Plant Developmental Biology
Prof. Shri Ram Yadav
Department of Biotechnology
Indian Institute of Technology, Roorkee

Lecture - 19
Cell-Cell Communication during Plant Development

Welcome back to the Plant Developmental Biology. So, this is going to be the final theory class of this course. Here we are going to study Cell-Cell Communication during Plant Development.

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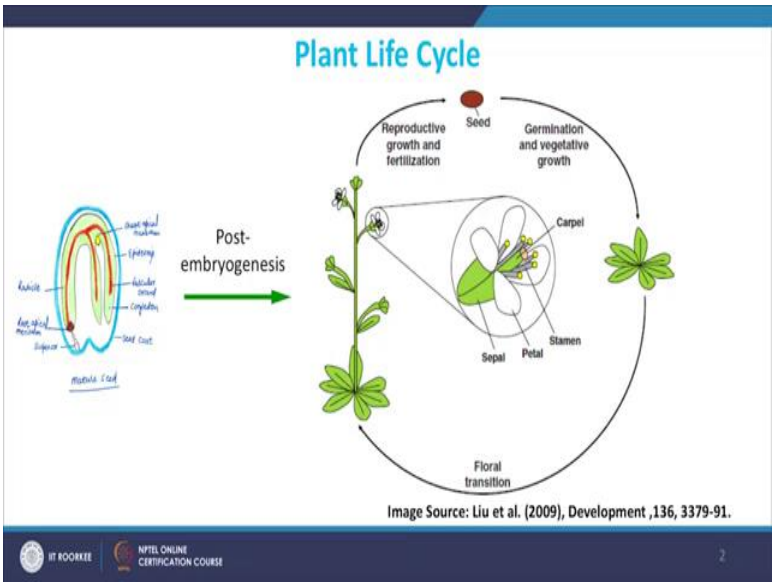

So, if you can recall early lectures where we have discussed that in case of plant growth and development to large extent depends on the cell to cell communication. Because in plant, there are two important thing, one is that plants are sessile in nature, which means that they cannot move from one place to another place. Second thing that they have a very tightly glued cells through the cell walls, so there are very rigid cell walls present between two cells. That is why it is important to have a plastic growth and development.

And in these cases the cell to cell communication becomes so important, because the positional information of a cell essentially helps in taking a proper developmental identity by a cell in a particular tissue. So, there are two major pathways through which cell communicate with each other from the neighboring cell, one is is peptide based signaling or receptor based signaling.

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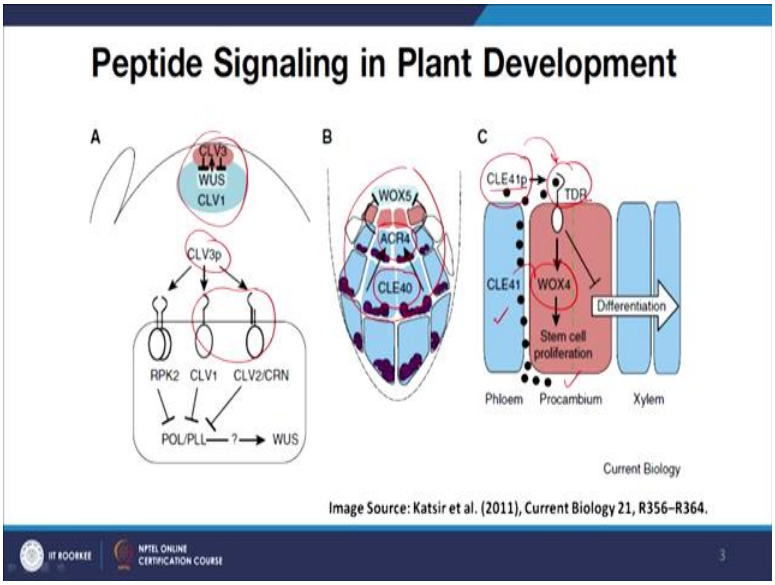

You have already studied some of the example in the previous classes. The classical and best studied example for receptor and ligand mediated signaling is WUSCHEL and CLAVATA signaling in the maintenance of the shoot apical meristem, and WOX5 and CLAVATA40 signaling in the root apical meristem.

So, this is just to recall you that in shoot apical meristem, CLAVATA1 and CLAVATA2, they act as receptors. And then CLAVATA3 is a signaling protein, which is getting received by this receptors molecules to initiate the signaling. On the other hand, if you took the example of root meristem, here CLAVATA40 is the signaling molecule and it is received by ACR4 and this initiate the signaling.

On the other hand, another very good example of receptor mediated signaling is CLE41 and TDR mediated signaling. So, CLE41 is produced by the phloem cells, but the receptor TDR is present in the procambium cells. Based on its position next to the phloem, it receives the signal and then activate the expression of procambium stem cell proliferation gene, which is WOX4. And then provides this procambium identity and ensures its stem cell property.

