Plant Developmental Biology
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Lecture - 11
Root Development

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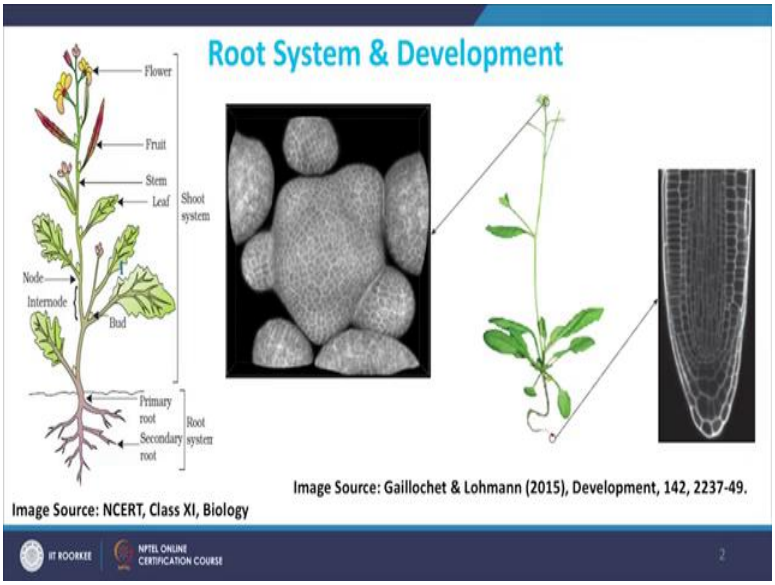

Welcome back to the course of Plant Developmental Biology. In today's class we are going to discuss about Root Development. If you take a mature plants, it can be divided into two system; the root system and shoot system. Root system is usually below the ground part of the plant; shoot system is above the ground part of the plant.

And if you look a closer view of the very tip of the plant, at shoot this is a scanning electron micrograph picture of growing shoot apical meristem which is basically inflorescence meristem. And at the very tip of the root, this structure is called root apical meristem which we will look in detail.

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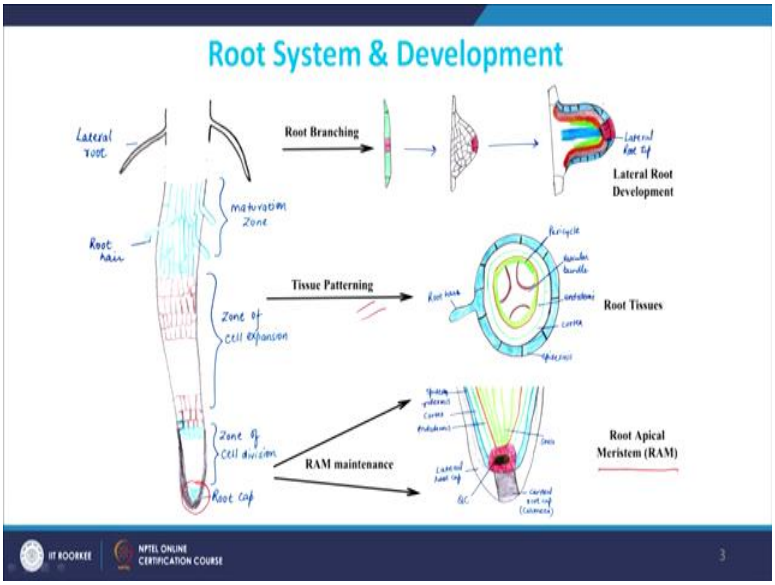

This slide also you have seen in one of the previous class. Root development in plant, there are three major developmental processes which is going on during the development. First process which is called root apical meristem maintenance which occurs at the very tip of the growing primary roots.

And if you look at the very tip this is the region which is called root apical meristem, and this is the region where some cells retain their identity as a stem cells. Stem cells means they can only undergo the process of cell division, they do not enter in the process of cell differentiation.

And this is very important for continuous growth of the root, for development of the root, for growth of the root or organogenesis, for which a continuous production of cells are required. And this is happening here in the root apical meristem. And then if you come slightly upper zone of the root, this zone is called zone of cell expansion. And during this zone mostly tissue patterning the second part of the development in the root takes place, where different tissues or different layers of the cells they take a very special identity.

And then if you come even higher up region of the root the process of proper differentiation of new organs or organogenesis occurs which in case of root is lateral roots or root branching. So, the process of root branching is visually initiated in the differentiation zone and a lot of lateral roots are developed in this region.

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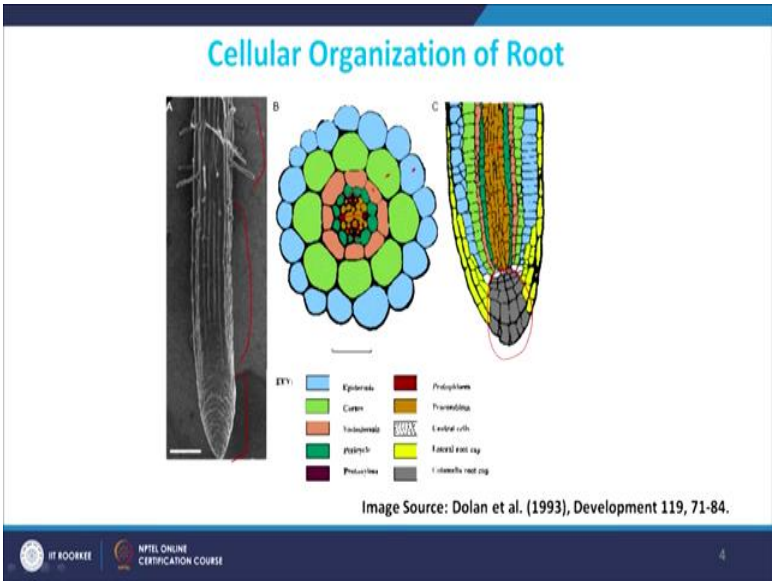

So, now in in this class, we will focus on the first aspect which is root apical meristem. What happens in the root apical meristem, what are the process, how the meristem is maintained. Because if you look the root tip carefully, you can see this is the region which is basically the meristematic region; meristematic region is the region which undergo the process of cell division. And this is the region of cell elongation and then here is the differentiation.

