Plant Developmental Biology
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Lecture - 12
Root Development. Cont.

Welcome to Plant Developmental Biology class. In last class we started Root Development which we will continue.

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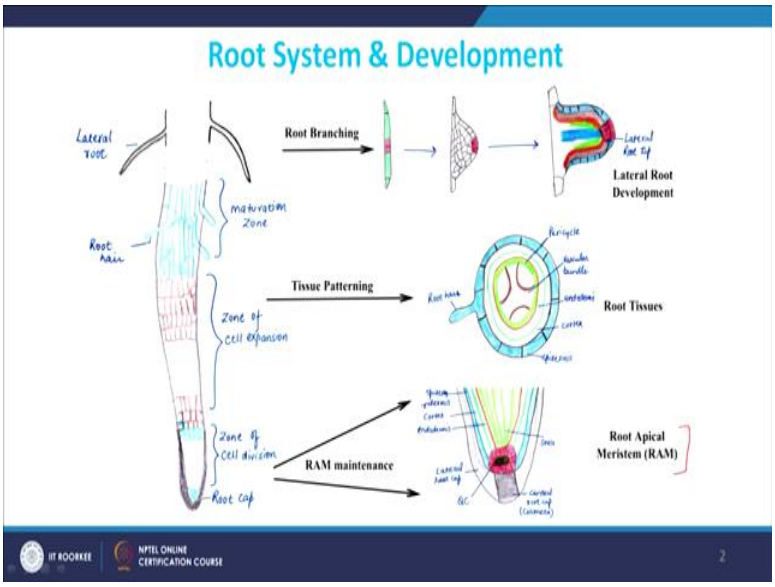

You have already seen this slide in previous lectures. There are three major steps which takes place during the process of root development; the root apical meristem, tissue patterning and root branching which is lateral root development. In last class what we discussed about how root apical meristem is maintained.

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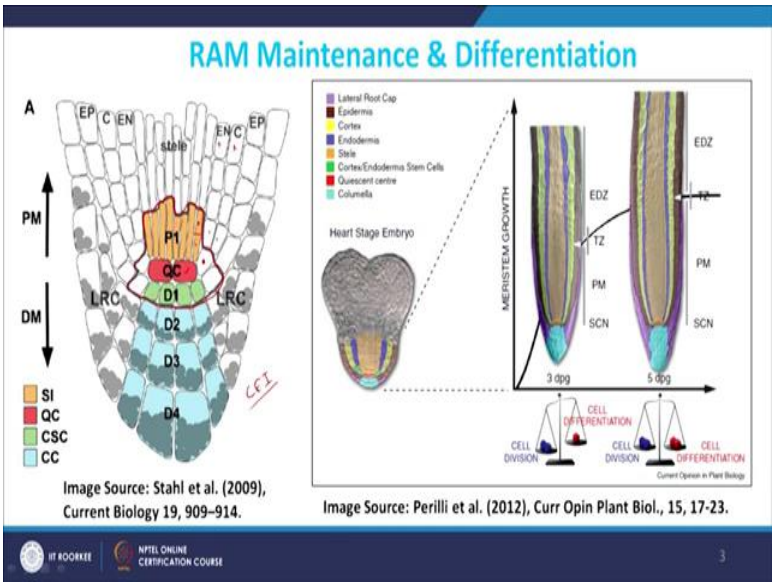

This is important to produce or to generate large number of stem cells. It is important to have a group of cells which should always divide and they give rise to the cells which is required for the process of differentiation.

And the first and important thing which happens for the maintaining apical root meristem is positioning stem cell niche. This is the region which is stem cell niche. The cells which are present in this domain, they are stem cells, they have stem cell property, they can divide and they can provide cells for later stage of differentiation and their position is relative with the QC.

The cells which are in direct contact with the QC they remains as a stem cells. Next step is the differentiation. The initial cells they depending on their position, their daughter cells after the cell division take different identity. Once this cells divide let us assume if this is the cell and it divides here. There are two daughter cells, the daughter cells which is towards the QC it remains as a stem cell or as initial, but the daughter cell which is away it enters in the process of differentiation. And just now I said that the fate of this cells; the differentiation program in this cells will depend on the relative position of the cells.