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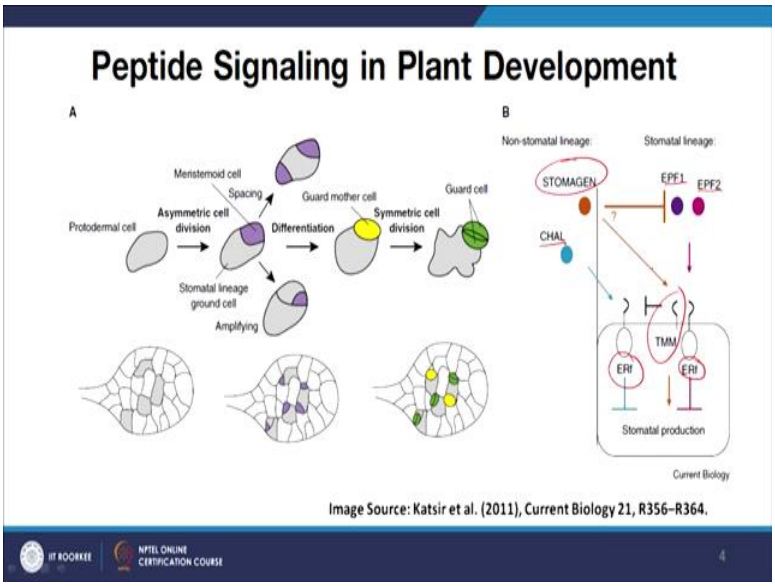

Next example for peptide mediated signaling is stomata development. Stomata are very special structure in the leaf, which differentiate through the process of asymmetric cell division differentiation and symmetric cell division. So, eventually if you have a leaf, you have some stomata, and in this stomata, you have guard cells.

In the stomata production is repressed by EPF1 and EPF2 and CHAL1, they are received by ERF receptors. So, there are two mechanism, one is receptor mediated mechanism which repress the identity. And another signaling which is STOMAGEN, it is received by TMM based signaling and this ensures proper stomata formation by repressing other negative regulators of the stomata formation.

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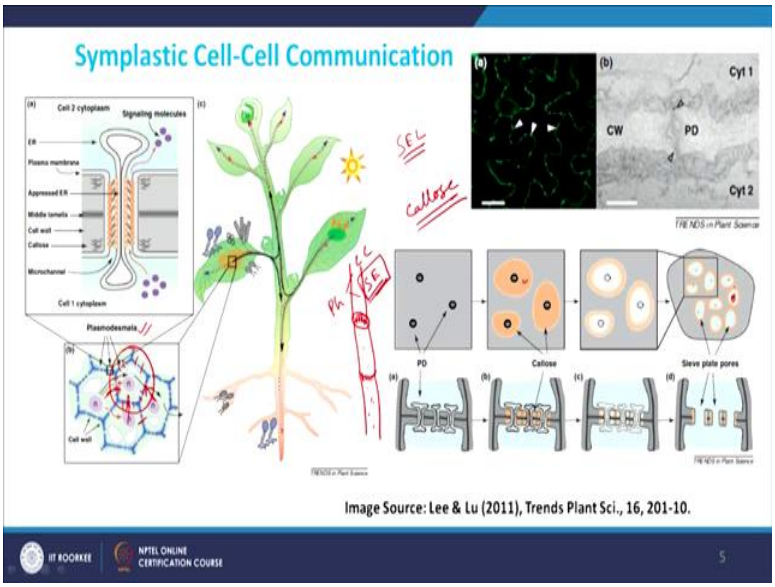

Apart from the receptor mediated signaling, one very strong signaling which is evolved in the plant system, is symplastic cell to cell communication. So, symplastic cell to cell communication is through a nanopore between two cells, which is called plasmodesmata. So, in plants almost every cells are connected through these nanopores also called plasmodesmata.

The trafficking between the cells from one cell to another cells, which occurs through the plasmodesmata is highly regulated. And size of plasmodesmata which is called size exclusion limit of plasmodesmata defines, which molecule to pass through the plasmodesmata, which molecule not to the plasmodesmata. So, this is a typical structure of plasmodesmata.

And if we look there is one polysaccharide, which is called callose, which has been shown to specifically accumulated at the plasmodesmata. If you look this structure, this is epidermis you can see that in the cell wall there are small dot like structure, which is localization of callose.

