Plant Developmental Biology
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Lecture - 14
Root Branching: Lateral Root Development

Welcome to Plant Developmental Biology course. In today's class we are going to discuss Root Branching particularly Lateral Root Development in plants. So, branching is very important or very critical events in the process of plant growth and development, because this helps in establishing a proper architecture in the plant body.

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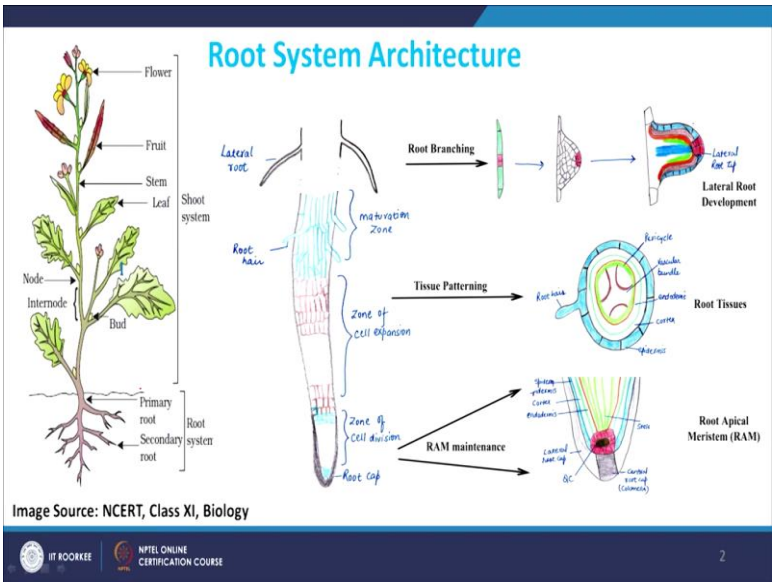

In a typical model plant here, you can see that the root system has some branching called root branching and then shoot system they also undergo the process of branching.

In today's lecture we will focus on root branching. And this is important because root branching increase the surface area of entire root system, it establishes a proper root architecture which is important for plant to be efficient in absorption and anchorage function of a plant.

If you recall previous lectures. We have discussed the meristem maintenance which happens at the tip of the meristem, then if you come slightly higher side during the stage of cell elongation and maturation, these are the stages or these are the places where tissue

patterning occurs where different tissues, different cell types, different identities are taken place, but if you come in the maturation zone or in the differentiation zone the visible branching start. So, we will cover this root branching in this lecture.

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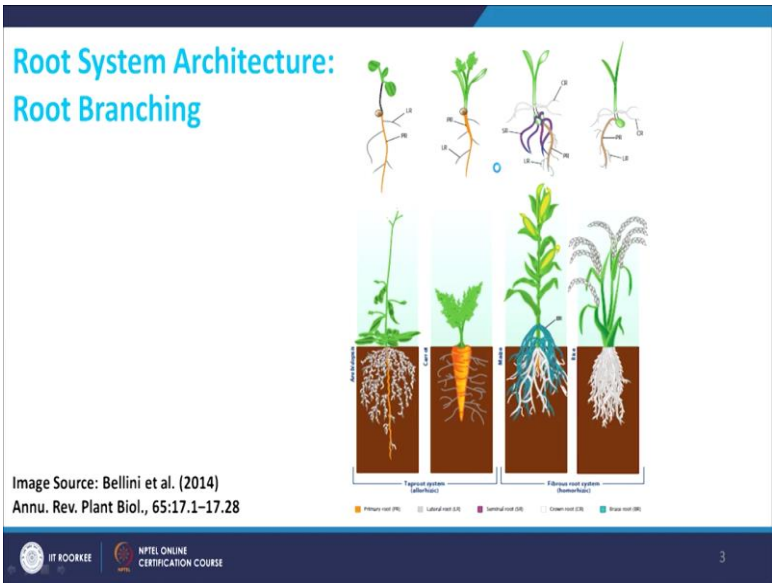

Root system architecture is important in the plant species and there is a huge variation in the root system architecture in different plants. But if you divide the total kind of root types based on the architecture, there are two major root types one is the tap root system another is fibrous root system.

In tap root system you have one primary roots which you can see here; this is typical *Arabidopsis* root system; this is carrot. Fibrous root system is present in the monocots whereas, tap root systems are present in dicot plants. In fibrous root system there are primary roots, but later on there are some additional roots starts coming which is called adventitious root or crown roots depending on the species.

So, this is a typical example of maize and rice; in rice you can see here these are the crown roots, this is the primary roots and then lot of lateral roots which are coming from both primary roots as well as the adventitious root and similar is the case of maize. So, the important thing here is that how the pattern or how this root system architecture is established in plant.