For example, if you look here. This is the initial which is a called CEI which is initial for cortex and endodermis. You can see here; both the layers cortex and endodermis they are

originated from the single cells; similarly this initial is for epidermis and lateral root cap. How this happens we will see later.

These initials are for columella cells they give raise to columella and these are the vascular cells initials they give raise the vascular tissues.

If you take a seed if you take any plant and germinate, at very early stage, as you can see this is at the embryogenic state the pattern is defined, but at early stage the rate of cell division is slightly more relative rate of cell division is more than the cell differentiation, but at certain time point there has to be a balance between rate of cell division and rate of cell differentiation.

This is extremely important to ensure that a proper amount of meristematic cells are maintained at the same time sufficient amount of cells required for the differentiation should be provided. One and very important step of differentiation is asymmetric cell division.

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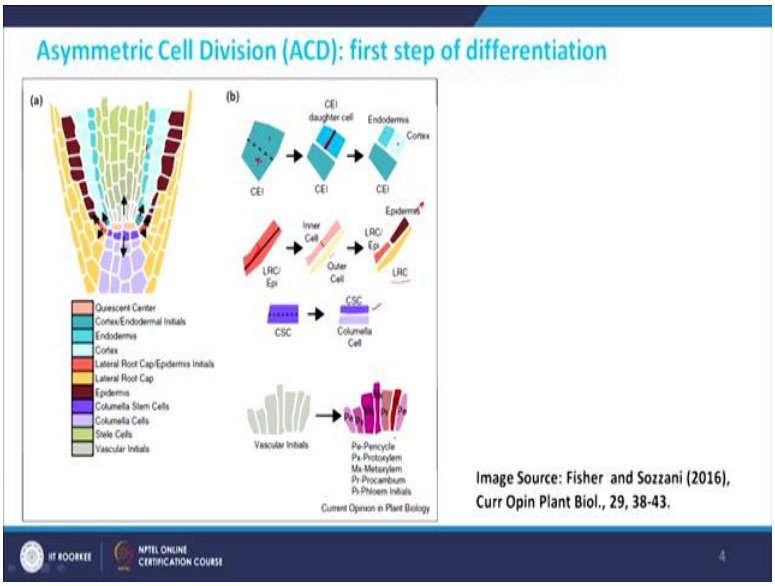

You can say this is the first step in many of the differentiation program in the life cycle of plant development, if you recall embryogenesis. The first cell division of zygote is asymmetric in nature. Asymmetric means producing two daughter cells which are not exactly similar. In this picture you can see that these are the initials which is for cortex and endodermis. After the cell division, it will generate two cells. The cell which is in

contact with QC will remain as CEI, but this cell will undergo the process of differentiation and what happens once the differentiation process start its undergo another round of cell division, this is the cell division and now this cell division is different in nature.

So, there is a reorientation of the plane of cell division. If this cell division is periclinal then this is called anticlinal and this cell division essentially generates two layers from here onwards. One layer which is going to give endodermis as you can see here and the outer layer which is going to make the cortex.

Similarly if you look this initial, it is epidermis and lateral root cap initials, this initials again undergo first round of cell division after cell division the inner cells which are in contact or which is a part of the stem cell niche will remain as a stem cells. But other inner layers this start another cell division and give raise to epidermis whereas, outer cells it will undergo the process of a specific cell differentiation program to give lateral root cap.

Third initials if you look these are the columella initial cells. This columella initial cells again undergo the process of cell division and they retain the stem cells property in the cells which are just below the QC, but the cells which are distal from the QC they undergo the process of columella specific differentiation program. Vascular initials based on their position can be pericycyle initials, they can be protoxylem initials, metaxylem initials and procambium initials or phloem initials.

In all this case the process of differentiation start at a first asymmetric cell division itself, then it undergo lot of programming lot of a cell division and cell differentiation programming together and lot of genes which are getting regulated or which are getting activated they are regulating these process.