One very important cell in the plant is phloem cell. In phloem cell, we have two cell types the companion cells and sieve elements. And sieve element is the cell, which is responsible for the transport, and this cell is responsible for the transport, and that is why the cell wall between these two cells has lot of pores which is called sieve pores. These cells are almost empty, nucleus is degraded, so that the transport can occur very efficiently, but if you look this cell plate here, it has a very high amount of callose accumulated. So, essentially these sieve pores are specialized plasmodesmata.

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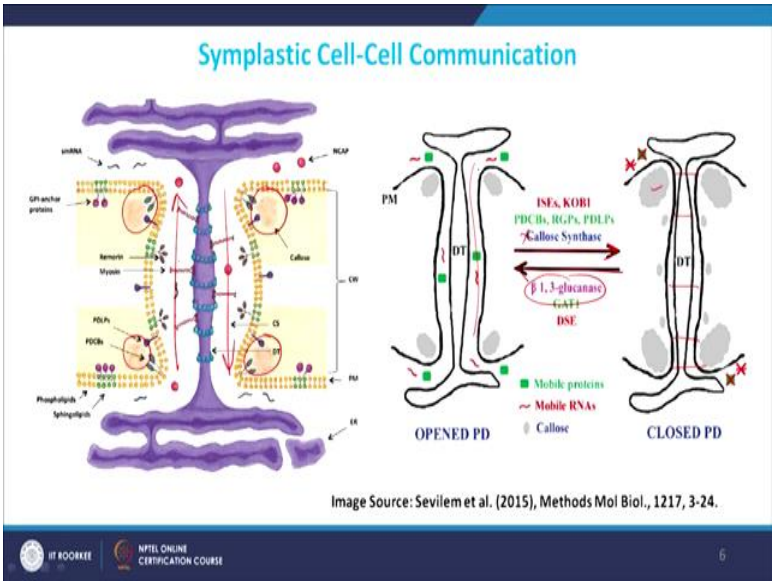

So, this is typical structure of plasmodesmata. You have cell wall, then you have endoplasmic reticulum. There are lot of proteins which has been identified specifically localized to the plasmodesmata. And if you look here, this is the site of callose accumulation and the amount of callose basically defines the molecular trafficking or the size exclusion limit of the plasmodesmata.

If you have high amount of callose the size exclusion limit of plasmodesmata is restricted. And then very small molecules can move from one cell to another cell through the plasmodesmata. On the other hand, if you have enzyme which is called beta-1,3-glucanase, these are the enzymes which can degrade callose. So, when you have less amount of callose at the plasmodesmata, the pores of plasmodesmata are more open and then molecular trafficking through plasmodesmata can occur very freely.

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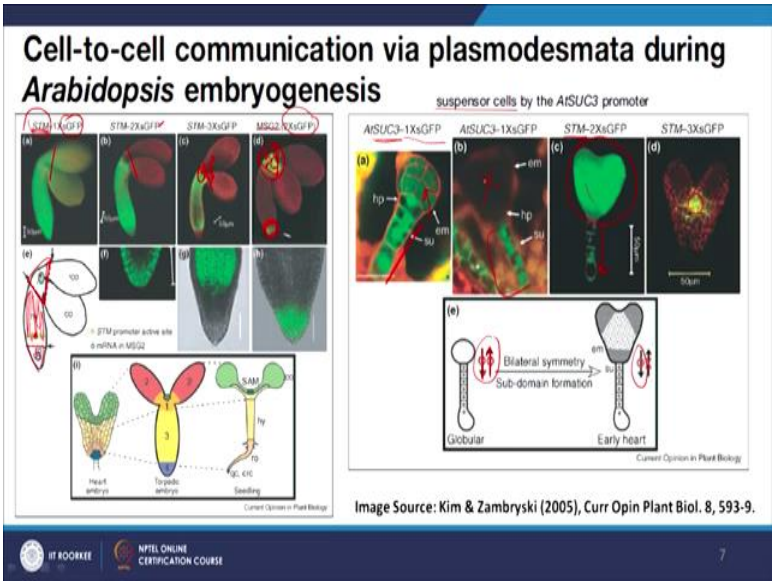

And these days number of mobile molecules has been identified. So, large number of proteins like transcription factor, messenger RNA, small RNAs, they have been identified as a mobile molecule, they get transported from one cell to another cell, and they can therefore, work in a non-cell autonomous manner.

If you look some of the initial studies, then you can find that cell-cell communication via plasmodesmata during embryogenesis. This is *STM* promoter driving GFP, and this free GFP is very small, which means that once GFP protein is synthesized, it can move freely. And if you are driving expression in the *STM* promoter, *STM* you recall this is one meristem specific promoter, which is basically active here. If we look the green region is *STM* promoter activity, either here or here in the hypocotyl region below the hypocotyl region. And what you can see here that you produce free GFP here, but you see signal everywhere.

