Plant Developmental Biology
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Lecture - 15
Shoot Development: SAM Maintenance

Welcome to Plant Developmental Biology course. So, in previous lectures we have finished some of the example of root development, today’s class we will start with Shoot Development and particularly we will focus today on Shoot Apical Meristem Maintenance. We have seen that during the development the basic body plan which is being established during the process of embryogenesis.

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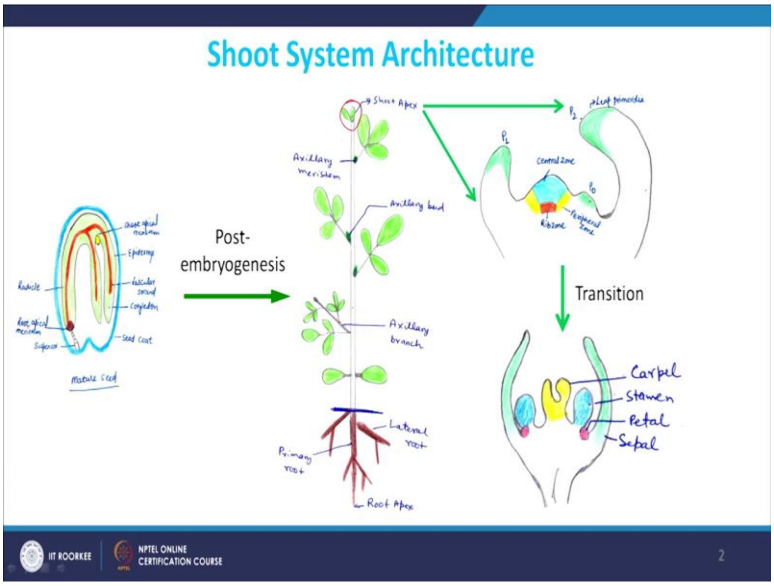

A typical mature embryo, here you can see that shoot and root system has been already the body plan the basic body plan for root and shoot system is already established. Body axis is established and then the root apical meristem and shoot apical meristems are positioned along the axis. And this is you have seen in root that this is very important for proper growth and development, particularly post embryonic development of different organs in both root system as well as in shoot system.

Shoot system after post embryonic development it makes mature plant. In mature plants you have many part or many organ depending on the plant species. In basic structure we have stem, some branches, some buds then later on after transition from vegetative phase to reproductive phase, we have some structure called inflorescence and in inflorescence we have flowers.

At the very apex called shoot apex, it has meristematic region or the region where stem cells are basically positioned. Stem cells which is responsible for primary growth of the shoot and this is called shoot apical meristem.

So, we will see in details what is happening and how the shoot apical meristems are maintained, an important thing here is that at the shoot apex there are coordinated process going on. So, the coordination between stem cell maintenance as well as cell differentiation. And, some cells basically the cells which is in blue in color this is stem cell, and this is being maintained throughout the life or till the plant is growing. And when these cells enters in this other regions, when it leave its place or when it leaves its domain it enters in the process of differentiation.

And in the differentiation depending on the identity of the meristem it takes different identity or different phase. For example, if the meristem identity is shoot then it will make lateral organ for shoot for example, leaf, but if the meristem identity is inflorescence then this will undergo the process of floral development. So, this is a typical example you can see this is primordia P0, P1, P2 different organs are developing and, but still a balance between cell division and cell differentiations are maintained.

And this shoot basically after certain time point, it transit to the reproductive phase, now it makes flowers. In flowers it makes sepals, petals, stamens and carpel and after making carpels in most of the flowers this process of stem cell maintenance is inhibited, it is totally suppressed. So, that is why there is no longer or there is no longer maintenance of stem cells and this is called determinate development. So, the indeterminacy get converted into determinate development. So, this is a view of shoot apical meristem and this is how shoot apical meristems are organized.