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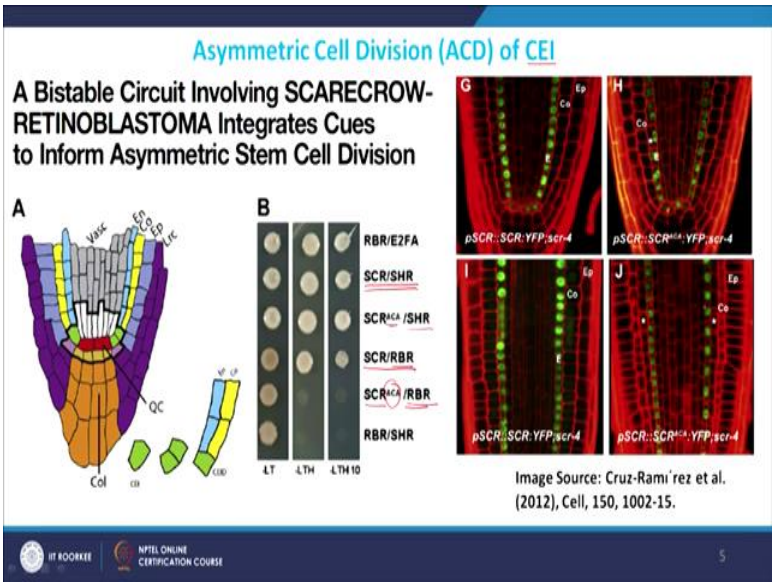

So, what we will do we will take few of the example and we will try to discuss how this happens. So, if you take the differentiation of cortex and endodermis, cortex endodermis initial cells it undergo the process of asymmetric cell division, but what are the regulators? So, if you recall your previous class you remember two proteins SHORTROOT protein and SCARECROW protein, they help in regulating not only stem cell maintenance, but they also help in giving a specific identity to endodermis.

SCARECROW protein has been found to physically interact with RETINOBLASTOMA like protein and SCARECROW protein is also interacting with SHORTROOT and RBR, but their interaction is totally different and these three important residues ACA it is important for interaction of SHORTROOT protein with RBR. If you mutate this residues the SCR will lose interaction with RBR, but interaction with SHORTROOT protein is not disrupted.

And how these regulates we are going to look here. This is a schematic diagram which we have already discussed. Here you have *SCARECROW* promoter driving SCARECROW:YFP fusion protein in *scarecrow-4* mutant background. This is close to wild type; you have *scarecrow* mutant, but this mutant phenotype is getting complemented by YFP fused SCARECROW protein, but if you mutate the interaction between SCR and RBR what happens that you can start looking some phenotype?

And one phenotype you can see here is that, there is an extra layer formation. Normally in wild type from CEI only two layers are formed and in this two layers the outer layers always remains as a cortex and inner layer differentiate into endodermis, but here if you look in this disrupted interaction mutants what is happening?

This is wild type you have only one cortex or equivalent wild type and you have only one endodermis, but when you mutate these residue when you disrupt this interaction between SCR and RBR you can see that between cortex and endodermis there is extra layer. This tells that this interaction between SCR and RBR is very important in regulating cell division pattern.

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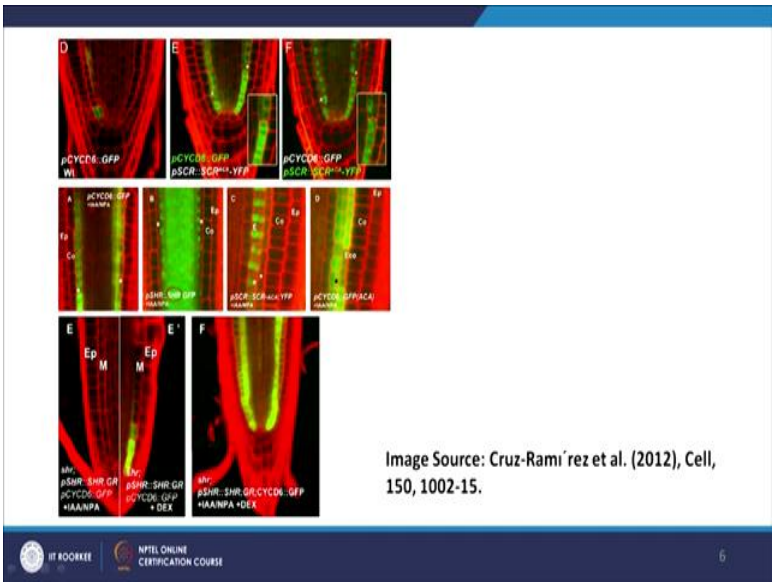

