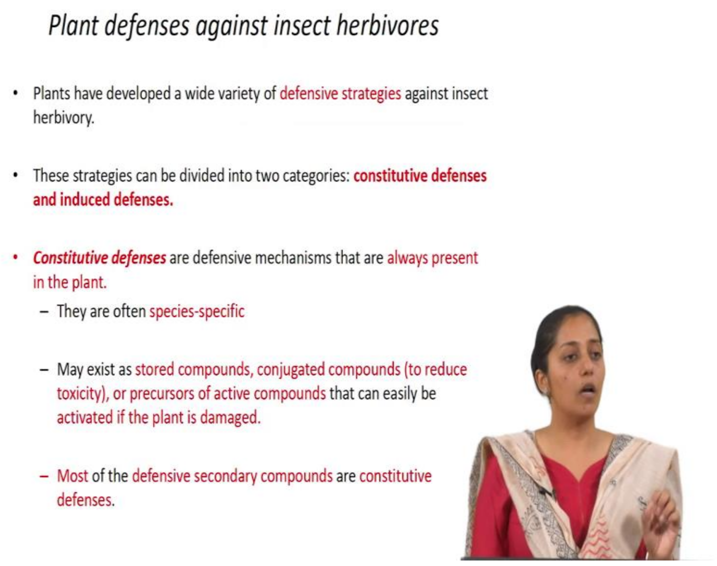


So, now we were also talking about the two different types of defence which are present in plants; one is constitutive defence and the other is induced defence. In order to conserve the recourses, the plant would not like to use all at once. So, there is first line of defence and only when needed there is a much stronger second line of defence; once it has been found that the second line of defence is needed. And what caters to this and what induces the second line of defence, we will see to that. So, where your signal cascade pathways are involved. So, let's first talk of the constitutive defence.

What is constituted defence in the plants? Now, this is species specific, the secondary metabolites which may be present inherently in that plant, may not be present in the other species. So, they may exist as stored compounds, conjugated compounds to reduce toxicity, or as precursors of the active compound which can easily be activated if the plant is damaged. Now, most of the defensive secondary metabolites are constitutive defence.

For example if you find that a particular secondary metabolite is present in lower amounts throughout the plant and irrespective of the season; then it is a constitutive line of defence, where the machinery , the enzymes needed to produce that are always present.

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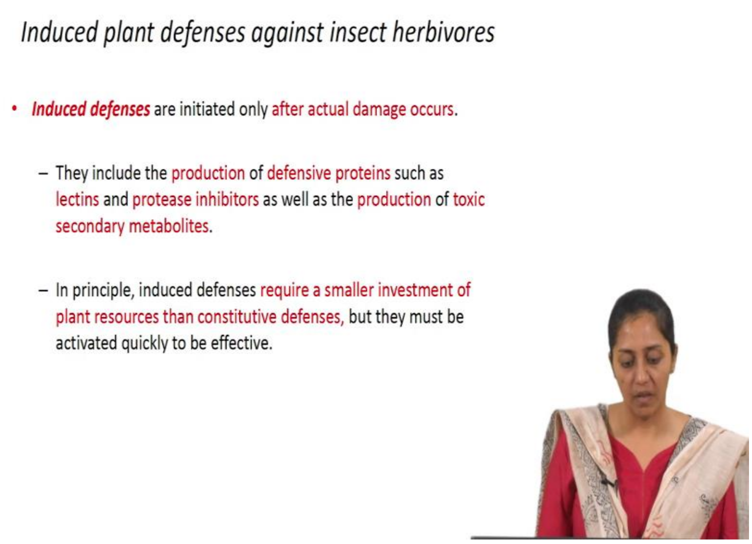


Now, induced defence on the other hand, it is initiated only after the actual damage has occurred; which means de novo synthesis happens; which means that, the proteins which are required to produce they are not present. But these compounds are only obtained de novo; which means transcription and translation begins only after the damage has happened.