And if you make a cross section, so you can clearly see that these are the different cell layers, the outermost layer is epidermis, then you have a layer of cortex, then you have endodermis, then this is pericycle. In pericycle there is a xylem axis. So, this is protoxylem, this is metaxylem.

And then at two poles you have phloem poles, where you have sieve elements as well as companion cells and then you have some procambium cells. This is a longitudinal view. So, you can see this is epidermis, cortex, endodermis pericycle and the vascular tissues. But apart from that you have some tissues here which is called columella cells and these are lateral root cap. So, all these are part of a particular growing roots.

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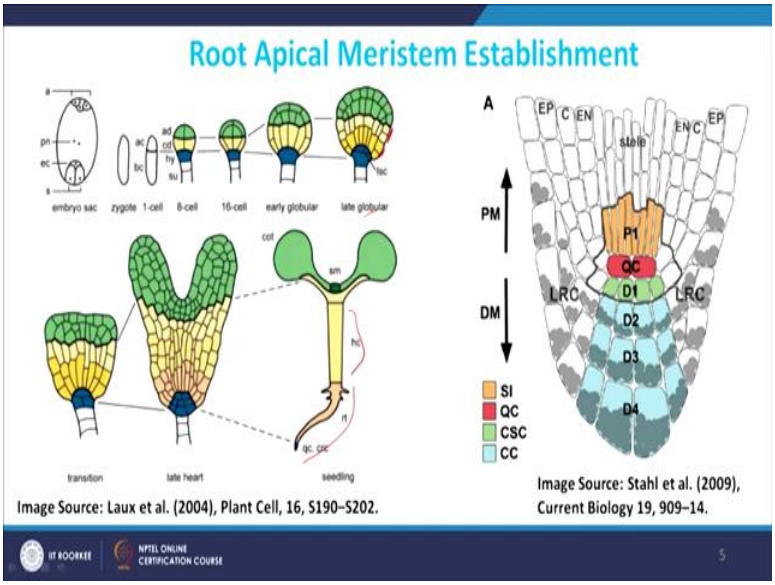

How the apical meristem is established, how this organization or proper organization of the root meristem takes place, and what is the importance of all these cells? This happens at a very early stage of the embryogenesis.

If you recall we have discussed that the first diploid cells after fertilization is zygote. Zygote undergo the process of embryogenesis where it undergo several round of mitotic cell division. And at some stage, if you look at late globular stage, you can see there are three distinct domains. This domain is the upper domain or apical domain which is eventually going to make the upper part of the plant. Then you have a middle domain here, this will make eventually the hypocotyl region of the seedling. And this lower domain is basically going to make the root.

The basic developmental program for root, shoot is established at the stage of embryogenesis itself. Here you can clearly see that this is the region of the root apical meristem, which is already established. Now, if you look the growing root once it is emerged and you can see this tip this is the very closer and high magnification view. So, few cells which you have to understand the entire process of root development.

So, if you look this is the root tip, and this red colour cell you can see here two, but actually they are four in numbers. These cells are called quiscent centre, this is QC. And the feature of these cells are that they themselves are very slowly dividing or almost not dividing, but they regulates the division capability of the cells which are close to them.

So, if you look this boundary domain, this is the region which is called stem cell niche, because the cells which are lying in these domains they will not undergo the process of cell differentiation, they will only divide, they are the stem cells. These are also called initial cells.

These initial cells or the stem cells, they are directly connected with the QC which means that the cells which are directly connected with the QC they might be receiving some kind of signals which allow these cells to remain as a stem cells or the cells which are dividing and it also helps them not undergoing the process of differentiation.

Then apart from that you can see these cells depending on their position the initials; these are stele initials, initial for cortex and endodermis,you have a common initial which is called C E I, which is Cortex Endodermis Initial. The D 1 layer which is just below the QC these are initial for columella cells. So, this entire region is columella.

This immediate down to the QC, they are the intial, they are the columella stem cells. But other cells which are in D 2 layer, D 3 layer and D 4 layer, they have undergone the process of columella specific differentiation. And these cells are basically making lateral root caps. Columella cells when they undergo the process of cell differentiation, they accumulate starch and there are way to detect the starch.

So, if you detect the starch, if you see the starch accumulation in the cell which is a marker that this cells has undergone the process of differentiation. These cells are no longer working as a stem cells. And then these different layers you can see clearly epidermis layer, cortex layer and endodermis layer and stele layers they are arranged in a radial manner.

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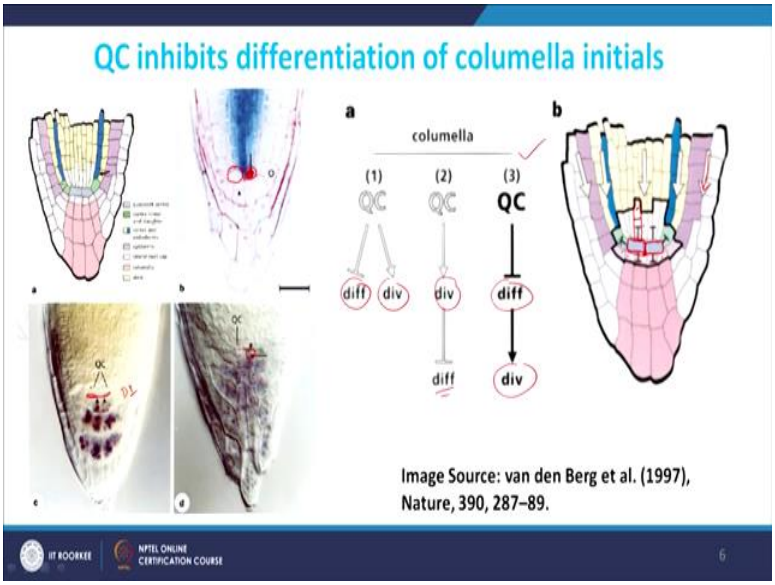

QC is sitting at a very central position and it is regulating stem cell property. And what is it's importance, how it is doing this function? And one very specific experiment called laser ablation through which you can specifically kill a particular cell in the growing root was used to study the function. And what happens if you kill the QC?