Another important gene which is called *CYCLIN D6;* here its promoter fusion construct has been used. This gene is specifically expressed in the daughter cells of CEI. So, when CEI undergo the first round of cell division you can see *CYCLIN D* gene is expresses, but immediately after that it its expression is totally disappearing. Which means that there is a possibility that the SCR RBR or this genetic regulators are activating expression of this which you will look later on in detail, and this might be interesting. Because when you disrupt this interaction between SCR and RBR you see that, there is an extra layer and the expression of *CYCLIN D6* expands.

So, it expands in both the layers. This tells that this interaction is not only important in maintaining the number of cell layers, but it is also important in restricting or in

regulating the expression domain of *CYCLIN D*. Another important thing if you look here. Auxin is an important plant hormone and regulates lot of processes. This is plant treated with auxin or increased level of auxin.

And here also particularly in roots auxin has an important role to play and if you induce this plants with auxin you can see there are extra layers and these extra layers have an induced expression of *CYCLIN D*.

But important thing here is that out of these extra layers your endodermis is only the inner most layer as you can see from the localization pattern of SHORTROOT protein, which is also always in the endodermis. Another important thing here if you look. This is SHORTROOT protein complemented with SHORTROOT GR fusion. If you recall we have discussed that glucocorticoid receptor fusion to a transcription factor make it inducible in a way that when you treat with dexamethasone then and only then this protein will enter in the nucleus and do its function.

But if you do not induce then the protein is there, but protein cannot go inside the nucleus and since it is a transcription factor and if it does not enter in the nucleus it will not perform the function. In *shortroot* mutant since this mutant we are not treating with dexamethasone that is why you do not see the complementation. So, this is a typical *shortroot* protein mutant and you do not see induction or expression of *CYCLIN D* gene.

But when you treat with dexamethasone; dexamethasone is basically allowing SHORTROOT protein to enter inside the nucleus and when SHORTROOT protein is enters inside the nucleus you can see *CYCLIN D* expression starts which means that SHORTROOT protein is activating expression of *CYCLIN D*. And when you treat dexamethasone plus auxin, then you can see that it is mimicking this phenotype where you have extra layers and extra layers are having more or expanded expression of *CYCLIN D*.

So, this all together suggest that *SHORTROOT SCARECROW* is important and it is working to activate auxin dependent activation of CYCLIN D proteins and SCR's interaction with RBR is also important in regulating.

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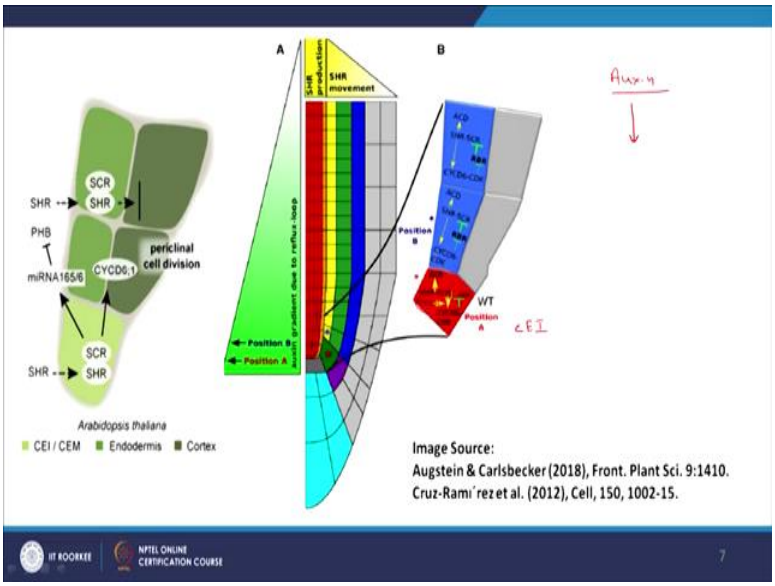

the asymmetric cell division of CEI and eventually a layer of endodermis and cortex formation. If I summarize whatever we have discussed here. So, there are two things to remember one is that SHORTROOT protein move from one cell to another cell and this movement is essentially generate a gradient. You can see the cells because you know SHORTROOT proteins are produced in the vascular tissue, the cells which is very close to vascular tissue it is having more SHORTROOT; and then similarly the auxin signaling path way, so, auxin is synthesized usually in highly dividing cells or in the young tissues and then there is a well established system that the auxin can be transported in a very specific manner. This is a process called polar auxin transport.