So, GFP can move freely across this one. But if you increase the size of GFP, basically you are increasing the size of mobile molecules, when you have 1x; mobility is very freely; when you have 2x, mobility is restricted only this domain; if we have 3x, it is further restricted.

Another important thing, if you use *MSG2*, *MSG2* is active in this region as well as in the root primordia. And if we took 2x of the GFP, you can clearly see that migration or movement is restricted in this region. So, based on that, you can clearly say that during the embryogenesis, all the regions are not equally allowing the molecular trafficking.

There are some domain of trafficking normally these domains are called symplastic domains.

And during embryogenesis through this experiment, you can clearly see that one symplastic domain is here where molecular trafficking is restricted. So, if you have some protein expressed here, it is not freely able to move in this domain. So, this domain is one isolated domain. Another symplastic domain, you can see here.

So, if you express something here, the migration can occur in a restricted manner. Similarly, if you express molecule in here, you can see that domain is restricted. So, you can have one domain here and here. But if you move, if you express your free GFP in this region, migration in the entire region is relatively more free that is why you can see that everywhere distribution.

At the early stage of embryo, you can clearly see that there are few symplastic domains. And these domains basically help in establishing axial and basal patterning in the embryogenesis. So, this suggest that this control of the molecular trafficking, control of the symplastic domain movement is very important in defining the developmental patterning.

Similarly, if you look here, so here you are driving GFP under *SUC3* promoter, *SUC3* promoter is basically suspensor cell specific promoter, which means that you are expressing in the suspensor. But at early stage you can see that GFP molecules can move in the apical region of the embryo. But slightly later stage, if you look, the GFP is only restricted in the suspensor and it is not able to move in the upper region.

On the other hand, if you take *STM* promoter and express GFP here, it can freely move everywhere in the apical domain and slightly in the suspensor domain. Which suggests that during this developmental, the symplastic communication is regulated in a way that in the early stage basically top to bottom movement and bottom to top movement both are occurring probably freely or maybe not so regulated.

But at slightly later stage the, the movement from apical region to the suspensor region is still happening little bit, but this movement from suspensor to the apical region is inhibited or restricted. So, this also helps in defining different symplastic domain at different stages.

This is important why? Because when it has to go the process of differentiation or it has to go the process of development, this domains basically allow some of the regulatory mechanism to function in a very specified domain, at the same time other regulatory mechanism to function in another specified domain.

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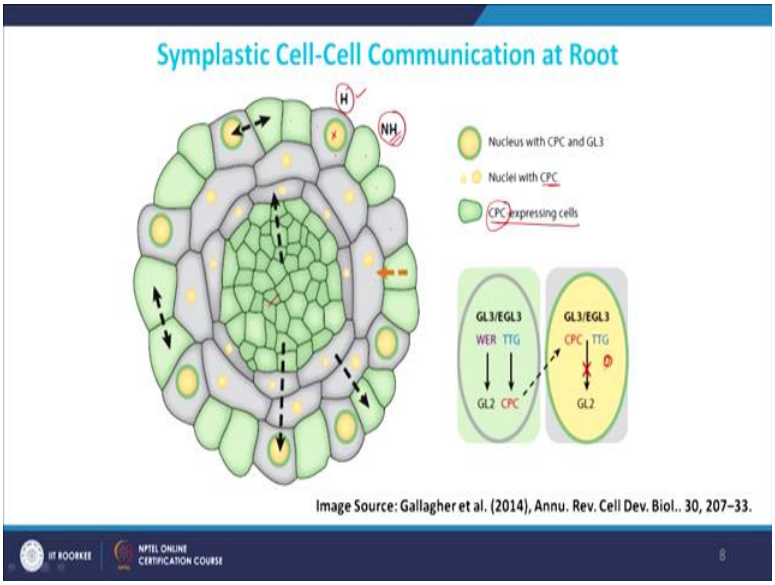

This is one another example of molecular movement which is CPC protein. So, this is a cross section of root; in root we have root hairs, but root hairs does not form everywhere. So, some of the epidermal cells, they have capability to make the hairs; some of the epidermal cells they do not have capability to make the hairs. And there is a pattern of root hairs coming.

But if we look the expression of CPC; CPC is expressed in the cells which are non-hair epidermis cells and the vascular tissue. But the protein, if we look CPC protein, CPC protein is localized to the nucleus of epidermis cells which can make the hairs as well as other cell's nucleus. And here it activates a program which is responsible for hair development. So, the movement of proteins or movement of CPC is important from one cell to another cell to ensure to position the root hair formation in the root.