This is a typical structure which you have seen in the previous slide. So, if you look this picture, this is your QC, one of the QC is here, but second QC which is now very small here this QC is ablated. And if you ablate the QC and look what happens to the cells which are in contact with this QC. You see here this is starch granules accumulation. So, if you see this starch granule accumulation which means that these cells are not stem cells, but these cells are undergoing the differentiation.

But if you look this is the QC and just below the QC the cells which is in D 1 layer, they do not accumulate the starch which means that they are they are basically stem cells, but the cell layers below the D 1 like D 2, D 3, D 4, they start accumulating the starch which means that they have lost the stem cell property and they have started cell differentiation.

But if you look this case where one of the QC was ablated, the QC which is intact the layer which is below the QC or the cell which is below the QC in the D 1 layer or which is called columella stem cells. This is still maintaining the stem cell identity, it has not accumulated starch. But the cell which is below the ablated QC, this has started accumulating starch which means that this cell has lost its capability of stem cells and it

has started differentiation, which tells that QC is important in maintaining a cell as a stem cell identity.

How this is possible, what could be the hypothesis? In first hypothesis, what can happen that QC is activating division and repressing differentiation simultaneously, independently. And second possibility is that QC is activating, division and the result is inhibition of differentiation. In third possibility, QC is repressing differentiation and result is activation of cell division.

But if you look this experiment, this suggests that the third pathway is working. Then on the basis of this, one model has been proposed. And the model here is that there are basically two types of signals. One signals which might be coming from the very mature cells of the upper region of the root, and they are the differentiation signal they are promoting the differentiation. And another signal which is coming from the QC, and this signal is basically inhibition of differentiation.

So, the balance between these two signals are important. This signal is short distance signal. So, the cells which are in direct contact with the QC they are receiving this differentiation inhibition signals, whereas other signals are coming from top. And what happens if you have inhibition signal, there the differentiation is inhibited.

But once this cells divide, if this is an initial cell and if this cell divide, the cell which is present at the upper side, now it loses the contact with the QC which means that it is not getting the inhibition signal or the signal which is responsible for differentiation inhibition. But at the same time it is getting signal from the top which is to promote the differentiation. Now, these cells will start the process of differentiation, whereas the lower cell which still remains in contact with the QC, it will retain the process of cell division and process of cell differentiation is inhibited in these cells.

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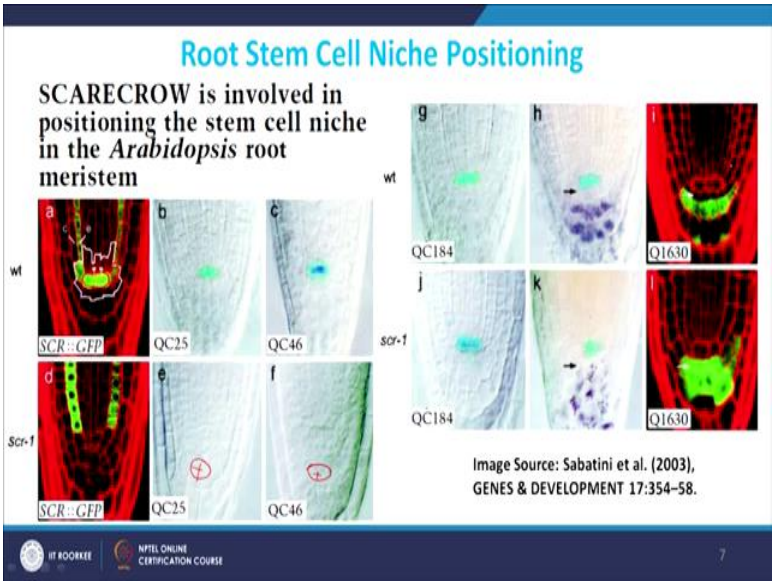

So, QC is important for maintaining cell differentiation a balance between cell division and cell differentiation. What could be the genetic pathway, what are the regulators, and, and how they helps in positioning these stem cell niche in the root apical meristem? And one of the pathway is SCARECROW SHORTROOT protein mediated pathway.

SCARECROW and SHORT ROOT, these are two GRAS family of transcription factor, they are a special transcription factor and they are expressed in a tissue specific manner. So, here if you look the expression pattern of *SCARECROW* promoter activity of *SCARECROW* driving GFP expression, so it expressed very well in the QC and the initials for cortex and endodermis. But at the later stage the expression is only restricted to the endodermis, and there is no expression in the cortex.

But in *scarecrow* mutant the expression in QC disappears in this region. Similarly, if you look other marker; these are the QC 25 and QC 46, they are the marker genes which are known to express in the QC. But in *scarecrow* mutant background you can see that the expression is totally lost, which means that in the *scarecrow* mutant QC identity is defective, which tells that the identity of QC is regulated by *SCARECROW*. But some of the QC markers they still get expressed in the QC. So, may be the identity is not completely lost or maybe this could be the position dependent expression of the genes.

If you look here the starch granule accumulation, so if you look in the wild type this is the QC and this is the columella stem cells there is no granule accumulation. But in absence of SCARECROW protein, the cells just below the QC they start accumulating

starch. So, this tells that the stem cell maintenance columella stem cell maintenance is defective in case of *scarecrow*.

Q1630 is marker for differentiated columella cells. In the wild type case, this is QC and this layer, the D 1 layer which has columella stem cells. This gene is not expressed here, but the D 2 layers which has the differentiated columella cells express Q1630. But in *scarecrow* mutant background, you can see that the cells which is just below the QC can start accumulating columella specific markers. This all together suggest that *SCARECROW* is important in not only for taking QC as a proper identity, but also positioning stem cell initial at the right position.

