

Plant Cell Bioprocessing
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Lecture – 19
Optimization strategies- Part 4

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Elicitor induced effects in plant cells

- 1) **Ca²⁺ metabolism** (it is an second messenger involved in many signal transduction pathways in plants)
- 2) **Massive variations in membrane integrities, protein and phosphate metabolism, ethylene production, peroxidase activity, etc.**
- 3) **Differential gene expression** (Gene expression that responds to signals or triggers), consequently forming enzymes concerned in the synthesis of polysaccharides as callose (plant polysaccharide), hydroxyproline-rich glucoproteins (HRGP) in cell walls via induction of proline hydroxylase, lignin and polyphenolics (deposited in cell walls), chitinases, protein inhibitors, specific proteins against pathogenic infections (PR), phytoalexins.



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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Mechanism of action for yield enhancement

- Removal of regulatory repressors, genetic manipulation of enzymes involved in biosynthetic pathways or as metabolic inducers for increased secondary metabolism
- Secondary biosynthetic capabilities of the plant cells are repressed in cell culture system and need a stimulus for expression. Providing the stimulus is the basis of exploiting the biotechnological potential of plant cells.
- A model for induction of plant defense responses: The elicitor binds to a specific receptor, probably located in the plant plasma membrane, and this binding indirectly leads to changes in the transcription activity of genes involved in the Ca^{+2} /c-AMP effect in plant cells.



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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- Ca^{2+} dependent enzymes in plant cells are:
 - NAD kinase
 - Quinate-NAD⁺-oxidoreductase
 - Protein kinase
 - Ca^{2+} transporting ATPases
 - H^{+} transporting ATPases
 - Membrane phospholipases
- Evidence of specific high affinity elicitor binding sites in plasma membrane.
- Inhibition of the plasma membrane ATPase that affects the proton electrochemical gradient across the membrane.
- The signal transduction chain between the elicitor receptor complex causes gene activation process. The signals are either transported locally by diffusion through intracellular and extracellular fluids, infection sites or systematically through the vascular system of the plant.
- Elicitors act by ionic communications which can be brought about by changes in pH, electrolyte leakage, inhibition of ion pump



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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- Optimum employment of elicitors depends upon factors like:
 - Elicitor specificity
 - Elicitor concentration
 - Duration of elicitor contact (time course of elicitation)
 - Growth stage of culture
- Elicitation of cell suspension culture can result in the following:
 - Different products may show high levels of accumulation at different times
 - New product accumulation which the plant cell line is not known to synthesize naturally
 - Excretion or leakage of product due to cell breakdown



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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Types of elicitors

- Elicitors formed inside or outside of plant cells are termed as endogenous or exogenous elicitors, respectively. Depending upon their nature they are classified as biotic or abiotic.
- Exogenous elicitors: **Originated outside the cell, inducing immediate reaction.**
- Endogenous elicitors: **formed via secondary rxns.** Induced by a signal of biotic or abiotic nature in the cell.



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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Biotic elicitors:

- Directly released by microorganisms and recognized by the plant cell (enzymes and cell wall fragments)
- Formed by the action of microorganisms on plant cell wall (fragments of pectins, etc.)
- Formed by the action of plant enzymes on microbial cell walls (chitosan and glucans)
- Compounds, endogenous and constitutive in nature, formed or released by the plant cell in response to various stimuli

Abiotic elicitors:

- Of physical or chemical nature, influence endogenously formed biotic elicitors
 - e.g. UV light, denatured proteins, freezing, thawing cycles, non-essential media components like agarose, etc. and heavy metals.
- Chemicals with membrane destroying activity like
 - Detergents: Xenobiochemicals
 - Fungicides: Butyl amine, Benomyl
 - Herbicides: Acifluorfen



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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- Biotic and abiotic elicitors also differ in their dose-effect relationship.
- Plant cells react to different stress factors by accumulating stress-specific compounds.
- Not all enzymes of a biosynthetic pathway necessarily respond to every elicitor.
- Concentration of elicitor used is critical for maximum effect on cultures as it is frequently observed that product accumulation reaches a peak at a specific elicitor concentration and then declines at higher values.



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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Biotic elicitors

- Involved in the interaction of plants and potential pathogens
 - Originate in the invading organism, in which case they are referred to as "exogenous"
 - "endogenous" elicitors are of plant origin and are generated by the interaction between micro-organism and plant.
- Biotic elicitors include:
 - Polysaccharides
 - Glycoproteins
 - Lipids
 - Lipopolysaccharides
 - Oligosaccharides
 - Active components from the cell walls of the pathogen or host



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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Abiotic elicitors

- Abiotic elicitors form a diverse collection of molecules that are not derived from natural sources, such as the tissues of the pathogen or host.
- The group includes compounds such as
 - Salts of heavy metals
 - Molecules involved in signal transduction pathway (methyl jasmonates, jasmonic acid etc)
 - Detergents
 - Factors that cause stress:
 - Repeated freezing
 - Thawing
 - Wounding
 - Exposure to UV light



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.