But the key here, whether the plant would be able to survive; which species is stronger than the other to survive , depends on how fast the plant is producing these compounds. So, they include the production of defensive proteins such as lectins and protease inhibitors as well as production of toxic secondary metabolites. In principle, the induced defences require a small investment; obviously, because they are not produced all the time, so it is like conserving the resource of the plant, the carbon and the energy.

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Now, how do the plants recognize? Plants recognize specific components in case of insects; they recognize specific components of insects which may also include saliva. So, now, the plant response to the damage by insect herbivores, it involves both the wound response and also the response towards insect bearing components.

Now, the recognition of certain insect derived compounds which we class as elicitors. Now these elicitors once recognize and they lead to induction of the signal cascade in the plants. Repeated mechanical wounding can also induce responses similar to that caused by the insect herbivory. Now molecules like insect saliva can act as enhancers or elicitors; elicitors are nothing, but stimulus to the defence, second line of defence.

Now plants recognise these elicitors and activate a complex signal transduction pathway. One of the products of this complex signal transduction pathway is jasmonic acid. Now, this jasmonic acid is produced through the phloem and it flows to the different parts of the plant, sometimes it gets conjugated as methyl jasmonate that is it gets methylated and becomes volatile; such that this becomes a signal for the nearing plants and they all flow through the phloem to different parts of the plant. So, insect derived elicitors can trigger the signalling pathways systematically, initiating defensive responses in distant regions of the plant or even the nearing plants.

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So, the major signalling pathway as I said is the jasmonic acid formation pathway which is called as octadecanoid pathway. Now in octadecanoid pathway it leads to the production of the plant hormone which we know as jasmonic acid. Now jasmonic acid levels steeply rise upon the pathogen attack, herbivore attack and trigger the production of many proteins which are called as pathogen related proteins. Now these proteins form the part of signal cascade mechanism.

So, either these proteins will directly be functioning as chitanases, or hydraulic enzymes or proteases which may damage the pathogen or they may induce the proteins in the biosynthetic pathway of the second line of defence; such that the production of much stronger and much specific secondary metabolites begins in the plants. So, two organelles which are involved in jasmonic acid formation; they are chloroplast and peroxisome, just for information.

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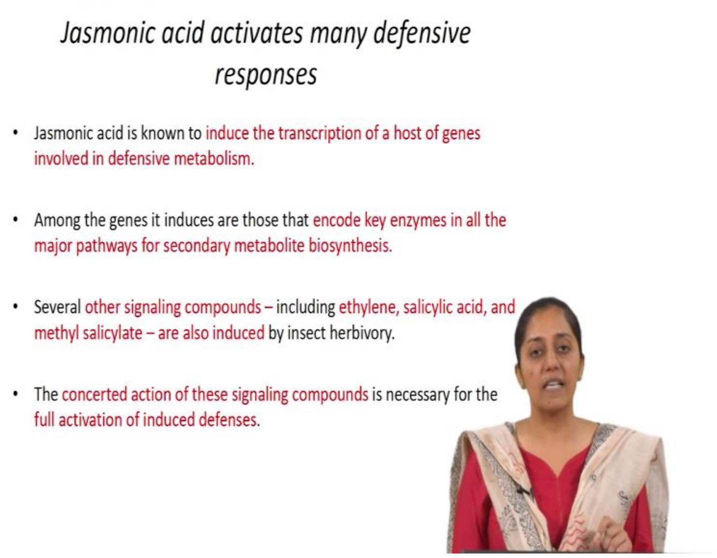


Now, jasmonic acid, it is known to induce the transcription of host of genes involved in the defensive mechanism. So, it is like umbrella or a big. So, it’s effect is very widespread and a number of transcription factors, a number of enzymes, expression get affected by jasmonic acid. Amongst the genes it induces those that encode key enzymes in all the major pathways for secondary metabolite biosynthesis.