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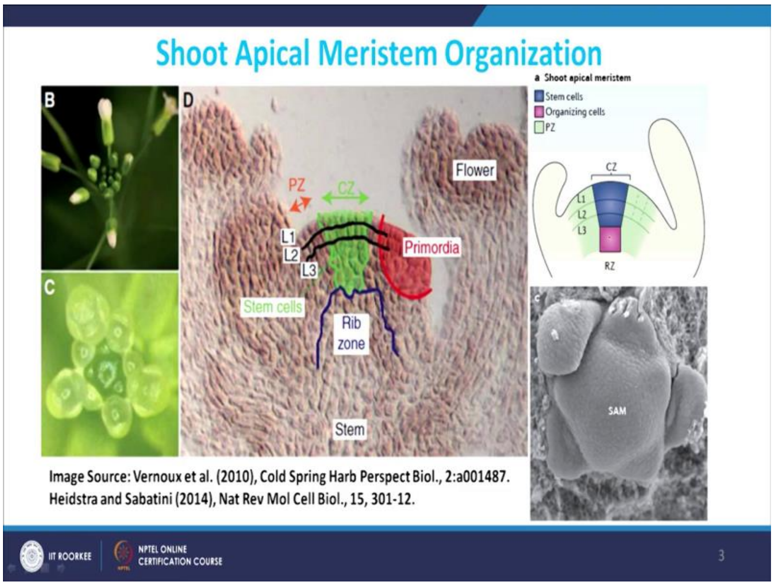

This is basically inflorescence meristem. If you look on the top, this is how growing, *Arabidopsis* inflorescence looks these are the different flowers, these are early flowers, these are mature flowers where you can see that they have already come if you look the longitudinal view. So, this is how the meristem is maintained, this is the central meristem and then you have the lateral organs coming. As I said depending on what is the nature of meristem if it is shoot meristem then the lateral organs will be leaf, when if it is inflorescence meristem the lateral organs will be flowers. Both are quite similar in process of establishment and differentiation.

If you look the shoot apical meristem there are certain domains. So, the domain which you look here this is called central zone, this is in the centre of the meristem then you have a layer just flanking the central region which is called peripheral zone. And then you have a domain or zone which is below the central domain which is called rib zone and this is the region the green color is basically stem cell region.

So, this domain is maintained and when the cells are present in the central zone or in the stem cell niche, they will have a property of cell division. But once they leave the central domain and enters in the peripheral region or enters in the rib zone, they basically loses its stem cell property and then initiate differentiation specific program.

Another important thing the shoot apical meristem also has different layers. So, you can have this is the first layer which is called L1, then the second layer which is below the L1 is called L2 and then the layer beneath it is called L3 layer. So, this is the organization of shoot apical meristem and this is the schematic view. So, as I said that the in the central zone this region is your stem cells and this region is also called organizing cells.

Positioning of organizing center is very important. Because organizing center is equivalent to QC of root apical meristem and it plays a very very important role in stem cell niche positioning and maintenance.

This is the scanning electron micrograph picture of the SAM you can see this is the central region and then these are the primordia or the lateral organs which are coming from this shoot apical meristem. If you look the establishment when the meristem shoot apical meristem is established it happens at very very early stage of embryogenesis.

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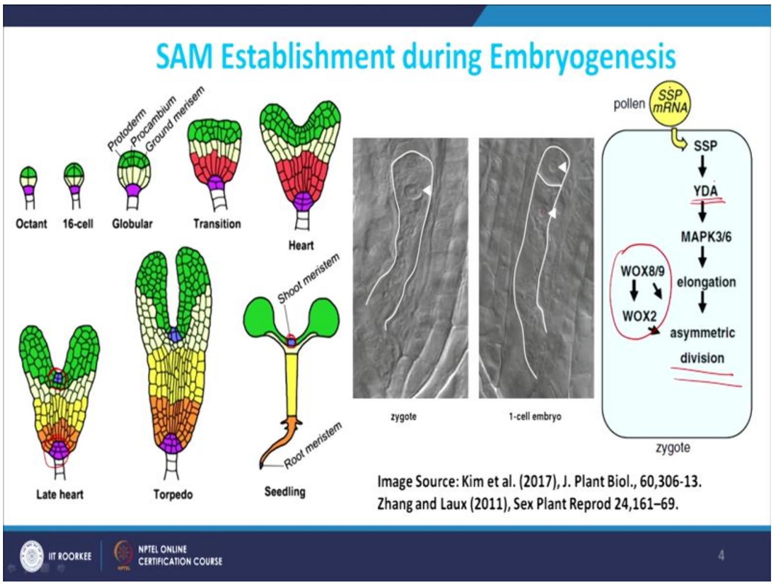

So, if you recall previous class you will find that the first cell which start cell division is zygote, and then zygote undergo the process of a series of cell division and it undergo different stages of embryo development. And during this stages at a particular stage if you look here this late state, this region is basically assigned as a shoot apical meristem and this region as a root apical meristem. And then you have a kind of in mature seedling if you look, shoot apical meristem is positioned here. If you look here, this is zygote, the single cell zygote and this is first cell embryo.