Auxin can be synthesized somewhere, but it can be transported to different places and this transportation ensures auxin concentration and generates a gradient in the auxin concentration, it generates maximum amount of auxin in some tissues minimum amount of auxin in another tissue and auxin works like a morphogen. If you have high amount of auxin it can induce a very specific genetic program at low amount of auxin, it can perform a different function.

The combination of all this kind of things if you look here. What happens in the position A? At position A is CEI in CEI we have high amount of auxin and then what happens that *SHORTROOT* and *SCARECROW* activates *CYLCLIN D* in auxin dependent manner and this process essentially induces asymmetric cell division and after asymmetric cell

division if you look in the inner cells the domain of *SHORTROOT* and *SCARECROW* is restricted only to the endodermis cells and how it is restricted is another different regulation which we are not going to talk here, but in inner cells since auxin level is going down.

So, you have *SHORTROOT SCARECROW* mediated cell fate determination of the endodermis. Overall the combination of auxin, *SHORTROOT, SCARECROW, RBR* ensures the first or the asymmetric cell division in CEI and then eventual process of layer differentiation.

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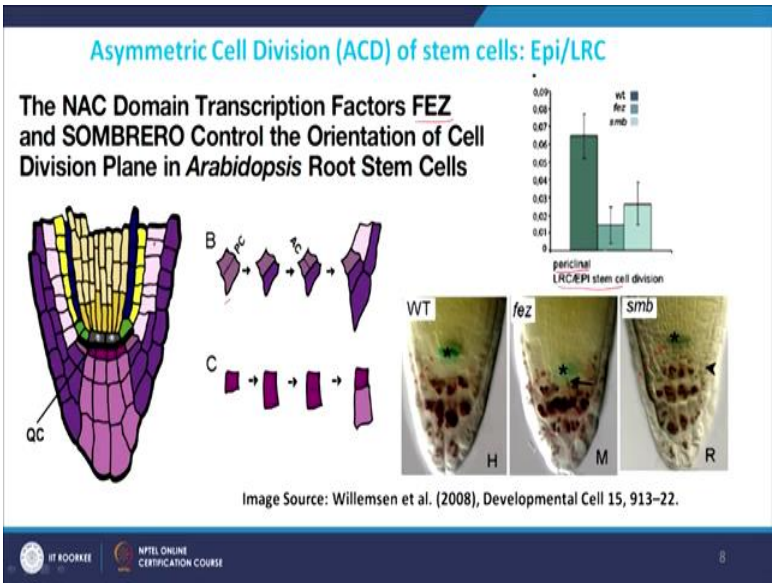

Similar kind of cell division is also important in differentiation of epidermis and lateral root cap. If you look this is epidermis and lateral root cap. The initials first undergo the process of periclinal cell division and then it undergo the process of anticlinal cell division and this essentially results in formation of epidermis and lateral root cap.

The origin of both the layers are from the same initial and there are two transcription factor both are NAC domain containing transcription factor one is called FEZ another is called SMB which is SOMBRERO and in mutant background you can see the frequency of periclinal cell divisions significantly decrease. So, this is the wild type level this is *fez* mutant and this is *smb* mutant.

So, which means that both are very important for making periclinal cell division and in the mature root in wild type this has eventual effectly on the lateral root cap development as you can see here in wild type there are four layers of the lateral root cap, but when you have *fez* mutant you have only three whereas, in *smb* mutants the number of cap layers are increased.