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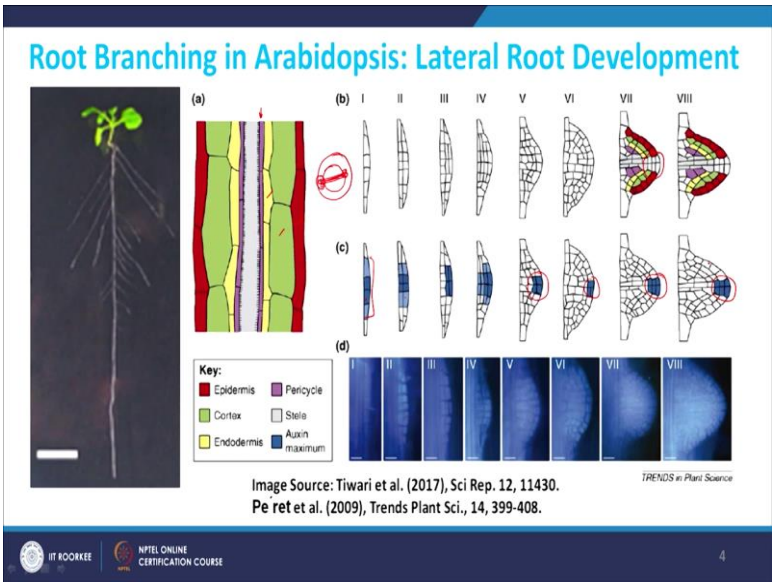

So, we will take here example of root lateral root development in model dicot plant *Arabidopsis thaliana*. So, if you look this is *Arabidopsis thaliana* seedling, you can see here this is the primary root. Primary root is growing and then if you look at the base you do not see any visible roots hairs because these are the regions where is meristem. Meristem is somewhere here then you have elongation zone and at higher side, you can start seeing that lot of emerged lateral roots has come.

So, these are the lateral roots which is coming in the maturation zone, but as I said the process or the program; the developmental program which is required for formation of this lateral root has started somewhere here. So, this is very important for us to understand. And if you look in *Arabidopsis* the process of lateral root development is well established and all branching happens post embryonically.

So, lateral roots are coming from the root. So, they are root borne root which means that you have already a differentiated root system, then suddenly a new branches or new roots are originated from that root. And this is only possible if there is new meristems which has been generated somewhere here in the root which is lateral meristem. Which means that there is a process. So, you have a differentiated cell then the differentiated cell has to undergo the process of cell division and then it will generate a meristem, and then that meristem will start the process of development.

In *Arabidopsis* it is known that the cell is next to the xylem, the pericycle cell. So, this is basically longitudinal view. So, you can look here this is epidermis, then below epidermis you have cortex then below cortex you have endodermis, and then inside endodermis this layer is called pericycle and then you have the vascular tissue.

If you look a cross section you will find that this pericycle layers are like this. But, the xylem makes a kind of axis here. So, there are pericycle cell which is close to the xylem and called as xylem pole pericycle cells. And it has been seen that in case of *Arabidopsis thaliana* these pericycle cells basically have competency and potential to initiate lateral root developmental specific program.

And when this happens; the mature or differentiated pericycle cells undergo the process of cell division. So, differentiated cell undergo the process of dedifferentiation then start dividing and then start redifferentiation of the lateral root. So, this entire process of lateral root development can be divided into different stages.

At very early stage there is one pericycle cells, when it starts more than one cell division and there are different orientation of cell division. Some are periclinal and some are anticlinal, and these results in growth of the primordia this is called lateral root primordia.

And then at later stage of the lateral root development different tissues start taking their identity like a typical root like QC and root meristem organization. So, new root meristem which is established here has QC then you have epidermis, you have cortex same as the layer of the root system.

And once this primordia start growing, then it will rupture and it will start coming out. This is called root emergence. So, the entire process of lateral root development can be divided into different stages; the first stage is the root meristem lateral root specification primordia specification, then the primordia differentiation where all this tissue patterning is happening and then finally, lateral root primordia emergence.

And one important thing which has been found that this process of lateral root initiation starting from the initiation, starting from the specification till the emergence; plant hormone auxin has been found to play a very important role in this entire process. And if

you recall we have seen that auxin functions as a morphogen it functions in the gradient dependent manner or the concentration dependent manner.

If you have high amount of auxin there is a full mechanism in plants which basically transport auxin. So, you can relocate auxin from one cell to another cell and this kind of mechanism is called polar auxin transport and this polar auxin transport basically helps in generating auxin maxima in some of the cells and auxin minima in another cells.