So, first cell division has occurred here and you can clearly see two nucleus, and if you look here even the first cell division is asymmetric. So, in development asymmetric cell division particularly in plant development, asymmetric cell division is one very very important process and which eventually give rise to the proper developmental program or establishing proper developmental program.

So, even in the zygote you can see here the first asymmetric cell division is basically establishing the process of later development. And this process are basically regulated by the first asymmetric cell division is regulated by two pathways. One path way which is mediated by YDA which is called YDA and another process is basically regulated by homeobox containing transcription factor WOX2, WOX8 and WOX9. And both of the pathway in the zygote they are basically regulating the process of asymmetric cell division. Interesting thing here is that this pathway is getting activated by SSP messenger RNA which is basically transmitted from the pollens.

So, the first step of shoot apical meristem initiation or you can see even the first state of embryogenesis begins with this activating these two pathways.

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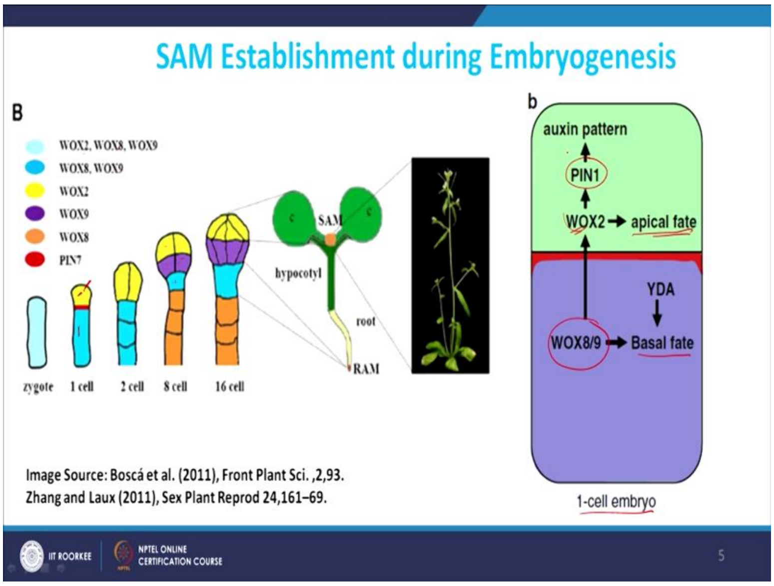

And then later on they play very important role. So, there is a dynamic expression and their transcript expression domain of these genes which basically helps in establishing apical fate of the cells or basal fate of the cells.

So, if you look here this is zygote. Zygote basically express all three transcription factor WOX2, WOX 8 WOX 9. But after first asymmetric cell division WOX2 is restricted to the apical cell which is smaller in size whereas, WOX 8 and WOX 9 they retain their expression in the basal cell. Later on you can see at this stage; 8 celled stage the apical most cells they retain WOX2 expression whereas, the basal side of the apical cells they basically have WOX 9 expression. And, this is the region which retains both WOX 9 and WOX 8, and WOX 8 alone is basically maintained in this cell.

So, this you can tell that that same transcription factors they are changing or their expression patterns are basically helping in establishing proper apical and basal patterning during the embryogenesis, and this is the schematic diagram you can see here.

So, in basal fate after cell division at first cell embryo, the basal cell fate is basically promoted by WOX 8 and WOX 9. And then WOX 8 and WOX 9 somehow restrict WOX2 expression to the apical fate, and WOX2 basically active PIN1; which is a kind of regulator of auxin signaling pathway.

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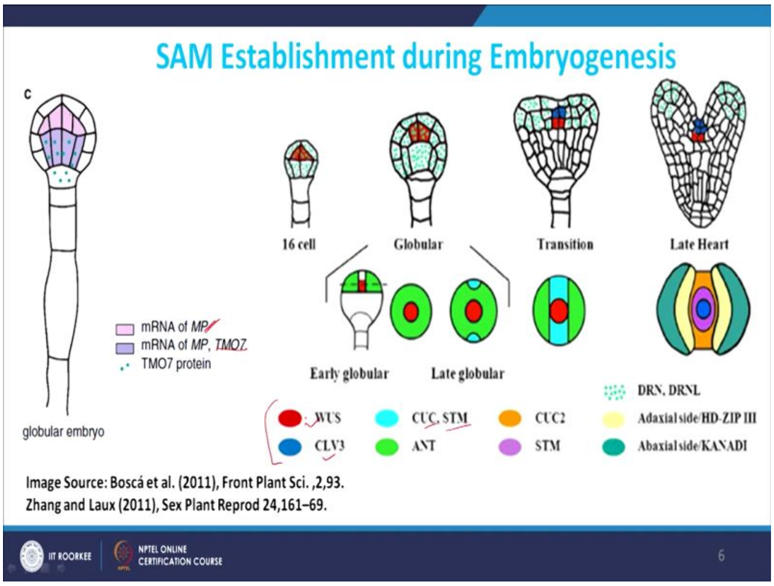

