Plant Cell Bioprocessing
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Lecture – 19
Optimization strategies- Part 4

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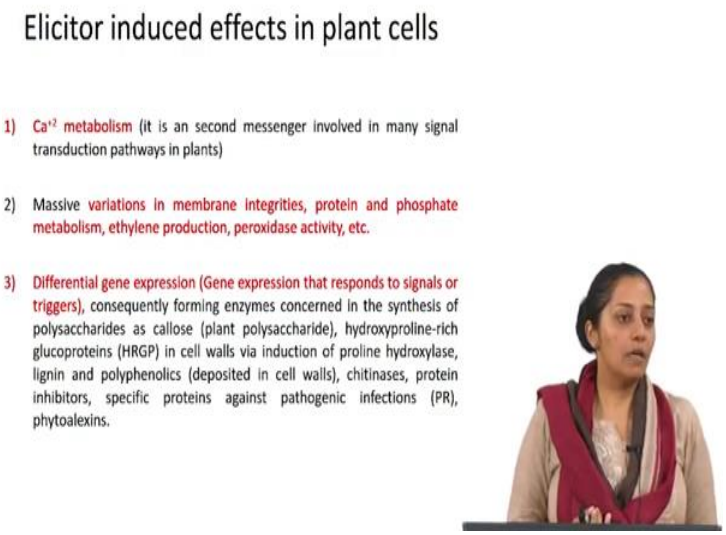

So, just to brush up, elicitors induced effects in plant cells include effect on calcium metabolism, because calcium is used as a second messenger and can play a role in transcription of certain proteins which may involve their participation in the secondary metabolism. So therefore, calcium metabolism can indirectly affect the secondary metabolite biosynthesis.

Now, there are reports where there are set receptors in the plasma membrane of plant cells, where these elicitors bind to. Once they bind there is a signal cascade mechanism which can lead to cyclic AMP formation and cyclic AMP burst leads to further calcium ion concentration increase in the cytoplasm. Which then binds to proteins thereby then affecting the transcription of certain proteins responsible for secondary metabolism.

So, massive variations in membrane integrities might happen, because of elicitors, protein and phosphate metabolism might happen, when I say protein or phosphate protein you can directly make out. And even with ions the permeability of the membrane may get disrupted, because of which the electrochemical gradient can get disrupted. And

because of the change in the ionic concentration, may be the pH can lead to further enhancement in the secondary metabolism or direct release of reactive oxygen species and differential gene expression where gene expression responds to signals or triggers.

So now, consequently enzymes concerned in the synthesis of polysaccharides, which means plant cell wall, or hydroxyproline-rich glucoproteins in the cell walls via induction of proline hydroxylase act. So, these are some examples by which different types of elicitors have been found to play a role and induce secondary metabolism.

Then polyphenolics which can deposit in cell walls may get altered. Then chitinases, these are certain enzymes or PR proteins. If you remember when we had studied they play a role in secondary metabolism and they are specific proteins against pathogenic infection. This is what is PR proteins, phytoalexins.

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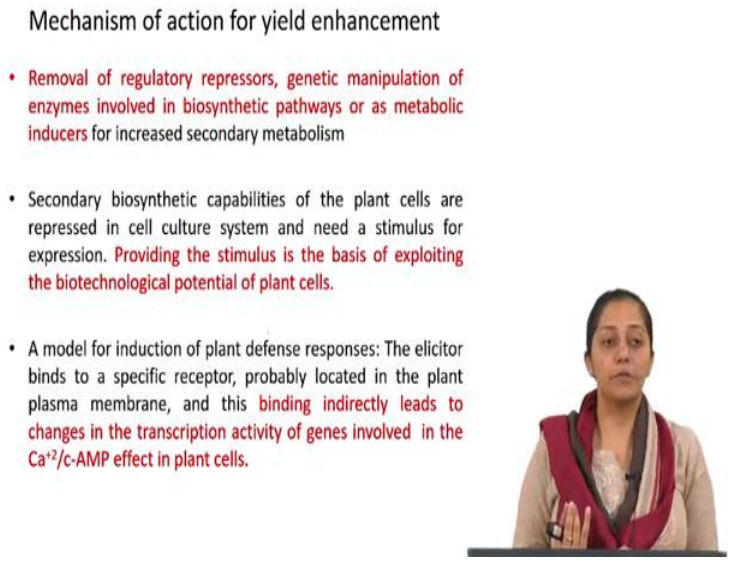