So, therefore, you notice that when plant cell bioprocess is optimised; jasmonic acid, salicylic acid which are part of these signal cascades are very well known elicitors used in plant biotechnology for enhancing the yield of the secondary metabolite; because their mode of action is so widespread that they are general elicitors and they are not species specific. So, in most of the cases if you add they work. Several other signalling compounds like ethylene; ethylene is also a plant hormone, but it also acts as a signalling molecule to induce secondary metabolism.

So, ethylene, salicylic acid, methyl salicylate; so methylated these components can become volatile which may act as volatile signals to the other parts of the plant or for the nearby plants. The concerted action of these signalling compounds is necessary for the full activation of induced defences. So, it is not that only once at a time this would happen. So, it is not that only jasmonic acid would be produced . Its because that everything is a part of that signal cascade. So, everything gets produced and everything in turn then induces a different line of defence. So, therefore, the plant is able to have such strong multiple mode of action based defence against the pathogens.

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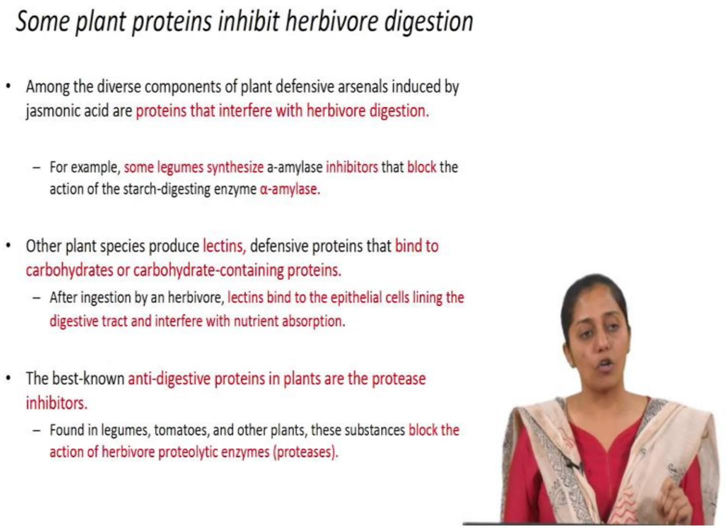


Now, among the diverse components of plant defensive arsenals, proteins that interfere with the herbivore digestions are also induced by jasmonic acid; like for example, legume synthesise, alpha amylase inhibitors. Now, this blocks the action of alpha amylase. Alpha amylase will be for starch so; obviously, it would block those pathogens to this kind of plant where the starch is stored and which would certainly have alpha amylase to utilise that as carbon source. So, therefore, they will produce alpha amylase inhibitors, so as to deter the feeding.

Other plant species produce lectins which are defensive proteins that bind to carbohydrates or carbohydrate containing proteins. Now in this what happens; they will bind to your epithelial cells of the digestive system and therefore, disturb the digestion of the food and that is also one of the ways for feeding deterrents.

Now the best known anti digestive proteins in plants are the protease inhibitors. So, that is like alpha amylase inhibitors, it is a protease inhibitor which the plants release.

Some of the plants you must have heard, our parents say stay away from it; I do not know whether you have heard. So, that is because these toxins are nothing, but secondary metabolites which may either directly act as toxins or may disturb the digestion of your system.

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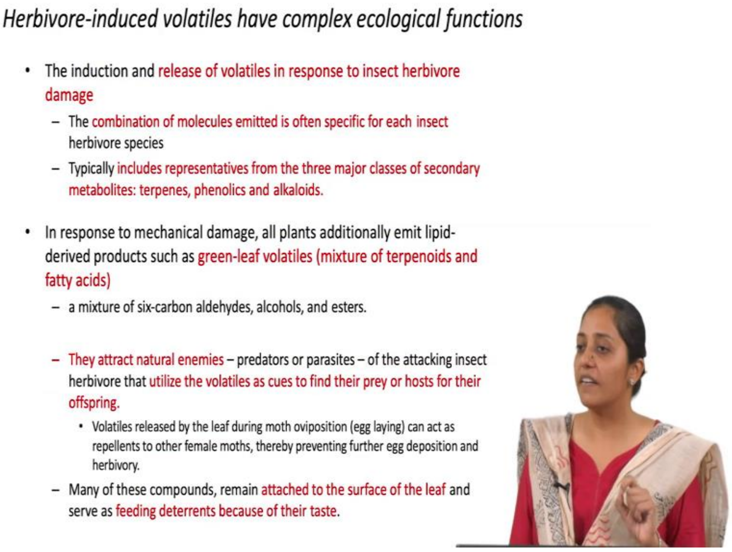