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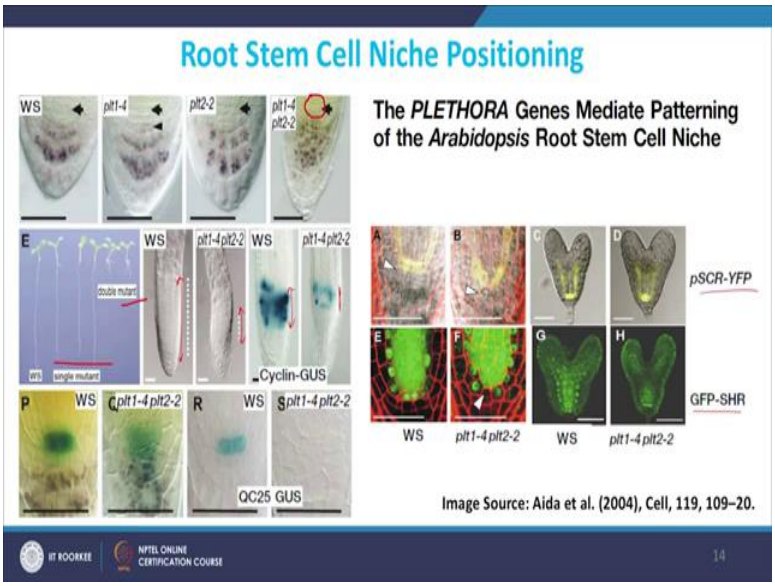

Another important pathway or genetic regulation of positioning root stem cell niche is mediated by another class of transcription factor which is PLETHORA. PLETHORA'S are AP2 domain containing plant specific transcription factor. And if you recall one of the class we have discussed that usually they work in a genetically redundant manner. When you have a single mutants, you do not see a strong phenotype, but when you combine *plethora1* and *plethora2* double mutant, you can see that root growth is inhibited. So, it is a very short root plant.

And if you look the differentiation pattern, this is the QC. But if you look the double mutant the cells just below the QC in the D 1 layer, it has started accumulating starch, which suggest that in double mutant *plethora1* and *2* double mutants the stem cell niche

is not getting maintained stem cells are losing their stem cell property. If you look here this is double mutant *plethora1* and *2* double mutant, this is wild type. And this is the basically size of meristem. So, in wild type, this is the meristem size and this meristem size is significantly reduced in the double mutant.

Here if you look the marker, cyclin gus marker, cyclin markers are basically markers which marks the dividing cells, it will express in the cells which is under process of cell division. And if you look in the wild type, you can clearly see that large number of cells are under the process of cell division, but in double mutant this number is significantly reduced. Same thing you can look by lack of the QC 25 markers. So, in wild type, you have QC 25 markers expressed in the QC, but in mutant this the expression is totally disappeared.

But then the question is that we know that *SCARECROW* and *SHORTROOT* pathways are regulating positioning of the root stem cells. Now, *PLETHORA'S* are also regulating. Are they working through the same pathway or they are working independently?

So, to check that if you look these mutants, this is the double mutant. And if you look the expression pattern of SCARECROW protein and SHORTROOT protein, you find that the expression looks quite normal. So, in the embryo stage between wild type and mutant here also wild type and mutant, so the expression pattern of both SHORTROOT and SCARECROW protein is not significantly affected in *plethora* mutants. This suggests that they might be working independently for regulating a stem cell niche positioning in case of root apical meristem ok.

So, if we combine, you can see that there are two pathways *SHORTROOT-SCARECROW* pathway and *PLETHORA'S* pathway. *SHORTROOT-SCARECROW* pathway are helping in positioning QC in radial manner.

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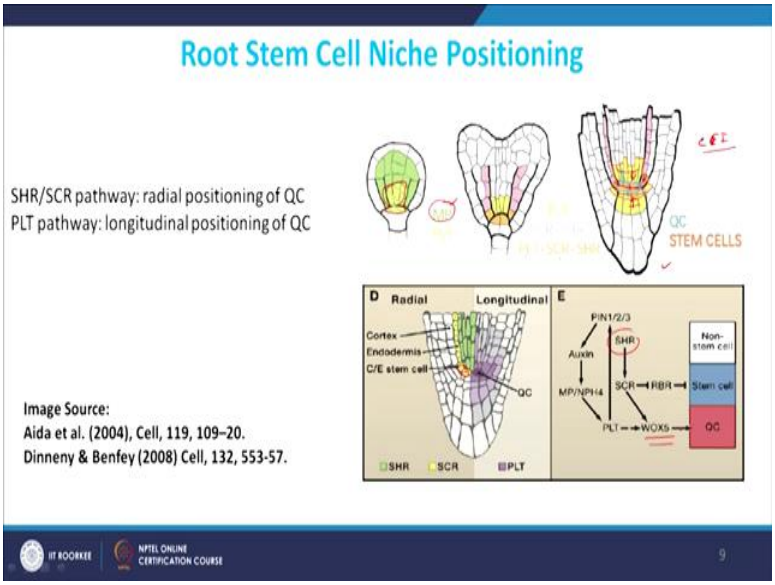

Radial manner: If you look this picture this is the expression pattern of the genes. *MP* we will talk maybe later this is a kind of genes which is regulated by auxin. And auxin is hormone which plays a very important role in the root development. But apart from that if you look the *PLETHORA* expression, *PLETHORA'S* are expressed here in this domain. But at the later stage, if you look or maybe in the growing meristem if you look so this is the cells where *PLETHORA* are expressed. This is the cell where you're *SCARECROW* and *SHORTROOT* proteins are expressed.

But if you look this cell, so this is QC as well as this CEI initials, so cortex endodermis initials. These are the cells where all three genes are expressed *PLETHORA*, *SCARECROW* and *SHORTROOT*. So, the hypothesis is that *SHORTROOT* and *SCARECROW*, *PLETHORAS* are helping in positioning the stem cell niche in the radial manner, whereas *PLETHORA'S* might be helping in positioning the stem cell niche in the longitudinal manner. Later on we will see that actually *SHORTROOT* activate expression of *SCARECROW*, and it activates the expression of *WOX* 5 which we will see in another slides.