And then this is another regulators and how they basically helps in positioning the stem cell or positioning or establishing shoot apical meristem during embryogenesis. MP is transcription factor which is AUXIN RESPONSE FACTOR 5.

So, if you look here the messenger RNA of MP is expressed here in the apical side whereas, messenger RNA overlap here with the TMO7 whereas, TMO7 protein is present here as well as here. And some other regulators of shoot apical meristem establishment and maintenance are getting also activated during the process of embryogenesis. For example, you will see later on this *WUSCHEL, CLAVATA 3, CUC, STM* all these activators are basically expressed or activated at particular stage in a particular cell. Some of them are very important which we will discuss in detail for example, this WUSCHEL-CLAVATA signaling; WUSCHEL CLAVATA signaling is very very important and if you look the pattern here *WUSCHEL* is getting activated and then *CLAVATA* is coming up. When *CLAVATA* is coming up, *WUSCHEL* is restricted here *CLAVATA* is on the upper layer. So, if you look at this stage of embryo. You can see that *CLAVATA3* are somewhere here and *WUSCHEL* is post here how this is happening we will see in the next slide, and SAM establishment is regulated.

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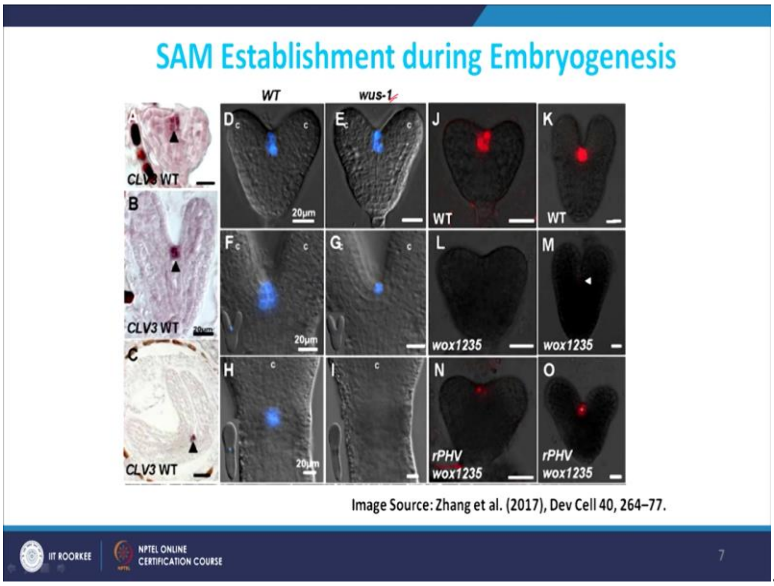

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WOX is playing very important role, but if you have just single *wox2* mutant we do not have a very strong phenotype. If you look the four mutants together *wox 1, 2, 3* and *5* then the effect is quite significant and what you can see here? This is the expression pattern this is *in-situ* hybridization of *CLAVATA3* see. So, if you look at the very early stage of embryogenesis you can see that it is expressed here at the tip then it basically remains here at the upper side of the shoot apical meristem. And here you can very clearly see with the fluorescent marker, at different developmental stages of the embryo.

And then if you have *wuschel* mutant, when *WUSCHEL* is not present; at early stage if you see *CLAVATA3* expression is ok, but later on the *CLAVATA3* expression is disappearing ok.

And if you look this mutant in this mutant basically what happens *CLAVATA3* is not present even from the start. So, *CLAVATA3* which is very important regulator of shoot apical meristem is missing in *wox* mutant background. But when you put *PHABULOSA* ; *PHABULOSA* if you recall some of the previous class we have little bit discussed about it. This is a class of homeobox domain containing transcription factor and it is regulated by micro RNA. So, if you put *PHABULOSA* this is basically resistance to the micro RNA.