The induction and release of volatiles in response to insect herbivore damage. Now the combination of molecules emitted is often specific for each insect herbivore, there can be so many different types of pathogens. So, either the plant should have a very generalized strong mode of action, but you will observe that sometimes the insects are also very specific to a particular variety of the plant; like for example, your Bt cotton.

So, it is always against a particular pest, which is generally known to attack that plant. So, you need that pest specific toxin. So, plants are also known to secrete or produce insect specific toxins and some are general. Now, typically it includes representatives from these three major classes; where terpenes, phenolics, and alkaloids are involved. Now, there are other forms of secondary metabolites which are called as green leaf volatiles. The green leaf volatiles are mixtures of terpenoids and fatty acids.

Now, you will also find that many of these are present near the leaf surface or will be present in the membranes of the cells. Because that is the first line which will be broken; anything which would try to attack would first try to lyse the cell through piercing through the cuticle, which is one of the barriers; then through the cell wall, so once it crosses cell wall, then comes to the cell membrane.

So, therefore, these secondary metabolites which can deter or which can be toxins are sometimes present on these surfaces; including your cuticle, leaves surface or your bark, or even your cell membranes like saponins are known to be present near the membranes.

So, green leaf volatiles attract the natural enemies of the pathogens. So, because they are volatiles they will send cues to the predators of these pathogens, who will then find out that through this cues that where the host is and this is how the plants operate. So, which means how intelligent is the machinery and how widespread is the plant defence in what different ways it protects, it is very interesting. They attract natural enemies which are predators or parasites of the attacking insect herbivore that utilize the volatiles as cues to find their prey or host, as I said.

Like there is an example, it is given here that moths when they lay eggs on the leaves. So, now, in order to deter further laying of eggs by other moths; moths produce such volatile compounds which will then give signals to the other moths, in these different ways not to come to that leaf or that plant for further egg laying. So, many of these compounds remain attached to the surface of the leaf and serve as feeding deterrents because of their taste; something the taste would be so bad that they will deter the feeding even to us, we do not prefer some fruits which are of bad taste.

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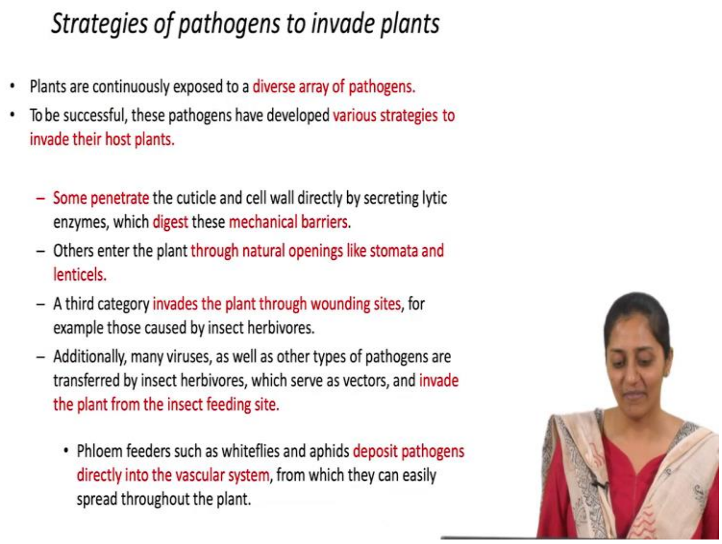


So, plants are continuously exposed to diverse array of pathogens. Now to be successful, these pathogens have developed various strategies, so both the sides the fight is going on;

the plant is increasing it's defence and the pathogens are also clever enough to create ways, new ways of breaking this line of defence. So, some penetrate through the cuticle, the cell wall directly by secreting lytic enzymes which can digest these mechanical barriers.