Now mechanism of action: Removal of regulatory repressors, genetic manipulation of the enzyme itself, which means that their expression level can get changed which may be involved in biosynthetic pathways as metabolic inducers for increase in secondary metabolism. Then they can provide stimulus which is the basis for exploiting the biotechnological potential of these plant cells. Then when we say stimulus; stimulus means in the form of over expression of the genes or the encrypted genes, because of the repressors they may get induced so these are the different ways.

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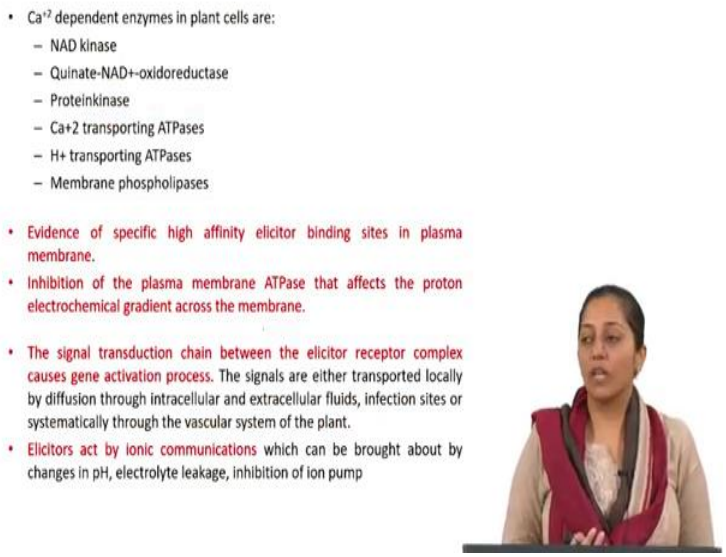

So, these are just example enzymes which get altered by the calcium ion concentration in the cell. NAD kinase then NAD oxidoreductase protein kinase. The protein which I was talking about where the calcium gets binds, these are generally kinases. Then calcium transport ATPases, ATPases also get affected because of which the electrochemical gradients will get affected, then the membrane phospholipases.

So, evidence of specific high affinity elicitor binding site, as I said in plasma membrane is available. Inhibition of the plasma membrane ATPase that affects the proton electrochemical gradient across the membrane. Therefore, the signal transduction chain between the elicitor receptor complex causes the gene activation process; one of the ways is calcium cyclic AMP pathway.

The signals are either transported locally by diffusion through intracellular or extracellular fluids, infection sites or they can be systematically transferred through your xylem elements. Which you remember we were talking about when we were discussing secondary metabolism in plants, that salicylic acid, ethylene or jasmonic acid were formed near the point of infection and then the signals were sent through xylem to other parts of the plant.

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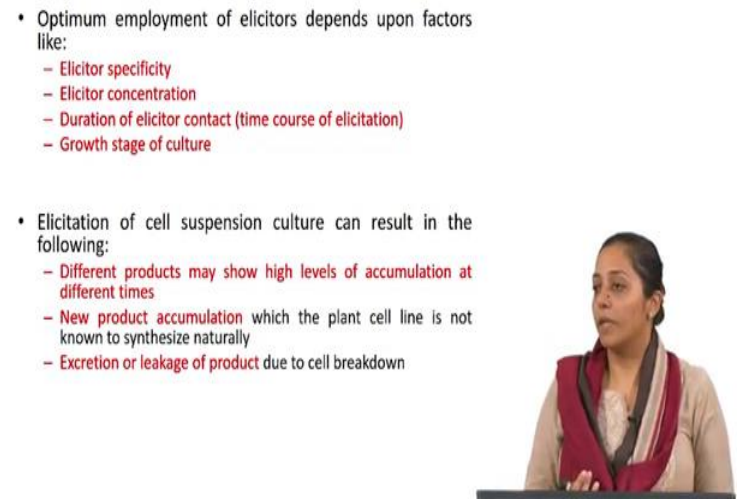