So, there is a correlation; if you look the auxin activity you can see that, these cells they start auxin maxima. So, these cell is undergoing the process of asymmetric cell division and the cell the smaller cell which is towards the centre they are having more auxin than the cells which are outer side. And then eventually in the development, the auxin maxima started getting accumulated only in this region very tip of the were the developing roots

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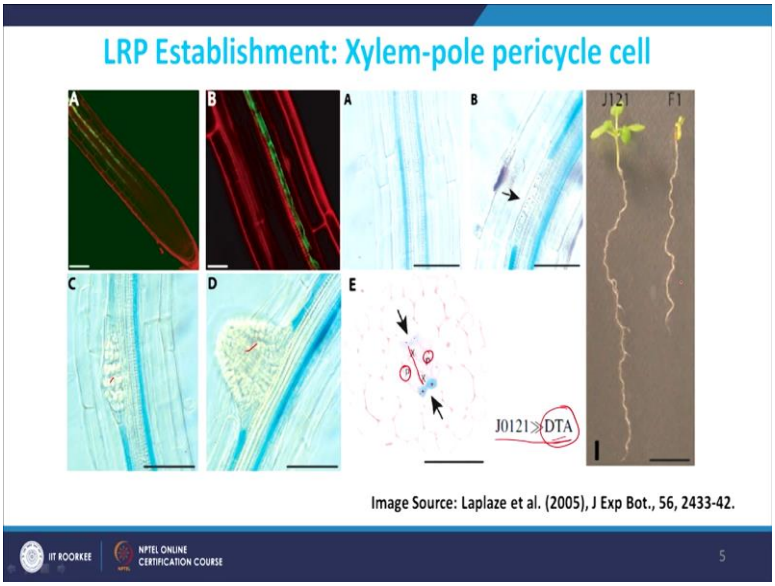

We will see this mechanism in details. This is just to show that lateral root primordia establishment initiate from xylem pole pericycle cell. J0121 is a marker which very specifically express in mature or differentiated xylem pole pericycle cells. So, if you look here this is driving GFP. If you look very carefully this is the xylem cells, you can identify xylem cells based on its structure because xylem has lot of modifications, lot of lignin and secondary cell wall modification.

Based on that you can clearly identify this is xylem and the cell which is just next to the xylem is pericycle cell; xylem pole pericycle cell. And these xylem pole pericycle cells is very specifically expressing this gene, and once these xylem pole pericycles they start the process of lateral root initiation. Xylem pole pericycle cell has started dividing at a particular time point and once it has started dividing, it is losing this identity of pericycle cells and it has acquired stem cell identity. And that stem cells eventually going to give rise to the primordia at early stage and this is emerging primordia; emerging lateral root primordia

This is cross section. So, this is driving GUS expression under J0121 promoter. If you make a cross section you can very clearly see this is the xylem axis, this is phloem pole and these two cells which are next to the xylem axis or which are in contact with the xylem axis, they are xylem pole pericycle cells they are very specifically expressing these markers.

Another way to test whether that xylem pole pericycle cells are directly involved in the lateral root development this experiment was performed. This DTA, it produce a kind of toxin. So, if you produce this toxins in a cell, the cell will be killed and then you see what is the effect.

So, here what is happening that these toxins are being produced very specifically in these cells which is xylem pole pericycle cells. And if you do that what you can see that in these plants when you kill the xylem pole pericycle cells, you do not see lateral root development here. So, this also suggest that; xylem pole pericycle cell is the cell which gives rise to the lateral root developmental program.

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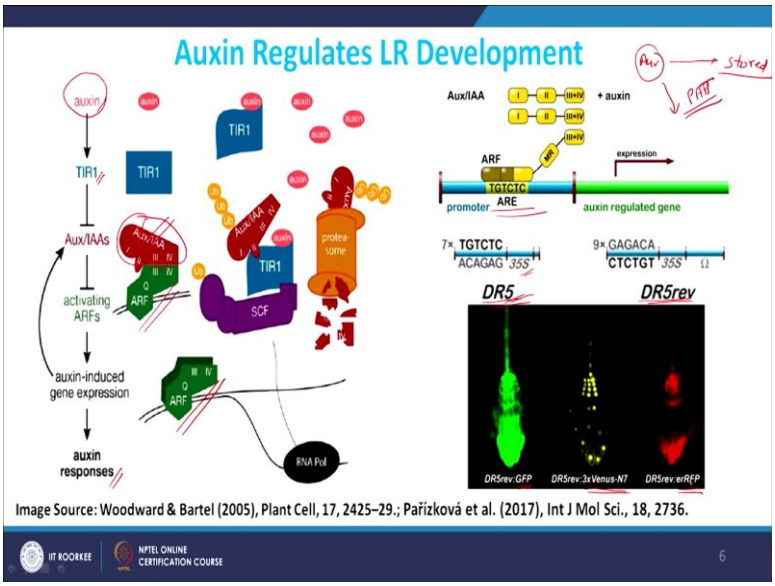

So, as I said that auxin is an important plant hormone which regulates almost every aspects of plant development. Here we will just have a quick look of auxin signalling.

Auxin is an important plant phytohormones which is being synthesized mostly in the young tissues. And when this auxin is synthesized, there are lot of auxin bio-synthesis pathway 3-4 auxin biosynthesis pathway, then you have lot of enzymes which are helping in auxin biosynthesis and once auxin is biosynthesized there is a mechanism to maintain its homeostasis.

High amount of auxin is not good for some of the development. If you have high amount of auxin, then auxin can be stored as inactive form. There is a mechanism to inactivate auxin that helps in maintaining auxin homeostasis in a particular cell.