So, if you know why is something happening in nature; obviously, you can get cues of what can be the potential applications of these. So, if you know that these pathogens are capable of producing lytic enzymes which can break down the plant cell wall; obviously, they would have enzymes which might be capable of lignin degradation, or cellulose degradation, hemicellulose degradation which can give you cues for its other applications. So, it is very important to understand, why is something happening in literature I mean in nature. Now, which digest these mechanical barriers?

Now others enter the plant through natural openings, stomata we know is one of the natural openings; it is also written that lenticels. Now lenticels, if you see some of these plants have a very rough surface; you go and type it on Google you will be able to see, that these are open spaces, specially in tree species. Because in tree species, there are many layers of cortex and all, so gas exchange is difficult. So, there are open spaces kept in the plant in the trunk which can lead to this gas exchange. Now these open spaces although they are meant for gas exchange, they also become an opening for pathogens to enter. So, there is always a balancing out between the merit and the demerit.

A third category invades the plant through wounding sites, whenever there is a wound this may enter; now, for example, those caused by the insect herbivores. Additionally, many viruses may enter also. Some of the insects they; suppose for even nectar, they pierce through the vascular bundle to get the food. Now, while they have pierced these vascular bundles for food, the virus will enter and then spread throughout the plant.

Now phloem feeders, for example, whiteflies and aphids. They deposit pathogens directly into the vascular system and from which they can easily get transmitted to the entire plant. So, now, plant has to cater to this problem also.

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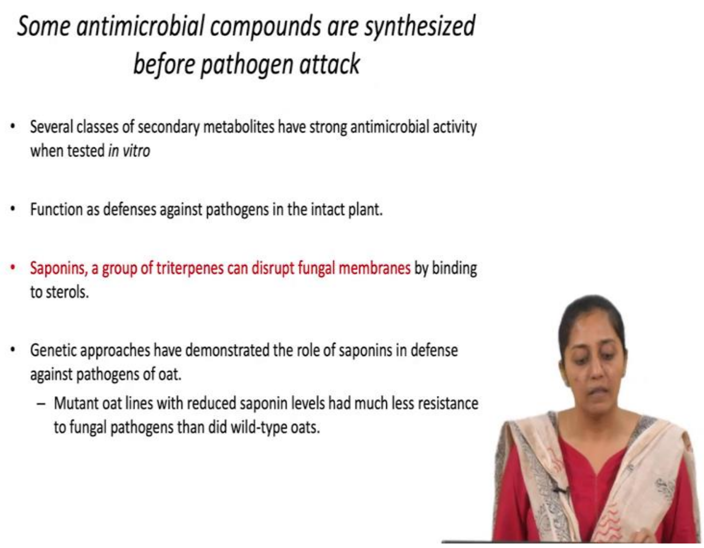


Now, several classes of secondary metabolites have strong antimicrobial activity. So, generally because most of it is for fungus, or for bacterial species, so there is majority of secondary metabolites you will find that; they will be having antimicrobial activity. Now saponins, is a group of triterpenes that disrupts fungal membranes by binding to sterol; sterols is a part of the phospholipid membranes. So, it disrupts the sterol in the membranes of fungal cultures.

Genetic approaches have demonstrated for example, here; in oat cell lines which were mutated to reduce the production of saponins, it was found that they were they became more susceptible to fungal attack; than the mutant lines which had higher amount of saponins.