So, if you create some kind of point mutation at the side of micro RNA binding, then what happens this gene is no longer regulated by micro RNA, then you can have only one regulation. And when you put back this *PHABULOSA* in *wox* mutant then you can see that the expression of *clavata3* is coming up. So, this basically tells that these critical regulators of stem cells they are basically playing role in shoot apical meristem.

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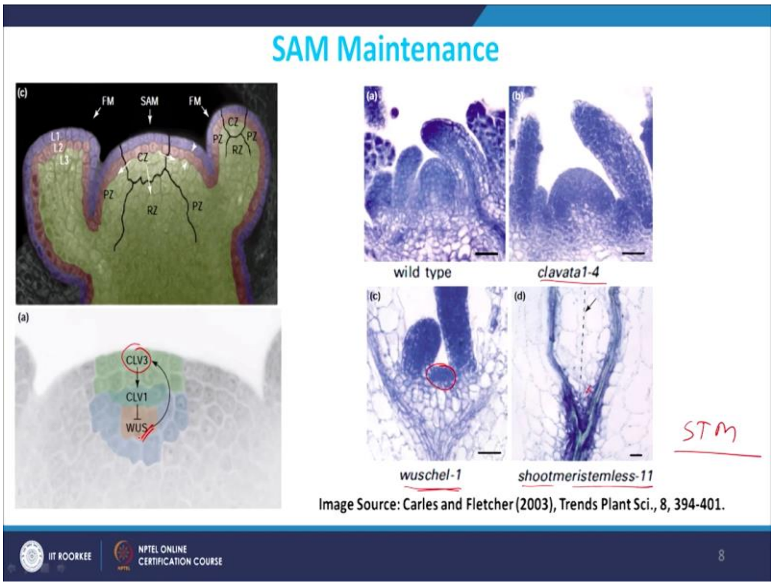

So, now if you look here what are the key regulators which are responsible for shoot apical meristem maintenance? So this is the central zone, this is the rib zone, this is the peripheral zone L1, L2 layer and this is the lateral organs coming and if you look some of the mutants. So, this is basically wild type you can see shoot apical meristem very nice, but if you have *clavata* mutant you can see that the shoot apical meristem or the meristem is slightly enlarged. So, there is more or bigger meristem size.

Which suggest that, *CLAVATA* is playing some role in may be restricting shape and may be size of the shoot apical meristem; whereas if you see *wuschel* mutant in *wuschel* background, you see meristem like structure, but it looks that it is terminated early. So, there is premature termination of the meristem here whereas here is the gain of meristem meristematic activity. But if you look this mutant this *stm; shoot meristem less* mutant here you do not see any meristem, so which suggest that *STM* is working at very early stage to even initiate shoot apical meristem specific program and if you do not have *STM*, the meristems are not getting formed.

But these genes they are more working slightly later stage, they are more important for maintaining the meristem. So, if you look here the very general way what happens that, this *CLAVATA3* is basically expressed here and actually the expression of *CLAVATA3* is activated by *WUSCHEL*. So, if you recall *WUSCHEL* is first expressed here. So, *WUSCHEL* is expressed early then wuschel is basically activating expression of *CLAVATA3,* but once it activate expression of *CLAVATA3*. Once *CLAVATA3* is on *CLAVATA3* is; so, basically *CLAVATA3* is a kind of signaling peptide, it is received by receptor which is *CLAVATA1* and once this signaling is activated it inhibit expression domain of *WUSCHEL.*

CLAVATA signaling pathway ensures that *WUSCHEL* expression domain or transcription of *WUSCHEL* is restricted to domain of expression, and if you recall this looks quite similar to the signaling modules, which we have during the root apical meristem development. There we have *CLE40* is regulating the expression of *WOX5* and this is quite similar. So, this is basically a negative feedback regulation. So, wuschel is activating *CLAVATA3* and *CLAVATA* 3 is repressing wuschel domain.