And the active pool of auxin which is able to initiate auxin signalling pathway can be distributed very properly through a process of polar auxin transport. Through this process you can distribute this auxin to a cell where auxin signalling has to be initiated. Let us assume that a cell has auxin. So, auxin is received by receptor; auxin receptor which is TIR1. Let us assume in a cell there is no auxin what happens? There is a class of transcription factor which is called auxin response factor. Auxin response factor regulates set of genes which are working downstream of auxin signalling pathway or you can say that auxin signalling pathway is regulating expression of many genes. And what

happens if there is no auxin there is a very high amount of negative inhibitor of auxin response factor which is Aux/IAA.

So, essentially Aux/IAA can pair with auxin response factor and it inactivate auxin response factor, it does not allow auxin response factor to go and activates the genes which is working downstream of auxin signalling pathway. But if you have auxin; if you have high amount of auxin, the auxin is received by the receptor and this complex of receptor and auxins target this Aux/IAA protein for degradation.

So, when there is auxin this protein will be degraded through a mechanism and then once this protein will be degraded the auxin response factor will be freely available. Now, this auxin response factor goes and it binds to auxin response element and then activate the gene or repress the gene depending on what is the nature of this auxin response factor and result is auxin responses.

These two construct which are very extensively used in plant developmental biology. Basically a synthetic promoter has been created where the auxin response element or DNA elements where auxin response factor can come and bind.

So, this has been cloned along with a basal minimum promoter and then this construct DR5 or DR5rev has been generated. Essentially a synthetic promoter is generated where we have put a basal expression element promoter, basal promoter along with auxin response elements. Which means that this protein will have a very low or very basal level expression under normal condition, but if there is auxin response factor if it comes and binds to the auxin response element, it can enhance the expression. This system is called DR5 based reporter system which reports auxin responses.

So, if you see high amount of signal or if you see high amount of reporter activity; DR5 reporter activity which means that, that is the cell where there is auxin signalling going on. This is a typical example if you take this construct and check in the root. This is the root cap, you can see very high amount of signal in root cap. And then reporter gene you can choose as per your choice, here the reporter gene is GFP, here is the nuclear localized YFP or VENUS, here you have endoplasmic reticulum localized RFP. So, based on that you tell exactly where is the auxin responses.

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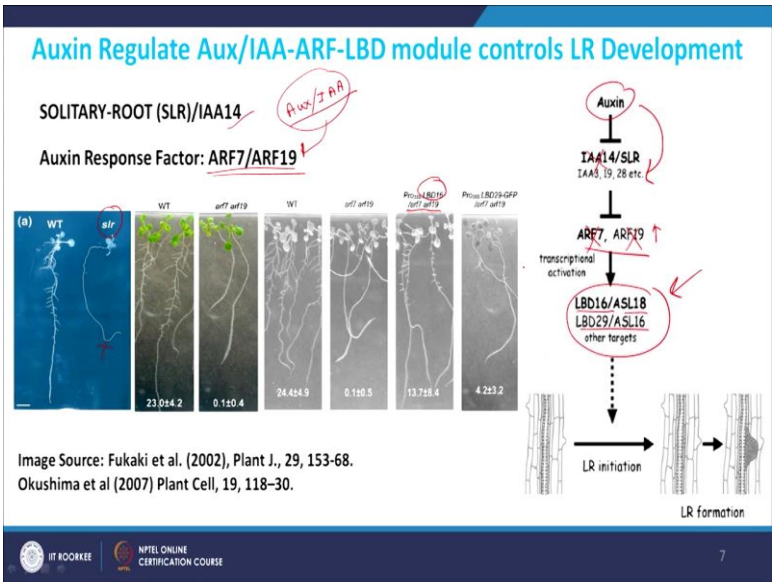

As auxin signalling is regulating lateral root development. What is the mechanism, how it regulates and what is the proof that auxin signalling is regulating lateral root development? If you recall previous, Aux/IAA is a negative inhibitor of auxin response factor.

So, if you have high amount of Aux/IAA it means that no auxin signalling will be activated, and here this is the mutant. In this mutant what happens that the level of this protein is stabilized; so, you have high amount of Aux/IAA. High amount of Aux/IAA means in this mutant there is no auxin signalling activated; no auxin signalling activated results in no lateral root formation. So, this suggests that activated auxin response signalling is important for lateral root formation.

Now these are two auxin response factor, *AUXIN RESPONSE FACTOR 7* and *AUXIN RESPONSE FACTOR 19*, if you look double mutant here you can also see that there is no lateral root formation. So, even if auxin is there, auxin signalling is started and this protein is degraded, but this auxin response factor is mutated. So, if you do not have auxin response factor, you cannot generate auxin response, and when there is no auxin response you cannot initiate lateral root developmental program.