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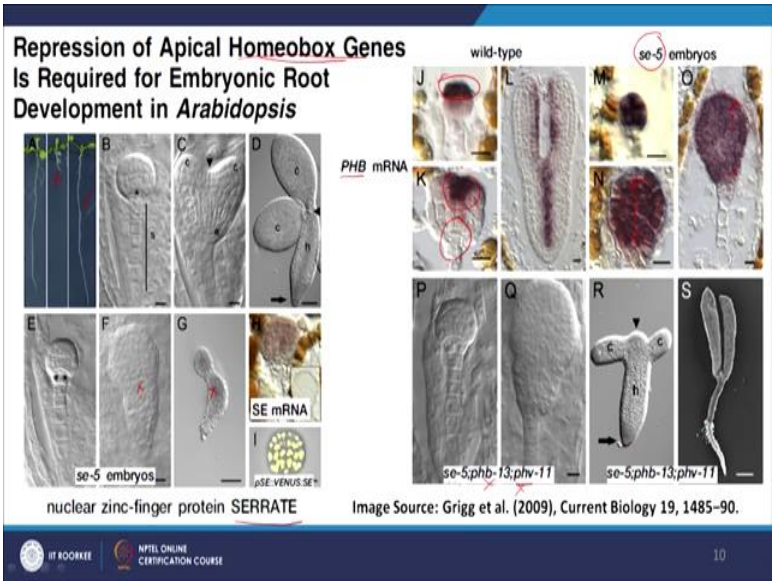

So, there are two pathways which are promoting stem cell niche formation and helping them to position in a right domain. But there are some repressions of this process. And those repressor need to be repressed to ensure that a proper stem cells are maintained and proper stem cell niche are positioned. And one of them belongs to another class of transcription factor which is called homeobox transcription factor.

If you look the mutant of *SERRATE* gene, *SERRATE* is a nuclear zinc finger protein. In this mutant what you observe that that the proper embryogenesis is totally defective, and there is no proper organization of the cells, there is no proper organization of apical meristems. But if you combine this mutant, *serrate* mutant with some of the homeobox genes like *PHABULOSA* and *PHABULOTA* , these are the homolog genes. If you make double mutant our triple mutant, this defect of *serrate* mutants is complemented. What does it mean? It means that somehow they are genetically interacting and they are basically suppressing the phenotype of *serrate* mutant.

How this happens? So, if you look the expression pattern of this box homeobox gene *PHABULOSA*, you can clearly see this is asymmetrically expressed or localized. You can see it is only present in the apical domain, it is not present in the basal domain or root apical meristem domain.This gene has to be repressed in the root apical meristem for proper meristem function.

But what happens in the *serrate* mutant? In *serrate* mutants this asymmetric expression pattern or transcript localization is lost for the *PHABULOSA* genes. Now, you can see

the *PHABULOSA* is expressed everywhere in the embryo and that is disturbing the pattern, that is not allowing a proper root apical meristem to form. But when you knock out this transcription factor in *serrate* mutant background, early there might be not a much complementation, but later stages this basically compliment the phenotype.

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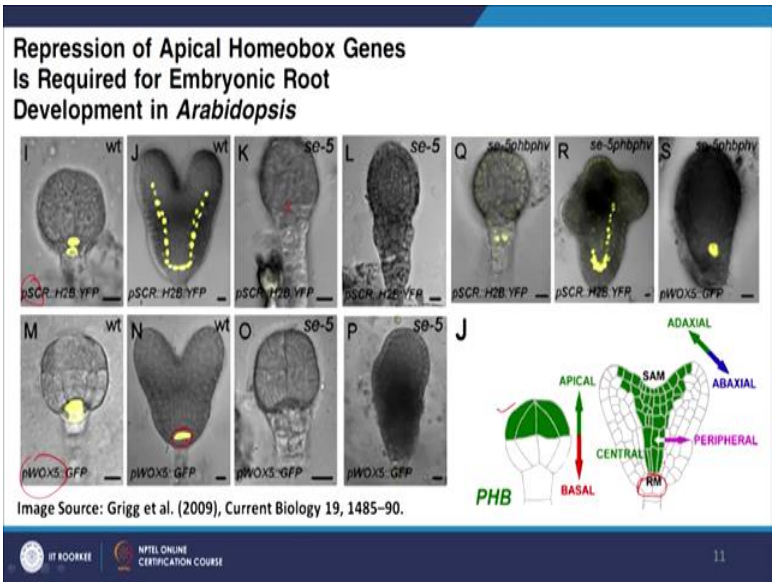

As you can see through the marker, so this is a *SCARECROW* marker. *SCARECROW* just now you have seen that it express in the QC as well as the endodermis initials. This is *WOX5*. *WOX5* is another homeobox protein, which is expressed specifically in the QC cells. But in the *serrate* mutant, both the markers lose their expression pattern, which means that there is no proper QC identity, there is no proper stem cell niche organization and positioning.

But when you make a double mutant or triple mutants, you can see that their expression reappear in the right domain which means that now if you mutate *PHABULOSA*, *PHABULOTA* there basically this mutant the *serrate* mutant or the triple mutant, they can reorganize their root stem cells niche and that is why they can rescue the phenotype.

See if you look this model, this *PHABULOSA* protein which is very which is highly expressed in the apical domain, and it is kept repressed in the basal domain. At this state particularly in root apical meristem, this needs to be totally kept repressed. So, there are two kind of mechanism. If you want to maintain root apical meristem, then some

activator has to be activated, at the same time the repressor has to be negatively regulated to ensure proper development.

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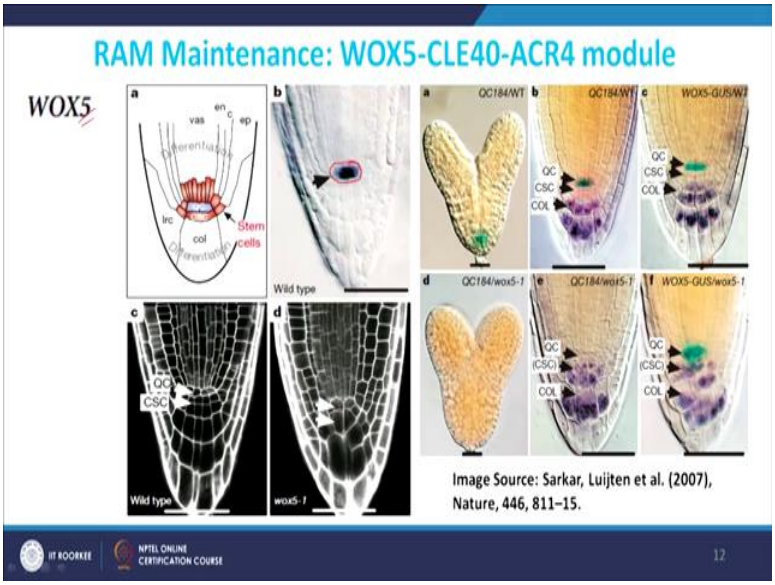

Another important regulatory pathways which is regulating root apical meristem maintenance is WOX-CLE-ACR4 module. So, we will go one by one. What are the interaction? If you recall our previous few classes where we have discussed in detail, what kind of approaches we are taking to study. Now, here what you are going to learn that how those approaches has been used and how the data has been interpreted to understand a particular developmental pathway.