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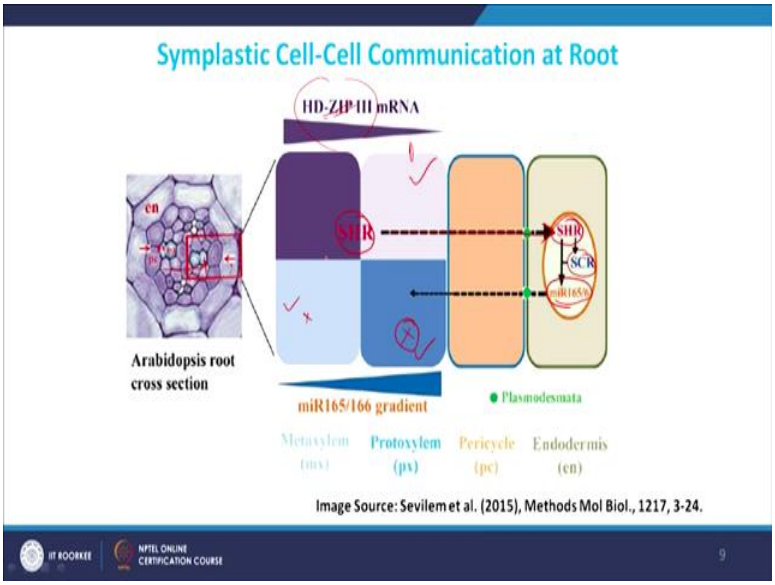

This example you would have already seen in one of the previous class, when we were discussing the, the patterning of xylem tissue. And what was important here, so this is the cross section of *Arabidopsis* root. Here you have endodermis, you have Pericycle, then you have Protoxylem and Metaxylem. And SHORTROOT protein basically this gene is expressed in the vascular tissues and then protein; SHORTROOT protein moves from vascular tissue to the endodermis, this is endodermis.

And in endodermis, this SHORTROOT proteins get localized to the nucleus. Together with *SER*, it activates a micro RNA 165 and 166, and this micro RNA moves back. And while moving back, it follows the pattern of diffusion and there is a gradient of micro RNA, which means that the cells, protoxylem cells has high amount of micro RNA, meta xylem cells has low amount of micro RNA. And the cells, protoxylem cells therefore which has high amount micro RNA has low amount of HD ZIP protein which is target of micro RNA.

And meta xylem cells which has low amount of micro RNA has high amount of HD ZIP. And this bidirectional signaling ensures a gradient of HD ZIP proteins and the amount of proteins helps in taking this cells as a protoxylem cell identity, this cell as a metaxylem cells identity. But the mechanism, how SHORTROOT protein is moving, how micro RNA is moving. So, this was identified through this study.

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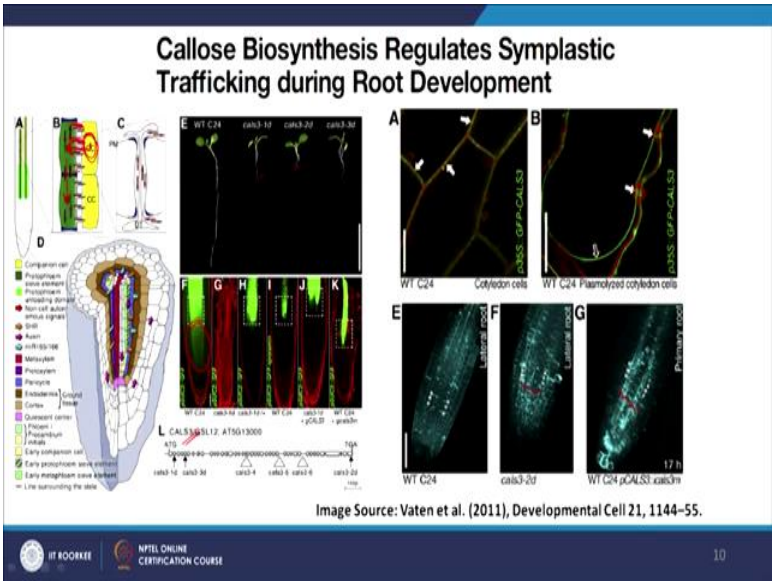

In this study callose was found to be a major regulator of molecular trafficking. If you look the root tip, this is your companion cell, this is sieve element. So, anything if you put in the companion cell, because there is a strong symplastic cell to cell communication between companion cell and sieve cells.

And what happens if you express GFP in the companion cells, this GFP can freely enter in the sieve element, through sieve elements it can transport, and you can see that here is the GFP signal coming. So, GFP signal the promoter is not active here promoter is only up in the in the companion cell, but GFP can move here; so, molecular movement is happening here. And there was three mutates has been identified, where the root was defective. There was defect in the growth, but at the same time, if you look the mutant phenotype, the unloading of the GFP or the molecular trafficking of the free GFP was strongly inhibited here.