It was also identified that this auxin response factors are regulating some other class of transcription factor, these are LOB domain or LBD domain containing transcription factor. As you can see here that if you take this *AUXIN RESPONSE FACTOR 7* and *19*

mutant and put *LBD 16* you can already rescue the phenotype. There is lateral root formation, which suggest that auxin is basically negatively regulating a negative regulator of auxin response factor.

So, Aux/IAA is basically negative regulator of auxin response factor. So, when there is auxin signalling, auxin signalling is degrading this factor which means that it is activating expression of *AUXIN RESPONSE FACTOR 7* and *19* and then *7* and *19* is going and activating *LBD 16 18 29* and *ASL16*. And once these genes are activated you can start lateral root initiation program.

In this mutant background you can clearly see that though these genes are not here they are basically mutant, but you are providing downstream gene. So, you are putting *LBD 16*. So, if you activate the signalling from here onwards, you can already see the lateral root specific developmental program, which means that they are working in the same pathway and they are regulating the process of lateral root initiation.

Another important role what auxin plays is, it regulates the positioning of lateral root. So, it is not only the lateral root development, but where in the primary roots lateral roots has to be that is also regulated by auxin signalling.

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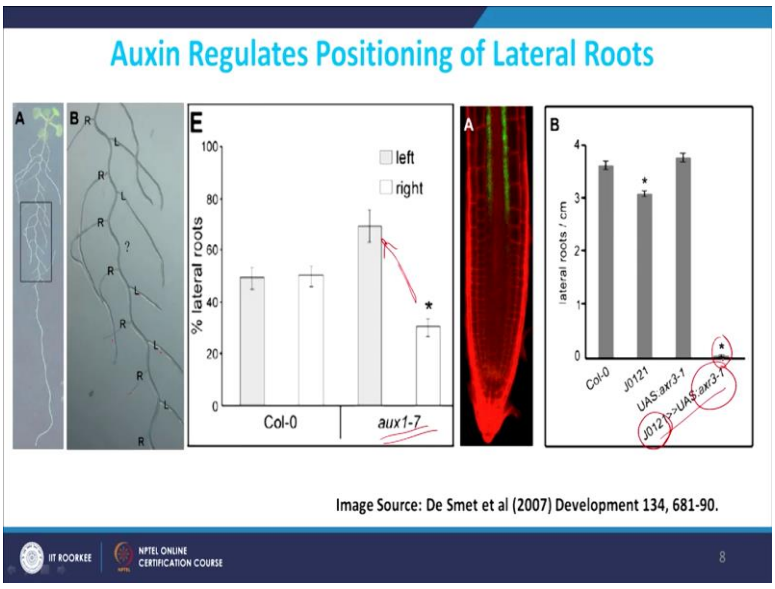

In growing *Arabidopsis* seedling here, you can see that the mode of lateral roots are alternates. So, one is on right then left, right left, right left and in normal wild type this

distribution is quite equal. So, you can see that around 50 percent lateral roots are coming towards left side, 50 percents are coming towards right side. But if you have, auxin signalling mutant or auxin response mutant you can see that this patterning is changed. So, now, you have more towards left less towards right. So, the balance is basically disturbed when you have the mutant background.

And here this is again proofs that auxin is important. This is a dominant negative mutant which suppress auxin signalling pathway. If you over express this in xylem pole pericycle cells, auxin signalling is suppressed and you do not have lateral root development. So, suppression of auxin signalling very specifically in the xylem pole pericycles almost stop lateral developmental program. This suggest that, activation of auxin signalling in xylem pole pericycles cell is important for lateral root development.

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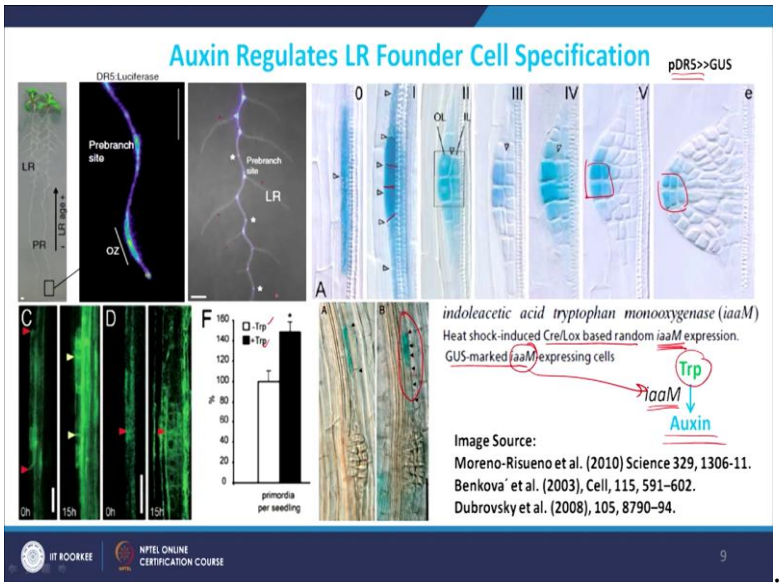

This is the DR5 construct which is auxin responsive construct, and if you take Luciferase assays, so wherever you have high amount of signals it means that there is high amount of auxin signalling, there you have lateral root primordia or lateral root development.