So, what is important when we are optimising elicitors is, what kind of elicitors what concentration and elicitor specificity. If an elicitor is working well or leading to maximum enhancement in a particular species there is no guarantee that it will work well with all the other species. So, there is elicitor specificity involved, elicitor concentration is one of the factors which has to be optimised. Duration of elicitor contact is crucial because these being elicitors, they are affecting the cell viability.

So, it has to be taken into account the exposure time, maybe if you expose it for a longer duration it may affect the cell viability and growth of the culture in turn leading to lesser productivity. Even if as I was discussing in the earlier classes, the ultimate aim is to maximize productivity, then growth stage of the culture, so, this indirectly involves the time of addition of elicitor.

So, elicitation of cell suspension culture can result in the following things: Different products may show high levels of accumulation at different times, if there are more than one product forming it is not necessary that all the products will get induced at the same time.

So, new product accumulation which might happen; as I said I give you an example of cyclotides, some of the novel cyclotides came up in the *in vitro* cultures which could be because of the stress under the *in vitro* conditions. Because you are growing them in a defined media under controlled conditions which could have been a stress to the plant

cells, leading to induction of certain genes or expression of certain genes which were encrypted under normal conditions. Then excretion or leakage of the product due to cell breakdown; it is not recommended that the elicitor should be used for product secretion you have set strategies, what was that which we studied in the last class, if you want the product to be out?

Student: Cell permeability enhancers.

Cell permeability enhancers; elicitors may lead to break down, but then that leads to compromise in the biosynthetic potential of the cell, the growth rate will be compromise or the viability of the cell.

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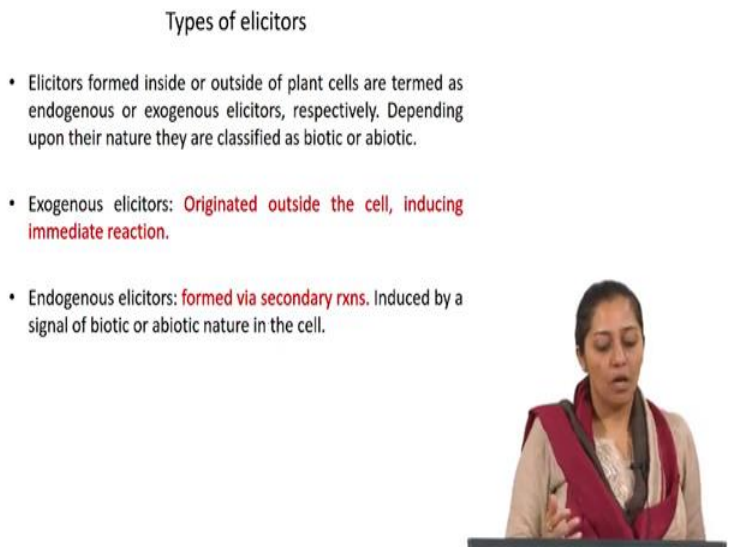

Now, what are the different types of elicitors: one which are originated outside the cell, which induces immediate reactions and these endogenous elicitors which are formed by secondary reactions. Which means once the infection has taken place outside then the subsequent changes or the signal transduction happens also form the class of secondary metabolites. These are called as endogenous elicitors. Exogenous elicitors impact the cell first.