And when this gene was mapped, it was basically one callose biosynthesis gene, which is *CAL3*. And if you look the localization, this protein is localized to the plasma membrane as well as the plasmodesmata. And then this mutant protein was used to develop system, which can be used here.

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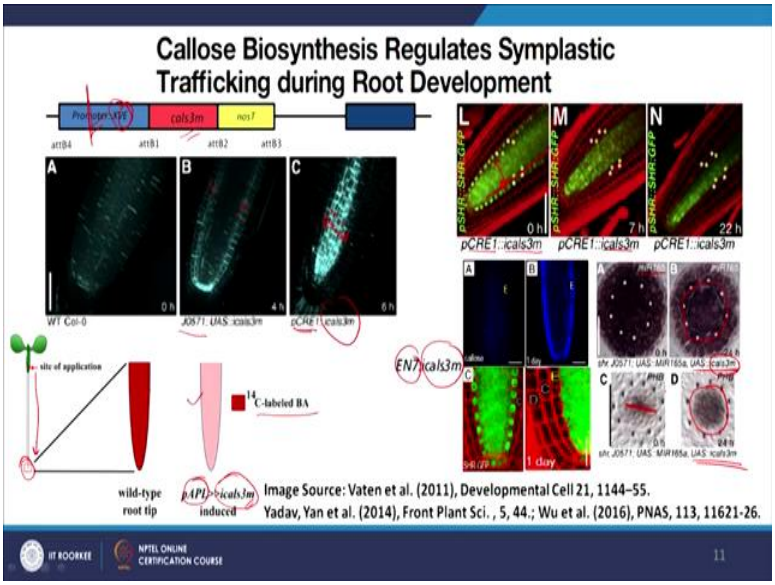

So, this is a genetically engineered system where *XVE* gene if you recall can be used to generate inducible expression. And here if you clone this mutant protein, this mutant protein is gain-of-function mutation. So, it is very active and it makes large amount of callose. And then you can put a promoter here, and since it has a estrogen receptor, you can have a double induction, one is the promoter based expression and the second is the estradiol based induction.

And if you look here when you use a different promoter, this promoter is active in this two cells, one is the cortex and endodermis cells. And when you induce this promoter here, you can see very high amount of callose, this blue color is aniline blue staining, it stains the callose. You can see very high amount of callose accumulation.

On the other hand, if you change the promoter, which is *CRE1* promoter, this promoter is active in the vascular tissue. And if you induce this callose synthesis gene, you can see a very high amount of callose getting accumulated. So, this is an engineered construct which is being very widely used to understand the cell-cell communication. Because through this mechanism you can very specifically block plasmodesmata and you can study what is the effect of cell to cell communication between the cells.

And when this system was used for example, if you look here *APL1*, *APL* is a gene, which express in the phloem cells both companion and sieve element cells. When you block movement through the phloem cells, and if you apply radio labeled cytokinin here, and measure the cytokinin activity radioactive activity in the root tip, in the normal

condition, you can see very high amount of transport of cytokinin from site of application to the root tip. But when you block molecular trafficking through the phloem, by inducing callose in the phloem, what you see that the amount of labelled cytokinin in the root tips are decreased. This suggest that cytokinin can move through the phloem.

Then if you use this construct to study that how SHORTROOT proteins and micro RNAs are moving, when you use the *CRE1* promoter and drive callose synthesis gene mutant callose synthesis gene. So, in normal condition SHORTROOT proteins are basically expressed in this region, and then it is protein is moving to the endodermis and you can see in endodermis, it is getting localized to the nucleus.

But when you induce *CALLOSE* gene, a different time point essentially you are blocking all the plasmodesmata which are present at this junction. And when you block this plasmodesmata, you can see that the SHORTROOT proteins cannot enter in the endodermis.

This is another experiment where if you use this construct in the endodermis, you can clearly see when you express in the endodermis very high amount of callose are getting produced in the endodermis. And this high amount callose are totally blocking movement of SHORTROOT protein from vascular tissue to endodermis; this is endodermis. You cannot see any signal whereas, if you look the wild type, you can clearly see that SHORTROOT proteins are moving. This suggest that SHORTROOT protein is actually moving through the plasmodesmata.

Similarly, when you express micro RNA in endodermis, you can see under normal condition, the micro RNAS are getting diffused and it is spread everywhere. But when you block through the callose, you can see that most of the micro RNAs are getting restricted here, other signals are going down, it will reflect in the homeodomain genes.

So, in normal condition you see that homeodomain genes, they are very restricted, because this region have more micro RNA, less homeodomain gene. This region has less micro RNA, more homeodomain gene. But when you induce when you block the micro RNA movement from endodermis, you can see that homeodomain genes are getting expressed everywhere uniformly. This suggest that micro RNA is actually moving through the plasmodesmata.