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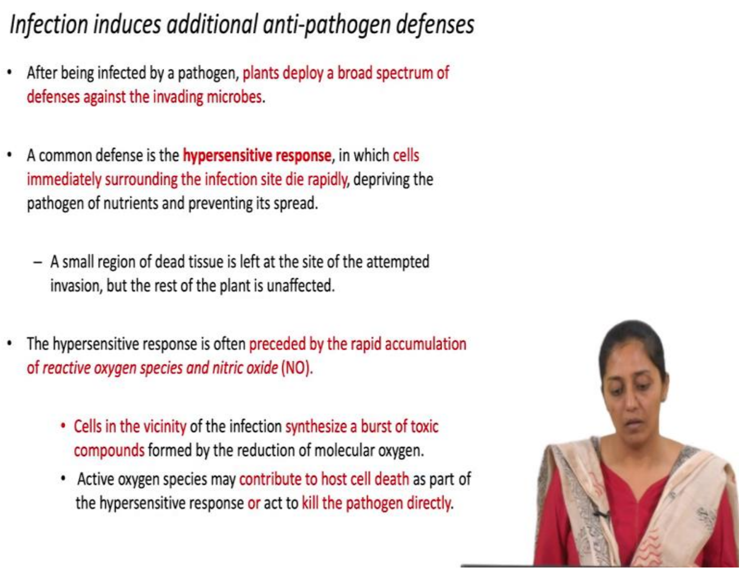


Now, after being infected by a pathogen, plant deploy a broad spectrum of defences against the invading microbes. Now, the common defence is called as a hypersensitive response. What is this? As soon as the pathogen attacks, a part of the plant, the nearing cells they die immediately. Now once they die, is to prevent any nutrients to be provided to that pathogen which has attack, so; which means that, it tries to deprive that pathogen of the nutrients.

So, there is a sacrifice involved. So, nearing the wounding site these cells die. And how do they die? As soon as the attack happens, there is a surge of toxic gases like nitric oxide accumulation or there will be higher accumulation of oxygen radicals, reactive oxygen species which will cause the death of the cells and this may also in turn help in direct damage to the pathogen also.

So, cells in the vicinity of the infection synthesize a burst of toxic compounds formed by the reduction of molecular oxygen, reactive oxygen species. Now active oxygen species may contribute to host cell death, as part of hypersensitive response or act to kill the pathogen directly.

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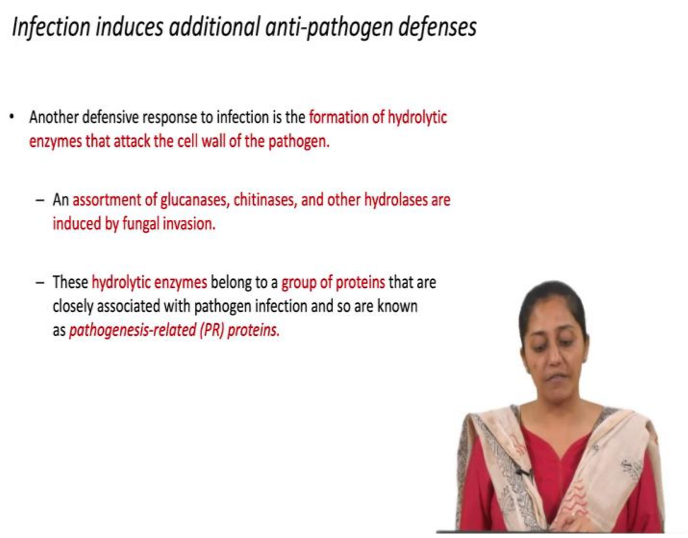


Now, another defensive response is formation of hydraulytic enzymes that attack the cell wall of the pathogen itself; like for example, chitinase in case of fungal cell walls or can even be your hydrolases, or your glucanases, or what else proteases. So, there can be different types of enzymes, lytic enzymes which are produced which can directly harm the pathogen. So, these hydrolytic enzymes belong to a group of proteins that are closely associated with the pathogen and they are called as pathogen related proteins, PR proteins.