WOX5 is another homeobox domain containing transcription factor, but it has more positive role on the root stem cell maintenance or QC identity unlike the *PHABULOSA* and *PHABULOTA*. If you look the expression pattern, *WOX5* promoter activity or RNA you can clearly see that it is very specifically expressed in the QC. And if you look this is wild type QC. Just below the QC you have columella stem cells. If you look in the *wox5* mutant background this is the QC, but the cell just below the QC, it looks defective, it is not normal cells. And if you check the markers, different markers of the QC, for example, QC 184, you can clearly see that though QC identity is not completely lost here, there might be maybe some of the markers are disappearing. For example, QC 184 is disappearing, but *WOX5* marker is still active. So, there is a defect in the identity.

In the wild type these columella stem cells identity is basically lost and it has differentiated. So, this has accumulated the starch granules, which tells that *WOX5* is important for stem cell maintenance for columella stem cell maintenance in the root.

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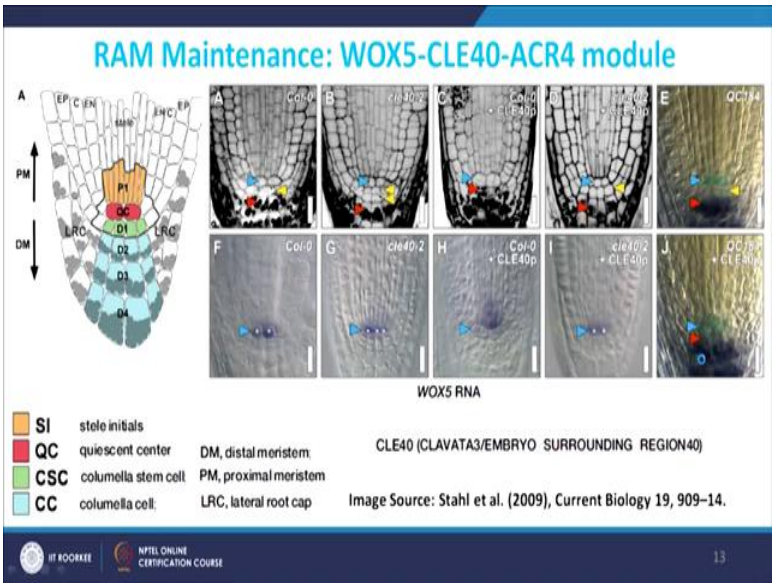

Then there is another peptides, which is called CLE peptides. In wild type, typically you have QC then you have one layer of D 1 layer which is your columella stem cells, and then next layer is differentiated columella cells. But if you mutate *CLE40* gene there is more than one layer of columella stem cells which means that in *cle40* mutants there is a gain of stem cell activity. So, there is more than the required stem cells activity.

And if you take this wild type and treat exogenously with CLE peptide, CLE peptide you can synthesize and then even the one layer of initial cells or stem cells has disappeared in wild type. So, in wild type, there is an endogenous CLE40 signalling is active, now you are putting more CLE40 protein from exogenously. So, it might be working in the dose dependent minor. And if you have more doses, even one layer of this activity is lost.

But if you look here when the endogenous CLE40 pathway is lost because of the *cle40* mutant, now you are supplementing CLE40 from outside it basically restores the phenotype to the wild type, here you can see only one layer of columella stem cells. And these are just the marker expression pattern for *WOX5* RNA and QC 184 RNA. So, in this way, you can say that *WOX5* is activator or positive regulator of stem cell activity,

whereas CLE40 might be negatively regulating the activity but we will see how exactly this is happening.

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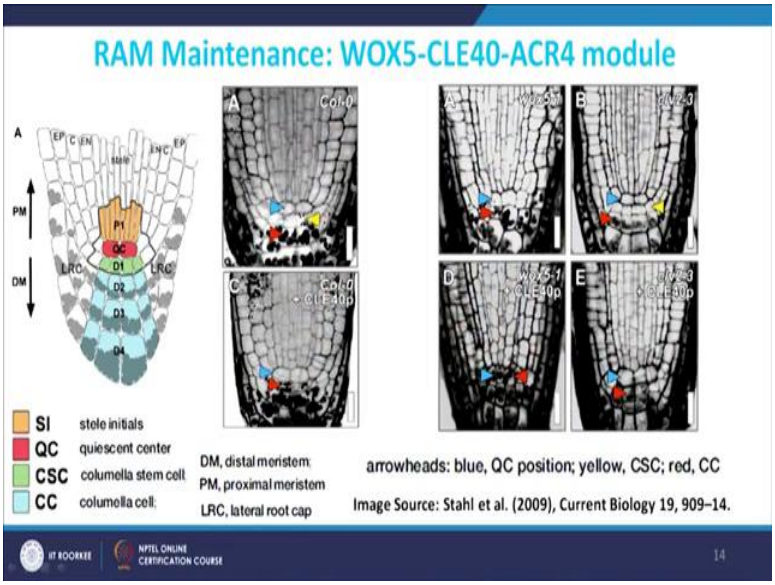

Then if you combine this mutant, if you look the *wox5* mutants as you have seen earlier in *wox5* mutant, you do not have columella stem cells. But when you treat with CLE40 proteins even the QC got differentiated. So, apart from the loss of columella stem cells, even QC has lost and has accumulated the grain, so there is a gain of differentiation.