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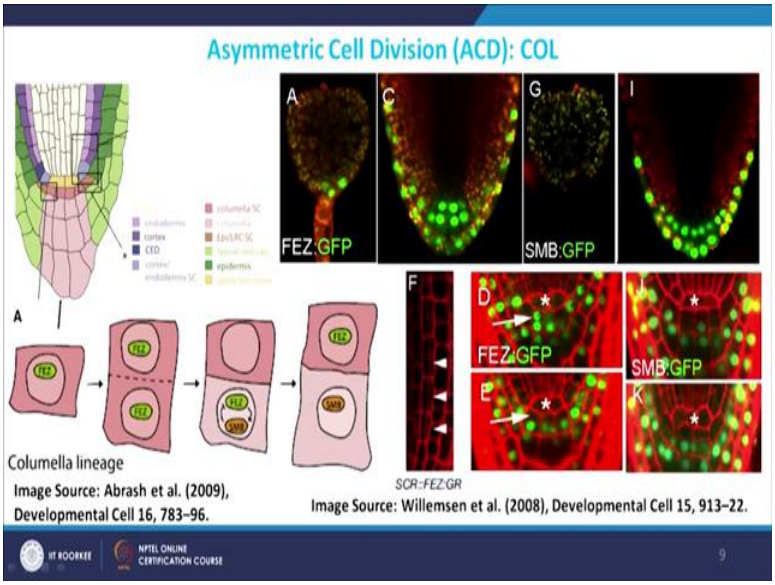

So, basically they are regulating lateral root cap development in a different manner. If you look here how columella initials undergo the process of differentiation the same regulators has been shown to play an important role.

If you look the expression pattern of FEZ and SMB protein, during embryogenesis the *FEZ* start expressing here at that stage *SMB* was not activated, but slightly later stage *SMB* is getting activated and when you overexpress this FEZ protein what happens in endodermis. So, you are using SCARECROW protein. You are over expressing FEZ protein and what you can see there is additional round of periclinal cell division.

This supports the previous observation that *FEZ* is important for periclinal cell division in mutant that cell division is decreased but when you overexpress there is additional cell division taking place. If you look the expression pattern and their regulatory network it is very important.

If you look this *FEZ*; *FEZ* is not expressed in the QC, but it express in the initials which is columella initials and when this columella initials undergo the process of cell division what happens? After this cell division the expression of *FEZ* remains in the lower cells, but it totally and immediately disappears from the initials which are close to the QC.

And *SMB* induction starts only in the lower layer which is your initial daughter cells of the initials. So, if you look all these expression pattern and mutant phenotype if you combine there is a regulatory mechanism. At the very early stage *FEZ* is getting activated in these cells then this initial cells undergo the process of cell division.

When cell division occurs in the lower cells *FEZ* activates the expression of *SMB*, but once *SMB* is activated it immediately repress the *FEZ* expression and there is differential expression pattern and this regulatory network ensures a proper stem cell identity for the columella stem cells and the distal cells to enter in the process of differentiation program.

If you look in detail how auxin regulates root cap formation there are other evidence. So, basically these are AUXIN RESPONSE FACTORS. AUXIN RESPONSE FACTOR are class of transcription factor which regulates the process downstream of auxin signaling.

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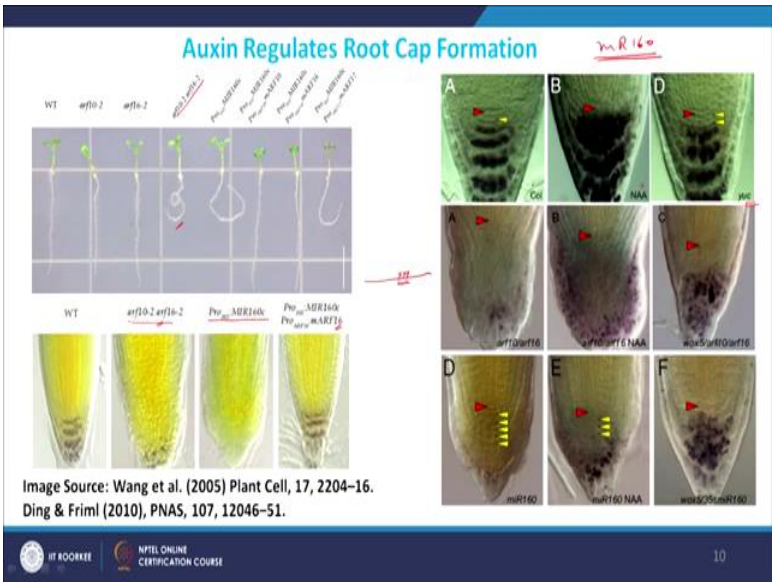