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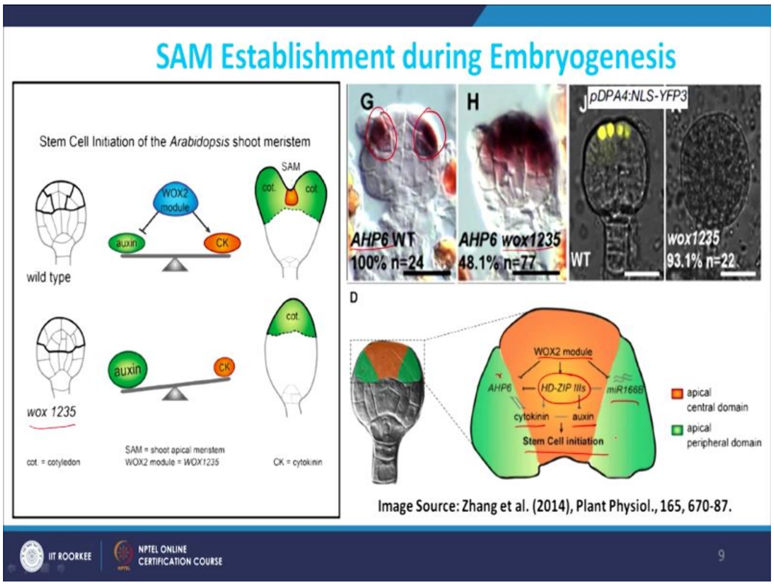

Another important regulator of the shoot apical meristem is auxin. The balance between auxin and cytokinin is important and this balance is embryogenesis is regulated by *WOX2* module.

So, if you have *WOX2* modules the balance between auxin and cytokinin is maintained, and this maintained balance is basically required or it is very important for regulating or coordinating and maintaining balance between cell division and cell differentiation. And if you have *WOX* mutants here the balance is basically disturbed and that is why you see the defect.

Another important thing in the apical domain so, in apical domains; this is apical domain in the peripheral of the apical domains, some of the genes are activated one of this gene is *AHP6* and *AHP6* basically is a negative regulator of cytokinin signaling. And if you look here it is very specifically expressed only in the peripheral domain of the apical regions and in *WOX* mutant this expression domain is disturbed and you have *AHP* expression everywhere.

Similarly, this is a kind of another marker which is known to express in the apical region and in mutant background this is disappear. So, this suggest that *WOX* mutants are very very important in assigning a proper fate to the apical region of the meristem. So, this is if you look the apical region what is happening, in the apical side there are peripheral domain and the central domain. So, in central domain *WOX2* module is basically getting activated *WOX2* module is regulating, homeodomain zip transcription factor through micro RNA micro RNA is also regulating we will see may be in the later slides and in here in this domain. So, basically *WOX2* is restricting expression of *AHP6* and micro RNA in the peripheral domain.

So, when you do not have *WOX2* that is why you can see that *AHP6* expression is coming in the central domain. And this kind of pathway regulates a critical amount of auxin and cytokinin signaling pathway and this critical balance is regulating stem cell initiation and a balance between stem cell division and cell differentiation program. Ok so, again this hormonal regulation is very important, it is extremely important for maintaining shoot apical meristem. If you look, this case; so, here is basically *STM* expression, *STM* express in the meristem then *CUC* is a kind of transcription factor which regulates at the boundary of the organs.

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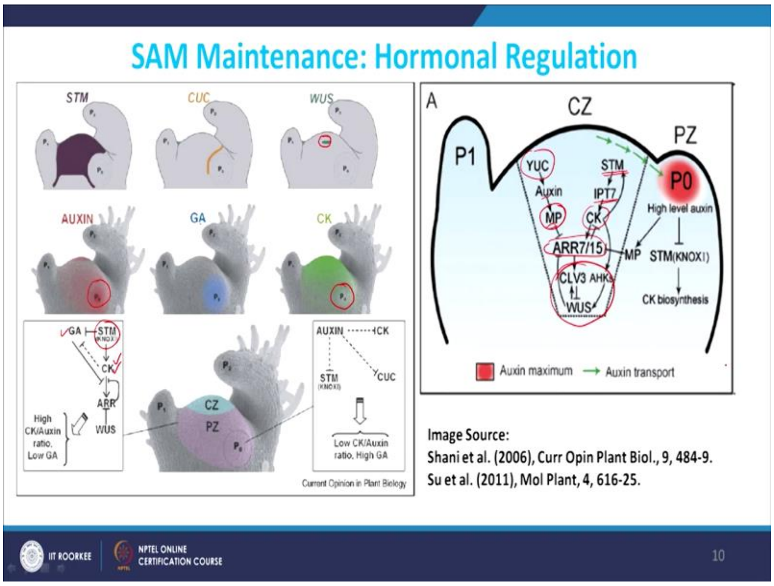

So, basically this is expressed here in the boundary between meristem and the organs this is very important. And this boundary has to be maintained very clearly and then you have *WUSCHEL*, *WUSCHEL* is expressed in the very center region of the meristem. If you look the responses of auxin cytokinin and gibberellic acid what you see that, cytokinin is very dominantly present in the meristematic region in the stem cell region whereas, the response of cytokinin in organ primordia is very very low, but auxin has about opposite pattern.