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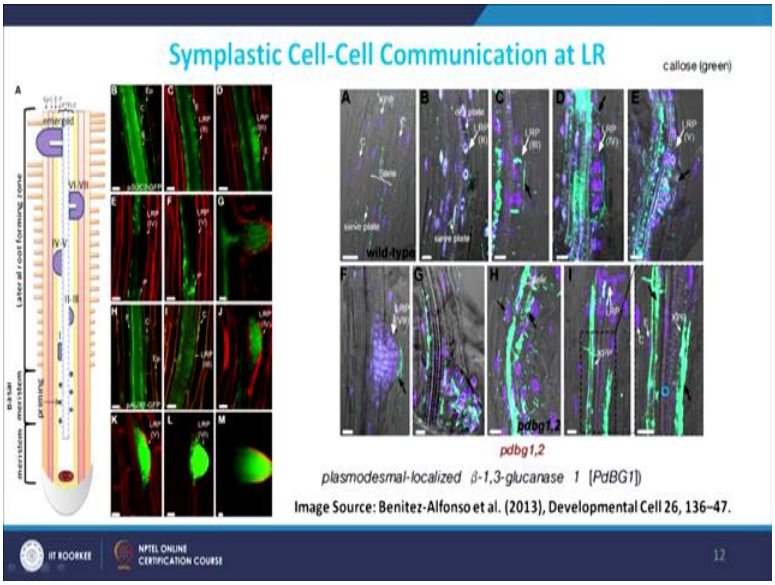

Cell to cell communication has also been shown to play important role in lateral root development. So, if you look the lateral root development, these are the typical lateral root development program. You have already seen in some in one of the previous class, where we have studied different stages of lateral root primordia initiation and development.

And if you just drive *SUC2:*GFP, you can see that there are some symplastic domain, which is being made either you use this GFP under OX1 promoter or SUC2 promoter. So, this suggests that there might be some correlation between Symplastic domain and lateral root development. So, does lateral root development follow some kind of symplastic domain.

And here is the callose localization. So, if you look at early stage of the lateral root primordia initiation, this is very early stage. You can see that there are some callose, but at later stage you can see it is restricted to some of the cell wall here in wild type. But if you have mutant, this mutant is basically mutant in the enzyme which is responsible for degrading the callose. So, if you have double mutant in the this enzymes, what happens that the overall callose level is going to be high, because the enzyme which is degrading is less and that is why you can see in the mutants high amount of callose.

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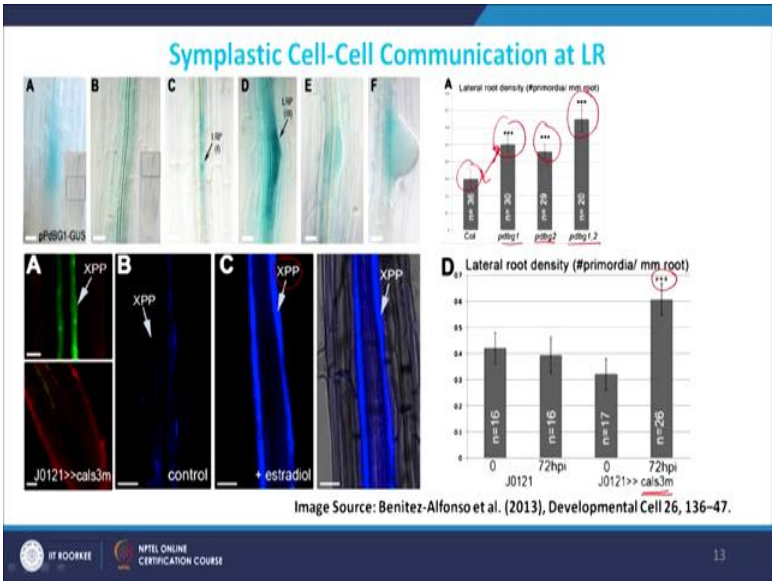

And if you look the expression pattern of this callose degrading enzymes, they are basically expressed at the different stages of lateral root primordia. And when you have mutant, when you do not have this genes, which means that you have we are going to have high amount of callose, then you can see that the density of lateral roots are increased. So, this is normal, wild type, then single mutant; single mutant, you can see that density of lateral roots are significantly increased. And when you have double mutant, it is even further improved.

So, this suggests that the presence of callose in the symplastic domain is playing very important in regulating number of lateral root primordia. Then if you take this and express callose very specifically in the xylem pole pericyclic cell. which is responsible for lateral root primordia formation.

If you express high amount of callose here, you can see that when you induce callose you can see high amount of lateral root primordia, the density is increased. This together suggests that callose is playing very, very important role in regulating lateral root formation.