Then there was another *CLAVATA* like genes which is *CLAVATA2*. We will see in the shoot development, similar kind of mechanism functions in maintaining shoot apical meristem. So, if you look *clavata2* mutant; *CLAVATA2* mutants basically it does not have any phenotype it looks very similar to the wild type Columbia.

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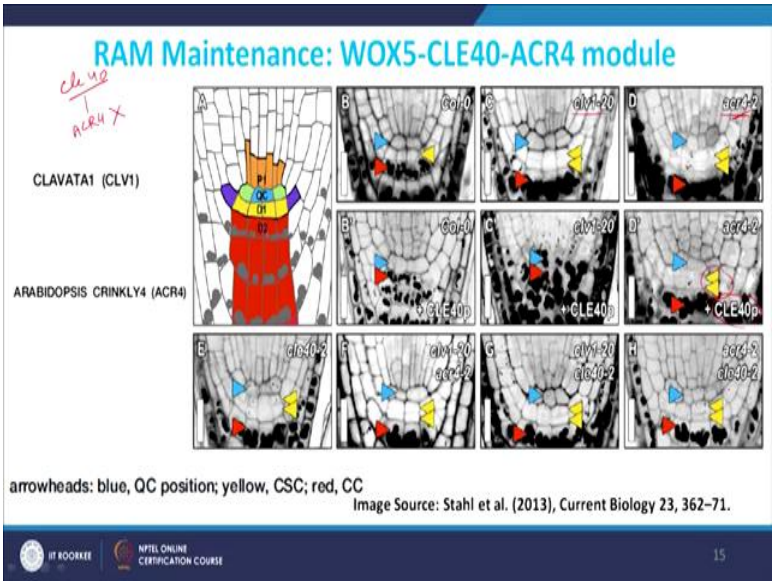

Then if you go again and look another mutants or if you see the genetic interaction with other genes, then what you can see there is another *CLAVATA* which is called *CLAVATA1*. In *clavata1* mutant, has a phenotype which is very similar to loss of *CLAVATA40*. Another gene which is called *ACR4*, which is basically *ARABIDOPSISCRINKLY4*. So, this also phenocopy *clavata40* mutant phenotype. And another important thing if you treat these mutants with the CLAVATA basically in *clavata*1 the the phenotype is lost and more differentiation has started.

But if you look in *acr4* mutant, even after CLAVATA40 treatment, there is two layer of columella stem cells. This suggests that they are working through the same pathway and *CLAVATA40* is upstream of *ACR4*. So, *CLAVATA40* is working through the *ACR4*. If you look here this is *CLE40* and then downstream is *ACR4*. If you do not have *ACR4*, even if you provide CLAVATA40, the phenotype cannot be rescued. So, *ACR4* is required for *CLAVATA40* to work here.

Then these are the double mutant phenotype. This is *cle40* alone there is two layers. When you combined *clavata1* and *acr4*, there is two layer. *clavata1* and *cle40* - two layers; *clavata4* and *cle40* - two layers. So, all are having similar phenotype which suggest that all of these might be working through the same genetic pathway.

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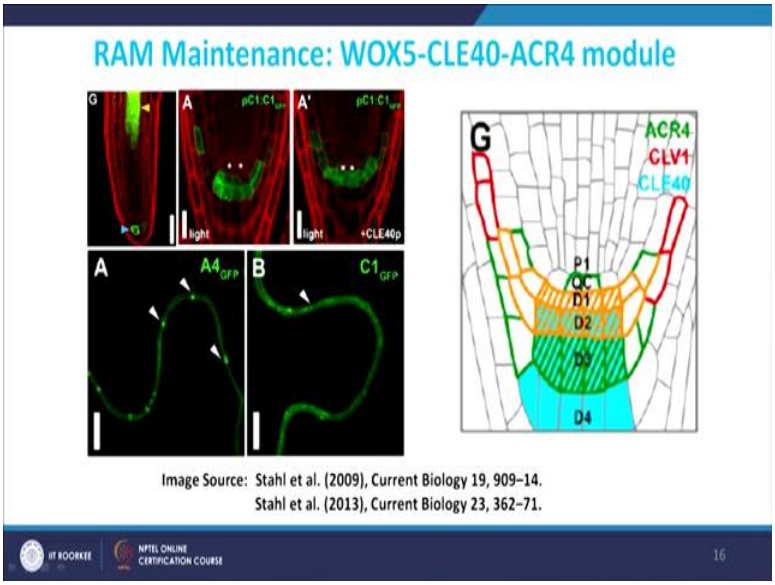

And then if you see the expression pattern of them. This green is *ACR4* expression pattern, and red is *CLAVATA1* expression pattern, and this is the *CLE40* expression pattern domain. And another important thing what has been seen that this *ACR4* is very specifically localized to the plasmodesmata. Plasmodesmata in some of the future class we will see that plasmodesmata is a channel through which lot of cell to cell communication takes place during the plant growth and development.

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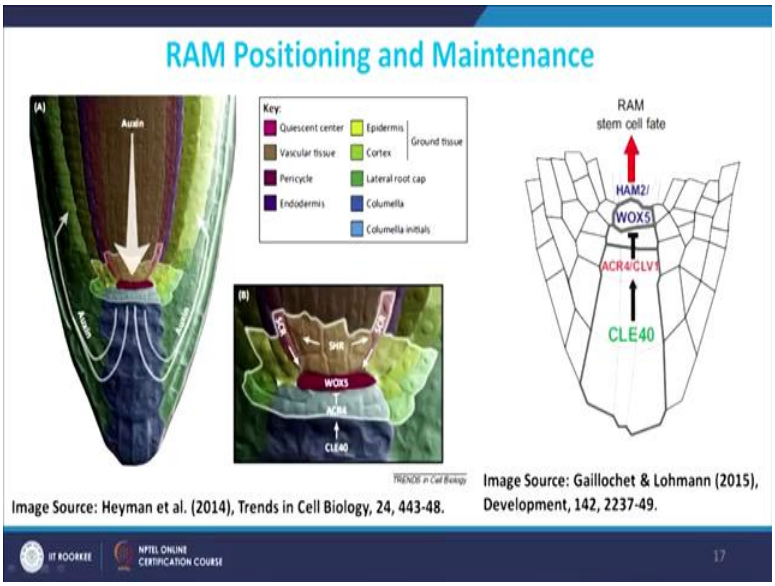

So, here if we combine all these information together, you can suggest that *CLAVATA40* is present here. This CLAVATA40, actually the biochemical nature of CLAVATA40 is

known, this is a kind of signalling peptide which works as a ligand and then these are the receptors.