So, if you look the meristematic region; most of the meristematic region they do not have very high auxin signaling whereas the primordia the organ primordia they have high amount of auxin signaling. Gibberellic acid is having quite similar to the auxin, but auxin is also having some responses here.

So, this suggest that maybe cytokinin is positively regulating or very important for regulating the meristematic activity in shoot apical meristem whereas, auxin is playing major role in the process of organogenesis starting new organ or the peripheral or the lateral organ initiation and that is how it happen. So, if you look this structure this is central zone peripheral zone.

So, in the peripheral zone you have high cytokinin to auxin ratio that is you have high cytokinin and low auxin. So, that is why and gibberellic acid is low and *STM* is present everywhere. *STM* is a positive regulator of cytokinin signaling and negative regulator of gibberellic acid signaling. *STM* is activating cytokinin signaling in the meristematic region, where gibberellic acid is low auxin is low, then *WUSCHEL* is getting activated. If you have high amount of cytokinin, it activates *ARR* and then *WUSCHEL* also regulate. So, there is a kind of feedback regulation going on here.

High amount of cytokinin and *STM* they ensures meristematic property, but if you come in this region where the organs are coming or the site where organogenesis has to start, there is low cytokinin and auxin ratio that is high auxin and low cytokinin. If you have high auxin, it is inhibiting *STM* activity there it is inhibiting cytokinin activity and it is probably inhabiting *CUC* activity.

And all this process is helping in initiation of organogenesis specific program and this is together ensuring that in the meristematic region or in the central region cell should be dividing, but in the region where primordia has to come their cell division has to be restricted or the stem cell property has to be restricted, and should have organogenesis specific property.

So, if you look the central region in central region *STM* is activating cytokinin biosynthetic enzyme which is *IPT7* and you have high amount of cytokinin and then cytokinin is regulating expression of cytokinin response regulators and then it is basically helping in establishing *WUSCHEL CLAVATA* signaling or regulating *WUSCHEL CLAVATA* signaling pathway.

On the other hand if you look *YUCCA*, auxin synthesis gene. Auxin is activating transcription factor auxin response factor, and they are basically inhibiting here *ARR* activity. Loop is establishing the stem cell identity. But in the peripheral zone or in the region where you have to have a primordia where *MP* is getting activated by high auxin and *STM* is getting repressed and this results in primordia formation in the peripheral zone.

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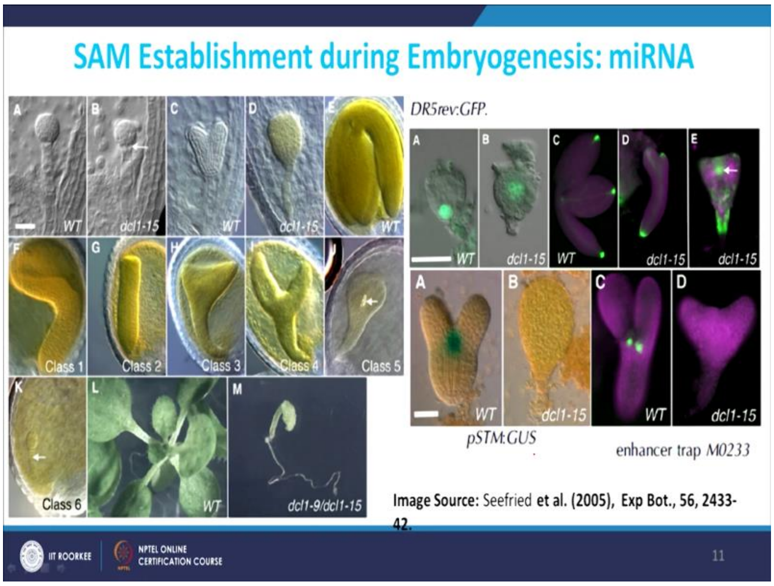

Apart from auxin and this key regulator of *WUSCHEL* and *CLAVATA* signaling pathway, micro RNA has been identified as a very important regulator of stem cell property or shoot apical meristem function. For example, if you have *dcl1* mutant, *DCL1* is basically a system which helps in biogenesis of micro RNA or small RNAs.