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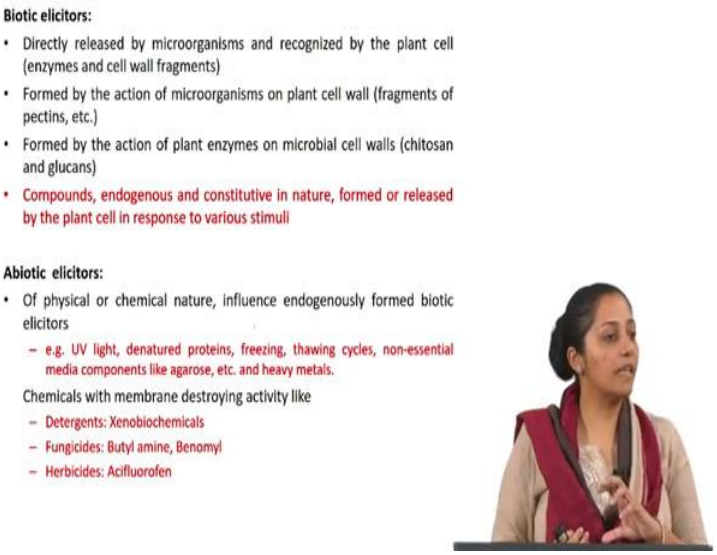

Now, even in that there are two classes, one is biotic elicitors and the other is abiotic elicitors depending on their origin. Now, biotic elicitors, these are directly released by microorganisms and recognised by the plant cells enzyme such as, cell wall fragments. Formed by the action of microorganisms on the plant cells like for example, fragments of pectins.

Then formed by the action of plant and enzymes on microbial cell walls, then if it is a fungus, then you can see chitin or carbohydrates cell wall components. Then compounds endogenous and constitutive in nature formed or released by the plant cell in response to various stimuli. Abiotic elicitors can be of physical or chemical in nature and they influence endogenously formed biotic elicitors.

So, the abiotic elicitors are physical factors such as UV light, then you freeze suddenly and then thaw, freeze thaw cycle can be a stress. Then medium components like agarose, agri gel like for example, I told you, once you bring in the culture under *in vitro* conditions you are growing it in the presence of such components, which may cause an impact on the secondary metabolism.

Now, chemicals which can disrupt membrane, for example your detergents, fungicides, herbicides anything which can cause stress.

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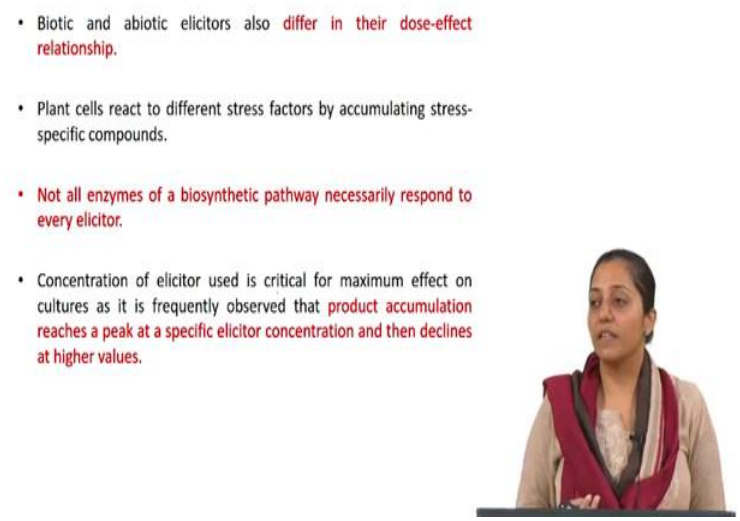

Now, biotic and abiotic elicitor, they differ in their dose effect relationship what does that mean; which means, concentration optimisation is necessary, at what concentration it is you cannot generalize for every biotic or abiotic elicitor. Because they are specific, they are concentration dependent, exposure time dependent, so optimisation is necessary. Not all enzymes as I said earlier will get affected by a particular elicitor, a particular elicitor might be affecting one particular enzyme, another elicitor might affect some other enzyme in the pathway.

Then concentration of the elicitor used is critical for maximum effect on the cultures. Product accumulation : Generally what is observed is as you would increase elicitor concentration, the product would increase, but beyond a certain concentration it will decrease, why?