And if you look the very early stage of lateral root development this is DR5 GUS expression. Auxin signalling is getting activated very specifically in these cells, once auxin signalling is activated this cells start undergoing the process of cell division, and then the process of primordia initiations begins. And once the process of initiation begins then auxin signalling is getting restricted at the tips.

And you can see here this is very interesting experiment which also proves that it is very important to activate auxin signalling very specifically in xylem pole pericycle cells. So, basically here what you do, you use Cre/Lox based random integration of a protein or a gene which is responsible for auxin biosynthesis. And then you look for a line where this has been integrated in the xylem pole pericycle, this you can do because it has been combined with GUS.

So, if you look this plant, you can see that these cells which is showing GUS activity it is xylem pole pericycle cell, because it is next to the xylem and it is expressing iaaM. This enzyme is basically responsible for converting tryptophan into auxin. If you do not provide tryptophan even though the enzyme is present, auxin is not synthesized.

Then you see that there is an enzyme expressing here, but you cannot initiate lateral root specific developmental program. But when you add tryptophan, these cells where this enzyme was present it has got the substrate now, it has produced auxin. And once auxin is produced in these cells, you can see that cell division starts and number of primordia per seedling is increased significantly in presence of tryptophan as compared to in absence of tryptophan.

Another interesting and important thing this is some of this we have already discussed that, auxin starts working at very first step of the lateral root differentiation.

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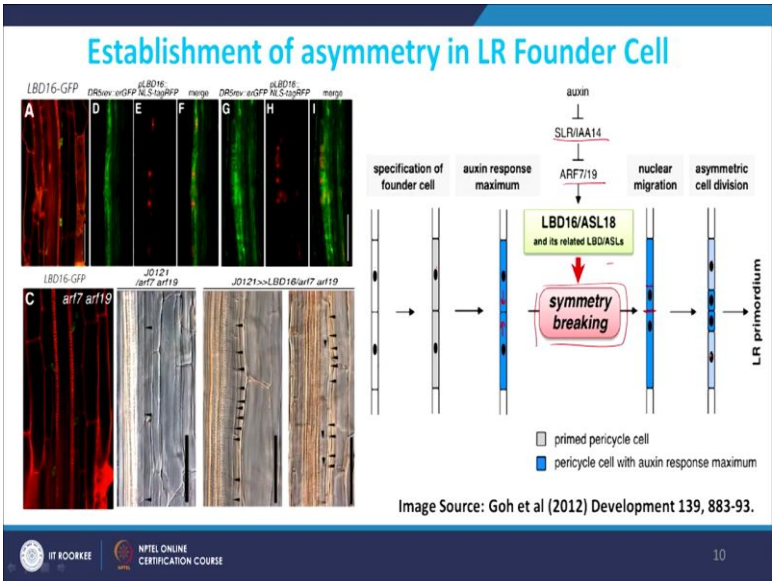

Even at the stage of lateral root founder cells specification and this happens through the process of asymmetric cell division. This is nuclear localized signal, you can clearly see that these are the cells which are having high amount of auxin, DR5 is basically telling the auxin activity, and the same cells they are expressing *LBD 16*. So, there is an overlap. Wherever the auxin signalling is highs *LBD16* are expressed there. And if you look in the *ARF7* and *9* mutant background you see *LBD* expression is disappeared.

So, all this experiment suggests that auxin is at very early stage. In the first cell auxin is repressing this pathway and specifically activating this which is basically starting the symmetry breaking. The symmetry breaking is very important for lateral root initiation. If this is the specification of founder cell and this is your pericycle cells, then in pericycle cells once there is auxin maxima, once there is high amount of auxin the nucleus start migrating towards the common pole. So, there is a process of nuclear migration.

Now, you can see that this nucleus are coming very close to the common cell wall. And when they reach to the common cell wall, these cells asymmetrically divide like this and then you have two small cells in the centre and then two cells which are outside. And if you look the auxin responses, auxin responses is very high here and it is slightly reduced in the lower cells.

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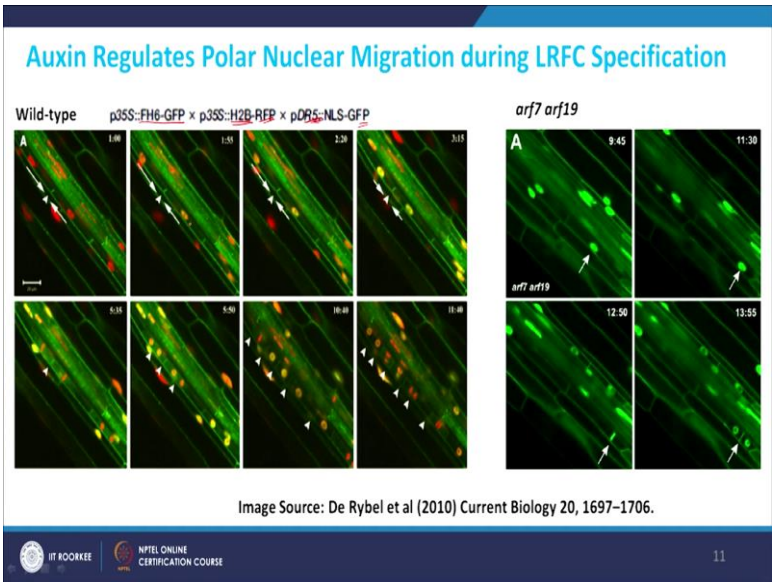