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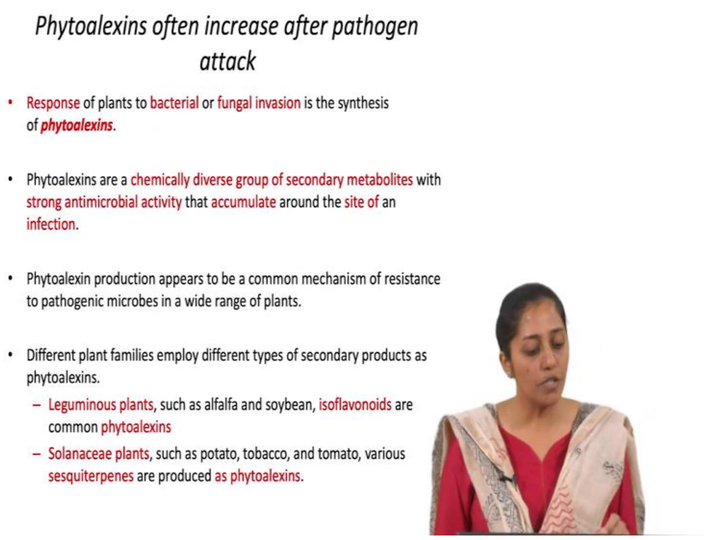

Now, response of the plants to bacterial and fungal invasion is the synthesis of phytoalexins. Now phytoalexins are not always present as the constitutive defence; but are produced as a result of the damage caused by a particular pathogen. Now, phytoalexins are chemically diverse group of secondary metabolites with strong antimicrobial activity that accumulate around the site of infection.

So, which means; now the speed matters, the speed at which the de novo synthesis of these phytoalexins will be taking place as soon as the damage has happened. So, that determines the survival capability of a particular plant species against that pathogen. So, that is why you will see that some plants survive and the others do not; because of the defence, how strong, how fast is the defence in a particular plant species against that particular pathogen.

Now, phytoalexin production appears to be common mechanism of resistance to pathogenic microbes in wide range of plants. Now for example, in leguminous plants such as alfalfa and soybean, isoflavonoids are known to act as phytoalexins. In Solanaceae plants which is; potato, tomato, or tobacco, various sesquiterpenes are produced as phytoalexins.

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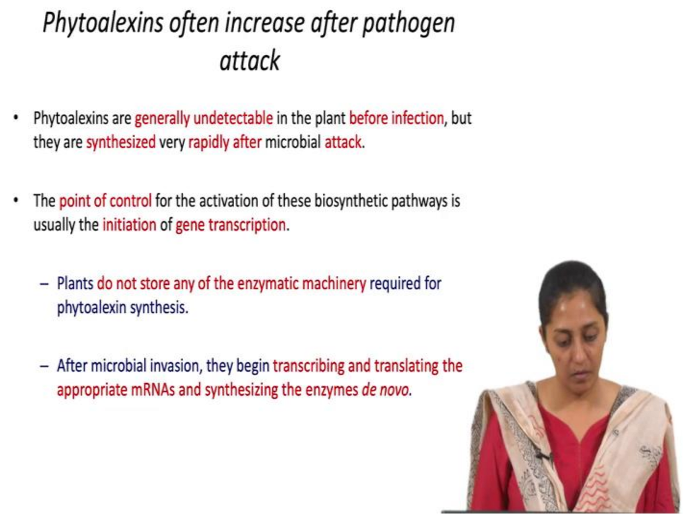


Now, phytoalexins as I said are generally undetectable when there is no attack; they begin to form at higher amounts or at very large amounts at fast rate once the attack has happened. So, the point of control for the activation of these biosynthetic pathways is usually the initiation of gene transcription. Now for this, de novo synthesis is involved. So, a signal cascade mechanism is involved, where your jasmonic acid, or salicylic acid, your ethylene everything is involved.

So, plants do not store any of the enzymatic machinery required for phytoalexin synthesis. After microbial invasion they begin transcribing and translating the appropriate mRNA for that particular synthesis.