So, this CLAVATA40 is received by *ACR4* and *CLAVATA1*. And then this signalling is very important to restrict the domain of *WOX5* to QC, so QC should not expand. And this is very important to ensure that a position of QC has to be fixed at a particular place. And once you fix the position of QC, eventually you are going to regulate or positioning of entire stem cell niche.

There are multiple pathways *PLETHORA* mediated pathway *SHORTROOT SCARECROW* mediated pathways, and then *WOX5 ACR4* CLE40 mediated pathway, all of them working together to ensure proper root apical meristem maintenance and the function.

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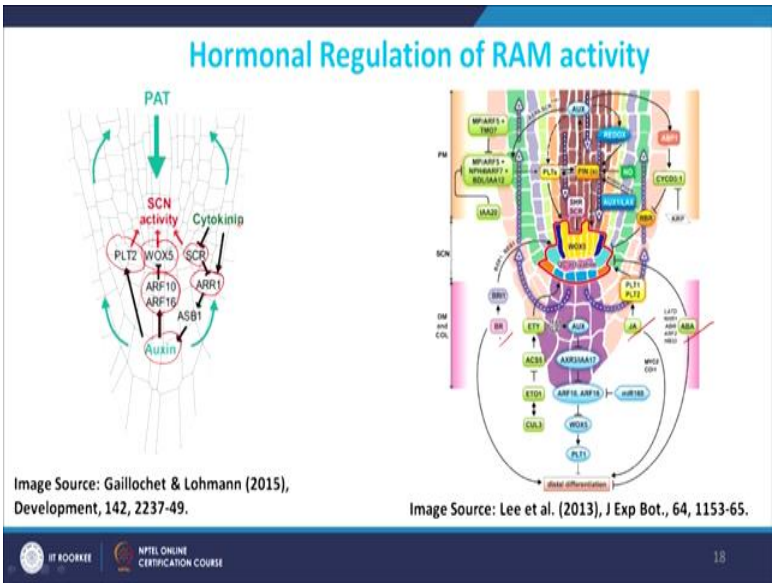

Apart from these regulators, plant hormones are important in growth and development. They almost regulate every aspects of growth and development. Auxins, cytokinins, Gibberellic acid all these hormones are very important. Here we will not go in detail, because this is a very complex process of hormonal signalling. Auxin, brassionosteroid all these hormones are also playing very very important role either directly or through the same genetic regulatory pathway which just now we have discussed to ensure the proper root apical meristem maintenance.

For example, if you look here this is *SCARECROW*. *SCARECROW* is negatively regulated by or repressed by cytokinin. This is response regulators which works in the cytokinin response. And then this is auxin; auxin is activating some of the auxin response factors. And these auxin response factors are regulating the activity of *WOX5*. Auxin is also regulating the activity of *PLETHORA'S.*

So, if you look in this picture, you can see that there is a cross talk between auxin and cytokinin together which ensures a proper root apical meristem and proper meristematic activity and a critical balance between cell division and cell differentiation during growth and development. Apart from auxin and cytokinin, ABA, JA, brassionosteroid, all other hormones are having role and functions in root apical meristem maintenance.

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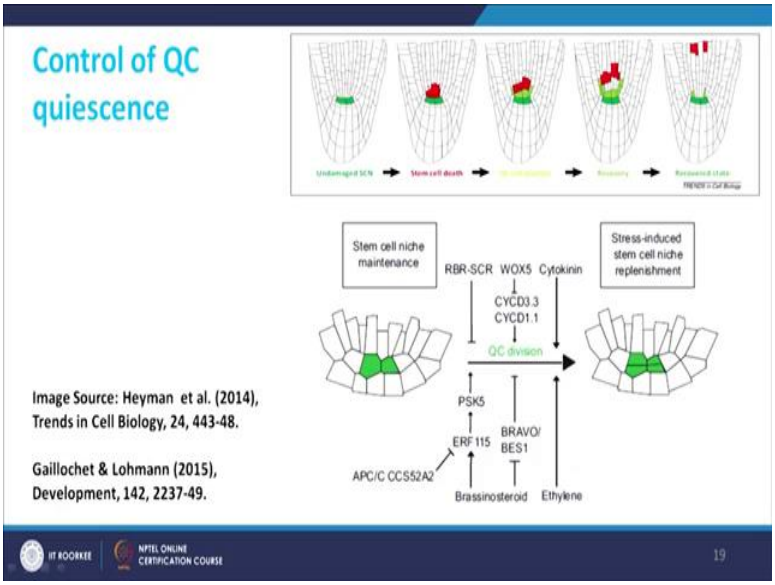

Then coming to the another very interesting thing here is control of QC. QC is important for maintaining root apical meristem activity, but QC itself does not divide very actively. So, could it be just that its function is only to regulate the activity or can it have more function? So, under normal condition it is only regulating this, but in some stress condition it has more functions. It can work as a reservoir for providing stem cells basically.

For example, if there is a damage of the growing root or if due to some reason these initial cells or the stem cells are damaged or they are dead, then under that condition this QC start dividing. So, there is an activation of cell division in the QC which is normally

silent. And this QC recovers entire stem cell niche here, so that root continue growing and this will lead to the recovered state of the root apical meristem.

Another thing you can see these pathways are also tightly regulated genetically, in normal condition if you look stem cell niche is maintained by this QC, but at this stage QC is not dividing. But if there is some stress induced signal or any issues, then what happens that this QC starts a process of cell division just to generate more ah stem cells.

And these processes are regulated by multiple pathways *WOX5* mediated pathway, cytokinin, *RBR-SCARECROW* mediated pathway and some of the other pathways, brassinosteroid and ethylene hormones are also playing an important role.

So, we will stop this class here. In next class, we will more move towards the balance between cell division and differentiation and particularly vascular tissue development.

Thank you very much.