Student: Inhibition due to toxicity

Right, because it is balancing between the yield enhancement and the toxic effect on the cell.

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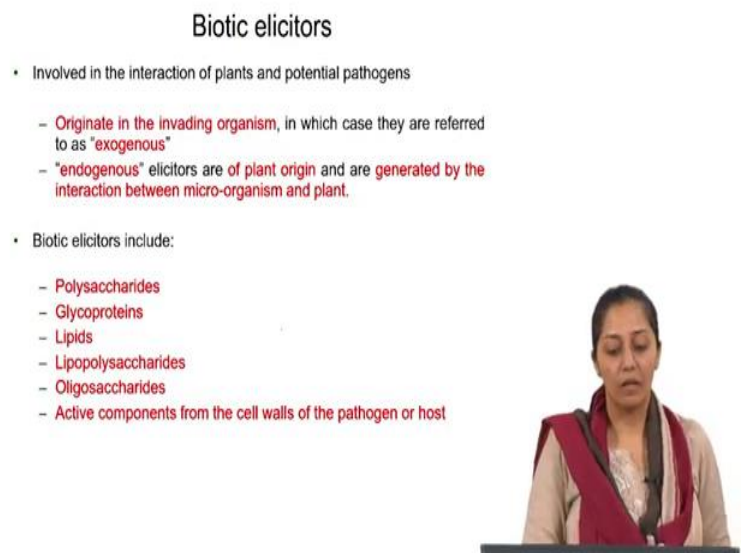

So, biotic elicitors are which originate in the invading organism in which case they are referred as exogenous. Endogenous elicitors, which are biotic in nature can be of plant origin and are generated by interaction between the microorganism; which means the pathogen and the plant which may be plant components. Then biotic elicitors; for example, polysaccharides, glycoproteins, lipids, lipopolysaccharides, oligosaccharides, any active component which is coming from of biological origin. If endogenous is coming after the attack from the plant cell itself. If coming from the pathogen, it is exogenous, but a biotic elicitor.

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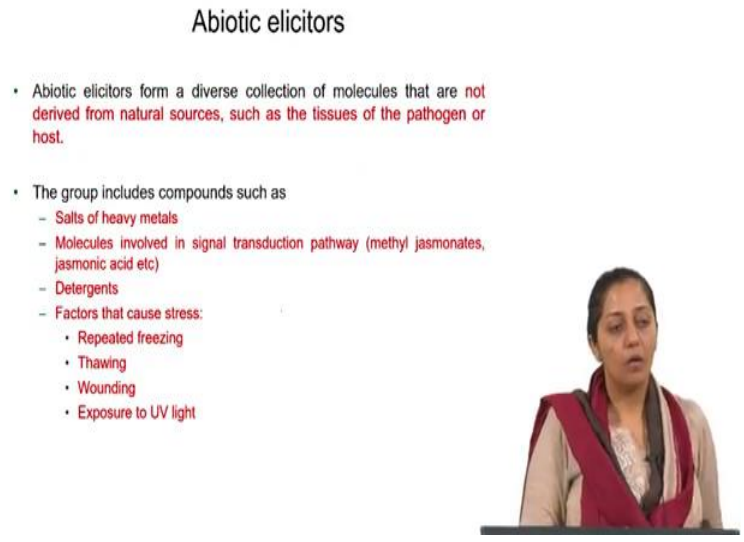

What are abiotic elicitors? Diverse collection of molecules that are not derived from natural sources, such as tissues of pathogen. These are from natural sources of biological origin. But abiotic for example, salts of heavy metals, these are very frequently used in literature as abiotic elicitors, molecules involved in signal transduction pathway.

If synthetically and exogenously add them they are considered as abiotic elicitors; for example, salicylic acid, jasmonic acid if you add them exogenously. Then detergents, factors that can cause stress physical factors such as repeated freezing, thawing cycles, wounding - creating wounds, then exposure to UV light, temperature shock which is heat shock can be a stress.