And if you mutate AUXIN RESPONSE FACTOR, in single mutant you do not see much phenotype, but when you make a double mutant of *AUXIN RESPONSE FACTOR 10* and *16* you see that there is a defect in the root growth and if you look in columella, in wild

type you have differentiated columella but in this mutant background most of the columella cells are not differentiated.

So, there is a problem in the differentiation. They are dividing and layers number of layers are also increased. Another important thing is that this *AUXIN RESPONSE FACTOR 10* and *16* they are regulated by micro RNA 160. So, micro RNA you know that they are small RNA and micro RNA, if they target a gene they silence its expression.

If you overexpress micro RNA, this gives a phenotype very similar to the loss of function of the *AUXIN RESPONSE FACTORS*. When you overexpress MIRNA there is more micro RNA which means more downregulation of *AUXIN RESPONSE FACTOR* resulting in a similar phenotype as the *auxin response factor* mutant.

Micro RNA are just 21 to 24 nucleotide molecules and they have some complementarity sequence with the target and if you mutate this target binding sequences. In overexpression of micro RNA lines if you complement with the mutant version of *auxin response factor* you do not see the phenotype which suggest that the micro RNA is regulating *AUXIN RESPONSE FACTOR 10* and *16* through the binding and this regulation is important in columella cell differentiation or root cap formation.

Similar kind of phenotype you can see here. If you have wild type this is the differentiation pattern if you treat with auxin. Here there was loss of auxin now we are having gain of auxin, when you have more auxin you can see the differentiation, even stem cell properties are lost. You have this *YUC*, *yuc* mutant is basically defect in auxin biosynthesis due to reduced internal biosynthesis.

So, in low amount of auxin you can see two layer of stem cells. Similarly, if you look this mutant background and if you look the complementation or if you make a double mutant triple mutant *auxin response factor 10, 16* if you combine with the *wox5* you can see here that these *AUXIN RESPONSE FACTOR*, micro RNA, auxin they all are working through the *WOX5* path way which means that the combination is not helping.

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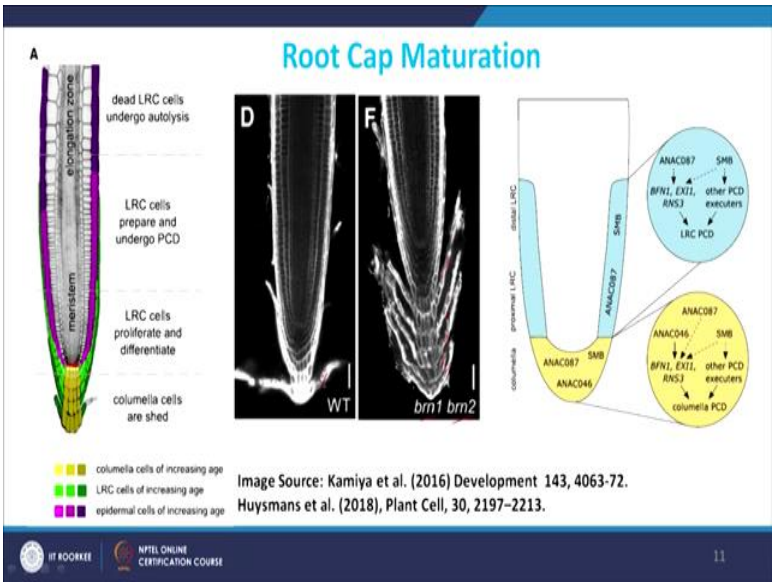

There are some more important mutants for the genes which are regulating later stage of root cap formation. The final stage of root cap formation is programmed cell death because by the process of program cell death these caps are removed at a certain frequency, but if you look this mutant background this lateral root caps are not getting removed.

So, these are the process of root cap maturation. This is also important and there are some another transcription factor which has been identified which regulate this process.