This is the process of for nuclear migration which is very important or you can say one of the first step which is required for lateral root initiation program. This is transgenic

plant where three reporter markers has been expressed in the same plant. This reporter marker is marking the plasma membrane so, you can see every cell lines plasma membrane. Then you have H2B which is histone 2 protein, histone 2 protein, it has RFP signal and it will localize to the nucleus because histone protein is nuclear protein, and then you have DR5 GFP which has auxin response.

So, at very early stage you can see here that you have only red signal means you have nucleus, but there is no DR5 activity yet there is no auxin response initiated that is why you have red cells. In a time-lapse video what happens, at slightly later stage the color of this red started turning to the pink which means that in the same cells where in the nucleus of the cells auxin responses are getting activated. When there is auxin responses then you will have GFP signal and when GFP and RFP will start merging the color will start changing from red to the yellow side.

And then you can see at later stage this becomes more yellow and this is the site when you can also see that nucleus has also started migrating. So, here nucleus was very far from this cell wall, but once auxin responses has started in these cells, nucleus has started coming very close to this common cell wall. And then once it is very close, you can see that there is a cell division here a nuclear division and cell division, and result is that you can see four cell stage and common cell wall and just after that there is a cytokinesis and every cell has their own nucleus.

This tells that, there are few things which is happening very critically and this process is regulated by auxin. The first thing is that there is high amount of auxin, auxin amount is going up. Once auxin amount is reaching to the auxin maxima nucleus start migrating, nucleus is coming close to the cell wall then the cells are dividing, this is the very first step of lateral look primordia initiation.

And this process is not happening when you have auxin signalling mutant. So, if you look auxin *arf 7* and *19* you can see that there is no clear nuclear migration, even if there is nuclear migration, cell division is not occurring. So, this also proves that auxin is important for it.

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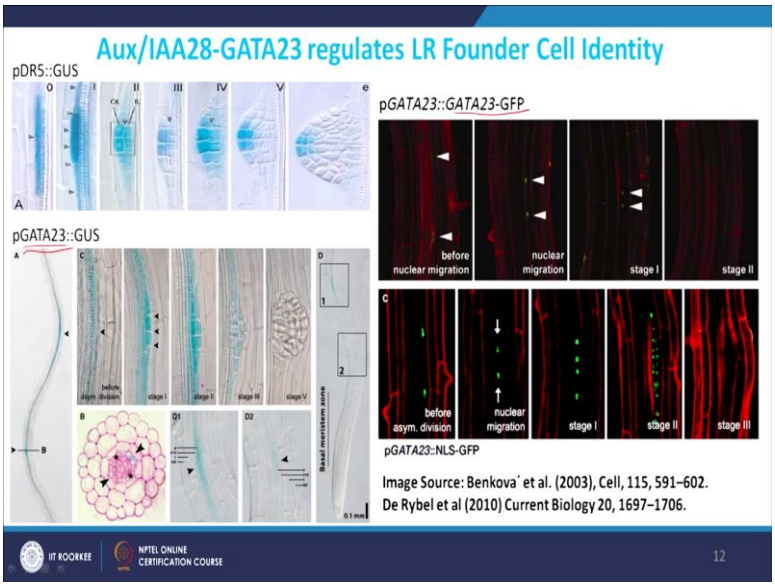

And then another gene which is called *GATA23*. GATA23 is another class of transcription factor and it was observed that it is showing overlapping expression pattern with the auxin. So, you can see before asymmetric cell division it is expressed in the xylem pole pericycle cells, and during stage 1, stage 2 , stage 3, but at very later stage the expression level is going down.

And if you look *GATA23* promoter driving GATA protein fused with GFP you can see the gene is or the *GATA23* is expressed even before nuclear migration and it continues during the nuclear migration. So, this could be one of the early regulator of nuclear migration or the process of LR founder cell specification. This is also clearly visible here before asymmetric cell division you can see here the signal then there is a nuclear migration and then at later stage there is cell division and all the cells are making the primordia.