This is the example of FT just now you have seen in the previous class that FT is moving from leaf to the shoot apex, then the question was what was the site of movement and the site is plasmodesmata here.

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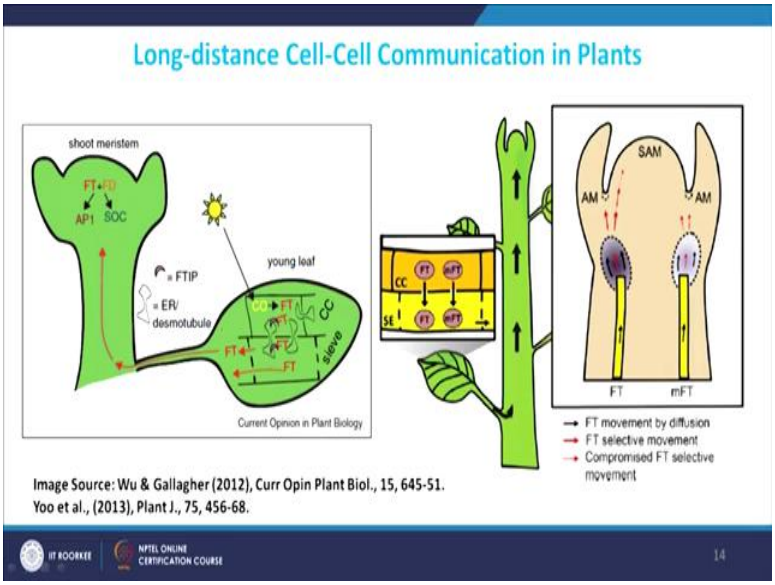

So, it has been shown that FT is basically transported or loaded to the phloem cells, then the through phloem, it is basically moving to the apex; and in apex, it is getting released from the phloem cells and performing the functions.

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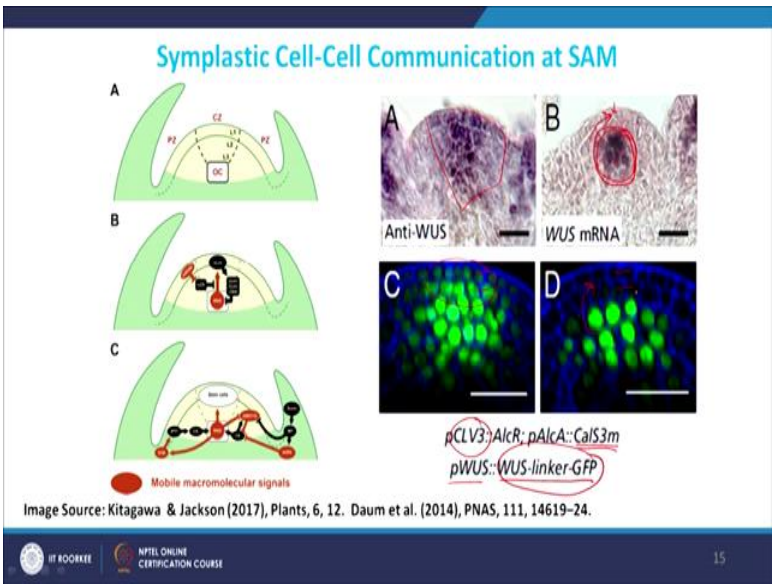

Symplastic cell communication is also important in shoot meristem. For example, if you recall WUSCHEL and CLAVATA signaling this is how it happens. This signals basically suggest the non-cell autonomous signaling. If you look the WUSCHEL messenger RNA, WUSCHEL messenger RNA is restricted in the organization center it is not present here. But it activates *CLAVATA* in L1 and L2 layer. This is a non-cell

autonomous activation, how it happens? When you, probe the WUSCHEL protein, this is antibody against the WUSCHEL protein, you can see that protein is present almost every layers, which means that messenger RNA is present in the restricted domain, protein is moving to the other region and that is why it is functioning.

Here you can prove that this is moving through the Plasmodesmata. You can use WUSCHEL promoter and drive WUSCHEL:GFP signal. Using this translational fusion construct you can clearly see that protein is getting localized here as well as in L1 and L2 layer. But when you block this movement by driving *callose* mutant in CLAVATA3 domain; CLAVATA3, if you recall it is here. So, you are basically producing high amount of callose in this region and then if you look here the L1 layer and L2, they do not get WUSCHEL protein distributed.

This suggest that first thing is that WUSCHEL protein is moving and second thing is that WUSCHEL protein is actually moving through the plasmodesmata. And when you block the plasmodesmata by overproducing callose, the presence of WUSCHEL protein disappears.

So, with this we will stop here. So, in this course, we have studied basically general or overview of plant development taking some of the example.

Thank you very much.