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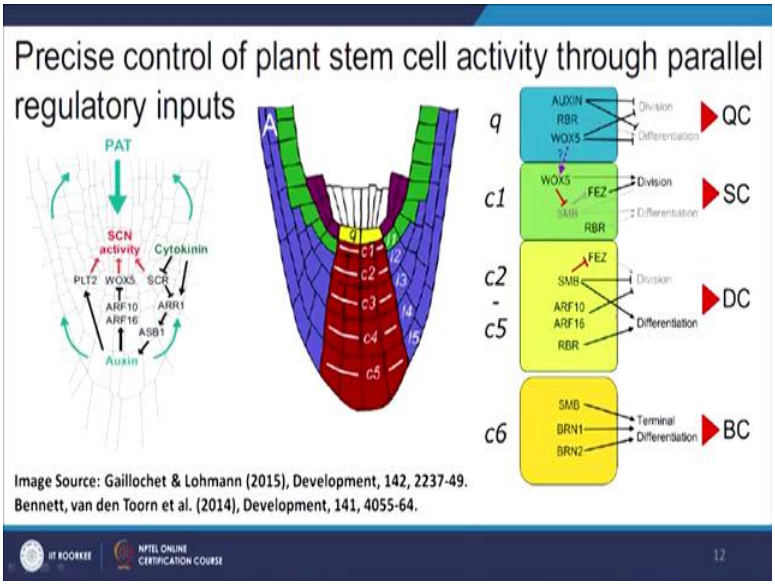

If we look in an overall regulation of the transition between cell division and cell differentiation. Auxin is regulating *ARF 10, 16* and these are working through the *WOX5* path way to restrict or to regulate the stem cell niche and allow a proper differentiation program. Here if you look layer wise how regulation is working. So, this is your QC this is c 1 c 2 c 3 c 4 c 5.

In QC there is a less division, less differentiation or you can say QC has no division no differentiation and *WOX* 5 is present. If you come one layer down this layer is basically stem cells; stem cell has to be divide. So, here division is dominating over the differentiation and *WOX* 5 is very active *RBR* is active and *FEZ* is active.

But in other layers c 2 to c 5 layer *ARF 10*, *ARF 16*, *SMB*, *FEZ* and *RBR* they interact and they shift the transition from cell division mode to cell differentiation mode and if you come in the last layer which is called terminal differentiation, the final layer differentiation there is another gene which is called *BRN1* and *BRN2* and *SMB* they initiate or activate the process of terminal differentiation.

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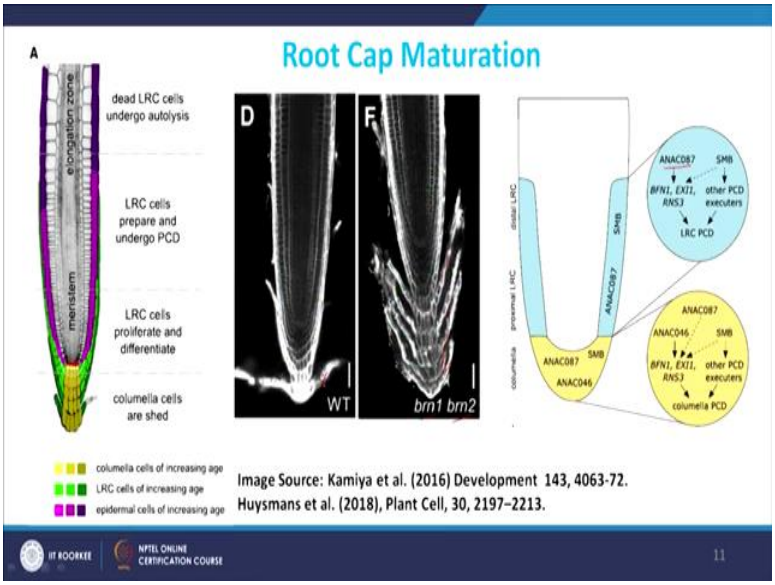

And that is why if you do not have *BRN1* and *BRN2* the terminal differentiation is defective and that is why you can see multiple layer of lateral root cap formation.

So, we will stop here and in next class we will discuss vascular tissue development.

Thank you very much.