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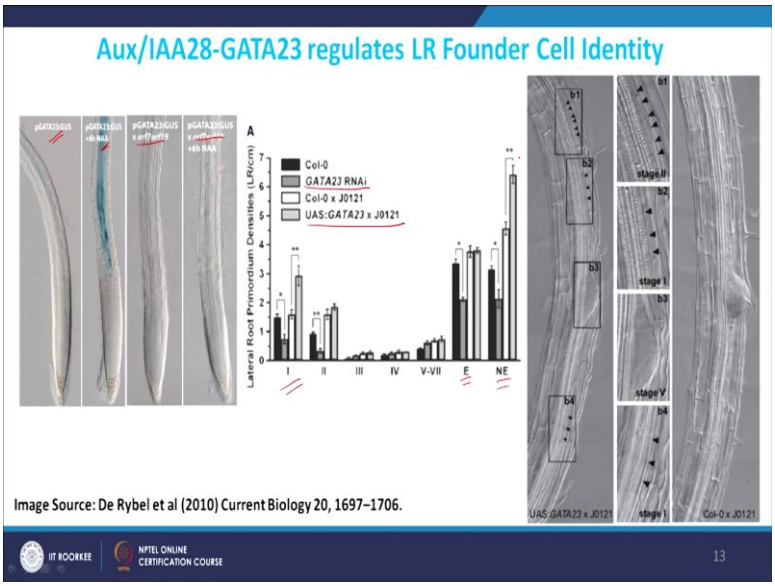

GATA23 expression is induced by auxin. If you treat with auxin you see very high amount of *GATA23* expression, but this induction is not happening when you have auxin response factor mutant. This also tells that GATA is working downstream of auxin signalling pathway. So if you have normal auxin signalling pathway, auxin signalling pathway is activating *GATA* and *GATA* might be regulating other gene.

So, if you have *gata* mutant, this is *gata* loss of function or plant over expressing *GATA23* in xylem pole pericycle. So, if you see different stage of primordia what happens? In early stage of primordia when you do not have *GATA23* you can see that number of lateral root primordia is decreased, but when you increase or overexpress it is increased. So, this tells that *GATA23* is important for defining lateral root primordia specific developmental program.

This is a picture which is showing that where it is present exactly. And if you look this is emerged primordia and these are the non-emerged primordia. You can see clearly that in *gata23* mutant, the emerged primordial numbers are significantly reduced and when you overexpress it is getting increased. Same is true for the non-emerged primordia; so, there are a lot of primordia which are being generated inside, but they are not emerged. If you count them or if you go through this and try to count you find that non emerged primordial number is also decreased when *GATA23* is mutated and when *GATA23* is induced you can see that number of primordia significantly induced.

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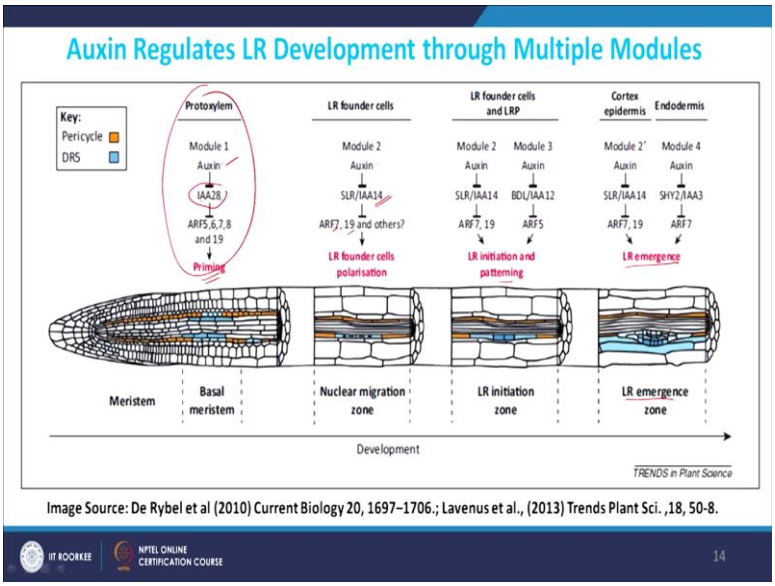

So, this all if I summarize in lateral root development. Auxin is regulating almost every expects of lateral root developmental program. And there are different set of modules of auxins regulating different program. Though we see lateral roots in the maturation zone, but the program or the developmental programming has started as early as in the basal meristem. So, this is meristematic zone at very tip of the root, in meristem we have this basal meristem.

So, the priming of lateral root program initiates in the in the basal meristem and here auxin basically is working through this module auxin *Aux/IAA 28 ARF 5, 6, 7, 8* and *19* and this auxin specific model is responsible for priming the developmental program for lateral root.

Then in the later stage this is second auxin signalling module where auxin is working through *Aux/IAA 14* and it is regulating *ARF 7* and *19* and they are basically helping in the lateral root founder cell polarization, here is the nuclear migration zone you can clearly see. And then if you come slightly later stage there are two modules which are working in parallel and they are regulating lateral root initiation and patterning development. And in the maturation zone, there is yet another modules of auxin or kind of similar modules, you can say this is module 2' and these moduels they are parallel working in regulating lateral root emergence or the zone where you can start seeing lateral root coming out.

So, here we stop lateral root development in next class we will take shoot development.

Thank you very much.