So, if you have mutant; micro RNA pathways are getting disrupted, and if you look here in the mutant background what happens that proper embryogenesis is not taking place and if you look at this stage the double mutant has unorganized growth or development. There are different types of phenotype or different classes of phenotype, but eventually what is happening that the proper organization and maintenance of shoot apical meristematic is not happening in this mutant background.

And if you look the auxin response or the expression of *STM* because this proper auxin response has to be initiated. So, proper auxin maxima has to be achieved where it should be achieved if that is not happening then organogenesis will not start. At the same time

STM which is very important regulator of meristematic activity has to be activated for maintaining. So, both the process has to be function properly in order to have a proper balance.

And if you look in this mutant background neither proper auxin response are being generated if you look here auxin response is very dis-localized or it is not at the right place. This is a kind of a typical auxin response in the wild type, but if you look in the *dcl* mutants, they are very diffused kind of response it is not getting properly organized.

At the same time if you look the *dcl* mutant and if you look the expression of *STM* promoter activity in wild type, it is very nicely positioned and express in the right domain, but in mutant background the expression is no longer here and this is true for another marker for shoot apical meristem.

So, this suggest that when you have disrupted micro RNA pathway, essentially neither stem cell is maintained nor proper organogenesis is taking place because auxin maxima not being generated or proper auxin responses are not getting generated here. So, this suggest that micro RNAs working as one of the critical pathway to regulate the steps and activity in shoot apical meristem.

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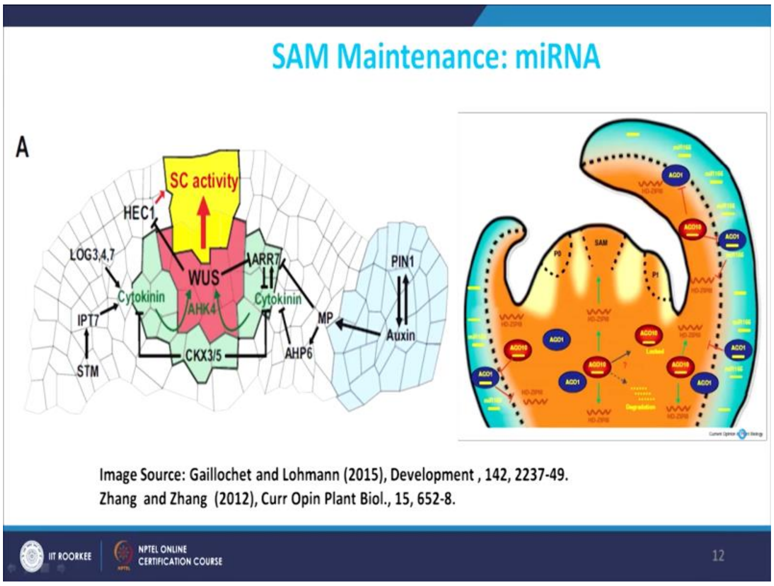

So, this is a typical shoot apical meristem, you have stem cell activity going on here then you have *WUSCHEL* expression domain; CLAVATA3-WUSCHEL pathways are working here. WUSCHEL is negatively regulating here ARR7. And this is cytokinin signaling pathway and then LOG is cytokinin biosynthetic enzyme.

So, STM is activating cytokinin biosynthesis through IPT7 and LOGs are getting activated here. Eventually in this region we are having a high amount of cytokinin and cytokinin is maintaining *WUSCHEL* and *ARRs* is maintaining here a proper stem cell property. But if you come in the in the lateral region or in the peripheral zone, where auxin is high it is activating transcription factor MP which is restricting the domain of cytokinin in the meristematic region, but here it should be repressed so, that the organogenesis process should start.

And if you look here, this is slightly complex slide, but just to generally tell you that how micro RNA is helping in establishing a proper polarity or stem cell maintenance. So, here you can see the asymmetric distribution. If you look this side which is basically abaxial side you have high amount of micro RNAs and that is why its target which is HD ZIP is low at this side, and if you look towards the adaxial side, it is having high amount of HD ZIP and low amount of micro RNA.

So, even the gradient or the amount of micro RNA and a particular transcription factor which is regulated by micro RNA are very important in positioning the stem cell activity and organogenesis together and maintaining a coordination between stem cell activity as well as organogenesis. So, we will stop here in next class we will continue shoot development part.

Thank you very much.